European Commission

Combined Draft (Renewal) Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

DODINE

Volume 1

December 2023

Rapporteur Member State: Spain
Co-Rapporteur Member State: Germany

Version History

When	What
May 2023	Initial DRAR – RMS Spain
October 2023	DRAR revised following receipt of applicant and Co-Rapporteur Member State comments
December 2023	DRAR revised following EFSA´s completeness check DRAR updated after ECHA Accordance check.

The RMS is the author of the Assessment Report. The Assessment Report is based on the validation by the RMS, and the verification during the EFSA peer-review process, of the information submitted by the Applicant in the dossier, including the Applicant's assessments provided in the summary dossier. As a consequence, data and information including assessments and conclusions, validated and verified by the RMS experts, may be taken from the applicant's (summary) dossier and included as such or adapted/modified by the RMS in the Assessment Report. For reasons of efficiency, the Assessment Report should include the information validated/verified by the RMS, without detailing which elements have been taken or modified from the Applicant's assessment. As the Applicant's summary dossier is published, the experts, interested parties, and the public may compare both documents for getting details on which elements of the Applicant's dossier have been validated/verified and which ones have been modified by the RMS. Nevertheless, the views and conclusions of the RMS should always be clearly and transparently reported; the conclusions from the applicant should be included as an Applicant's statement for every single study reported at study level; and the RMS should justify the final assessment for each endpoint in all cases, indicating in a clear way the Applicant's assessment and the RMS reasons for supporting or not the view of the Applicant.

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Level 1

DODINE

1 STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1 CONTEXT IN WHICH THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the draft assessment report was prepared

According to this Regulation "An application for renewal shall be submitted electronically via a central submission system using the format as set out in Article 7 by a producer of the active substance no later than three years before the expiry of the approval."

The Company Arysta LifeScience Benelux SPRL notified its intention to support Dodine for Annex I renewal to the Commission, the Rapporteur, the co-Rapporteur and EFSA in order to prepare for the upcoming expiry of Annex I inclusion, in May 2018.

Arysta LifeScience Benelux SPRL applied for the renewal of the active substance Dodine into the list of approved substances according Regulation (EC) No 1107/2009. This draft assessment report was prepared by Spain as Rapporteur Member State for the renewal of the approval of the active substance Dodine according the Regulation (EC) No 1107/2009

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

1.1.3 EU Regulatory history for use in Plant Protection Products

The existing active substance Dodine was included in Annex I of Council Directive 91/414/EEC (Commission Directive 2011/9/EU).

With Commission Implementing Regulation (EU) No 540/2011 implementing Regulation (EC) No 1107/2009 as regards the list of approved active substances, Dodine was included in the list of approved active substances according to Regulation (EC) No 1107/2009.

With Commission Implementing Regulation (EU) No 2020/2007, the expiry date of the approval of Dodine was set to 31.08.2024.

Commission Implementing Regulation (EU) No 2020/1740 sets out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

1.1.4 Evaluations carried out under other regulatory contexts

No applicable

1.2 APPLICANT INFORMATION

1.2.1 Name and address of applicant(s) for approval of the active substance

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Name:	Arysta Lifescience Benelux SPRL
Address:	Rue de Renory 26, Boîte 1 Postal code: 4102 Ougrée (Seraing) Belgium

Person to contact

Name and address: [redacted]
Rue de Renory 26, Boîte 1
Postal code: 4102
Ougrée (Seraing)
Belgium

Phone [redacted]

E-mail: [redacted]

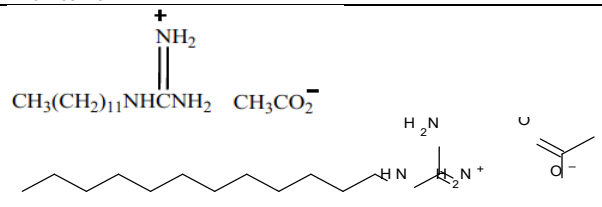
1.2.2 Producer or producers of the active substance

CONFIDENTIAL information - data provided separately (DRAR, Volume 4)

1.2.3 Information relating to the collective provision of dossiers

Not relevant. The Company Arysta LifeScience Benelux SPRL is the only notifier of the active substance Dodine.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	Dodine
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	1-dodecylguanidinium acetate
CA	Guanidine, N-dodecyl-, acetate (1:1)
1.3.3 Producer's development code number	none
1.3.4 CAS, EEC and CIPAC numbers	
CAS	2439-10-3
EEC	219-459-5
CIPAC	101
1.3.5 Molecular and structural formula, molecular mass	
Molecular formula	$C_{15}H_{33}N_3O_2$
Structural formula	
Molecular mass	287.4 g/mol
1.3.6 Method of manufacture (synthesis pathway) of the active substance	CONFIDENTIAL information - data provided separately (DRAR, Vol 4)
1.3.7 Specification of purity of the active substance in g/kg	The minimum purity of the active substance Dodine proposed by the applicant is 980 g/kg Purity for the first approval (Commission Directive 2011/9/EU): 950 g/Kg FAO specification (101/TC/S (1988) (AGP:CP/236)):

	Dodine: min. 950 g/kg
1.3.8 Identity and content of additives (such as stabilisers) and impurities	
1.3.8.1 Additives	***CONFIDENTIAL information - data provided separately (DRAR, Vol 4)***
1.3.8.2 Significant impurities	***CONFIDENTIAL information - data provided separately (DRAR, Vol 4)***
1.3.8.3 Relevant impurities	The active substance as manufactured does not contain impurities that are particularly undesirable because of their toxicological, ecotoxicological or environmental properties
1.3.9 Analytical profile of batches	CONFIDENTIAL information - data provided separately (DRAR, Vol 4)

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1 Applicant	Arysta LifeScience Benelux SPRL
1.4.2 Producer of the plant protection product	[redacted]
1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product	Trade name for the formulation: Dodine 544 SC
1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product	
1.4.4.1 Composition of the plant protection product	**content of pure active substance :** **544 g/L** **(52.88% w/w)** limits : 519 – 569 g/L [50.45 – 55.3] (% w / w) *d = 1.0287 g/mL* **content of technical active substance :** **555.1 g/L** **(53.96% w/w)** limits : 529.6 – 580.6 g/L [51.48 – 56.44] (% w / w) at a minimum purity of the technical active substance of 98%.
1.4.4.2 Information on the active substances	**Type** / **Name/Code Number** ISO common name: Dodine CAS No.: 2439-10-3 EC No.: 219-459-5 CIPAC No.: 101 Salt, ester anion or cation present: -- **RMS comment:** Cation: 1-dodecylguanidinium Anion: acetate
1.4.4.3 Information on safeners, synergists and co-formulants	CONFIDENTIAL information (DRAR, Vol 4)
1.4.5 Type and code of the plant protection product	Suspension Concentrate [Code: SC]

1.4.6 Function	Dodine is a fungicide with protectant and some curative activity.
1.4.7 Field of use envisaged	Dodine is a fungicide to be used against scab on apples and pears, against cherry leaf spot on cherry and against peach leaf curl on peaches. Dodine is a fungicide with protectant and some curative activity. Dodine is fungitoxic in action preventing disease infection and establishment. Dodine is currently used as a fungicide against scab (*Venturia inaequalis*/*Venturia pyrina*) on pome fruits (Apple/Pear/quince/medlar/loquat), leaf spot (*Blumeriella jaapii*) and leaf scorch (*Gnomonia erythrostoma*) on cherry, leaf curl (*Taphrina deformans*) on peach, nectarine, leaf spot of olives (*Cycloconium oleaginum*), anthracnose of walnut, leaf spot of chestnut and pistachios, leaf blotch of almonds and leaf spot of poplar (*Drepanopeziza punctiformis*).
1.4.8 Effects on harmful organisms	Dodine is fungitoxic in action preventing disease infection and establishment. Dodine is intended to be used as a fungicide against scab *(Venturia inaequalis/Venturia pyrina*) on pome fruits (Apple/Pear), leaf spot (*Blumeriella jaapii*) on cherry and leaf curl (*Taphrina deformans*) on peach, nectarine. Dodine has a translaminar action. Dodine penetrates partially in the leaves and stops the disease. It is a multisite inhibitor acting mainly on the fungus membranes.

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1 Details of representative uses

Dodine is currently used as a fungicide on pome fruits (apple/pear/quince/medlar/loquat), on cherry, peach and nectarine, olives, walnut, chestnut and pistachios, almonds, and poplar.

Representative uses for this application are pome fruit, cherry, and peach.

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min-max (l)	Water L/ha min-max	kg a.s./ha min-max (l)		
Apples / Pear	SEZ, CEZ, NEZ	Dodine 544 SC	F	Scab *(Venturia ineaqualis* [VENTIN] / *Venturia pyrina* [VENTPI])	*SC*	*544*	Foliar sprayi ng	BBCH 01-till 60 days before harvest	a) 1-2 b) 2	21 d	a) 1.25 b) 2.5	a) 0.68 b) 1.36	500-1500	60	
Cherry	SEZ, CEZ, NEZ	Dodine 544 SC	F	Cherry leaf spot *(Blumeriella jaapii* [BLUMJA] = *Coccomyces hiemalis)*	*SC*	*544*	Foliar sprayi ng	BBCH 60-BBCH 79 and/or BBCH 91-BBCH 97	a) 1-2 b) 2	21 d	a) 1.25 b) 2.5	a) 0.68 b) 1.36	500-1500	14	
Peach	CEZ, SEZ	Dodine 544 SC	F	Peach leaf curl *(Taphrina deformans* [TAPHDE]*)*	*SC*	*544*	Foliar sprayi ng	BBCH 01-BBCH 69 and/or BBCH 95-97	a) 1-2 b) 2	21 d	a) 1.65 b) 3.3	a) 0.9 b) 1.8	600-1500	Cover ed by vegat ation perio d	

* F: professional field use, G: professional greenhouse use, I: indoor application

(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)
(c) *e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated
(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**
(j) Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of applications possible under practical conditions of use
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha

	(m) PHI - minimum pre-harvest interval

Summary of additional intended uses for which MRL applications have been made, that in addition to the uses above, have also been considered in the consumer risk assessment (*name of active substance or the respective variant*)

Regulation (EC) N° 1107/2009 Article 8.1(g))

1.5.2 Further information on representative uses

Composition of the formulation:

- Active substance: Dodine : 544 g/L (519 - 569 g/L) (values above are calculated for pure Dodine Technical conten (min. 95%): Typical concentration: 572.6 g/L Concentration range: 546.2 - 598.9 g/L)
- other components: Confidential (Please see DODINE_DRAR_22_Volume_4, C.1.3.2)
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Application rate:

Crop	Application rate per treatment		Max. annual appliation rate (a.s.)	Water amount / spray volume	Conc. of formulation in dilution
	product	active substance			
Malus domestica (Apple) (MABSD) ; Pyrus communis (Common pear) (PYUCO)	1.25 L/ha	0.68 kg/ha	1.36 kg/ha	>=500 - <=1500 L/ha	>=0.083 - <=0.25 L/hL
Prunus avium (Cherry) (PRNAV) ; Prunus cerasus (Amarello cherry) (PRNCE)	1.25 L/ha	0.68 kg/ha	1.36 kg/ha	>=500 - <=1500 L/ha	>=0.083 - <=0.25 L/hL
Prunus persica (Peach) (PRNPS)	1.65 L/ha	0.9 kg/ha	1.8 kg/ha	>=600 - <=1500 L/ha	>=0.11 - <=0.275 L/hL
Prunus persica (Peach) (PRNPS)	1.65 L/ha	0.9 kg/ha	1.8 kg/ha	>=600 - <=1500 L/ha	>=0.11 - <=0.275 L/hL

Method of application

Crop	Type of method	Target
Malus domestica (Apple) (MABSD) ; Pyrus communis (Common pear) (PYUCO)	air assisted broadcast spraying [spray] ; broadcast [spray]	foliage/plant

Prunus avium (Cherry) (PRNAV) ; Prunus cerasus (Amarello cherry) (PRNCE)	air assisted broadcast spraying [spray] ; broadcast [spray]	foliage/plant
Prunus persica (Peach) (PRNPS)	air assisted broadcast spraying [spray] ; broadcast [spray]	foliage/plant
Prunus persica (Peach) (PRNPS)	air assisted broadcast spraying [spray] ; broadcast [spray]	foliage/plant

Number of applications, treatment intervals and crop growth stage

Crop	No applications	Re-treatment interval (d) and treatment window for dispensers	Growth stage of crop		
			first application	last application	season
Malus domestica (Apple) (MABSD) ; Pyrus communis (Common pear) (PYUCO)	1 - 2	21	01 - Beginning of seed imbibition; Beginning of bud swelling (P, V)	- Application till 60 days before harvest	
Prunus avium (Cherry) (PRNAV) ; Prunus cerasus (Amarello cherry) (PRNCE)	>=1 - <=2	21	60 - First flowers open (sporadically)	79 - Nearly all fruits have reached final size - and/or after harvest	
Prunus persica (Peach) (PRNPS)	>=1 - <=2	21	01 - Beginning of seed imbibition; Beginning of bud swelling (P, V)	69 - End of flowering: fruit set visible - and /or later from 50% leaf falling till after leaf falling (autumn)	
Prunus persica (Peach) (PRNPS)	>=1 - <=2	21	01 - Beginning of seed imbibition; Beginning of bud swelling (P, V)	69 - End of flowering: fruit set visible - and /or later from 50% leaf falling till after leaf falling (autumn)	

Compatibility with IPM Strategies

Not relevant

NECESSARY WAINTING PERIODS OF OTHER PRECUATIONS TO AVOID PHYTOTOXICITY EFFECTS ON SUCCEEDING CROPS

This data point is not applicable; no waiting periods need to be defined.

Succeeding crops are of no relevance for the intended use of Dodine 544 SC.

Limitations on choice of succeeding crops:

Not relevant, Dodine 544 SC is applied in orchards.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

1.5.4 Overview on authorisations in EU Member States

Dodine containing products are widely authorised in European countries. For details, please refer to the following table LIST OF CURRENTLY AUTHORIZED USES AND EXTENT OF USE.

LIST OF CURRENTLY AUTHORIZED USES AND EXTENT OF USE.

Currently registered uses of Dodine:

Country	**Product name**	**Active substance content**	**Registration number**	**Authorized uses (crops, Harmful organisms, Rates and no of applications, Timing, growth stage)**	
Austria	Syllit 450 SC	Dodine 450 g/L SC	971-0	Apple/Pear	Scab (Venturia sp.), 1.5 L/ha in 4 applications (max. 2 after flowering) from the bud opening till 60 days before harvest.
				Cherry	Leaf spot (*Blumeriella jaapii*), 1.5 L/ha in 2 applications from petal fall till 2 weeks before harvest. Post-harvest applications possible on infected trees.
Belgium	Syllit 400 SC	Dodine 400 g/L SC	8418/B	Apple/Pear	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Sweet or sour cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
	Syllit 544 PRO	Dodine 544 g/L, SC	10597P/B	Apple/Pear	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Sweet or sour cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
Bulgaria	Syllit 544 SC	Dodine 544 g/L SC	01507-PPP-2/17 04 2014	Apple/Pear	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Peach/Nectarine	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud opening till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Olive	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn, 7 days before harvest.
Croatia	Syllit 544 SC	Dodine 544 g/L SC	UP/I-320-20/17-03/383	Apple/Pear	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Peach/Nectarine	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud opening till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.

Country	Product name	Active substance content	Registration number	Authorized uses (crops, Harmful organisms, Rates and no of applications, Timing, growth stage)	
				Olive	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn, 7 days before harvest.
Cyprus	Syllit 544 SC	Dodine 544 g/L SC	3330	Apple/Pears	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Peach/Nectarine	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud opening till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
				Cherry and sour cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Olives	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn, 7 days before harvest
				Almonds/Pistachios/Walnut/Chestnut	Antracnose of walnut, leaf spot of chestnut and pistachios, leaf blotch of almonds at 2 applications of 0.68 kg as/ha at 120 days of harvest.
Denmark	Syllit 544 SC	Dodine 544 g/L SC	36129	Apple/Pear	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
Estonia	Syllit 544 SC	Dodine 544 g/L SC	0535/21.11.14	Apple/Pear	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
Finland	Syllit 544 SC	Dodine 544 g/L SC	3191	Apple/Pear	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
France	Syllit 544 SC	Dodine 544 g/L SC	2160756	Apple/Pear	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Peach/Nectarine	Leaf curl (Taphrina deformans), at 2 applications of 0.9 kg as/ha from bud opening till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
				Olive	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn, 7 days before harvest.

Country	Product name	Active substance content	Registration number	Authorized uses (crops, Harmful organisms, Rates and no of applications, Timing, growth stage)	
Germany	Syllit	Dodine 400 g/L SC	025427-00	Apple/Pear	Scab (*Venturia sp*.) at 1 application of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Sweet or sour cherry	Anthracnosis of cherry *Blumeriella jaapii* at 1 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
Greece	Syllit 544 SC	Dodine 544 g/L SC	60592	Apple/Pears	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Peach/Nectarine	Leaf curl (Taphrina deformans), at 2 applications of 0.9 kg as/ha from bud opening till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
				Cherry and sour cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Olives	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn, 7 days before harvest
				Almonds/Pistachios/Walnut/Chestnut	Antracnose of walnut, Leaf spot of chestnut and pistachios, leaf blotch of almonds at 2 applications of 0.68 kg as/ha at 120 days of harvest.
Ireland	Syllit 544 SC	Dodine 544 g/L SC	PCS-04739	Apple/Pear:	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
Italy	Syllit Flo	Dodine 400 g/l SC	7369	Apple/Pears	Scab (*Venturia sp.)* at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry and sour cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Peach/Nectarine	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud opening til petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
				Olives	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn, 7 days before harvest
				Poplars	Leaf spot of poplar (*D. punctiformis*) at 2 applications of 0.9 kg as/ha, between June and August
		Dodine 544 g/L SC	15748	Apple/Pear/Medlar/Loquat	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest

Country	Product name	Active substance content	Registration number	Authorized uses (crops, Harmful organisms, Rates and no of applications, Timing, growth stage)	
	Syllit 544 SC			Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Peach/Nectarine	Leaf curl (Taphrina deformans), at 2 applications of 0.9 kg as/ha from bud swelling till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
				Olives	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn, 7 days before harvest
				Poplars	Leaf spot of poplar (*D. punctiformis*) at 2 applications of 0.9 kg as/ha, between June and August
	Syllit 65	Dodine 65% WG	3412	Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Peach/Nectarine	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud swelling till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
				Olives	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn, 7 days before harvest
				Apple/Pear/Medlar/Loquat	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
Latvia	Syllit 544 SC	Dodine 544 g/L SC	477	Apple/Pears	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
Lithuania	Syllit 544 SC	Dodine 544 g/L SC	AS2-4F/2015	Apple/Pears	Scab (*Venturia sp.)* at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
Netherlands	Syllit Flow 400 SC	Dodine 400 g/L SC	11647 N W 3	Apple/Pear	Scab (*Venturia sp*.) at 1 application of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Sweet or sour cherry	Anthracnosis of cherry *Blumeriella jaapii* at 1 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
Poland		Dodine 544 g/L SC	R246/2017	Apple/Pear/Medlar/Loquat	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest

Country	Product name	Active substance content	Registration number	Authorized uses (crops, Harmful organisms, Rates and no of applications, Timing, growth stage)	
	Syllit 544 SC			Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Peach/Nectarine	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud swelling till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
Portugal	REPIMAX Syllit 544 SC	Dodine 544 g/L SC	1605 1250	Apple/Pear/Medlar/Quince	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Peach/Nectarine	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud swelling till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
				Olives	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn, 7 days before harvest. For tables olive, in Autumn treatment, only after harvest.
	Syllit 400 SC	Dodine 400 g/L	3667	Apple/Pear/Medlar/Quince	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
Romania	Syllit 400 SC	Dodine 400 g/L SC	2154	Apple	Scab (*Venturia sp*.) at 2 applications 0.13% (1.95 L/ha), from bud opening until 60 days before harvest
				Plum	*Polystigma rubrum* at 2 applications at 0.13% (1.3 L/ha)
				Peach	Leaf curl (*Taphrina deformans*), at 2 applications of 0.2% (2 L/ha) from bud swelling till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
Slovakia	Syllit 544 SC	Dodine 544 g/L SC	20-00727-AU	Apple/Pear/Quince	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Peach/Nectarine	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud swelling till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
	Syllit 400 SC	Dodine 400 g/L SC	12-02-1236	Apple	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest

Country	Product name	Active substance content	Registration number	Authorized uses (crops, Harmful organisms, Rates and no of applications, Timing, growth stage)	
				Peach	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud swelling till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
Slovenia	Syllit 544 SC	Dodine 544 g/L SC	U34330-229/14/3	Apple/Pear/Quince	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Peach/Nectarine	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud swelling till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
	Syllit 400 SC	Dodine 400 g/L SC	U34330-64/13/13	Apple/Pear/Quince	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Peach/Nectarine	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud swelling till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
				Olives	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn.
Spain	Syllit Flow	Dodine 400 g/L SC	23392	Apples/pear/Medd lars/loquat/quince	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Peach/Nectarine	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Cherry	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud swelling till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.
				Olives	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn, 7 days before harvest. For tables olive, in Autumn treatment, only after harvest
	Syllit MAX	Dodine 544 g/L SC	ES-00390	Apples/pear/Medd lars	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest
				Peach/Nectarine	Leaf curl (*Taphrina deformans*), at 2 applications of 0.9 kg as/ha from bud swelling till petal fall at the latest 75 days before harvest and/or later from 50% leaf falling till autumn.

Country	Product name	Active substance content	Registration number	Authorized uses (crops, Harmful organisms, Rates and no of applications, Timing, growth stage)	
				Cherry	Anthracnosis of cherry *Blumeriella jaapii* at 2 applications of 0.68 kg as/ha, from flower opening till 2 weeks before harvest and/or after harvest.
				Almonds	Leaf blotch of almonds at 2 applications of 0.68 kg as/ha at 120 days of harvest the latest.
				Olives	Leaf spot of olives (*Cycloconium oleaginum*) at 2 applications of 0.9 kg as/ha, from the beginning development of leaves till the end of flowering and / or autumn, 7 days before harvest. For tables olive, in Autumn treatment, only after harvest.
Sweden	Syllit 544 SC	Dodine 544 g/L SC	5216	Apples/pear	Scab (*Venturia sp*.) at 2 applications of 0.68 kg as/ha, from bud opening until 60 days before harvest

On-going extension of uses of Dodine

Country	Product name	Active substance content	Registration number	Authorized uses (crops, Harmful organisms, Rates and nb of applications, Timing, growth stage)	
France	Syllit 544 SC	Dodine 544 g/L SC	2160756	Banana	*Black sigatoka*, at 2 applications of 0.408 kg as/ha, BBCH 13-98, until the day of harvest
Greece	Syllit 544 SC	Dodine 544 g/L SC	60592	Almonds	Leaf blotch (*Polystigma ochraceum*), Almond red leaf blotch, at 2 applications of 0.680 kg/ha from BBCH 60 until 30 days before harvest
Portugal	REPIMAX Syllit 544 SC	Dodine 544 g/L SC	1605 1250	Citrus fruits	*Altenaria alternata* at 2 application of 0.68 kg as/ha, from bud burst until 21 days before harvest
Spain	Syllit MAX	Dodine 544 g/L SC	ES-00390	Citrus fruits	*Altenaria alternata* at 2 application of 0.68 kg as/ha, from bud burst until 21 days before harvest
				Almonds	Leaf blotch (*Polystigma ochraceum*), Almond red leaf blotch, at 2 applications of 0.680 kg/ha from BBCH 60 until 30 days before harvest

Level 2

DODINE

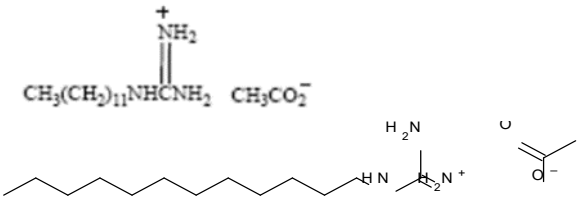
2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

Summary of methodology proposed by the applicant for literature review and for all sections

2.1 IDENTITY

2.1.1 Summary or identity

For CONFIDENTIAL information, please refer to DODINE_DAR_22_Volume_4.

Chemical name (IUPAC)	1-dodecylguanidinium acetate
Chemical name (CA)	dodecylguanidine monoacetate
CIPAC No	101
CAS No	2439-10-3
EC No (EINECS or ELINCS)	219-459-5
Identity of relevant impurities (of toxicological, environmental and/or other significance) in the active substance as manufactured (g/kg)	No relevant impurities
Molecular formula	$C_{15}H_{33}N_3O_2$
Molecular mass	287.4 g/mol
Structural formula	

According to the SANCO/12248/2010 and EFSA Journal 2010; 8(6):1631, the minimum purity for Dodine shall comply with the FAO specification (101/TC/S (1988) (AGP: CP/236)): Dodine: min. 950 g/kg, Water: max. 10 g/kg.

The minimum purity of the technical active substance Dodine is in agreement with the FAO specification.

The representative formulation with trade name Dodine 544 SC is designated as a Suspension Concentrate [Code: SC] and contains 52.88% w/w (544 g/L) of technical dodine.

2.2 PHYSICAL AND CHEMICAL PROPERTIES [EQUIVALENT TO SECTION 7 OF THE CLH REPORT TEMPLATE]

2.2.1 Summary of physical and chemical properties of the active substance

Table 1: Summary of physicochemical properties of the active substance

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid at 20 °C and 101.3 kPa Form: solid: particulate/powder Colour: yellow Intensity: light	[redacted] 1998 R-97-57-part A DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.1.1/01)	Visual assessment, organoleptic assessment
Melting/freezing point	Melting point: 133.2°C at 101.3 kPa	[redacted] 1998 R-97-57-part A DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.1.1/01)	EEC Method A.1
Boiling point	Decomposition: 200.5°C - No boiling before decomposition of the substance	[redacted] 1998 R-97-57-part A DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.1.1/01)	EEC Method A.2 (DSC)
Relative density			Not reported
Vapour pressure	< 5.49×10^{-6} Pa at 50°C	[redacted] 1999a R-97-57-part F DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.3.1/01)	Vapour pressure at 50 ºC could not be exactly determined using the OECD 104 gas saturation method and it was estimated from the LOD of the analytical method (1.3 mg/L). Vapour pressure at 20 ºC could not be exactly determined from the preliminary test at 50ºC

Property	Value	Reference	Comment (e.g. measured or estimated)
Surface tension	50.6 mN/m at 20.1 ºC (90% solubility)	[redacted] 2008 DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.14/01)	EEC Method A5/ OECD 115 (Ring method)
Water solubility	Water solubility at 20°C pH=4.9, 0.87 g/L pH=6.9, 0.93 g/L pH=9.1, 0.79 g/L	[redacted] 1999b R-97-57-part C DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.6/01)	EEC method A6 (OECD 105) (Shake¬ flask method)
Partition coefficient n-octanol/water	log Pow. Partition coefficient: 1.28. Temp: 20°C. pH: 4.9. log Pow. Partition coefficient: 1.25. Temp: 20°C. pH: 6.9. log Pow. Partition coefficient: 1.32. Temp: 20°C. pH: 9.1. No pH dependence	[redacted] 2006 R-450045 DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.8/02)	Estimated, since the a.s. is tensioactive and the shake flask method is not applicable to surface active material
Henry's law constant	< 1.69x10^{-6} Pa.m^3.mol^{-1} (at 20ºC)	[redacted] 2007 And [redacted] 1999b R-97-57-part C And [redacted] 1999a R-97-57-part F DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.3.2/01 and B.2.3.2/02)	Calculated
Flash point	--	--	Not required (dodine is not a liquid at temperatures below 40°C)
Flammability	Technical Dodine is not "highly flammable"	[redacted] 2001 R-327083 DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.11.1/02)	962 g/kg TGAS Method EC A.10 (Flammability for solids)

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td>Explosive properties</td><td>Shock test: not shock sensitivity to explosion.
Friction test: not sensitive to friction using a friction load of 360 N
Thermal sensitivity - Koenen test: has been determined not to have thermal sensibility to explosion.</td><td>[redacted] 1998 R-98-131

DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.11.1/01)</td><td>983 g/kg TGAS Method EC A.14</td></tr>
<tr><td>Self-ignition temperature</td><td>No self-ignition temperature</td><td>[redacted] 1998 R-98-131

DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.11.1/01)</td><td>983 g/kg TGAS Method EC A.16</td></tr>
<tr><td>Oxidising properties</td><td>Dodine has no oxidizing properties</td><td>[redacted] 2000 R-00-330-SEC

DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.15/01)</td><td>982 g/kg TGAS Method EC A.17</td></tr>
<tr><td>Granulometry</td><td></td><td></td><td>Not reported</td></tr>
<tr><td>Solubility in organic solvents and identity of relevant degradation products</td><td><table><tr><th>Organic solvent</th><th>Solubility at 20°C</th></tr><tr><td>Acetonitrile</td><td>0.044 g/L</td></tr><tr><td>n-octanol</td><td>16.54 g/L</td></tr><tr><td>Dichloromethane</td><td>0.015 g/L</td></tr><tr><td>Ethyl acetate</td><td>0.015 g/L</td></tr><tr><td>Acetone</td><td>0.048 g/L</td></tr><tr><td>Xylene</td><td>0.004 g/L</td></tr><tr><td>Ethanol</td><td>57 g/L</td></tr><tr><td>n-Heptane</td><td>0.018 g/L</td></tr></table></td><td>[redacted] 1999b R-97-57-part C

DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.6/01)</td><td>1000 g/kg TGAS Method EC A.6</td></tr>
<tr><td>Dissociation constant</td><td>Not determinable. No pKa value could be associated to Dodine : By carrying out the acidic titration of dodine a pKa value was reached corresponding to acetic acid and of course not characteristic of dodine. In the basic titration of dodine no pKa of the test substance could be determined by this method</td><td>[redacted] 1999d R-97-57-part B

DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.9.4/01)</td><td>literature data exist for the pKa of dodine and for the guanidine group in other compounds. The notifier should clarify this and try to determine the pKa value of dodine by an adequate</td></tr>
</table>

Property	Value	Reference	Comment (e.g. measured or estimated)
			method, if possible.
Viscosity	Not relevant for a solid		
Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity	**UV/VIS at 25°C:** Recorded in the range of 200 to 800 nm Acid medium (9 mL methanol soln.+ 1 mL HCl – 1N) and neutral medium (9 mL methanol soln + 1 mL deionised water) at 0.7 x 10^{-3} mol/L of dodine: An absorption max. at 200 nm with ε = 2600 L/mol cm, at λ ≥ 290 nm highest ε <1.5 L/ mol cm Basic medium: 9 mL Methanol soln + 1 mL NaOH – 1N at 0.7 x 10^{-3} mol/L of dodine : Possible interaction with the solvent and no absorption in range 200-210 nm **IR spectrum (4000-400 cm-1):** Spectrum is in agreement with proposed structure. **NMR (^{1}H and ^{13}C)** The chemical shifts and integrals as presented in the study report are in agreement with the proposed structure. **Mass spectra (MS)** Major signals in the fragment pattern-ESI-positive mode: m/z 228.2 (cationic part of dodine technical) Product-ions of m/z 228.2 / possible assignment / Explanation 211.0 / $[M_{cation}-NH_3]^+$ / Loss of ammonia 186.0 / $[M_{cation}-CN_2H_2]^+$ / loss of H-N=C=N-H 112.7 / unknown 84.9 / unknown 70.8 / unknown The test substance showed no response in the negative ion mode.	[redacted] 1994 R-94-140 (CA_2.4_05 in the dossier for renewal) DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.5.1/01) [redacted] 2009 (CA_2.4_04 in the dossier for renewal) DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.5.1/07) [redacted], 2002, R-343204 and [redacted], 2009, R-490448 (CA_2.4_03 and CA_2.4_02 in the dossier for renewal) DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.5.1/03 and B.2.5.1/06) [redacted] 2002 R-343215 (CA_2.4_01 in the dossier for renewal) DAR Vol. 3 Annex B, Additional Report rev. 1, 2009 (B.2.5.1/04)	In-house method OECD 101 (1000 g/kg) FT-IR, (MKII Golden Gate Single Reflection Diamond ATR System in combination with KRS-5 lenses, 5000-300 cm-1) (994.2 g/kg) ^{1}H-NMR and ^{13}C-NMR Standard methodology (985 g/kg and 994.2 g/kg) Solvent: Deuterated methanol In-house method ESI in positive mode (985 g/kg)

2.2.1.1 Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

2.2.1.1.1 Explosives *[equivalent to section 8.1 of the CLH report template]*

Table 2: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
Method EC A.14 (983 g/kg TGAS)	Shock test: not shock sensitivity to explosion. Friction test: not sensitive to friction using a friction load of 360 N. Thermal sensitivity - Koenen test: has been determined not to have thermal sensibility to explosion.	Method EC A.14 is not a standar technique for assessing explosive properties according to CLP criteria.	[redacted] 1998 R-98-131

2.2.1.1.1.1 Short summary and overall relevance of the provided information on explosive properties

Shock test: not shock sensitivity to explosion.

Friction test: not sensitive to friction using a friction load of 360 N

Thermal sensitivity - Koenen test: has been determined not to have thermal sensibility to explosion.

2.2.1.1.1.2 Comparison with the CLP criteria

No explosivity classification is required. According to point 2.1.4.3 of Annex I of CLP Regulation the acceptance procedure for the hazard class ‘explosives’ does not apply for dodine taking into account that the molecule does not contain chemical groups associated with explosivity considering the examples given in Table A6.1 of Appendix 6 of the UN RTDF Manual of Tests and Criteria.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

No classification proposed based on data conclusive but not sufficient for classification.

2.2.1.1.2 Flammable gases (including chemically unstable gases) *[equivalent to section 8.2 of the CLH report template]*

Table 3: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
Not relevant for a solid			

2.2.1.1.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Not relevant for a solid

2.2.1.1.2.2 Comparison with the CLP criteria

Not relevant for a solid

2.2.1.1.2.3 Conclusion on classification and labelling for flammable gases

Not relevant for a solid

2.2.1.1.3 Oxidising gases *[equivalent to section 8.3 of the CLH report template]*

Table 4: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
Not relevant for a solid			

2.2.1.1.3.1 Short summary and overall relevance of the provided information on oxidising gases

Not relevant for a solid

2.2.1.1.3.2 Comparison with the CLP criteria

Not relevant for a solid

2.2.1.1.3.3 Conclusion on classification and labelling for oxidising gases

Not relevant for a solid

2.2.1.1.4 Gases under pressure *[equivalent to section 8.4 of the CLH report template]*

Table 5: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
Not relevant for a solid			

2.2.1.1.4.1 Short summary and overall relevance of the provided information on gases under pressure

Not relevant for a solid

2.2.1.1.4.2 Comparison with the CLP criteria

Not relevant for a solid

2.2.1.1.4.3 Conclusion on classification and labelling for gases under pressure

Not relevant for a solid

2.2.1.1.5 Flammable liquids *[equivalent to section 8.5 of the CLH report template]*

Table 6: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
Not relevant for a solid			

2.2.1.1.5.1 Short summary and overall relevance of the provided information on flammable liquids

Not relevant for a solid

2.2.1.1.5.2 Comparison with the CLP criteria

Not relevant for a solid

2.2.1.1.5.3 Conclusion on classification and labelling for flammable liquids

Not relevant for a solid

2.2.1.1.6 Flammable solids *[equivalent to section 8.6 of the CLH report template]*

Table 7: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EC A.10	Technical Dodine is not "highly flammable"		[redacted] 2001 R-327083

2.2.1.1.6.1 Short summary and overall relevance of the provided information on flammable solids

The test material did not ignite and did not propagate combustion. It is not highly flammable by ignition according to the test method EC A.10.

2.2.1.1.6.2 Comparison with the CLP criteria

According to RAC/62/2022/04 documment *Assessing physical hazards as part of CLP* if the result of the A.10 test method is "not highly flammable", no more testing is necessary following REACH Guidance on Information Requirements Chapter R.7a: Endpoint specific R.7.1.10.3.

2.2.1.1.6.3 Conclusion on classification and labelling for flammable solids

No classification proposed based on data conclusive but not sufficient for classification.

2.2.1.1.7 Self-reactive substances *[equivalent to section 8.7 of the CLH report template]*

Table 8: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
EEC Method A16 (Relative self-ignition temperature for solids)	No self-ignition temperature		[redacted] 1998 R-98-131

2.2.1.1.7.1 Short summary and overall relevance of the provided information on self-reactive substances

No classification proposed based on the results of the test method A.16.

2.2.1.1.7.2 Comparison with the CLP criteria

No classification for self-reactivity is required. According to CLP point 2.8.4.2 of Annex I of CLP Criteria, the hazard class does not apply if there are no chemical groups in the molecule associated with explosive or self reactive properties. Dodine molecule does not contain chemical groups associated with explosive or self-reactive considering the examples given in Table A6.1 and Table A6.3 of Appendix 6 of the UN RTDF Manual of Tests and Criteria.

2.2.1.1.7.3 Conclusion on classification and labelling for self-reactive substances

No classification proposed based on data conclusive but not sufficient for classification.

2.2.1.1.8 Pyrophoric liquids *[equivalent to section 8.8 of the CLH report template]*

Table 9: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
Not relevant for a solid			

2.2.1.1.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Not relevant for a solid

2.2.1.1.8.2 Comparison with the CLP criteria

Not relevant for a solid

2.2.1.1.8.3 Conclusion on classification and labelling for pyrophoric liquids

Not relevant for a solid

2.2.1.1.9 Pyrophoric solids *[equivalent to section 8.9 of the CLH report template]*

Table 10: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
No data			

2.2.1.1.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

Dodine is unlikely to be pyrophoric and the test for pyrophoricity according to UN Test N.3 described in Part III, Section 33 of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria should not be performed.

2.2.1.1.9.2 Comparison with the CLP criteria

Experience in manufacture and handling shows that the substance dodine does not ignite spontaneously on coming into contact with air at normal temperature.

2.2.1.1.9.3 Conclusion on classification and labelling for pyrophoric solids

Dodine is not a pyrohoric solid.

2.2.1.1.10 Self-heating substances *[equivalent to section 8.10 of the CLH report template]*

Table 11: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EC A.16	No self-ignition temperature	Dodine Technical Grade (Batch 6012) Purity 983 g/kg	[redacted] 1998 R-98-131

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances

No self ignition temperature was determined for dodine according to the results of the test method A.16.

2.2.1.1.10.2 Comparison with the CLP criteria

Dodine is not classified as a self-heating substance according to the test method A.16. Although the recommended method in CLP Regulation is the UN Test. N.4 described in Part III, Section 33 of the UN Recommendations of the Transport of Dangerous Goods, Manual of Tests and Criteria, according to RAC/62/2022/04 document *Assessing physical hazards as part of CLP* the test method A.16 can be considered conclusive when the result is negative.

Besides, according to the ECHA Guidance on the Application of the CLP Criteria (Version 5.0 – July 2017): *substances or mixtures with a low melting point (< 160 °C) should not be considered for classification in this hazard class since the melting process is endothermic and the substance-air surface is drastically reduced. However, this criterion is only applicable if the substance or mixture is completely molten up to this temperature.* The melting point of dodine is 133.2°C at 101.3 kPa and consequently this hazard class does not apply according to CLP criteria.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

No classification proposed based on data conclusive but not sufficient for classification.

2.2.1.1.11 Substances which in contact with water emit flammable gases *[equivalent to section 8.11 of the CLH report template]*

Table 12: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
No data			

2.2.1.1.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

No data available.

2.2.1.1.11.2 Comparison with the CLP criteria

According to CLP Regulation 2.12.4.1, no classification is required if:

a) There are no metals or metalloids in the chemical structure, OR
b) Experience in production or handling shows that the substance does not react with water, e.g. the substance is manufactured with water or washed with water, OR
c) The substance is known to be soluble in water and form stable mixture.

Since dodine does not contain in its chemical structure metals or metalloids, no classification is required.

2.2.1.1.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

No classification proposed based on data conclusive but not sufficient for classification.

2.2.1.1.12 Oxidising liquids *[equivalent to section 8.12 of the CLH report template]*

Table 13: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Not relevant for a solid			

2.2.1.1.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Not relevant for a solid.

2.2.1.1.12.2 Comparison with the CLP criteria

Not relevant for a solid.

2.2.1.1.12.3 Conclusion on classification and labelling for oxidising liquids

Not relevant for a solid.

2.2.1.1.13 Oxidising solids *[equivalent to section 8.13 of the CLH report template]*

Table 14: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EEC Method A17	No oxidising properties		[redacted], 2000 R-00-330-SEC

2.2.1.1.13.1 Short summary and overall relevance of the provided information on oxidising solids

Dodine has not oxidising properties according to the results of the test method A.17.

2.2.1.1.13.2 Comparison with the CLP criteria

According to criteria included for organic substances in point 2.14.4.1 of Annex I of CLP Regulation, dodine is not an oxydising solid since it does not contain chlorine or fluorine and it contains oxygen but chemically bounded to carbon and hydrogen.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids

No classification proposed based on data conclusive but not sufficient for classification.

2.2.1.1.14 Organic peroxides *[equivalent to section 8.14 of the CLH report template]*

Table 15: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
No data			

2.2.1.1.14.1 Short summary and overall relevance of the provided information on organic peroxides

No data available. However, since the peroxide group (O-O) is absent in the chemical structure of dodine, the hazard class is not applicable.

2.2.1.1.14.2 Comparison with the CLP criteria

Dodine has not contain the peroxide group (O-O) and according to CLP criteria classification is not required.

2.2.1.1.14.3 Conclusion on classification and labelling for organic peroxides

No classification proposed based on data conclusive but not sufficient for classification.

2.2.1.1.15 Corrosive to metals *[equivalent to section 8.15 of the CLH report template]*

Table 16: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
No studies available			

2.2.1.1.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No data provided.

2.2.1.1.15.2 Comparison with the CLP criteria

According to the ECHA Guidance on the Application of the CLP Criteria (Version 5.0 – July 2017) only solids with a melting point below 55°C need to be tested for this hazard class (2.16.4.1) and no classification can be proposed. Since the melting point of dodine is 133.2°C at 101.3 kPa classification is not required.

2.2.1.1.15.3 Conclusion on classification and labelling for corrosive to metals

No classification proposed based on data conclusive but not sufficient for classification.

2.2.2 Summary of physical and chemical properties of the plant protection product

The appearance of the product is that of an opaque white liquid with a small clear liquid layer on the top and a small sediment on the bottom (claying) homogeneous after gentle shaking, and with a faint chemical odour. It is not explosive, and has no oxidising properties. It has a self-ignition temperature of 404°C. The stability data indicate that after 14 days at 54°C, no decrease of Dodine content is seen (0.0%). It is noted that the product is

more viscous after accelerated storage. Its technical characteristics are acceptable for such a liquid formulation (SC). There is no effect of low temperature on the stability of the formulation since after 7 days at 0°C, neither the appearance nor the technical properties had changed. Dodine 544 SC in its commercial packaging has a shelf-life of at least 2 years and it was also demonstrated stability for 3 years.

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

Dodine is currently used as a fungicide against scab (*Venturia inaequalis/Venturia pyrina*) on pome fruits (apple/pear/quince/medlar/loquat), leaf spot (*Blumeriella jaapii*) and leaf scorch (*Gnomonia erythrostoma*) on cherry, leaf curl (*Taphrina deformans*) on peach and nectarine, leaf spot of olives (*Cycloconium oleaginum*), anthracnose of walnut, leaf spot of chestnut and pistachios, leaf blotch of almonds and leaf spot of poplar (*Drepanopeziza punctiformis*). Representative uses are scab (*Venturia inaequalis/Venturia pyrina*) in apple/pear, leaf spot (*Blumeriella jaapii*) in cherry and leaf curl (*Taphrina deformans*) on peach. Dodine is currently used as a fungicide on pome fruits (apple/pear/quince/medlar/loquat), on cherry, peach and nectarine, olives, walnut, chestnut and pistachios, almonds and poplar. Representative uses for this application are pome fruit, cherry and peach.

Dodine based formulations for foliar spray applications are already authorized for many years in member states of the European Union. No new data is submitted within the framework of this applicationn for the renewal of the approval of the active substance Dodine.

RMS agrees with the data and information submitted by the applicant regarding the Guidance SANCO/2012/11251 Guidance Document on the renewal of approval of active substances to be assessed in compliance with Regulation (EU) No 844/2012 (The Renewal Regulation):

"The dossier should include an overview of the efficacy information concerning representative and supported uses already authorised in Member States according to the format provided in MCA section 3 (see GD SANCO/10181/2013). Information as regards the representative uses and the supported uses has to be reported as part of chapter C 3.3 (MCP section 3). Information about their current authorisation status is reported in Doc D-2. Considering that the substance is approved and authorisations of plant protection products containing the substance have already been evaluated according to the Uniform Principles (Regulation (EC) No 546/2011), no other efficacy documentation is deemed to be necessary at this stage".

However, **RMS considers** useful to add summary tables with results of efficacy trials developed during the last years by the notifier, mainly for the representative uses proposed in this submission for the renewal of the active substance. The purpose is to show clearly the efficacy of the active substance. In the Table 1, summary data provided by the notified have been added.

2.3.2 Summary of information on the development of resistance

According to FRAC, (group-u12-(guanidines) ---dodine-recommendations-22th-of-december-2020)[1] for recent years no cases of resistance towards Dodine have been found. Nevertheless, resistance cases are known for *Venturia inaequalis* (apple scab) from Canada and the North-Eastern part of the USA. These were reported in the 1970s and 1980s. Some cases of resistance have also been identified from resistance-monitoring programs in Poland and New Zealand. However, these cases are attributed to very intensive, often exclusive use of Dodine (mainly in the USA and Canada) or to the use of Dodine as rescue applications to burn out established scab lesions (Poland). Since that period, the use of Dodine against apple scab has dropped drastically in the USA and Canada. FRAC reports that in New Zealand, sensitivity towards guanidines has not increased since the 1990s and may have actually decreased.

Furthermore, FRAC does not report information on resistance to Dodine for any other diseases or crops even not on pear scab (*Venturia pyrina*).

[1] document available at https://www.frac.info/frac-teams/other-fungicide-recommendations accessed 6th May 2021

FRAC evaluates the general risk for development of resistance against Dodine as low to medium.

2.3.3 Summary of adverse effects on treated crops

The crop safety of the representative uses has already been evaluated under Uniform Principles for national registration and found acceptable. Therefore, no specific data on phytotoxicity is required.

2.3.4 Summary of observations on other undesirable or unintended side-effects

The representative uses have already been evaluated under Uniform Principles for national registration and found acceptable. Therefore, no information regarding observations on other undesirable or unintended side effects is required.

2.4 FURTHER INFORMATION

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

Precautions for safe handling

Appropriate engineering controls: Ensure good ventilation of the workstation.

Hand protection: protective gloves

Eye protection: Safety glasses

Skin and body protection: Wear suitable protective clothing

Respiratory protection: In case of inadequate ventilation, wear respiratory protection.

Do not breathe dust/fume/gas/mist/vapours/spray.

Avoid contact with skin and eyes. Wear personal protective equipment.

Storage conditions

Store locked up. Store in a well-ventilated place. Keep container tightly closed.

Store at room temperature

Maximum storage period: 2 year(s)

Transport (ADR, IMDG, IATA)

Transport of dangerous goods by land: with regard to ADR 2015 and RID 2015

Hazard Class 6.1

UN Number: 2588

Packing Group: II

Shipping Name: PESTICIDE, SOLID, TOXIC, N.O.S. (Dodine)

Firefighting measures

Extinguishing media:

Suitable extinguishing media: Water spray. Dry powder. Foam.

Specific hazards:

Explosion hazard: Dust may form explosive mixture in air. Hazardous decomposition products in case of fire: Toxic fumes may be released

Protection during firefighting: Do not attempt to take action without suitable protective equipment.

Self-contained breathing apparatus.

Complete protective clothing.

2.4.2 Summary of procedures for destruction or decontamination

For containment: Collect spillage.

Methods for cleaning up: Mechanically recover the product.

Other information: Dispose of materials or solid residues at an authorized site.

2.4.3 Summary of emergency measures in case of an accident

Description of first aid measures

First-aid measures general: Call a physician immediately.

First-aid measures after inhalation: Remove person to fresh air and keep comfortable for breathing. Call a physician immediately. Call a doctor.

First-aid measures after skin contact: Wash skin with plenty of water. Take off contaminated clothing. If skin irritation occurs: Get medical advice/attention.

First-aid measures after eye contact: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Call a physician immediately.

First-aid measures after ingestion: Rinse mouth. Call a poison center or a doctor if you feel unwell. Treat symptomatically.

Personal precautions and protective equipment

For non-emergency personnel: Do not breathe dust/fume/gas/mist/vapours/spray. Only qualified personnel equipped with suitable protective equipment may intervene.

For emergency responders: Do not attempt to take action without suitable protective equipment (safety glasses, suitable protective clothing, and protective gloves.

Environmental precautions

For containment: Collect spillage.

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

2.5.1.1 Methods for the analysis of the active substance as manufactured

Determination of the active ingredient Dodine in the TGAS: The active substance content is determined after dilution in water and methanol / water 9/1 v/v by HPLC/UV. But the specificity of the HPLC-UV method was not demonstrated and a confirmatory method is required.

Analyte	Method	Specificity	Linearity	Accuracy (Recovery)	Repeatability (Precision)	Reference
Dodine	HPLC-UV	no interferences were detected in blank chromatograms, confirmation with FT-IR spectra	50 to 120 mg/L (6 levels, equivalent to 500 to 1200 g/kg) r^2 >0.99	Not required	The relative standard deviation (RSD) was found to be 0.18% (at 983 g/kg).	[redacted] R (2016)

The active substance as manufactured does not contain relevant impurities. Therefore analytical methods for the determination of impurities are not required.

For determination of significant impurities LC-MS/MS and GC-MS/MS methods are used. More information in the confidential Vol 4 of the DRAR.

2.5.1.2 Methods for the analysis of the formulation

Determination of the active ingredient Dodine in the formulation Dodine 544 SC: The active substance content is determined after dispersion in water and methanol and dilution in a mixture acetonitrile - water containing heptane sulfonic acid. The separation is achieved using HPLC-ion-pair chromatography with ultra-violet detection.

Analyte	Method	Specificity	Linearity	Accuracy (Recovery)	Repeatability (Precision)	Reference
Dodine	HPLC-ion-pair chromatography	Since no interferences were detected in blank chromatograms, the specificity requirements were met and the analytical method was found to be specific for Dodine	the range 49-203 µg dodine/mL (±50% of the nominal concentration) r = 1.000 r^2 = 1.000	The mean recovery for Dodine in Dodine 544 SC was determined to be 100.4%.	The relative standard deviation (RSD) was found to be 0.35%.	[redacted] (2011)

Adequate analytical methods are available for the determination of Dodine in the representative formulation.

Methods for determination of relevant impurities, co-formulants or components of co-formulants in the plant protection product are not required.

2.5.1.3 Methods for risk assessment

GC/MS/MS methods after derivatization of dodine and HPLC/MS/MS methods were used for the determination of dodine in support of e-fate, toxicological, ecotoxicological and residues studies.

2.5.1.3.1 Environmental fate

Dodine in soil

Extraction with metanolic solutions, Derivatization with hexafluoroacetyl acetone (HFAA), analysis GC-MSD
LOQ : 10 ppb (ng/g)
Validated working range : 20 – 150 ng/mL.

2.5.1.3.2 Toxicology

In brief the methods are as follow:

Dodine in animal diet

- Extraction with MeOH, derivatization with hexafluoroacetyl acetone (HFAA), analysis by HPLC/UV or by HPLC/FL.

- Derivatization with trifluoroacetic anhydride (TfAA), analysis by GC/FID. Validated in Corning Hazleton Study No. 6157-186 (old study, no longer available)

For operator exposure studies

Determination of Dodine on glass fiber air sampling filters, T-shirts/long underpants/head bands, socks, gloves and coveralls: Extraction with 1% HCl in methanol/water 50/50 v/v% and analysis by HPLC-MS/MS using ESI+.
LOQ : approximately 5 µg/L
Working range : 2 to 100 µg/L (in 0.1 acetic acid in methanol/water 50/50 v/v%), r^2 >0.99

2.5.1.3.3 Residues

Dodine in Dislodgeable Foliar Residues

Dilution with MeOH, analysis by LC-MS/MS

LOQ: 0.5 µg/L

Validated working range: 0.075 ng/mL to 10 ng/mL equivalent to 0.15 µg/L to 20 µg/L in wash solutions

Dodine in plant matrices

Method MEREDODINE based on CHIMAC-AGRIPHAR method RPA/DOD/97112 ([redacted] 1998): Extraction with MeOH, derivatization with hexafluoroacetyl acetone (HFAA), analysis by GC-MS (quantitation ion 244, qualifiers ions 245 and 399.20), calibration with matrix matched solution, fortified with dodine standard and derivatised prior to analysis.
LOQ in apple: 0.005 mg/kg
Validated working range: 0.0025 µg,/mL to 0.5 µg/mL expressed as dodine (equivalent to 0.005 to 1 mg/Kg in apple).

Rhône Poulenc Method 45137, 1996:
Extraction with MeOH, derivatization with hexafluoroacetyl acetone (HFAA), analysis by GC-MS (quantitation ion 243.95, qualifiers ions 244.95 and 399.20), and calibration with matrix matched solution, fortified with standard of the derivative.
LOQ in apple: 0.05 mg/kg

Method in report P/B 1272 G:
Extraction with MeOH, analysis by LC-MS/MS (transition at 228 m/z → 187 m/z of dodine for primary quantification and a 2nd MRM transition 228 m/z → 60 m/z for qualitative confirmation).
LOQ in cherry: 0.01 mg/kg
Validated working range: 0.10 to 50 ng/mL (equivalent to 0.002 to 1 mg/Kg in cherry).

2.5.1.3.4 Ecotoxicology

Determination of dodine in feeding solutions for honey bee

[redacted], 2017:

Feeding solution: Extraction with ice-cold (-20 °C) mixture of methanol/acetone (80/20; v/v), analysis by LC-MS/MS. LOQ: 0.026 g Dodine/L. Validated working range: 0.175 to 7.0 mg/L.

Stock solutions: dilution of the sample with methanol/acetone (80/20; v/v), analysis by HPLC-UV. LOD: 0.8 g Dodine/L. Validated working range: 0.431 to 5.17 mg/L.

[redacted], 2016:

Feeding solution and stock solutions: Dilution of the sample with water/acetonitrile (80/20; v/v), analysis by HPLC-UV. LOQ (sugar solution): 0.0014 g Dodine/L. Validated working range: 0.132 - 4.82 mg/L in water/acetonitrile (80/20; v/v). Complete validation is required. Applicant informed that a new chronic bee study has been launched with new method of analysis.

Determination of dodine in water media

[redacted], 2020:

Dilution with acetonitrile and acetonitrile/water (1/1, v/v), analysis by LC-MS/MS. LOQ: 0.0302 µg/L. Validated working range: 0.00506 – 0.504 µg test item/L.

[redacted], 2008:

Dilution and analysis by LC-MS/MS. LOQ: 0.135 µg a.i./L (LOQ should be 1.98 µg a.i./L according to RMS assessment). Validated working range : 0.500 – 12.5 µg a.i./L.

[redacted], 1989, 1990 and 1991

Determined spectrophotometrically after ion-pair partitioning into Chloroform containing 10% n-Butanol. LOQ: not determined. Validated working range: 0 to 10 ppm in water. The method is not accepted (Method not specific; calibration not correct; results not reliable.

[redacted], 1992:

Extraction with ethyl acetate, derivatization with 1,1,1,5,5,5-hexafiuoro-2,4-pentanedione, analysis by GC/NPD. LOQ: 0.0024 ng. Validated working range: 505 to 25.3 µg/L.

[redacted], 2022:

Dilution of sample and analysis by UHPLC-MS. LOQ: 0.25 mg/mL, Validated working range: 0.300 to 4.80 µg/mL

Determination of dodine in larvae and beetles of *Tenebrio molitor*

Extraction with MeOH, dilution and analysis by LC-MS/MS. LOQ: 0.3 mg/kg. Validated working range: 0.2 to 100 ng/mL equivalent to 0.08 to 100 mg/Kg.

2.5.1.3.5 Physico-chemistry

No individual studies available for methods for risk assessment in support of physical and chemical properties studies.

2.5.1.3.6 Other studies (unclassified)

Determination of dodine in soil (report no EC-97-384):

Extraction with metanolic solutions, derivatization with hexafluoroacetyl acetone (HFAA), analysis GC-MSD and calibration with matrix matched solution, fortified with standard of the derivative. LOQ: 10 ppb (ng/g) = 0.01 mg/kg. Validated working range: 20 – 150 ng/mL.

2.5.2 Methods for post control and monitoring purposes

2.5.2.1 Plants and plant products

Residue definition for monitoring was set as follow in the EFSA Journal 2010; 8(6):1631, Conclusion on pesticides peer review of Dodine:

- For plants and plant products residues definition is Dodine.

Adequate analytical methods are available to monitor Dodine residues in Hight Water content plants (GC-MSD with LOQ of 0.05 mg/kg), Acidic Crop Matrices (LC/MS/MS with LOQ of 0.01 mg/kg), High Oil content plants (LC/MS/MS with LOQ of 0.01 mg/kg) and Dry Crop Matrices (LC/MS/MS with LOQ of 0.01 mg/kg).

2.5.2.2 Food of animal origin

Residue definition for monitoring was set as follow in the EFSA Journal 2010; 8(6):1631, Conclusion on pesticides peer review of Dodine:

- For food and animal origin: an analytical method for food of animal origin is not required due to the fact that no residue definition is proposed.

However, since the EFSA Journal 2010; 8(6):1631 the MRL for food and animal origin has now been set, therefore new methods are provided. Adequate analytical methods are available to monitor Dodine residues in Food/feed of animal origin, milk, muscle, egg and fat (LC/MS/MS with LOQ of 0.01 mg/kg). The efficiency of the extraction

procedure was not reported but it is not reqired, according to SANTE 2017/10632, Rev. 3, 2017, considering, that the expected residues in food of animal origin are very low (< 0.01 mg/kg) (please see Vol. 3, B.7.4).

- A new MRL is proposed for **honey**, 0.3 mg/kg for the NEU/SEU use *versus* the previous default value of 0.05 mg/kg. An adequate analytical method is available to monitor Dodine residues in honey: LC-MS/MS with a transition at 228 m/z → 186 m/z of dodine for primary quantification and a 2nd MRM transition (228 m/z → 60 m/z) for qualitative confirmation. LOQ of 0.01 mg/kg. **An ILV is required according to SANTE/2020/12830 rev. 2**

2.5.2.3 Soil

Residue definition for monitoring was set as follow in the EFSA Journal 2010; 8(6):1631, Conclusion on pesticides peer review of Dodine:

- For soil residues definition is Dodine.

Adequate analytical methods are available to monitor Dodine residues in soil (GC/MS with LOQ of 0.01 mg/kg and LC/MS/MS with LOQ of 0.01 mg/kg).

2.5.2.4 Water

Residue definition for monitoring was set as follow in the EFSA Journal 2010; 8(6):1631, Conclusion on pesticides peer review of Dodine:

- For water residues definition is Dodine.

Adequate analytical methods are available to monitor Dodine residues in surface water (LC/MS/MS with LOQ of 0.008 µg/L and LC/MS/MS with LOQ of 0.05 µg/L).

ILV is required for drinking water.

2.5.2.5 Air

Residue definition for monitoring was set as follow in the EFSA Journal 2010; 8(6):1631, Conclusion on pesticides peer review of Dodine:

- For air the residues definition is Dodine

Analytical methods are available to monitor Dodine residues in air (LC/MS/MS with LOQ 0.00850 mg/absorber (0.1xC-level)). Nevertheless the specificity was not demonstrated according to SANCO/825/00 rev.8.1 for monitoring since only one MS transition was validated. In addition the LOQ (0.0085 mg/absorber) of the method is not low enough to cover the trigger value of 0.00173 mg dodine / absorber. Therefore the validation of the method does not fully comply the requirements of the guidance SANTE/2020/12830 rev. 1, 2021 regarding specificity and LOQ and it is not adequately validated.

2.5.2.6 Body fluids and tissues

Residue definition for monitoring was set as follow in the EFSA Journal 2010; 8(6):1631, Conclusion on pesticides peer review of Dodine:

- For body fluids the residues definition is Dodine

But residue definition for body fluids and tissues was set as dodine and the metabolite hydroxy-dodecylguanidine in the current evaluation for renewal (please see Vol. 1, section 2.6.1.1).

Adequate analytical methods are available to monitor Dodine residues in tissues (liver) by LC/MS/MS with LOQ of 0.01 mg/kg.

In body fluids (human blood and urine) dodine is determined by LC/MS/MS with LOQ of 2 µg/L. A confirmatory method for dodine and the metabolite hydroxy-dodecylguanidine at the LOQ of 0.002 mg/kg in urine and blood is required. A confirmatory method for the metabolite hydroxy-dodecylguanidine at the LOQ of 0.005 mg/kg in liver is required.

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals *[equivalent to section 9 of the CLH report template]*

Table 17: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Absorption, distribution, metabolism and excretion GLP: Yes Guideline EPA OPP 85-1 Sprague-Dawley (Crl:CD BR) rats (♂/♀) ^{14}C-Dodine (n-dodecylguanidine monoacetate), Purity > 99%, Activity: 56.5 mCi/mmol Dodine (n-dodecylguanidine monoacetate), Purity > 99.8%, -*Single oral dose* (oral gavage) of 40 or 400 mg/kg bw (5♂/5♀). -*Repeated oral dose* (oral gavage): 14 single oral daily doses (40 mg/kg body weight) of non-labelled dodine followed by the 15th oral dose (40 mg/kg body weight) of ^{14}C-dodine (5♂/6♀). Deviations from current test guideline (OECD TG 417, 2010): -Biliary excretion was not assessed. -Metabolic profiles were only assessed in one animal/sex/group. **Study acceptable**	Absorption & Excretion: (% of dose) -*Single low dose* (40 mg/kg bw): ▪ At 48 h: 39%/39% for ♂/♀ in urine, and 56%/51% for ♂/♀ in feces. ▪ At 120 h: 41%/42% for ♂/♀ in urine, and 60%/55% for ♂/♀ in feces. -*Repeated low dose*: ▪ At 48 h: 43%/42% for ♂/♀ in urine, and 53%/50% for ♂/♀ in feces. ▪ At 120 h: 45%/45% for ♂/♀ in urine, and 56%/54% for ♂/♀ in feces. -*Single high dose* (400 mg/kg bw): ▪ At 48 h: 24%/20% for ♂/♀ in urine, and 18%/15% for ♂/♀ in feces. ▪ At 120 h: 42%/43% for ♂/♀in urine, and 50%/48% for ♂/♀ in feces. Distribution: (% of dose) - Residual radioactivity was located mainly in muscle (0.20-0.61%), skin (0.06-0.21%), and GI tract (0.16-1.14%) after 120 h. Metabolic profile: Four main metabolites: M2, M3, M4 y M5 only found in urine and the parental (M1) only in faeces. Profile (% of dose): - M1: Dodine, 40-55%. - M2: Hydroxy-dodecylguanidine, 11- 23%. - M3: Not identified, 7- 11%. - M4: Identified as a mixture of acidic products of β-oxidation, 3 - 13%. - M5: Urea, 3-5% Expired $^{14}CO_2$ was a minor route of elimination.	Less than 50% of ^{14}C-Dodine was absorbed, bio-transformed, and excreted in urine (estimated oral absorption: 43.6% based on the amounts recovered in urine, carcass and tissues at 120 h after single low dose). At low dose, excretion mainly in 48 h, at similar proportion in urine and faeces. At high dose, excretion slower but completed at 120 h, also with similar proportion in urine/faeces. No differences in excretion between males and females. No potential for bioaccumulation was observed. The recovery of radioactivity was low in all tissues. Most of the dodine-derived radioactivity in the urine was eliminated as metabolites, where the parent compound was no detected. Four metabolites were mainly observed in urine, being hydroxy-dodecylguanidine, an omega-oxidation product, the major metabolite (up to 23% of dose).	[redacted] (1992) **(AS) B.6.1.1.1**
Toxicokinetics study GLP: Yes OECD 417 (1984) Sprague-Dawley (Crl:CD (SD) (SPF)) rats (♂/♀) ^{14}C-Dodine (Dodecylguanidine acetate), Purity > 98%, Activity: 27 mCi/mmol. Dodine (Dodecylguanidine acetate), Purity: 96.61% -*Single oral dose* (oral gavage) of 40 or 400 mg/kg bw (5♂/5♀) Deviations from current test guideline (OECD TG 417, 2010):	*Low dose group* (40 mg/kg bw) - T_{max} (h) : 12/4 for ♂/♀. - T_{last} (h): 96/96 for ♂/♀. - $T_{1/2}$ (h): 17.28/16.50 for ♂/♀. - C_{max} (mg/Kg): 0.417/0.836 for ♂/♀. - AUC $_{last}$ (h x mg/kg): 13.9/20.07 for ♂/♀. - AUC $_{\infty}$ (h x mg/kg): 14.39/20.66 for ♂/♀. *High dose group* (400 mg/kg bw) - T_{max} (h) : 4/2 for ♂/♀. - T_{last} (h): 120/120 for ♂/♀. - $T_{1/2}$ (h): 60.92/36.14 for ♂/♀. - C_{max} (mg/Kg): 2.155/2.530 for ♂/♀.	Oral absorption was relatively rapid for both sexes, peak plasma concentration at 2-4 hours, except in low dose male group (12 h) but already a peak was seen at 4 h. The apparent terminal half-lives ($T_{1/2}$) were 17 h for low dose group and 36 h (♀) and 61 h (♂) for high dose group.	[redacted] (2006) **(AS) B.6.1.1.2**

Method	Results	Remarks	Reference
-Intravenous administration of the test substance was not performed. **Study acceptable**	- AUC $_{last}$ (h x mg/kg): 195/225 for ♂/♀. - AUC $_{\infty}$ (h x mg/kg): 290/266 for ♂/♀.	Clinical signs were observed in the high dose group.	
Absorption and excretion study GLP: Yes OECD TG 417 Sprague-Dawley Crl:CD(OFA) rats (♂/♀) ^{14}C-Dodine acetate, Purity = 99.2%. Activity: 46.8 mCi/mmol. Dodine technical, Purity: 98.6% -*Single oral dose* (oral gavage) of 5 mg/kg bw (4♂/4♀). Deviations from current test guideline (OECD TG 417, 2010): -Only one dose tested. -Expired air was not collected. **Study acceptable**	Absorption & Excretion: Cumulative excretion (% of dose): -*Single oral dose* (5 mg/kg bw): ▪ At 24 h: 31%/31% for ♂/♀ in urine, 43.4%/38.1% for ♂/♀ in feces, and 3.5%/1.8% for ♂/♀ in bile. ▪ At 48 h: 33.6%/33.9 for ♂/♀ in urine, 58.6%/59.9% for ♂/♀ in feces, and 3.6%/1.8% for ♂/♀ in bile. ▪ At 72 h: 34%/34.7% for ♂/♀ in urine, 60.9%/62% for ♂/♀ in feces, and 3.6%/1.8% for ♂/♀ in bile. No evidence of bioaccumulation. Remaining radioactivity was 0.17% in the carcass and 0.23% in GI tract after 72 hours.	Oral absorption of 39 % based on urine, bile, cage wash and carcass recoveries within 72 hours. Excretion completed in 72 h. Excretion was almost complete (96%) within 48 h (34% urinary, 59% faecal and 3% bile). No relevant sex differences found. Low radioactivity recovered in bile (~3.6% in ♂ and 1.8% in ♀) at 72h.	[redacted] (2019) **(AS)** **B.6.1.1.3**
Absorption, distribution, metabolism and excretion (oral and i.v.) GLP: Yes Guideline not stated Sprague-Dawley CD rats (3♂/3♀) ^{14}C-dodecylguanidine acetate; Purity: >95%; specific activity 2 mCi/mmol. Unlabelled dodine. Purity: not stated -*Single low oral administration* (5 mg/kg bw *via* gastric gavage). -*Single intravenous* (i.v.) *administration* (5 mg/kg bw). -*Repeated single oral dose*: 7 daily single oral doses at 5 mg/kg bw (gastric gavage). -*Single high oral administration* (50 mg/kg bw *via* gastric gavage). Deviations from current test guideline (OECD TG 417, 2010): - The label is not located in the molecule core. - The unlabelled test substance was not properly characterized - 3 males and 3 females were used. - Blood concentrations were measured only in plasma. -Identification of the metabolites was not done. - For repeated dosing, animals received seven daily oral doses of ^{14}C-dodine. - Volatile ^{14}C was measured only in 4 animals (2 rats from each 2 groups oral and intravenous). - Rationale for the choice of vehicle was not provided.	Absorption & Excretion: Recovery of total rad. (% of dose): -*Single oral dose* (5 mg/kg bw): ▪ At 24 h: 40.2%/45.6% for ♂/♀ in urine, and 37.8%/38.3% for ♂/♀ in feces. ▪ At 96 h: 42.7%/48.5 for ♂/♀ in urine, and 49.7%/47.2% for ♂/♀ in feces. - *Single iv. dose* (5 mg/kg bw) ▪ At 24 h: 57.1%/51.2% for ♂/♀ in urine, and 8%/9.9% for ♂/♀ in feces. ▪ At 96 h: 70.2%/66.6% for ♂/♀ in urine, and 12.7%/15.6% for ♂/♀ in feces. Distribution: No relevant accumulation was observed in tissues, although some radioactivity remained in fat (mainly), ovaries, thyroid and skin. Multiple dosing caused a slower elimination than single dosing. Metabolic profile: Analysis of the metabolites indicates that ^{14}C-dodine was rapidly metabolised to a number of unidentified polar components, which were chromatographically dissimilar to dodine, dodecylamine and dodecylurea.	Peak plasma radioactivity levels were reached earlier after i.v. administration (30 and 5 min. for ♂ and ♀, respectively), than observed after oral administration (~4 h for both sexes). Elimination of ^{14}C-dodine by oral route was similar through urine and feces (~45%). Elimination by i.v. route was mainly through urine (up to 70%) in 96 h. No relevant sex differences were found. Expired $^{14}CO_2$ was a minor route of elimination. Residual radioactivity in carcass was higher after i.v. dosing (8%) than after oral (0.6%). Oral absorption was considered to be about 45%.	[redacted] (1985) **(AS)** **B.6.1.1.4**

Method	Results	Remarks	Reference
- Tissue residues were not characterised. **Supporting information**			
Toxicokinetic Calculations* GLP: Not applicable No guideline applicable *The data supplied for this study originated from the study B.6.1.1.4 ([redacted], 1985), so same information and deviations could be derived. **Supporting information**	*Oral low dose group (5 mg/kg bw)* - T_{max} (h) : 4/4 for ♂/♀. - *$T_{1/2}$ (h): ranged 8-10.7/5.5-7.4 h for ♂/♀. - C_{max} (µg eq./ml): 0.137/0.254 for ♂/♀. - AUC_{24} (µg eq.h/ml): 1.59/2.34 for ♂/♀. - F: 36.1%/40.3% for ♂/♀. *Oral high dose group (50 mg/kg bw)* - T_{max} (h) : 8/8 for ♂/♀. - *$T_{1/2}$ (h): 8.8/7.6 h for ♂/♀. - C_{max} (µg eq./ml): 1.13/1.6 for ♂/♀. - AUC_{24} (µg eq.h/ml): 18.2/25.2 for ♂/♀. - F: 41.4%/43.4% for ♂/♀. *Oral repeated single dose group (5 mg/kg bw)* - T_{max} (h) : 6/4 for ♂/♀. - *$T_{1/2}$ (h): ranged 10-23.1/9.8-13.1 h for ♂/♀. - C_{max} (µg eq./ml): 0.169/0.249 for ♂/♀. - AUC_{24} (µg eq.h/ml): 2.49/3.93 for ♂/♀. *Intravenous dose group (5 mg/kg bw)* - T_{max} (h) : 0.25/0.083 for ♂/♀. - *$T_{1/2}$ (h): 9.1/7.8-11.9 h for ♂/♀. - C_{max} (µg eq./ml): 1.54/1.48 for ♂/♀. - AUC_{24} (µg eq.h/ml): 4.4/5.81 for ♂/♀. **Estimated in accordance with acceptance study criteria, T½ must be interpreted carefully.*	The oral bioavailability of radioactivity following single doses was in the range 36.1-43.4% and were independent of dose and sex. Peak plasma concentration ranged from 4-8 hours after oral administration, and 5/15 min. for ♀/♂ in the i.v. dose group. The AUC_{24} values were approximately proportional to doses after a single oral dose of dodine.	[redacted] (2020) **(AS) B.6.1.1.5**
***In vitro* comparative metabolism (rat, dog and human liver microsomes)** No guideline applicable GLP: Yes Human, dog and rat liver microsomes (mix gender) Concentration: 10 µM Dodine acetate, [guanidine-^{14}C]; purity: 99.9%. Activity: 38.8 mCi/mmol. Unlabelled dodine (dodine technical), purity: 98.6% Vehicle: Acetonitrile (ACN): milli Q water (MQ) 1:1 **Study acceptable**	• Four radioactivity peaks were detected (M1, M2, M3 and ^{14}C-dodine). <u>Presence in Incubation Samples after 60 min</u> (% of total integrated radioactivity of the chromatogram): *Rat*: M1: 7.9-9.8%; M2: 22.3-28.8%; M3: n.d., ^{14}C-dodine: 63.3-67.9% *Dog*: M1: 6.3-6.8%; M2: 10.0 - 10.1%; M3: 4.5%; ^{14}C-dodine: 79.2-83.1% *Human*: M1: 3.5-5.3%; M2: 32.6-33.5%; M3: 18.3-19.0%, ^{14}C-dodine: 42.1-45.7% *n.d.: non detected* • -<u>M2 and M3 peaks</u> were further subjected to MS analysis for metabolite identification: -MS analysis of **M2** revealed two possible metabolites: **M2a**: *m/z* 246.241 (oxidation product) and **M2b**: *m/z* 244.226 (oxidation and desaturation product). -MS analysis of **M3** revealed two possible metabolites: **M3a**: *m/z* 246.241 (oxidation product) and	No specific human metabolites were detected. The four radioactive metabolite peaks detected in human liver microsomes were also detected in at least one of the animal species. Metabolite M3 was mainly detected in human liver microsomes (>4-fold) and was not detected in rat. Metabolic reactions observed included oxidation (-OH (hydroxyl)), desaturation and oxidation (=O (ketone)) and a combination of both.	[redacted] (2018) **(AS) B.6.1.1.6**

Method	Results	Remarks	Reference
	M3b: *m/z* 260.222 (desaturation and two oxidations).		

2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

A total of five studies have been submitted for the renewal of approval of the active substance dodine, only two were previously evaluated in the original DAR (2009), and three new studies (including the *in vitro* comparative metabolism study) have been submitted for the renewal process. In addition, an evaluation report with toxicokinetic calculations using the data from the [redacted] 1985 study was provided.

Oral route

- An *in vivo* single-and repeated dose study in rats was provided (Vol.3, AS, B.6.1.1.1). In this study, absorption, excretion, distribution and metabolism were assessed after oral (gavage) administration to Sprague-Dawley rats. The authors used a single low dose of 40 mg/kg bw, and a single high dose of 400 mg/kg bw. On the other hand, a multiple dose group based on 14 single oral daily doses (40 mg/kg bw) of non-labelled dodine followed by the 15th oral dose (40 mg/kg bw) of ^{14}C-dodine was tested. Dodine-derived radioactivity was eliminated within 120 h in both male and female rats. The major portion of the radioactivity in the single and multiple low oral dose (40 mg/kg bw) groups was eliminated in the first 48 h, almost equally in urine and feces for both sexes, whereas in the single high dose group, the excretion was slower than observed at low dose, but completed within 120 h.

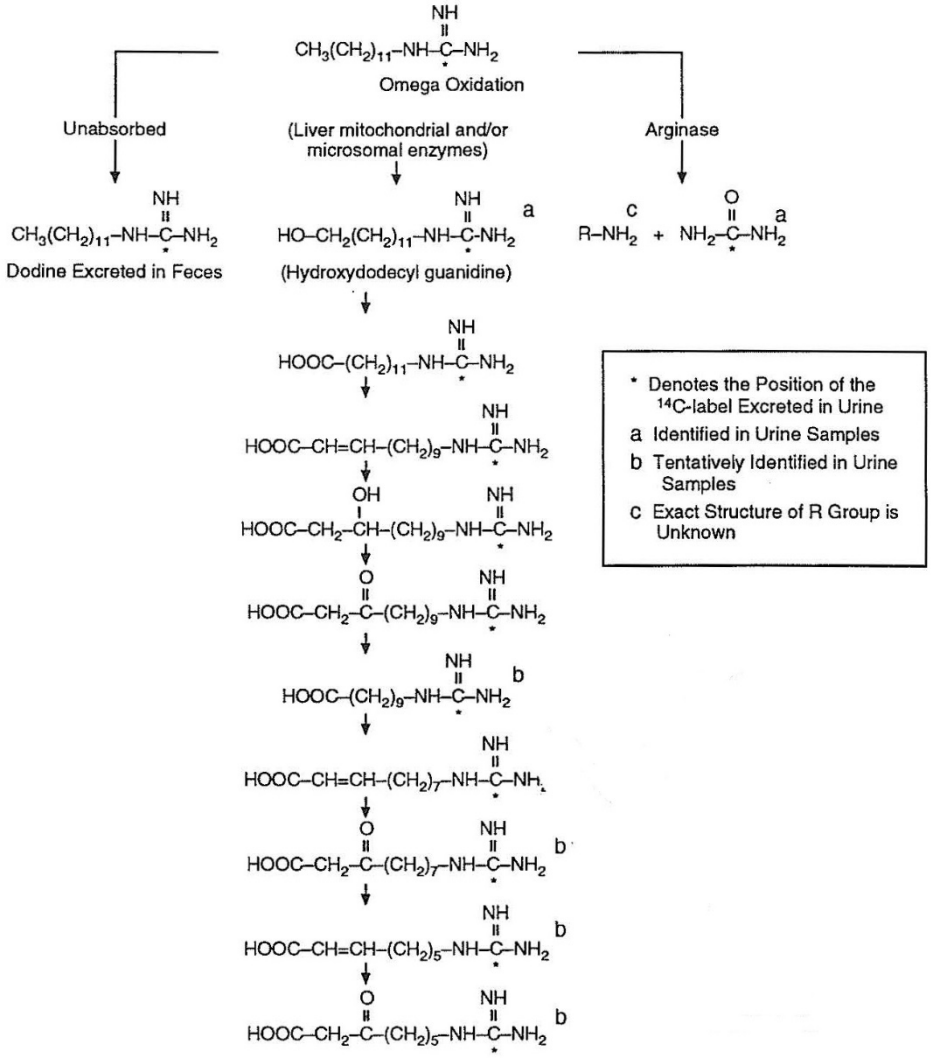
At 120 h, in the single dose groups, dodine-derived radioactivity recovered in urine was 42% and 43% for low and high dose groups, respectively; and in faeces 60% and 50% for low and high dose groups, respectively. Furthermore, ^{14}C-dodine elimination in multiple dose group was 45% and 56% in urine and feces, respectively, at 120 h. Faecal elimination (aprox. 50-60% of the administered dose) was higher than urine excretion (40-45% of the administered dose) in all dose groups at 120 h. No relevant amounts of radioactivity were detected in blood and tissues. The recovery of radioactivity in all tissues together ranged from 0.67% to 3.33%, and the overall distribution pattern at 120 h was similar in both sexes of all dose groups. Oral absorption of ^{14}C-dodine in rats accounts for less than 50% (approx. 43.6% at 120 h after single dose of 40 mg/kg bw).

On the other hand, most of the dodine-derived radioactivity in the urine was eliminated as metabolites, where the parent compound was no detected. Four metabolites were mainly observed in urine, being hydroxy-dodecylguanidine, an omega-oxidation product, the major metabolite (up to 24% of dose). In faecal extracts, the parent compound was found to be the major component.

Because of the presence of the hydroxy dodecylguanidine (M2) and the other tentatively identified acids in the M4 peak, the author of the study postulated that, the metabolism of dodine follows a beta oxidation pathway similar to that of medium- or long-chain fatty acids. Upon entering the liver cell, dodine may be activated by formation of a CoA derivative. With the help of a carrier (similar to carnitine) it may be entering the mitochondrial matrix, and being oxidized by a sequence of reactions in which the alkyl chain of dodine is shortened by two carbon atoms at a time (beta oxidation). This series of reactions may also be catalyzed by a monooxygenase that requires NADPH, O_2, and cytochrome P450.

The absorbed dodine probably enters the liver through the portal circulation and is metabolized to hydroxydodecylguanidine and other intermediate products with shorter chain lengths which are then eliminated through the urine. Urea may also be formed in the liver as a result of the action of arginase on dodine and/or one or more of its metabolites and eliminated through the urine.

Figure 2.6.1.1/1. Proposed metabolic pathway of dodine in Sprague-Dawley rats



- Another *in vivo* study tested the toxicokinetics of dodine in plasma after a single oral gavage administration in Sprague-Dawley rats (Vol.3, AS, B.6.1.1.2) at 40 mg/kg or 400 mg/kg bw. Dodine was rapidly absorbed, with T_{max} values ranging from 2-4 hours. However, T_{max} was 12 hours for low male dose group, but at t = 4 already a peak in concentration data was observed, which could be related to the absorption phase. Some sex differences in toxicokinetics parameters were also appreciated within each tested group. In the low dose group, C_{max} value of females (0.836 mg/kg bw) was 2-fold the males' value (0.417 mg/kg bw), whereas T_{max} of males was 12 hours compared with the 4 h. in females. In the high dose group, T½ value of males (61 h.) was almost 2-fold the females´ value (36 h.). On the other hand, clinical signs were observed in the high dose group after dodine single oral administration. Piloerection was observed in all animals, whereas other signs such as lethargy, hunched posture, chromodacryorrhoea, and mucous feces were observed throughout experiment.

- A recent *in vivo* study that tested the absorption, distribution and excretion of ^{14}C-dodine in Sprague-Dawley rats after a single oral dose of 5 mg/kg bw, was provided for the renewal process of dodine (Vol.3, AS, B.6.1.1.3). The present study showed that dodine-derived radioactivity was eliminated within 72 h in both male and female rats Excretion of radioactivity via bile was a minor route of excretion. Excretion was rapid and almost complete (96%) within 48 h (34% urinary, 59% faecal and 3% bile). No relevant sex differences were appreciated. Regarding clinical signs after single oral administration, piloerection was observed in 50% of males (2/4) and 75% of females (3/4). Overall, oral absorption after ^{14}C- dodine administration in rats accounts for 39% for both sexes.

- Another *in vivo* study tested the absorption, distribution, metabolism and excretion of ^{14}C-dodine in Sprague-Dawley rats after oral (single dosing, 5 and 50 mg/kg bw, repeated dosing, 5 mg/kg bw) and intravenous administration (i.v, 5 mg/kg bw) (Vol.3, AS, B.6.1.1.4). Regarding plasma radioactivity levels, a peak was reached earlier after i.v. administration (30 and 5 min. for males and females, respectively), than observed after oral administration (~4 h for both sexes). On the other hand, after single oral dose (5 mg/kg bw), major portion of radioactivity excretion occurred in 24 h, at similar proportion in urine and feces, whereas after single i.v.

administration, excretion was observed mainly through urine at 24 h. At 96 h, radioactivity recovery was >90% for both oral and i.v. routes. No differences in excretion were recorded between males and females. Additionally, no relevant accumulation was observed in tissues, and $^{14}CO_2$ recovery was low (~0.5%). Radioactivity remaining in the residual carcass was higher by intravenous injection (8%) compared to oral administration (0.6%). Analysis of the metabolites indicates that ^{14}C-dodine was rapidly metabolised to a number of unidentified polar components, which were chromatographically dissimilar to dodine, dodecylamine and dodecylurea.

-A toxicokinetic report that re-analysed previous data from [redacted] study (Vol. 3, B.6.1.1.4), has also been provided (Vol. 3, B.6.1.1.5). This review used the available data with the aim to calculate toxicokinetic parameters such as bioavailability (F), that original authors did not calculated. Due to this report is based on the B.6.1.1.4 study data, and such study presented methodological deviations, the B.6.1.1.5 ([redacted], 2020) study is hence considered as supporting information, and the (F) and other toxicokinetic parameters could be taken such as rough estimation. The current report showed that Tmax value was shorter (5 and 15 min. for females and males, respectively) after i.v. administration than oral (ranged from 4 to 8 h for both sexes) administration. The AUC_{24} values increased approximately proportionately to the dose increment after a single oral dose of ^{14}C-dodine. On the other hand, Cmax values were higher in the i.v. group than in the single oral low dose group at similar dose (5 mg/kg bw). An estimated oral bioavailability of radioactivity following single doses value was in the range 36.1- 43.4%.

Comparative metabolism

-An *in vitro* comparative study has been presented for the renewal purpose of dodine (Vol. 3, B.6.1.1.6). This study evaluated the metabolic profile of dodine, and identified the main metabolites formed using rat, dog and human liver microsomes as test system. No human specific metabolites were detected as the four radioactivity peaks detected (including dodine peak) in human liver microsomes were also detected in at least one of the animal species. Of these, three of them were found in the three species tested, and one metabolite was mainly detected in human (>4-fold). The metabolism of ^{14}C-dodine included oxidations and desaturations.

Other routes

No studies of dodine by dermal or inhalation routes have been submitted. There is no concern for toxicity following dermal exposure to dodine as acute dermal toxicity is expected low compared to acute toxicity following oral exposure. As for the inhalation route, dodine is not volatile (vapour pressure: < 5.49 x 10^{-6} Pa at 50°C). Therefore. no studies are required using dermal or inhalation routes of exposure.

Residue definition for body fluids and tissues

Considering the available information, residues in body fluids it could be applied to active substance dodine and the metabolite hydroxy-dodecylguanidine identified as a major metabolite in urine of rats exposed to dodine.

2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template]

Table 18: Summary table of animal studies on acute oral toxicity

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance, dose levels, duration of exposure</th><th>Value
LD50</th><th>Reference</th></tr>
<tr><td>Acute oral toxicity study in albino rats
OECD TG 401
Data on the preliminary study not provided.
GLP: Yes
Acceptable</td><td>Albino Crl:CD rats
5 males and 5 females /group</td><td>Dodine technical (Purity: 96.61%) in 0.5% methylcellulose
Oral by gavage
Single dose
3 dose groups: 450, 761 and 1285 mg/kg bw
14-day observation period</td><td>Results (no. death/no. animals treated):
<table>
<tr><th>Dose (mg/kg bw)</th><th>Females</th><th>Males</th><th>Combined</th></tr>
<tr><td>450</td><td>0 / 5</td><td>0 / 5</td><td>0 / 10</td></tr>
<tr><td>761</td><td>3 / 5</td><td>1 / 5</td><td>4 / 10</td></tr>
<tr><td>1285</td><td>4 / 5</td><td>5 / 5</td><td>9 / 10</td></tr>
</table>
Clinical signs:
Abnormal defecation in all three dose groups (mucoid faeces, ↓defecation, diarrhoea and/or white material in faeces). Coloured materials in several areas due to discharges/excretions.
Hypoactivity: all animals at 761 and 1285 mg/kg bw (reversible only in surviving animals).
Impaired muscle coordination at 1285 mg/kg bw (2 ♂and 3♀).</td><td>[redacted] (1999a) (CA) B.6.2.1.1</td></tr>
</table>

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Value LD_{50}	Reference
			Penis prolapse: mid and high dose (2 and 4 males, respectively). Surviving animals appeared normal by day 12 and thereafter (except for discoloured areas and hair loss). **Oral LD_{50} female rats: 817 (501-1333) mg/kg bw** LD_{50} males: 830 (731-942) mg/kg bw LD_{50} combined = 851 (658 – 1100) mg/kg bw	
Acute oral toxicity study in mice OECD TG 426 (up and down) GLP: Yes **Acceptable**	Swiss female mice, 9 animals	Dodine technical (Purity: 95.06%) in distilled water Oral by gavage Single dose: 970 mg/kg bw (2 ♀) 1290 mg/kg bw (3 ♀) 1750 mg/kg bw (3 ♀) 2300 mg/kg bw (1 ♀) 14-day observation period	Mortality results by day of administration (O=survival and X=death): **Step** / **Dose (mg/kg b.w.)** / **Result** / **Time of death** 1 / 1750 / O / 2 / 2300 / X / D3 3 / 1750 / X / D3 4 / 1290 / X / 24h 5 / 970 / O / 6 / 1290 / X / 5h 7 / 970 / O / 8 / 1290 / O / 9 / 1750 / X / D4 Mortality results by dose group: **Dose level (mg/kg bw)** / **Mortality % (# dead/total)** 970 / 0% (0/2) 1290 / 67% (2/3) 1750 / 67% (2/3) 2300 / 100% (1/1) Clinical signs were observed from the 1290 mg/kg bw dose and higher, except for little noise at breathing which was observed in one animal at 970, 1290 and 2300 mg/kg bw, with reversibility at the next observation time point. Decreased spontaneous activity was observed in all animals treated with 1290, 1750 and 2300 mg/kg bw, showing reversibility in those animals which survived until the end of the study (one at 1290 and one at 1750 mg/kg bw) and in two females at 1750 and 2300, respectively. One animal at each dose of 1290, 1750 and 2300 mg/kg bw showed also decreased Preyer's reflex and righting reflex. **Oral LD_{50} female mice = 1354 mg/kg bw** (software program AOT425 statpgm)	[redacted] (2008) (CA) B.6.2.1.2
Acute oral toxicity study in mice Similar to OECD TG 401 Deviations: The study is a range finding, tested doses were chosen in order to avoid mortality GLP: No **Supportive**	SPF-bred albino mice 2 groups with 10 animals/sex	Dodine technical (Purity: 98%) in Propylene glycol (10% w/v) Oral by gavage Single dose: 250 mg/kg bw 500 mg/kg bw 14-day observation period	No deaths occurred. No signs of toxicity were recorded for any of the animal tested **Oral LD_{50} > 500 mg/kg bw for male and female mice**	[redacted] (1985) (CA) B.6.2.1.3

Table 19: Summary table of human data on acute oral toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data on acute oral toxicity available				

Table 20: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies relevant for acute oral toxicity available				

2.6.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

All the available acute oral toxicity studies performed with dodine technical were included and assessed in the previous DAR (2009). Two of the three animal studies were performed according to guidance test methods and are considered acceptable: one in rats ([redacted] 1999; B.6.2.1.1) and the other in mice ([redacted], 2008; B.6.2.1.2). The LD_{50} values obtained in these studies were 817 mg/kg bw for female rats and 1354 mg/kg bw for female mice.

The last study in mice ([redacted] 1985; B.6.2.1.3) is considered supportive information, since it was performed as a range-finding study, to determine the maximum dose level of dodine that does not cause mortality in a subsequent mutagenicity study. Selected doses for the study were 250 and 500 mg/kg bw based on the available data at the moment of the study, that suggested an oral LD_{50} value for mice between 500 and 1000 mg/kg bw. No deaths occurred and therefore, the resulting LD_{50} value of this study is > 500 mg/kg bw, supporting the results obtained in the other study in mice (LD_{50} = 1354 mg/kg bw).

Harmonized classification and labelling of dodine is listed in Annex VI of Regulation (EC) No. 1272/2008 (modified for the last time by Commission Directive 98/73/EC of 18th September 1998). Classification regarding acute oral toxicity is included as Acute (oral) toxicity, category 4 (Acute Tox. 4*; H302).

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

The lowest oral LD_{50} value obtained for dodine was 817 mg/kg bw, obtained in the study in rats ([redacted] 1999; B.6.2.1.1). This value is between the threshold values of 300 and 2000 mg/kg bw established in Regulation (EC) No. 1272/2008 for classification of a substance as Acute (oral) Toxicity in category 4 (Acute Tox.4; H302).

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

According to the criteria under Regulation (EC) No. 1272/2008, dodine is classified as **acute (oral) toxicity, category 4, Acute Tox. 4 (H302)** with an **ATE (oral) = 817 mg/kg bw** based on the lowest LD_{50} obtained in the available acute oral toxicity studies.

2.6.2.2 Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template]

Table 21: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance, Dose levels, duration of exposure	Value LD_{50}	Reference
Acute dermal toxicity study in albino rats OECD TG 402 GLP: Yes **Acceptable**	Albino Crl:CD rats 5 males and 5 females	Dodine technical (Purity: 96.7%) moistened with 1.1 ml of deionized water Dermal single application Limit test 5000 mg/kg bw 24-h exposure (occlusive) 14-day observation period	No deaths occurred. No signs of toxicity were recorded for any of the animal tested. Dermal observations in all animals included severe erythema, very slight to slight oedema, eschar, exfoliation and desquamation. Dermal findings that persisted by day 14 included erythema of grades 1 and 4 (on 2 ♀), desquamation (3 ♂ and 2 ♀) and exfoliation (1♀) **Dermal LD_{50} > 5000 mg/kg bw for male and female rats**	[redacted] (1999b) (CA) B.6.2.2

Table 22: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data on acute dermal toxicity available				

Table 23: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies relevant for acute dermal toxicity available				

2.6.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Animal data provided to address the acute dermal toxicity of dodine consisted in one acceptable study ([redacted] 1999b; B.6.2.2), which was already assessed and accepted in de previous DAR (2009). This study complies with the guidance test methods and no deviations from the guideline were observed.

The resulting LD_{50} of this study is > 5000 mg/kg bw for male and female rats.

Harmonized classification and labelling of dodine is listed in Annex VI of Regulation (EC) No. 1272/2008 (modified for the last time by Commission Directive 98/73/EC of 18th September 1998) and no classification regarding acute dermal toxicity is included.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

The LD_{50} of dodine is greater than 5000 mg/kg bw (according to the study of [redacted] 1999b; B.6.2.2), which is above the threshold value of 2000 mg/kg bw established in Regulation (EC) No. 1272/2008 for triggering acute dermal toxicity classification.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

Data available indicates that dodine does not require classification for acute dermal toxicity.

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

Table 24: Summary table of animal studies on acute inhalation toxicity

<table>
<tr><th>Method, guideline, deviations[1] if any</th><th>Species, strain, sex, no/group</th><th>Test substance, form and particle size (MMAD) Dose levels, duration of exposure</th><th>Value LC_{50}</th><th>Reference</th></tr>
<tr>
<td>Acute inhalation toxicity study in rats
OECD TG 403
Deviations: No individual data is available for clinical signs.
GLP: Yes
Acceptable</td>
<td>Rats
Sprague-Dawley
4 dose groups of 5♂ and 5♀, each</td>
<td>Dodine technical (Purity: 96.7%)
Test atmosphere:
<table>
<tr><th rowspan="2">Concentration (mg/L air)</th><th colspan="2">Particle size distribution</th></tr>
<tr><th>MMDA (µm)</th><th>GSD</th></tr>
<tr><td>0</td><td>-</td><td>-</td></tr>
<tr><td>0.25 ± 0.019</td><td>3.2</td><td>2.43</td></tr>
<tr><td>0.34 ± 0.035</td><td>3.3</td><td>2.59</td></tr>
<tr><td>0.51 ± 0.0035</td><td>3.0</td><td>2.62</td></tr>
</table>
Exposure duration: 4h (nose-only)</td>
<td>Mortality:
<table>
<tr><th rowspan="2">Group</th><th rowspan="2">Concentration (mg/L air)</th><th colspan="2">Mortality / animals treated Time of death*</th></tr>
<tr><th>Males</th><th>Females</th></tr>
<tr><td>1 (control)</td><td>0</td><td>0/5</td><td>0/5</td></tr>
<tr><td>3</td><td>0.25 ± 0.019</td><td>0/5</td><td>0/5</td></tr>
<tr><td>4</td><td>0.34 ± 0.035</td><td>1/5
D2</td><td>0/5</td></tr>
<tr><td>2</td><td>0.51 ± 0.0035</td><td>3/5
1h, 2h, D1</td><td>4/5
D1, D1, D4, D5</td></tr>
</table>
*in hour (h) or day (D) post exposure

LC_{50} (♀) = 0.44 mg/L (4h)
LC_{50} (♂) = 0.47 mg/L (4h)
LC_{50} (combined) = 0.45 mg/L (4h)</td>
<td>[redacted] (1999) (CA) B.6.2.3</td>
</tr>
</table>

Table 25: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data on acute inhalation toxicity available				

Table 26: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies relevant for acute inhalation toxicity				

2.6.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Animal data provided to address the acute toxicity of dodine by inhalation consisted in one acceptable study ([redacted] 1999; B.6.2.3), which was already assessed and accepted in de previous DAR (2009). This study complies with the guidance test methods and no relevant deviations from the guideline were observed.
The resulting 4-hour LC_{50} value of this study is 0.44 mg/L for female Sprague-Dawley rats (nose-only exposure).

Harmonized classification and labelling of dodine is listed in Annex VI of Regulation (EC) No. 1272/2008 (modified for the last time by Commission Directive 98/73/EC of 18th September 1998). No classification regarding acute inhalation toxicity is included.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

Dodine LC_{50} value, obtained after 4-hour nose-only exposure in rats, is 0.44 mg/L ([redacted] 1999; B.6.2.3). This value is between the threshold values of 0.05 and 0.5 mg/L (for dusts and mists) established in Regulation (EC) No. 1272/2008 for classification of a substance as Acute (inhalation) Toxicity in category 2 (Acute Tox. 2; H330).

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the available data on dodine, and according to the criteria under Regulation (EC) No. 1272/2008, this active substance is classified as **acute (inhalation) toxicity, category 2, Acute Tox. 2 (H330)** with an **ATE (inhalation) of 0.44 mg/L (dust/mist)** based on the lowest LC_{50} obtained in females in the available acute inhalation toxicity study in rats.

2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template]

Table 27: Summary table of animal studies on skin corrosion/irritation

<table>
<tr><th>Method, guideline, deviations[1] if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels, duration of exposure</th><th>Results
- Observations and time point of onset[2]
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr>
<td>Acute dermal irritation study in albino rabbits
OECD TG 404
GLP: Yes
Acceptable</td>
<td>New Zealand White rabbits (2♂ and 1♀)</td>
<td>Dodine technical (Purity: 96.7%) 0.5 g dodine moistened with 0.4 ml of deionized water</td>
<td>Dermal single application
0.5 g
4-h exposure (occlusive)
14-day observation period</td>
<td>Results:
<table>
<tr><th rowspan="2">Observation time</th><th colspan="6">Rabbit No. (sex)</th></tr>
<tr><th colspan="2">26320 (M)</th><th colspan="2">26321 (M)</th><th colspan="2">26353 (F)</th></tr>
<tr><th>E*</th><th>O*</th><th>E</th><th>O</th><th>E</th><th>O</th></tr>
<tr><td>0.5 – 1 h</td><td>0</td><td>0</td><td>1</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>24 h</td><td>2</td><td>0</td><td>1</td><td>0</td><td>2</td><td>1</td></tr>
<tr><td>48 h</td><td>2</td><td>0</td><td>1</td><td>0</td><td>2</td><td>0</td></tr>
<tr><td>72 h</td><td>2</td><td>0</td><td>2</td><td>0</td><td>2</td><td>0</td></tr>
<tr><td>Mean 24/48/72h</td><td>2</td><td>0</td><td>1.33</td><td>0</td><td>2</td><td>0.33</td></tr>
<tr><td>14 D</td><td>1d</td><td>0</td><td>0</td><td>0</td><td>1d</td><td>0</td></tr>
<tr><td>Reversibility</td><td>N</td><td>-</td><td>Y</td><td>-</td><td>N</td><td>Y</td></tr>
</table>
* E: erythema, O: oedema
[d] Desquamation

The test material induced slight to moderate erythema and desquamation on all animals. One animal was observed with very slight oedema at 24 hours. The erythema and desquamation persisted though study termination, with the exception of one animal in which both subsided by day 14 (no reversible).
There were no other dermal findings.</td>
<td>[redacted] (1999c) (CA) B.6.2.4</td>
</tr>
</table>

Table 28: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data on skin corrosion/irritation				

Table 29: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Acute dermal toxicity study in albino rats OECD TG 402 GLP: Yes **Acceptable**	Dodine technical (Purity: 96.7%) moistened with 1.1 ml of deionized water	Albino Crl:CD rats 5 males and 5 females Dermal single application Limit test: 5000 mg/kg bw 24-h exposure (occlusive) 14-day observation period	Dermal observations in all animals included severe erythema, very slight to slight oedema, eschar, exfoliation and desquamation. Dermal findings that persisted through day 14 included erythema of grades 1 and 4 (on 2 ♀), desquamation (3 ♂ and 2 ♀) and exfoliation (1 ♀).	[redacted] (1999b) (CA) B.6.2.2

2.6.2.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Animal data provided to address the acute skin irritation properties of dodine consisted in one acceptable study ([redacted] 1999c; B.6.2.4), which was already assessed and accepted in de previous DAR (2009). This study complies with the guidance test methods and no relevant deviations from the guideline were observed.

Under the conditions of the study, dodine technical caused inflammation in the application site in the form of erythema (reaching grades 2 and 3) that lasted in two of the three animals until the end of the 14-day observation period (not reversible).

Moreover, in the acute toxicity study by dermal route ([redacted] 1999b; B.6.2.2), erythema, desquamation and exfoliation were observed in several animals at the end of the 14-day observation period, confirming the no-reversibility of the skin lesions provoked by the substance.

Harmonized classification and labelling of dodine is listed in Annex VI of Regulation (EC) No. 1272/2008 (modified for the last time by Commission Directive 98/73/EC of 18th September 1998). Classification regarding skin corrosion/irritation is included as Skin irritation, category 2 (Skin Irrit. 2; H315).

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

According to the current EU Criteria (Regulation (EC) No 1272/2008), classification as skin irritant is required if inflammation persists to the end of the observation period in at least 2 animals.

Available animal data on dodine described erythema (grade 1) and desquamation that lasted until the end of the observation period in two of the three animals of the skin corrosion/irritation study ([redacted] 1999c; B.6.2.4), together with erythema (grades 1 and 4), desquamation and exfoliation in several animals of the acute dermal toxicity study by dermal route ([redacted] 1999b; B.6.2.2).

Therefore, the assessed information confirms the actual classification of dodine in Annex VI of Regulation (EC) No 1272/2008 as skin irritant category 2 and, therefore, no modification of this classification is proposed.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No modification of the actual classification of dodine as **skin irritation, category 2 (Skin Irrit. 2, H315)** is proposed.

2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template]

Table 30: Summary table of animal studies on serious eye damage/eye irritation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance</th><th>Dose levels duration of exposure</th><th>Results
- Observations and time point of onset[2]
- Mean scores/animal
- Reversibility</th><th>Reference</th></tr>
<tr>
<td>Acute eye irritation study in albino rabbits
OECD TG 405
Deviations: Examination results of the left (control) eye at 48 hours were inadvertently not recorded.

GLP: Yes
Acceptable</td>
<td>New Zealand White rabbits (1♀)</td>
<td>Dodine technical (Purity: 96.7%)</td>
<td>47 mg (equivalent to a volume of 1 ml)

Study duration: 7 days (animal euthanized)</td>
<td>Results:
<table>
<tr><th></th><th>Corneal opacity</th><th>Iris lesion</th><th colspan="2">Conjunctiva</th></tr>
<tr><th></th><th></th><th></th><th>redness</th><th>chemosis</th></tr>
<tr><td>1 hr</td><td>0</td><td>0</td><td>1</td><td>4</td></tr>
<tr><td>24 hrs</td><td>4*</td><td>2*</td><td>2</td><td>4</td></tr>
<tr><td>48 hrs</td><td>4*</td><td>2*</td><td>3</td><td>4</td></tr>
<tr><td>72 hrs</td><td>4</td><td>2</td><td>3</td><td>4</td></tr>
<tr><td>4 days</td><td>4</td><td>2</td><td>3</td><td>4</td></tr>
<tr><td>7 days</td><td>4</td><td>2</td><td>3</td><td>4</td></tr>
<tr><td>Revers.</td><td>no</td><td>no</td><td>no</td><td>no</td></tr>
<tr><td>Average (24-72h)</td><td>-*</td><td>-*</td><td>2.7</td><td>4</td></tr>
</table>
*unable to be determined due to severe chemosis. Maximum score applied.

Maximum scores were noted, even 7 days after instillation, for corneal opacity (4), iris lesion (2), conjunctival redness (3) and chemosis (4).
Other findings observed in the treated eye included purulent discharge from 24 h until the end of the study, 25% of cornea retaining stain at 72 h and day</td>
<td>[redacted] (1999d) (CA) B.6.2.5</td>
</tr>
</table>

				7, petite haemorrhage on days 4 and 7 and corneal neovascularization on day 7.	

Table 31: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data on serious eye damage/eye irritation				

Table 32: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies relevant for serious eye damage/eye irritation				

2.6.2.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Animal data provided to address the acute eye irritation properties of dodine consisted in one acceptable study ([redacted] 1999d; B.6.2.5), which was already assessed and accepted in de previous DAR (2009). This study complies with the guidance test methods and no relevant deviations from the guideline were observed.

Under the conditions of the study no reversibility of the lesions were observed, with maximum scores noted for corneal opacity (4), iris lesion (2), conjunctival redness (3) and chemosis (4) even 7 days after instillation. Moreover, corneal neovascularization was also noted at day 7, confirming the serious eye damage caused by dodine in the rabbit.

Harmonized classification and labelling of dodine is listed in Annex VI of Regulation (EC) No. 1272/2008 (modified for the last time by Commission Directive 98/73/EC of 18th September 1998). Classification regarding skin corrosion/irritation is included as Eye irritation, category 2 (Eye Irrit. 2; H319).

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

According to the current EU Criteria (Regulation (EC) No 1272/2008), classification of substances within hazard class Category 1 (serious eye damage), includes persistent lesions (those which are not fully reversible within an observation period of normally 21 days).

Available animal data on dodine ([redacted] 1999d; B.6.2.5) described maximum scores for corneal opacity (4), iris lesion (2), conjunctival redness (3) and chemosis (4) until day 7, when the study was finalised. These scores were mantained from 24 hours post-instillation.

Mean values could not be obtained for corneal and iris lesions since at 24 and 48 hours after instillation, it was not possible to determine these grades due to severe chemosis (and maximum scores were applied).

Moreover, the grades of corneal opacity and iritis remaining by day 7 are considered severe in the three rabbits, since these scores (4 and 2, respectively) exceed the value established as CLP criteria (mean 24/48/72 h values ≥ 3 for corneal opacity and/or >1.5 for iritis) for classification of substances as Category 1.

Altogether, the assessed information suggests the actual classification of dodine in Annex VI of Regulation (EC) No 1272/2008 (as eye irritant category 2) is underestimated and, therefore, classification in a higher category (serious eye damage, category 1) is proposed.

2.6.2.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on the available data on dodine, and according to the criteria under Regulation (EC) No. 1272/2008, classification of this active substance in category 2 should be modified to: serious eye damage, category 1, Eye Dam. 1 (H318).

2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template]

Table 33: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
No data available					

Table 34: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data on respiratory sensitisation available				

Table 35: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies relevant for respiratory sensitisation available				

2.6.2.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data available for dodine.
Harmonized classification and labelling of dodine is listed in Annex VI of Regulation (EC) No. 1272/2008 (modified for the last time by Commission Directive 98/73/EC of 18th September 1998) and no classification regarding respiratory sensitization is included.

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

No data available for dodine.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

In the absence of any data, no classification for respiratory sensitisation can be drawn for dodine.

2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template]

Table 36: Summary table of animal studies on skin sensitisation

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance Dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Skin sensitization test in guinea-pigs
OECD TG 406 (Maximization M&K)

Deviations: skin reactions after induction applications not recorded.

GLP: Yes
Acceptable</td><td>Dunkin-Hartley guinea-pigs

Preliminary test: 1/sex
Main test: 15/sex</td><td>Dodine technical (Purity: 96.7%)

<u>Preliminary study</u>:
-i.d.: 0.1% w/w in corn oil (maximum practicable concentration)
-Topical: 0.5 ml of 20 and 40% dodine in corn oil

<u>Main study</u>:
Induction:
-i.d.: 0.1% dodine in</td><td>Results:

<u>Preliminary study</u>: Selection of the doses to be used in the main study.
<table>
<tr><th rowspan="3">Anim.</th><th colspan="4">Intradermal route</th><th colspan="4">Cutaneous route</th></tr>
<tr><th rowspan="2">% Test subst.</th><th colspan="3" rowspan="2">Scoring after treatment</th><th rowspan="2">% Test subst.</th><th rowspan="2">Site</th><th colspan="4">Scoring after removal of dressings</th></tr>
<tr><th colspan="2">24 h</th><th colspan="2">48 h</th></tr>
<tr><th></th><th></th><th>24h</th><th>48h</th><th>6d</th><th></th><th></th><th>E</th><th>O</th><th>E</th><th>O</th></tr>
<tr><td rowspan="2">Male 01</td><td>0.1 + FCA</td><td>I</td><td>I</td><td>-</td><td>40</td><td>RF</td><td>0S</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>0.1</td><td>I</td><td>LI</td><td>-</td><td>20</td><td>LF</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table></td><td>[redacted]
(1999)
(CA) B.6.2.6</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any</th><th>Species, strain, sex, no/group</th><th>Test substance Dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>corn oil
-Topical (previous day: 0.5 ml of sodium lauryl sulfate (10% w/w) in vaseline): 0.5 ml of 40% dodine in corn oil

Challenge: 0.5mL of 40% dodine in corn oil in right flank and vehicle only in the left flank</td><td>
<table>
<tr><td rowspan="2">Fem. 01</td><td>0.1 + FCA</td><td>I</td><td>I</td><td>I</td><td>40</td><td>RF</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>0.1</td><td>I</td><td>LI</td><td>LI</td><td>20</td><td>LF</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
</table>
FCA: Freund's Complete Adjuvant

I: Irritant
LI: Slightly irritant
- Dead animal

E: Erythema
O: Oedema
RF: Right flank
LF: Left flank
S: dryness of the skin

Main study:
No skin reactions were observed in any of the animals (control and treated group) at 24 and 48 hours after the removal of the dressings. Scoring values were 0 for erythema and oedema at 24 and 48 hours and in both flanks (vehicle was applied on the left flank and test substance on the right flank).</td><td></td></tr>
</table>

Table 37: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data on skin sensitisation available				

Table 38: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies relevant for skin sensitisation available				

2.6.2.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Animal data provided to address the skin sensitization properties of dodine consisted in one acceptable study ([redacted] 1999; B.6.2.6), which was already assessed and accepted in de previous DAR (2009). This study complies with the guidance test methods and, although skin reactions after induction applications were not recorded, sodium lauryl sulfate (10% w/w) in vaseline was applied the previous day and no relevant deviations from the guideline were observed.

No skin sensitization responses were provoked by dodine under the conditions of the study.

Harmonized classification and labelling of dodine is listed in Annex VI of Regulation (EC) No. 1272/2008 (modified for the last time by Commission Directive 98/73/EC of 18th September 1998) and no classification regarding skin sensitization is included.

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

Available animal data showed negative results in an acceptable maximization (Magnusson and Kligman) study in guinea pigs.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

Data available indicates that dodine does not require classification for skin sensitization.

2.6.2.8 Phototoxicity

Table 39: Summary table of studies on phototoxicity

Method, guideline, deviations[1] if any	Test substance	Dose levels duration of exposure	Results	Reference
No data available.				

Table 40: Summary table of human data on phototoxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 41: Summary table of other studies relevant for phototoxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

No phototoxicity study with dodine was provided. However, this testing is not required by Regulation (EU) No 283/2013, since the results of UV/Visible spectroscopy performed with the active substance dodine showed no absorption with wavelength > 290 nm.

2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template]

Table 42: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

No evidence of aspiration hazard of dodine was found in the provided data.

Harmonized classification and labelling of dodine is listed in Annex VI of Regulation (EC) No. 1272/2008 (modified for the last time by Commission Directive 98/73/EC of 18th September 1998) and no classification regarding aspiration hazard.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

Dodine is presented in a solid (particulate/powder) form. According to CLP Regulation, "Although the definition of aspiration in section 3.10.1.2 includes the entry of solids into the respiratory system, classification according to point (b) in Table 3.10.1 for Category 1 is intended to apply to liquid substances and mixtures only". Hence, this point does not apply for dodine.

Besides, according to point (a) of Table 3.10.1 of CLP Regulation, a substance can be classified for aspiration toxicity based on reliable and good quality human evidence. Dodine is not a hydrocarbon compound and no data associated with this hazard class have been reported in humans for dodine.

Taking into account points (a) and (b) of classification criteria for aspiration toxicity included in Table 3.10.1 of CLP Regulation, criteria for classification for this hazard class are not fulfilled for dodine.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

No classification proposed based on data conclusive but not sufficient for classification.

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template]

Table 43: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Acute oral toxicity study in albino rats OECD TG 401 Data on the preliminary study not provided. GLP: Yes **Acceptable** Albino Crl:CD rats 5 rats/sex/group	Dodine technical (Purity: 96.61%) in 0.5% methylcellulose Oral by gavage Single dose 3 dose groups: 450, 761 and 1285 mg/kg bw 14-day observation period	*Only effects relevant for STOT SE are presented (see also section 2.6.2.1. for more study details)* **Clinical signs:** ▪ Hypoactivity: all rats from 761 mg/kg bw (reversible only in surviving animals). ▪ Impaired muscle coordination: 2♂ and 3♀ at 1285 mg/kg bw. ▪ Penis prolapse: 2 and 4 rats at 761 and 1285 mg/kg bw, respectively. LD_{50} female rats: 817 (501-1333) mg/kg bw LD_{50} males: 830 (731-942) mg/kg bw LD_{50} combined = 851 (658 – 1100) mg/kg bw	[redacted] (1999) (CA) B.6.2.1.1
Acute oral toxicity study in mice OECD TG 426 (up and down) GLP: Yes **Acceptable** Swiss ♀ mice	Dodine technical (Purity: 95.06%) in distilled water Oral by gavage Single dose: 970 mg/kg bw (2 ♀) 1290 mg/kg bw (3 ♀) 1750 mg/kg bw (3 ♀) 2300 mg/kg bw (1 ♀) 14-day observation period	*Only effects relevant for STOT SE are presented (see also section 2.6.2.1 for more study details)* **Clinical signs**: ▪ Little noise at breathing: 1 animal at 970, 1290 and 2300 mg/kg bw, with reversibility at the next day. ▪ Decreased spontaneous activity: all animals treated with 1290, 1750 and 2300 mg/kg bw, with reversibility in 1 at 1290 mg/kg bw, 2 at 1750 mg/kg bw and 1 at 2300 mg/kg bw. ▪ Decreased Preyer's reflex and righting reflex: 1 animal at each 1290, 1750 and 2300 mg/kg bw. LD_{50} female mice: 1354 mg/kg bw.	[redacted] F. (2008) (CA) B.6.2.1.2
Acute inhalation toxicity study in rats OECD TG 403 Deviations: No individual data is available for clinical signs. GLP: Yes **Acceptable** Sprague-Dawley rats 5 rats/sex/group	Dodine technical (Purity: 96.7%) Exposure duration: 4h (nose-only): 0 mg/L 0.25 mg/L 0.34 mg/L 0.51 mg/L	*Only effects relevant for STOT SE are presented (see also section 2.6.2.1 for more study details)* **Clinical signs**: ▪ Brown staining around snout and/or jaws: from 0.25 mg/L in ♂ and ♀. ▪ Matted fur: from 0.34 mg/L in ♂ and ♀. ▪ Wet fur: from 0.25 mg/L in ♂ and from 0.34 mg/L in ♂. ▪ Pilo-erection: at 0.51 mg/L in ♂ and ♀. ▪ Gasping: from 0.25 mg/L in ♂ and ♀. ▪ Cold body to touch: at 0.51 mg/L in ♂ and ♀. ▪ Lethargy: from 0.34 mg/L in ♂. ▪ Ataxic: at 0.34 mg/L in ♀, with recovery. **Bodyweight:** ▪ (↓) bw at day 7 in ♂ [20.3% at 0.25 mg/mL, 26.9% at 0.34 mg/mL and 26.6% at 0.51 mg/mL] and ♀ [9.8% at 0.25 mg/mL, 12.8% at 0.34 mg/mL and 17.1% at 0.51 mg/mL]. ▪ (↓) bw gain between day 0-7 in ♂ [112% at 0.25 mg/mL,	[redacted] (1999) (CA) B.6.2.3

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		160% at 0.34 mg/mL and 158% at 0.51 mg/mL] and ♀ [126.7% at 0.25 mg/mL, 180% at 0.34 mg/mL and 246.7% at 0.51 mg/mL]. **Organs´ weight:** ▪ Lungs: (↑) wt in ♂ decedents [75% at 0.34 mg/mL and 129.9% at 0.51 mg/mL] and ♀ [120.8% at 0.51 mg/mL]. ▪ Liver: (↓) abs wt in ♂ decedents [32.5% at 0.34 mg/mL and 17.2% at 0.51 mg/mL], ♂ survivors [12.7% at 0.25 mg/mL, 15.2% at 0.34 mg/mL and 12.6% at 0.51 mg/mL] and ♀ decedents [31.7% at 0.51 mg/mL]. ▪ Kidney: (↓) abs wt in ♂ decedents [22.8% at 0.34 mg/mL and 12% at 0.51 mg/mL], ♂ survivors [17.2% at 0.25 mg/mL, 21% at 0.34 mg/mL and 12% at 0.51 mg/mL] and ♀ decedents [16.4% at 0.51 mg/mL]. **Gross pathology:** ▪ (↑) Congestion in lung lobes in ♂ (0/5 in controls, 1/5 at 0.34 mg/mL, 3/5 at 0.51 mg/mL) and in ♀ (0/5 in controls, 4/5 at 0.51 mg/mL). ▪ (↑) Congestion in intestines in ♂ (0/5 in controls, 1/5 at 0.34 mg/mL, 3/5 at 0.51 mg/mL) and in ♀ (0/5 in controls, 2/5 at 0.51 mg/mL). ▪ (↑) Enlarged heart: in ♀ (0/5 in controls, 2/5 at 0.51 mg/mL). LC_{50} (♀) = 0.44 mg/L (4h) LC_{50} (♂) = 0.47 mg/L (4h) LC_{50} (combined) = 0.45 mg/L (4h)	
Mammalian chromosome aberrations in somatic cells (Micronucleus test) Similar to OECD TG 474. Some deviations from OECD TG 474 (2016): Negative control group sampled only at 24 h, minimal information about HCD, just 1000 PCE per animal. Test system: ♂/♀ Mice (ICR strain) 3 mice/sex/dose in dose-range finding study 5 mice/sex/dose in main study GLP: yes	Dodine (batch: KG 303/90; purity 94%) Vehicle: Corn oil Dosage: 100, 200 and 400 mg/kg bw (oral by gavage) Sampling: 24, 48 and 72 h after administration.	*Only effects relevant for STOT SE are presented (see also section 2.6.4 for more study details)* **Toxicity:** *Dose range finding test*: No effects immediately after dosing. 41 h after dosing, 1♂ at 387.5 mg/kg bw and 1♂ at 500 mg/kg bw died. All remaining mice at 387.5 and 500 mg/kg bw had rough hair coats. Prior to euthanasia, all remaining mice at 387.5 and 500 mg/kg bw languid and with rough hair coats. *Micronucleus assay*: No effects immediately after dosing. 6 h after dosing, 1♂ at 400 mg /kg bw died. Prior to the 24-h harvest, 1♂ at 400 mg /kg bw died. Prior to the 48-h harvest, 1♂ at 200 mg/kg bw and 1♂ at 400 mg/kg bw with distended abdomen. Prior to the 72-h harvest, 1♂ at 400 mg/kg bw with distended abdomen.	[redacted] (1992) (CA) B.6.4.2.1-02

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Acceptable			

Table 44: Summary table of human data on STOT SE (specific target organ toxicity-single exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No human data on STOT SE available				

Table 45: Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies relevant for STOT SE				

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. Relevant information for STOT SE is covered by acute toxicity studies in form of clinical observations and macroscopic and microscopic pathological examination that can reveal hazards that may not be life-threatening but could indicate functional impairment. Effects of other single dose studies or repeated dose studies (first dosing effects) are also considered for STOT SE.

During the analysis of the classification of dodine as STOT SE, effects potentially relevant for STOT SE have been found in acute toxicity studies and genotoxicity/germ cell mutagenicity studies (from sections 2.6.2 and 2.6.4, respectively).

In the acute oral toxicity study in rats (B.6.2.1.1), the effects observed relevant for STOT SE were hypoactivity in all rats from 761 mg/kg bw (reversible in surviving animals), impaired muscle coordination in some animals at 1285 mg/kg bw and penis prolapse from 761 mg/kg bw.

In the acute oral toxicity study in female mice (B.6.2.1.2), the effects observed relevant for STOT SE were clinical signs like little noise at breathing with reversibility at the next day from 970 mg/kg bw, decreased spontaneous activity in all mice from 1290 mg/kg bw and decreased Preyer's reflex and righting reflex from 1290 mg/kg bw.

In the acute inhalation toxicity study in rats (B.6.2.3), the effects relevant for STOT SE were found among clinical signs, organs´ weights and gross pathology parameters. Brown staining around snout and/or jaws was observed from 0.25 mg/L, matted fur from 0.34 mg/L, wet fur from 0.25 mg/mL, pilo-erection at 0.51 mg/L, gasping from 0.25 mg/L, cold body to touch: at 0.51 mg/L, lethargy from 0.34 mg/L and ataxia at 0.34 mg/L. Lungs weight increased from 0.34 mg/mL, liver weight decreased from 0.25 mg/mL and kidney weight decreased from 0.25 mg/mL. Increased incidence of congestion in lung lobes was observed from 0.34 mg/mL and in intestines from 0.51 mg/mL. Furthermore, the incidence of enlarged heart was incremented in females from 0.51 mg/mL.

In an in vivo micronucleus test (B.6.4.2.1-02), a dose-range finding study and a main study were carried out. In the dose-range finding study, no effects were seen immediately after dosing. 41 hours after dosing, some mice from 387.5 mg/kg bw/day died and the remaining had rough hair coats. Prior to euthanasia, all remaining mice from 387.5 mg/kg bw were languid and with rough hair coats. In the main microcucleus study, neither effects immediately after dosing were reported. Prior to the 48-hour and 72-hour harvests, one remaining male showed distended abdomen from 200 and 400 mg/kg bw, respectively.

Prior to the 72-h harvest, 1♂ at 400 mg/kg bw with distended abdomen.

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

<u>STOT SE 1 and 2</u>

STOT-SE Category 1 and 2 is assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context

'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature with significant impact on health. Both factors have to be evaluated by weight of evidence and expert judgement.

Effects in the range of STOT SE 1 (guidance value for classification by inhalation: ≤ 1 mg/L/4h) were observed in the acute inhalation study in rats. They were observed mainly at doses close to the LC_{50} and they were covered by the classification proposal for dodine as Acute (inhalation) Tox. 2 (H330).

Effects in the range of STOT SE 2 (guidance value for classification by oral, ≤ 2000 mg/kg bw and >300 mg/kg bw) were observed in acute oral studies in rats and mice and in an *in vivo* micronucleus test. They were observed mainly at doses close to the LD_{50} (therefore, they were covered by the classification proposal for dodine as Acute (oral) Tox. 4; H302) and/or did not indicate the presence of a clear target organ.

Therefore, dodine does not require classification for STOT SE 1 or 2.

STOT SE 3

STOT SE 3 includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2.

Although some of the acute effects observed after the administration of dodine could be considered as narcotic effects (i.e. hypoactivity, loss of reflex, lethargy or ataxia) and respiratory tract irritation signs (congestion of the lung lobes), they were all observed only at doses covered by the classification proposal for dodine as Acute Tox. (H302 and H330).

Therefore, dodine does not require classification for STOT SE 3.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

Dodine does not require classification for STOT SE according to CLP Regulation.

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE) [equivalent to section 10.12 of the CLH report template]

Table 46: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL** **[Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]**	**Reference**
Rat toxicity studies			
28-day oral (gavage) study in rat. Guideline: US EPA FIFRA F-82-1. GLP: Yes Rat strain: Crl:CD® (SD) BR No. animals 10 rats/sex/dose Deviations from OECD TG 407	Dodine (batch no. APA 303/90 and purity of 94.07%). Vehicle: 0.5% methylcellulose. Doses: 0, 75 and 100 mg/kg bw/day for 28 days. 200 mg/kg bw/day for less than 2 weeks.	**Mortality**: ♂: 10/10 at 200 mg/kg bw/day died ♀: 1/10 at 75 mg/kg bw/day, 4/10 at 100 mg/kg bw/day and 10/10 at 200 mg/kg bw/day died. **200 mg/kg bw/day** Clinical signs *(no statistical analysis performed):* ▪ (↑) Respiratory problems in ♂ (9/10 *vs* 1/10 in controls) and ♀ (5/10 *vs* 0/10 in controls). ▪ (↑) Salivation in ♂ (10/10 *vs* 1/10 in controls) and ♀ (9/10 *vs* 0/10 in controls). ▪ (↑) Staining of head in ♂ (10/10 *vs* 1/10 in controls) and ♀ (9/10 *vs* 1/10 in controls). Histopathological findings *(no statistical analysis*	[redacted] (1994a) **B.6.3.1.1 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
(2008): - There should not be mortality. - Functional observation not performed. - Reticulocyte count and determination of T3, T4 and TSH hormones not performed. - Epididymides, prostate + seminal vesicles with coagulating glands as a whole, thymus, spleen and heart not weighed. - Histopathology not in all the recommended tissues or at high dose only. **Study acceptable as supportive information.** *Guideline value for classification: STOT RE 2 ≤ 300 mg/kg bw/day STOT RE 1 ≤ 30 mg/kg bw/day (Haber's rule from 90- to 28-day value)*		*performed):* ▪ (↑) Adrenals haemorrhage in ♂ (6/10 *vs* 0/10 in control) and in ♀ (6/10 *vs* 0/10 in control). ▪ (↑) Duodenum vacuolization in ♀ (2/10 *vs* 0/10 in control) and necrosis in ♂ (4/10 *vs* 0/10 in control). ▪ (↑) Heart degeneration and/or fibrosis in ♀ (4/10 *vs* 0/10 in control). ▪ (↑) Ileum necrosis in ♂ (2/10 *vs* 0/10 in control). ▪ (↑) Jejunum necrosis in ♂ (4/10 *vs* 0/10 in control). ▪ (↑) Lung haemorrhage in ♂ (2/10 *vs* 1/10 in control) and in ♀ (3/10 *vs* 0/10 in control) and oedema in ♀ (2/10 *vs* 0/10 in control). ▪ Prostate and seminal vesicle atrophy (3/10 each one). Unknown in controls. ▪ (↑) Spleen atrophy in ♂ (10/10 *vs* 0/10 in control) and in ♀ (9/10 *vs* 0/10 in control). ▪ (↑) Stomach hyperkeratosis in ♂ (5/10 *vs* 0/10 in control) and in ♀ (5/10 *vs* 0/10 in control); hyperplasia in ♂ (7/10 *vs* 0/10 in control) and in ♀ (6/10 *vs* 0/10 in control); and ulcer in ♂ (7/10 *vs* 0/10 in control) and in ♀ (7/10 *vs* 0/10 in control). ▪ Thymus haemorrhage and lymphoid necrosis in ♂ (2/10 each one, unknown in controls). **100 mg/kg bw/day** Clinical signs *(no statistical analysis performed):* ▪ (↑) Respiratory problems in ♂ (9/10 *vs* 1/10 in controls) and ♀ (9/10 *vs* 0/10 in controls). ▪ (↑) Salivation in ♂ (10/10 *vs* 1/10 in controls) and ♀ (10/10 *vs* 0/10 in controls). ▪ (↑) Staining of head in ♂ (8/10 *vs* 1/10 in controls) and ♀ (6/10 *vs* 1/10 in controls). Bodyweight and food consumption: ▪ (↓) bw in ♂ [at day 28 (24.8%)] and ♀ [at day 28 (15.5%)]. ▪ (↓) bw gain week 0-4 in ♂/♀ (53.9/29.9%) *(no*	

		statistical analysis reported for this period). ▪ (↓) food consumption in ♂ [between days 0-28 (27%)] and ♀ [between days 0-28 (17%)] *(no statistical analysis reported for this period)*. <u>Haematology:</u> ▪ (↑) WBC in ♂ (63%) and in ♀ (40%, ns). ▪ (↑) Neutr. segm. in ♂ (204%) and ♀ (257%). ▪ (↓) Lymph. in ♂ (11%) and ♀ (25%). ▪ (↑) RDW in ♂ (12%) and in ♀ (7%). ▪ (↑) RBC in ♂ (8%). ▪ (↑) Plt in ♀ (9.6%, ns, ndr). ▪ (↓) MPV in ♀ (1.2%, ns, ndr). ▪ (↓) MCHC in ♀ (1.1%). ▪ (↑) Hb in ♂ (8.8%). ▪ (↑) Ht in ♂ (7.7%). <u>Clinical chemistry:</u> ▪ (↓) glucose in ♂ (26.8%) and in ♀ (31.4%). ▪ (↑) ALT in ♂ (212.5%) and in ♀ (202.6%). ▪ (↓) globulin in ♂ (14.7%) and ♀ (20.6%). ▪ (↓) albumin in ♂ (9.4%) and ♀ (9.1%). ▪ (↑) A/G ratio in ♀ (17.7%). ▪ (↑) Na in ♂ (1.1%). ▪ (↓) Ca in ♂ (3.3%). ▪ (↓) Total protein in ♂ (10.6%) and ♀ (14.9%). <u>Organs´ weight:</u> ▪ Liver: (↓) abs wt in ♂ (21.8%) and (↑) rel-to-body wt in ♀ (27.2%). ▪ Lungs: (↓) abs wt in ♂ (11.1%) and (↑) rel-to-body wt in ♂ (17.4%). ▪ Brain: (↓) abs wt in ♂ (5.3%) and in ♀ (6.6%); (↑) rel-to-body wt in ♂ (24.6%) and in ♀ (10.6%, ns). ▪ Thyroid + parathyroid: (↓) abs wt in ♂ (14.3%, ns) and (↑) rel-to-body wt in ♂ (16.7%, ns) and in ♀ (14.3%, ns). ▪ Adrenals: (↑) abs wt in ♀ (15%, ns); (↑) rel-to-body wt in ♂ (33.3%) and in ♀ (36.7%); (↑) rel-to-brain wt in ♀ (23.7%). ▪ Kidneys: (↓) abs wt in ♂ (16.6%, ns); (↑) rel-to-body wt in ♂ (10.2%, ns) and in ♀ (14.8%). ▪ Testis: (↑) rel-to-body wt (31.2%). <u>Gross pathology</u> *(no statistical analysis performed):* ▪ (↑) Adrenals enlargement in ♂ (1/10 *vs* 0/10 in controls) and in ♀ (1/6 *vs* 0/10 in controls). ▪ (↑) Duodenum thickening in ♂ (3/10 *vs* 0/10 in controls) and in ♀ (1/6 *vs* 0/10 in controls). ▪ (↑) Area dark in lungs in ♂ (3/10 *vs* 0/10 in controls). ▪ (↑) Stomach thickening in ♂ (9/10 *vs* 1/10 in controls) and in ♀ (6/6 *vs* 0/10 in controls). ▪ (↑) Area raised in stomach in ♂ (2/10 *vs* 0/10 in controls). ▪ (↑) Small thymus in ♂ (2/10 *vs* 0/10 in controls) and in ♀ (4/6 *vs* 0/10 in controls). <u>Histopathological findings</u> *(no statistical analysis performed):* ▪ (↑) Stomach hyperkeratosis in ♂ (9/10 *vs* 0/10 in control) and in ♀ (6/10 *vs* 0/10 in control); hyperplasia in ♂ 10/10 *vs* 0/10 in control) and in ♀ (5/10 *vs* 0/10 in control); oedema in ♂ (9/10 *vs* 0/10 in control) and in ♀ (6/10 *vs* 0/10 in control); infiltration in ♂ (9/10 *vs* 0/10 in control) and in ♀ (6/10 *vs* 0/10 in control); and haemorrhage in ♂ (1/10 *vs* 0/10 in control) and in ♀ (1/10 *vs* 0/10 in control).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		75 mg/kg bw/day Clinical signs *(no statistical analysis performed):* ▪ (↑) Respiratory problems in ♂ (6/10 *vs* 1/10 in controls) and ♀ (4/10 *vs* 0/10 in controls). ▪ (↑) Salivation in ♂ (10/10 *vs* 1/10 in controls) and ♀ (10/10 *vs* 0/10 in controls). ▪ (↑) Staining of head in ♂ (6/10 *vs* 1/10 in controls) and ♀ (2/10 *vs* 1/10 in controls). Bodyweight and food consumption: ▪ (↓) bw in ♂ [at day 28 (10.2%)]. ▪ (↓) bw gain week 0-4 in ♂/♀ (23/22.8%) *(no statistical analysis reported for this period).* ▪ (↓) Food consumption in ♂ [between days 0-28 (11%)] and ♀ [between days 0-28 (14%)] *(no statistical analysis reported for this period).* Haematology: ▪ (↑) WBC in ♂ (19%, ns) and in ♀ (29%, ns). ▪ (↑) RDW in ♂ (6%). ▪ (↑) Plt in ♀ (11.3%, ndr). ▪ (↓) MPV in ♀ (3.5%, ndr). Clinical chemistry: ▪ (↑) ALT in ♂ (47.6%) and in ♀ (115.9%). ▪ (↓) globulin in ♂ (8.8%) and ♀ (8.8%). ▪ (↓) albumin in ♀ (6%). ▪ (↓) Total protein in ♂ (6.1%) and ♀ (7.5%). Organs´ weight: ▪ Brain: (↓) abs wt in ♀ (4.3%). ▪ Adrenals: (↑) abs wt in ♀ (11.7%, ns). Gross pathology *(no statistical analysis performed):* ▪ (↑) Duodenum thickening in ♂ (1/10 *vs* 0/10 in control). ▪ (↑) Area dark in lungs in ♂ (1/10 *vs* 0/10 in control). Histopathological findings *(no statistical analysis performed):* ▪ (↑) Stomach hyperplasia in ♂ (1/10 *vs* 0/10 in control) and in ♀ (1/10 *vs* 0/10 in control); oedema in ♂ (3/10 *vs* 0/10 in control) and in ♀ (4/10 *vs* 0/10 in control); and infiltration in ♂ (2/10 *vs* 0/10 in control). **LOAEL**: **75 mg/kg bw/day**, based on mortality in ♀, increased incidence of clinical signs in ♂/♀ (respiratory problems, salivation and staining of the fur), reduction of bw in ♂, reduction in bw gain and food consumption in ♂/♀ and increase in alanine aminotransferase in ♂/♀.	
28-day oral (diet) study in rat. Guideline: US EPA FIFRA F-82-1. GLP: Yes Rat strain: Crl:CD® (SD) BR No. animals 10 rats/sex/dose	Dodine (batch no. APA 303/90 and purity of 94.07%). Doses: 0, 500, 750, 1000 ppm for 28 days, equivalent to 0, 47, 71 and 87 mg/kg bw /day in ♂ and 0, 50, 72 and 92 mg/kg bw /day in ♀.	**Mortality**: ♂: no deaths. ♀: 1/10 in control and 1/10 at 750 ppm died. **1000 ppm** (87♂/92♀ mg/kg bw/day) Bodyweight and food consumption ▪ (↓) bw at day 28 [in ♂/♀ (14.3/12.3%)]. ▪ (↓) bw gain between day 1-28 [in ♂/♀, (30.2/35.6%), *no statistical analysis reported for this period*]. ▪ (↓) food consumption between days 1-28 [in ♂/♀, (20/19%), *no statistical analysis reported for this*	[redacted] (1994b) **B.6.3.1.2 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
Deviations from OECD TG 407 (2008): - Functional observation not performed. - Reticulocyte count and determination of T3, T4 and TSH hormones not performed. - Epididymides, prostate + seminal vesicles with coagulating glands as a whole, thymus, spleen and heart not weighed. - Histopathology not performed in all recommended tissues in control and high dose groups and not extended to all dosage groups, if treatment-related changes are observed. **Study acceptable.** *Guideline value for classification: STOT RE 2 ≤ 300 mg/kg bw/day STOT RE 1 ≤ 30 mg/kg bw/day (Haber´s rule from 90- to 28-day value)*		*period*]. Haematology: ▪ (↑) Hb in ♀ (3.2%). Clinical chemistry: ▪ (↓) creatinine in ♂ (9.8%). ▪ (↓) glucose in ♂ (14.7%). ▪ (↓) ALT in ♂ (18.3%, ndr) and in ♀ (17.5%). ▪ (↓) AST in ♀ (21.9%, ndr). ▪ (↓) albumin in ♂ (0.6%, ns, ndr). ▪ (↑) Na in ♂ (1.1%) and in ♀ (0.9%, ndr). ▪ (↑) Cl in ♂ (1.7%, ndr). Organs´ weight: ▪ Lungs: (↓) abs wt in ♂ (9.1%) and (↑) rel-to-body wt in ♀ (9.3%). ▪ Brain: (↑) rel-to-body wt in ♂ (12.9%) and in ♀ (15.1%). ▪ Thyroid + parathyroid: (↓) abs wt in ♂ (19%, ns) and in ♀ (20%, ns). ▪ Kidneys: (↓) abs wt in ♂ (13.9%) and in ♀ (15%); (↓) rel-to-body wt in ♂ (12%) and in ♀ (15.9%). ▪ Testis: (↑) rel-to-body wt (23%). Gross pathology *(no statistical analysis performed):* ▪ (↑) Lung area dark in ♂ (2/10 *vs* 1/10 in control) and in ♀ (4/10 *vs* 0/10 in control). ▪ (↑) Small thyroid in ♀ (1/10 *vs* 0/10 in control). Histopathological findings *(no statistical analysis performed):* ▪ (↑) Kidney mineralization of cortico-medullary junction in ♀ (5/10 *vs* 2/10 in control). ▪ (↑) Findings in lungs in ♀ (3/10 *vs* 0/10 in control). **750 ppm** (71♂/72♀ mg/kg bw/day) Bodyweight and food consumption: ▪ (↓) bw at day 28 [in ♂ (8.3%)]. ▪ (↓) bw gain between day 1-28 [in ♂/♀, (17.4/16.5%), *no statistical analysis reported for this period*]. ▪ (↓) food consumption between days 1-28 [in ♀, (14%), *no statistical analysis reported for this period*]. Clinical chemistry: ▪ (↓) creatinine in ♂ (8.2%). ▪ (↓) ALT in ♂ (23.3%, ndr) and in ♀ (13.5%, ns). ▪ (↓) AST in ♀ (13.2%, ns, ndr). ▪ (↓) albumin in ♂ (3.9%, ndr). ▪ (↑) Na in ♀ (1.1%, ndr). ▪ (↑) Cl in ♂ (2.4%, ndr). Organs´ weight: ▪ Testis: (↑) rel-to-body wt (12%). **500 ppm** (47♂/50♀ mg/kg bw/day) Bodyweight and food consumption: ▪ (↓) bw gain between days 1-28 [in ♀ (10.5%), *no*	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		statistical analysis reported for this period]. Clinical chemistry ▪ (↓) ALT in ♂ (18.8%, ncdr) and in ♀ (13.5%, ns). ▪ (↓) AST in ♀ (27.5%, ndr). Organs´ weight ▪ Testis: (↑) rel-to-body wt (12.2%, ncdr). **LOAEL**: 500 ppm (equivalent to **47 and 50 mg/kg bw/day** in ♂ and ♀, respectively), based on reduction of the bw gain in ♀.	
28-day oral (diet) study in rat. Guideline: Not stated. GLP: Yes Rat strain: Sprague-Dawley. No. animals 10 rats/sex/dose Deviations from OECD TG 407 (2008): - Less than 3 doses tested. - Functional observation, haematology and clinical biochemistry not performed. - Organs not weighed: adrenals, testes, epididymides, prostate + seminal vesicles with coagulating glands as a whole, thymus, spleen, brain and heart. - Histopathology not carried out in all recommended tissues. **Study acceptable as supportive only** *Guideline value for classification: STOT RE 2 ≤ 300 mg/kg bw/day STOT RE 1 ≤ 30 mg/kg bw/day*	Dodine (batch no. 1174 and purity of 98.6%). Doses: 0, 200 and 800 ppm for 28 days, equivalent to 0, 17.66 and 67.7 mg/kg bw/day in ♂ and 0, 19.17 and 76.71 mg/kg bw/day in ♀.	**Mortality**: Not reported. **800 ppm** (67.7♂/76.71♀ mg/kg bw/day) Bodyweight and food consumption: ▪ (↓) bw in ♂ [at day 8 (5.5%), at day 15 (6.6%) and at day 28 (6.6%, ns)]. ▪ (↓) bw gain at day 8 [in ♂/♀ (24.2/23.4%)] and between days 1-28 [in ♂/♀, (14.3/16.6%), *no statistical analysis reported for this period*]. ▪ (↓) food consumption in ♂ [at day 8 (10.4%), at day 15 (7.7%) and at day 28 (7.8%, ns)]. Organs´ weight: ▪ Liver: (↓) in abs wt in ♀ (17%) and in rel-to-body wt in ♀ (12.8%). ▪ Kidneys: no change in rel-to-body wt in ♀ **200 ppm** (17.66♂/19.17♀ mg/kg bw/day) Organs´ weight: ▪ Kidneys: (↑) in rel-to-body wt in ♀ (5.6%, ndr). **NOAEL**: 200 ppm **(17.66 and 19.17 mg/kg bw/day** in ♂/♀, respectively). **LOAEL**: 800 ppm **(67.7**♂ and **76.61** ♀ **mg/kg bw/day** in ♂/♀, respectively), based on reduction of bw gain in ♂/♀, reduction of food consumption in ♂ and decrease in absolute and relative-to-body liver weight in ♀.	[redacted] (1997) **B.6.3.1.3 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
(Haber's rule from 90- to 28-day value)			
7- and 28-day oral, in diet, gut motility study in rat. Guideline: Not stated. GLP: Yes Rat strain: Sprague-Dawley. No. animals 10 rats/sex/dose (5 rats/sex/dose *per* time point). Deviations from OECD TG 407 (2008): - Less than 3 doses tested. - Functional observation, haematological examination and clinical biochemistry not performed. - Organs not weighed and histopathology not examined. **Study acceptable as supportive only** *Guideline value for classification:* *STOT RE 2 ≤ 300 mg/kg bw/day* *STOT RE 1 ≤ 30 mg/kg bw/day* *(Haber's rule from 90- to 28-day value)*	Dodine (batch no. 1174 and purity of 98.6%). Doses: 0, 200 and 800 ppm for 7 and 28 days.	**Mortality**: Not reported. **800 ppm** Bodyweight and food consumption (*no statistical analysis reported):* ▪ (↓) bw gain between days 1-8 [in ♂/♀ (36.5/40.7%)], between days 1-15 [in ♂/♀ (17.3/9.3%)], between days 1-22 [in ♂/♀ (9.5/10.6%)] and between days 1-28 [in ♂/♀ (7.2/11.3%)]. ▪ (↓) food consumption in ♂ [at day 8 (18.3%) and at day 15 (11.7%)]. **200 ppm** Bodyweight and food consumption (*no statistical analysis reported):* ▪ (↓) bw gain between days 1-8 [in ♀ (9.9%)]. **NOAEL** not derived.	[redacted] (1996) **B.6.3.1.4 (AS)**
90-day oral (diet) study in rat. Guideline: Not stated. GLP: Yes Rat strain:	Dodine (batch no. 196.53 and purity of 95%). Doses: 0, 50, 200 and 800 ppm for 90 days, equivalent to 0,	**Mortality**: Not deaths reported. **800 ppm** (55.84♂/60.44♀ mg/kg bw/day) Bodyweight and food consumption: ▪ (↓) bw in ♂ [between days 14-35 (between 9.2-10.3%)] and ♀ [between days 7-84 (between 4.2-9.4%)]. ▪ (↓) bw gain days 0-91 in ♂/♀ (10/11.2%) *(no*	[redacted] (1982) **B.6.3.2.1 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
SPF-bred, Cpb:WU, Wistar. <u>No. animals</u> 10 rats/sex/dose <u>Deviations from OECD TG 408 (2018):</u> - Sensory reactivity to stimuli, grip strength and motor activity not analysed. - Functional observation not performed. - Several haematological and biochemistry parameters not measured. - Epididymides, prostate + seminal vesicles with coagulating glands and uterus not weighed. - Not all recommended tissues with histopathological examination and not extended to all dose groups when observed at high dose. - Oestrus cycle not determined. **Study acceptable** *Guideline value for classification: STOT RE 2 ≤ 100 mg/kg bw/day STOT RE 1 ≤ 10 mg/kg bw/day*	3.59, 14.09 and 55.84 mg/kg bw/day in ♂ and 0, 3.87, 14.94 and 60.44 mg/kg bw/day in ♀.	*statistical analysis reported for this period).* ▪ (↓) food consumption in ♀ [between days 14-84 (between 8.2-17.1%)]. <u>Haematology:</u> ▪ (↓) MCHC in ♂ (3.4%). ▪ (↑) Neutrophils in ♂ (67.5%) and ♀ (12.5%, ns). ▪ (↓) Lymphocytes in ♂ (6.5%). <u>Clinical chemistry:</u> ▪ (↓) Total bilirubin in ♂ (8.3%, ns, ndr). ▪ (↓) Ca in ♂ (7.1%, ndr). ▪ (↓) ALT in ♀ (20.5%). <u>Organs´ weight:</u> ▪ Kidneys: (↓) abs wt in ♀ (10.6%); (↑) rel-to-body wt in ♂ (8.1%, ndr). ▪ Testis: (↑) rel-to-body wt (12.7%). ▪ Heart: (↑) rel-to-body wt in ♂ (9%). ▪ Thymus: (↓) abs wt in ♂ (15.8%, ns) and in ♀ (11.7%, ns, ndr); (↓) rel-to-body wt in ♂ (10.2%, ns) and in ♀ (3.9%, ns, ndr). ▪ Thyroid: (↓) abs wt in ♀ (16.7%, ns) and rel-to-body wt in ♀ (7.3%, ns); (↑) rel-to-body wt in ♂ (17.7%, ns). ▪ Spleen: (↓) abs wt in ♀ (10.1%, ns). <u>Histopathological findings:</u> ▪ (↑) Heart myocardial degeneration in ♂ (3/10 *vs* 0/10 in control). ▪ (↑) Spleen extra-medullary haematopoiesis in ♂ (2/10 *vs* 0/10 in control). ▪ (↑) Testes tubular atrophy (1/10 *vs* 0/10 in control). ▪ (↑) Proteinaceous material in urinary bladder in ♂ (6/10 *vs* 4/10 in control) and in ♀ (9/10 *vs* 0/10 in control). ▪ (↑) Luminal dilation in uterus (6/10 *vs* 3/10 in control). **200 ppm** (14.09♂/14.94♀ mg/kg bw/day) <u>Bodyweight and food consumption:</u> ▪ (↓) food consumption in ♀ [between days 14-28 (between 7.6-11.2%)]. <u>Clinical chemistry:</u> ▪ (↓) Total bilirubin in ♂ (11.1%, ns, ndr). ▪ (↓) Ca in ♂ (7.4%, ndr). <u>Organs´ weight:</u> ▪ Kidneys: (↑) rel-to-body wt in ♂ (8.5%, ndr). ▪ Thymus: (↓) abs wt in ♀ (14.7%, ns, ndr) and rel-to-body wt in ♀ (13.5%, ns, ndr). **50 ppm** (3.59♂/3.87♀ mg/kg bw/day) ▪ (↓) food consumption in ♀ [at day 21 (6.3%)]. <u>Clinical chemistry:</u> ▪ (↓) Total bilirubin in ♂ (19.4%, ndr). ▪ (↓) Ca in ♂ (6%, ndr). **NOAEL**: 200 ppm **(14.09♂ and 14.94♀ mg/kg bw/day).** **LOAEL**: 800 ppm **(55.84♂** and **60.44♀ mg/kg bw/day),** based on reduction in the bw gain in ♂/♀, decrease in	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		food consumption in ♀, the increment in neutrophils in ♂ and the decrease in ALT in ♀.	
90-day oral (gavage) study in rat. Guideline: Not stated. GLP: No Rat strain: Wistar. No. animals 6 rats/sex/dose Deviations from OECD TG 408 (2018): - Test substance not characterised. - Unknown age. - Data for bw and food consumption not reported. - Not all clinical biochemistry and haematological parameters measured. - Not all organs weighed, not all tissues with histopathology and data not shown. - No oestrus cycle. - 6 rats/sex/dose. **Study acceptable as supportive information.**	Dodine (unknown batch no. and purity of 99%). Doses: 0, 5, 10 or 20 mg/kg bw/day.	**Mortality**: Not deaths reported. **20 mg/kg bw/day** Bodyweight *(data not shown).* ▪ (↑) bw gain Haematology *(data not shown).* ▪ (↓) Leukocytes in ♀ (49%, ndr). Clinical chemistry *(data not shown).* ▪ (↑) Urea in ♂ (26%, ns, ndr). ▪ (↓) Bilirubin in ♂ (65%). ▪ (↓) AST in ♂ (54%). Histopathological findings *(data not shown).* ▪ (↑) Vascular congestion in heart in ♂/♀ (ndr). ▪ (↑) Vascular congestion in kidneys in ♂/♀ (ndr). ▪ (↑) Portal inflammation in ♂/♀ (ndr). **10 mg/kg bw/day** Bodyweight *(data not shown).* ▪ (↑) bw gain Haematology *(data not shown).* ▪ (↓) Leukocytes in ♀ (34%, ndr). Clinical chemistry *(data not shown).* ▪ (↑) Urea in ♂ (19%, ns, ndr). Histopathological findings *(data not shown).* ▪ (↑) Vascular congestion in heart in ♂/♀ (ndr). ▪ (↑) Vascular congestion in kidneys in ♂/♀ (ndr). ▪ (↑) Portal inflammation in ♂/♀ (ndr). **5 mg/kg bw/day** Bodyweight *(data not shown).* ▪ (↑) bw gain Haematology *(data not shown).* ▪ (↓) Leukocytes in ♀ (65%, ndr). Clinical chemistry *(data not shown).* ▪ (↑) Urea in ♂ (58%, ndr). Histopathological findings *(data not shown).* ▪ (↑) Vascular congestion in heart in ♂/♀ (ndr). ▪ (↑) Vascular congestion in kidneys in ♂/♀ (ndr). ▪ (↑) Portal inflammation in ♂/♀ (ndr). **NOAEL** not derived.	Mitjans, M. *et al* (1999) **B.6.3.2.2 (AS)**
100-day oral (diet) study in rat. Guideline: Not stated. GLP: No Rat strain: CFN. No. animals: Treated: 20 ♂ and 18 ♀. Controls: 19/sex.	Dodine (unknown batch no. and purity of 97%). Doses: 3200 ppm.	**Mortality**: Not deaths reported. **3200 ppm** Bodyweight *(data not shown).* ▪ (↓) bw gain **NOAEL** not derived.	Levinskas, G.J., *et al* (1961) **B.6.3.2.3 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
Deviations from OECD TG 408 (2018): - Test substance not characterised. - Data not reported. - 1 dose tested. - Measured parameters not fully described. **Study acceptable as supportive information.**			
28-day dermal study in rat. Guideline: EPA OPPTS 870.3200 GLP: Yes Rat strain: Crl:CD® IGS(SD) BR. No. animals 10 rats/sex/dose Deviations from OECD TG 410 (1981): - Ornithine decarboxylase not measured. **Study acceptable** *Guideline value for classification: STOT RE 2 ≤ 600 mg/kg bw/day STOT RE 1 ≤ 60 mg/kg bw/day (Haber's rule from 90- to 28-day value).*	Dodine (batch no. OP750142 and purity of 98%). Vehicle: deionized water. Doses: 0, 50, 125 or 200 mg/kg bw/day for 28 days (6 h/day, 5 days/week).	**Mortality**: Not deaths reported. **200 mg/kg bw/day** Clinical signs *(no statistical analysis performed):* ▪ (↑) Yellow urogenital area in ♀ on day 16 (5/10 *vs* 0/10 in controls), unwrapped overnight. Dermal irritation *(no. observations/total observations, no statistical analysis performed):* ▪ (↑) Erythema in ♂ (very slight, 7/40; slight, 2/40; moderate, 3/40; severe, 8/40) and in ♀ (very slight, 5/40; slight, 6/40; moderate, 11/40; severe, 16/40) *vs* 0/40 in controls. ▪ (↑) Oedema in ♂ (very slight, 13/40; slight, 1/40) and in ♀ (very slight, 23/40; slight, 1/40) *vs* 0/40 in controls. ▪ (↑) Fissuring in ♀ (1/40 *vs* 0/40 in control). ▪ (↑) Desquamation in ♂ (35/40 *vs* 0/40 in control) and in ♀ (38/40 *vs* 0/40 in control). ▪ (↑) Eschar in ♂ (8/40 *vs* 0/40 in control) and in ♀ (16/40 *vs* 0/40 in control). ▪ (↑) Exfoliation in ♂ (5/40 *vs* 0/40 in control) and in ♀ (5/40 *vs* 0/40 in control). ▪ (↑) Blanching in ♂ (4/40 *vs* 0/40 in control). ▪ (↑) Encrustation in ♂ (11/40 *vs* 0/40 in control) and in ♀ (14/40 *vs* 0/40 in control). Bodyweight and food consumption: ▪ (↓) bw gain week 0-1 in ♂ (37%), week 0-4 in ♂/♀	[redacted] (1999e) **B.6.3.4.1.1 (AS)**

		(22.5%, ns/17.5%, ns, ndr). Haematology: ▪ (↓) RBC in ♂ (0.6%, ns, ndr). ▪ (↓) Hb in ♂ (1.2%, ns, ndr). ▪ (↓) Ht in ♂ (1.8%, ns, ndr). ▪ (↑) Neutrophils in ♂ (20%, ns) and ♀ (40%, ns, ndr). ▪ (↓) Lymphocytes in ♀ (25%, ns, ndr). Clinical chemistry: ▪ (↑) ALP in ♂ (13.5%, ns) and in ♀ (202.6%). ▪ (↓) Cholesterol in ♂ (25.9%, ns). Urinalysis: ▪ (↑) Osmolality in ♂ (46.2%, ns) and in ♀ (21.8%, ns). Organs´ weight: ▪ Brain: (↓) abs wt in ♀ (5.3%); (↑) rel-to-body wt in ♂ (8.5%). ▪ Thymus: (↓) abs wt in ♂ (18.4%, ns) and in ♀ (14.1%, ns, ndr); (↓) rel-to-body wt in ♂ (13.7%, ns) and in ♀ (10.7%, ns, ndr). ▪ Uterus: (↑) abs wt (23%, ns); (↑) rel-to-body wt (27.7%, ns). ▪ Kidneys: (↑) rel-to-brain wt in ♀ (10%). Gross pathology *(no statistical analysis performed):* ▪ (↑) Treated skin scabbing in ♂ (3/10 *vs* 1/10 in controls) and in ♀ (4/10 *vs* 0/10 in controls). ▪ (↑) Skin red matting in ♂ (2/10 *vs* 0/10 in controls). ▪ (↑) Haemorrhagic thymus in ♂ (1/10 *vs* 0/10 in controls). Histopathological findings on treated skin *(no statistical analysis performed):* ▪ (↑) Ulcer in ♀ (5/10 *vs* 0/10 in control). ▪ (↑) Exudate in ♂ (4/10 *vs* 1/10 in control) and in ♀ (7/10 *vs* 0/10 in control). ▪ (↑) Suppurative inflammation in ♀ (3/10 *vs* 0/10 in control). ▪ (↑) Parakeratosis in ♂ (2/10 *vs* 0/10 in control) and in ♀ (4/10 *vs* 0/10 in control). ▪ (↑) Epidermal hyperplasia ♂ (6/10 *vs* 0/10 in control) and in ♀ (6/10 *vs* 0/10 in control). ▪ (↑) Hyperkeratosis in ♂ (2/10 *vs* 0/10 in control). ▪ (↑) Subacute inflammation in ♂ (1/10 *vs* 0/10 in control) and in ♀ (2/10 *vs* 0/10 in control). **125 mg/kg bw/day** Dermal irritation *(no. observations/total observations, no statistical analysis performed):* ▪ (↑) Erythema in ♂ (very slight, 3/40; slight, 6/40; moderate, 6/40; severe, 8/40) and in ♀ (very slight, 7/40; slight, 6/40; moderate, 10/40; severe, 7/40) *vs* 0/40 in controls. ▪ (↑) Oedema in ♂ (very slight, 14/40) and in ♀ (very slight, 20/40; slight, 1/40) *vs* 0/40 in controls. ▪ (↑) Fissuring in ♂ (1/40 *vs* 0/40 in control) and in ♀ (1/40 *vs* 0/40 in control). ▪ (↑) Desquamation in ♂ (34/40 *vs* 0/40 in control) and in ♀ (37/40 *vs* 0/40 in control). ▪ (↑) Eschar in ♂ (8/40 *vs* 0/40 in control) and in ♀ (7/40 *vs* 0/40 in control). ▪ (↑) Exfoliation in ♂ (1/40 *vs* 0/40 in control) and in ♀ (3/40 *vs* 0/40 in control). ▪ (↑) Blanching in ♂ (2/40 *vs* 0/40 in control) and in ♀ (4/40 *vs* 0/40 in control).	

		▪ (↑) Encrustation in ♂ (13/40 *vs* 0/40 in control) and in ♀ (18/40 *vs* 0/40 in control). Bodyweight and food consumption: ▪ (↓) bw gain week 0-1 in ♂ (25.7%), week 0-4 in ♂/♀ (13.5%, ns/7.5%, ns, ndr). Haematology: ▪ (↓) RBC in ♂ (0.2%, ns, ndr). ▪ (↓) Hb in ♂ (0.6%, ns, ndr). ▪ (↓) Ht in ♂ (0.6%, ns, ndr). ▪ (↑) Neutrophils in ♀ (80%, ndr). ▪ (↓) Lymphocytes in ♀ (12%, ndr). Clinical chemistry: ▪ (↓) Cholesterol in ♂ (16.7%, ns). Urinalysis: ▪ (↑) Osmolality in ♂ (16%, ns, ndr); (↓) osmolality in ♀ (11.2%, ns, ndr). Organs´ weight: ▪ Thymus: (↓) abs wt in ♂ (10.2%, ns) and in ♀ (16.5%, ns, ndr); (↓) rel-to-body wt in ♀ (15%, ns, ndr). ▪ Uterus: (↓) abs wt (19.7%, ns, ndr); (↓) rel-to-body wt (17.2%, ns, ndr). Gross pathology *(no statistical analysis performed):* ▪ (↑) Treated skin scabbing in ♂ (3/10 *vs* 1/10 in controls) and in ♀ (2/10 *vs* 0/10 in controls). Histopathological findings on treated skin *(no statistical analysis performed):* ▪ (↑) Ulcer in ♀ (4/10 *vs* 0/10 in control). ▪ (↑) Exudate in ♂ (7/10 *vs* 1/10 in control) and in ♀ (7/10 *vs* 0/10 in control). ▪ (↑) Suppurative inflammation in ♀ (1/10 *vs* 0/10 in control). ▪ (↑) Parakeratosis in ♂ (6/10 *vs* 0/10 in control) and in ♀ (3/10 *vs* 0/10 in control). ▪ (↑) Epidermal hyperplasia ♂ (6/10 *vs* 0/10 in control) and in ♀ (3/10 *vs* 0/10 in control). ▪ (↑) Hyperkeratosis in ♂ (2/10 *vs* 0/10 in control). ▪ (↑) Subacute inflammation in ♂ (1/10 *vs* 0/10 in control) and in ♀ (5/10 *vs* 0/10 in control). **50 mg/kg bw/day** Dermal irritation *(no. observations/total observations, no statistical analysis performed):* ▪ (↑) Erythema in ♂ (very slight, 6/40; slight, 1/40; severe, 2/40) and in ♀ (very slight, 14/40; slight, 7/40; severe, 1/40) *vs* 0/40 in controls. ▪ (↑) Oedema in ♂ (very slight, 2/40) and in ♀ (very slight, 4/40) *vs* 0/40 in controls. ▪ (↑) Fissuring in ♀ (1/40 *vs* 0/40 in control). ▪ (↑) Desquamation in ♂ (22/40 *vs* 0/40 in control) and in ♀ (29/40 *vs* 0/40 in control). ▪ (↑) Eschar in ♂ (2/40 *vs* 0/40 in control) and in ♀ (1/40 *vs* 0/40 in control). ▪ (↑) Exfoliation in ♀ (1/40 *vs* 0/40 in control). ▪ (↑) Blanching in ♂ (4/40 *vs* 0/40 in control) and in ♀ (6/40 *vs* 0/40 in control). ▪ (↑) Encrustation in ♂ (3/40 *vs* 0/40 in control) and in ♀ (2/40 *vs* 0/40 in control). Bodyweight and food consumption: ▪ (↓) bw gain week 0-4 in ♀ (17.5%, ns, ndr). Haematology: ▪ (↓) RBC in ♂ (7.6%, ndr). ▪ (↓) Hb in ♂ (6%, ndr).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		▪ (↓) Ht in ♂ (7.4%, ndr). Urinalysis: ▪ (↑) Osmolality in ♂ (19%, ns, ndr) and in ♀ (10.8%, ns, ndr). Organs´ weight: ▪ Thymus: (↓) abs wt in ♀ (15%, ns, ndr); (↓) rel-to-body wt in ♀ (12.6%, ns, ndr). ▪ Uterus: (↑) abs wt (16.4%, ns, ndr); (↑) rel-to-body wt (19.1%, ns, ndr). Gross pathology *(no statistical analysis performed):* ▪ (↑) Treated skin scabbing in ♀ (1/10 *vs* 0/10 in controls). Histopathological findings on treated skin *(no statistical analysis performed):* ▪ (↑) Exudate in ♀ (1/10 *vs* 0/10 in control). ▪ (↑) Parakeratosis in ♀ (1/10 *vs* 0/10 in control). **NOAEL**: 50 mg/kg bw/day (equivalent to **35.7 mg/kg bw/day** due to 5 day/week dosing). **LOAEL**: 125 mg/kg bw/day, based on reduction in the bw gain in ♂. **LOAEL for local effects**: 50 mg/kg bw/day (equivalent to **35.7 mg/kg bw/day** due to 5 day/week dosing), based on findings of dermal irritation in ♂/♀.	
21-day dermal study in rat. Guideline: EPA OPP 82–2 21-day dermal GLP: Yes Rat strain: Sprague-Dawley CD®. No. animals 5 rats/sex/dose Deviations from OECD TG 410 (1981): - Different substance. - Age at initiation not reported. **Study not acceptable** *Guideline value for classification: STOT RE 2 ≤ 867 mg/kg bw/day STOT RE 1 ≤ 86.7 mg/kg bw/day (Haber´s rule from 90- to 28-day value).*	1-dodecylguanidinium hydrochloride (CT-334-87) (unknown batch no. and purity). Vehicle: water. Doses: 0, 12.5, 25 or 50 mg/kg bw/day for 21 days (6 h/day, 5 days/week).	**Mortality**: Not deaths reported. **50 mg/kg bw/day** Dermal irritation *(no statistical analysis performed):* ▪ (↑) Erythema (10/10 *vs* 0/10 in control). ▪ (↑) Oedema (2/10 *vs* 0/10 in control). ▪ (↑) Atonia (3/10 *vs* 0/10 in control). ▪ (↑) Desquamation (10/10 *vs* 1/10 in control). ▪ (↑) Fissuring (3/10 *vs* 0/10 in control). ▪ (↑) Eschar (3/10 *vs* 0/10 in control). ▪ (↑) Exfoliation (2/10 *vs* 0/10 in control). ▪ (↑) Necrosis (1/10 *vs* 0/10 in control). Bodyweight and food consumption: ▪ (↓) bw gain week 0-3 in ♀ (37.1%) *(no statistical analysis for this parameter*). ▪ (↓) food consumption in ♀ [between days 7-14 (14.3%)]. Haematology: ▪ (↑) Platelets in ♂ (15.9%, ns, ndr) and ♀ (19.9%, ns, ndr). ▪ (↑) WBC in ♂ (35.9%, ns) and ♀ (78.7%, ns). Clinical chemistry: ▪ (↑) AST in ♂ (19.8%, ns, ndr); (↓) AST in ♀ (14.3%, ns). ▪ (↑) ALT in ♂ (31.3%). ▪ (↓) Albumin in ♂ (8.6%). ▪ (↓) Total bilirubin in ♀ (33.3%). Organs´ weight ▪ Liver: (↑) abs wt in ♀ (11.1%, ns); (↑) rel-to-body wt in ♀ (15.5%, ns). Histopathological findings on treated skin *(no statistical*	[redacted] (1989) **B.6.3.4.1.2 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		analysis performed): ▪ (↑) Inflammatory cells on surface in ♂ (4/5 *vs* 0/5 in control) and in ♀ (3/5 *vs* 0/5 in control). ▪ (↑) Hyperkeratosis in ♂ (5/5 *vs* 0/5 in control) and in ♀ (5/5 *vs* 0/5 in control). ▪ (↑) Parakeratosis in ♂ (5/5 *vs* 0/5 in control) and in ♀ (5/5 *vs* 0/5 in control). ▪ (↑) Squamous cell hyperplasia in ♂ (5/5 *vs* 0/5 in control) and in ♀ (5/5 *vs* 0/5 in control). ▪ (↑) Epithelium necrosis in ♂ (1/5 *vs* 0/5 in control). ▪ (↑) Erosion/ulcer in ♂ (3/5 *vs* 0/5 in control) and in ♀ (3/5 *vs* 0/5 in control). ▪ (↑) Subacute/chronic inflammation in ♂ (4/5 *vs* 0/5 in control) and in ♀ (5/5 *vs* 0/5 in control). **25 mg/kg bw/day** Dermal irritation *(no statistical analysis performed):* ▪ (↑) Erythema (7/10 *vs* 0/10 in control). ▪ (↑) Desquamation (8/10 *vs* 1/10 in control). ▪ (↑) Fissuring (2/10 *vs* 0/10 in control). Bodyweight and food consumption: ▪ (↓) bw gain week 0-3 in ♀ (17.1%, ndr) *(no statistical analysis for this parameter*). Haematology: ▪ (↑) Platelets in ♂ (19.5%, ns, ndr) and ♀ (27.3%, ndr). ▪ (↑) WBC in ♀ (32.6%, ns). Clinical chemistry: ▪ (↓) AST in ♂ (1%, ns, ndr). ▪ (↑) ALT in ♂ (6.3%). **12.5 mg/kg bw/day** Dermal irritation *(no statistical analysis performed):* ▪ (↑) Erythema (2/10 *vs* 0/10 in control). ▪ (↑) Desquamation (5/10 *vs* 1/10 in control). Bodyweight and food consumption: ▪ (↓) bw gain week 0-3 in ♀ (31.4%, ndr) *(no statistical analysis for this parameter*). Haematology: ▪ (↑) Platelets in ♀ (13%, ns, ndr). Clinical chemistry: ▪ (↑) AST in ♂ (25%, ndr). ▪ (↑) ALT in ♂ (18.8%, ns, ndr). **NOAEL** not derived.	
106-week oral study in rats GLP: Yes Method: OECD 453 (1981) and US-EPA FIFRA 83-5 (1984) Rat strain: Sprague-Dawley	Test substance: Dodecylguanidine acetate. Purity: 98.6% Oral (diet) **Doses:** Males: 0, 200, 400 and 800 ppm (equivalent to 0,	*Only effects relevant for STOT RE are presented (see also section 2.6.5)* **Mortality**: No significant differences detected, only a slight decrease in males at 800 ppm after 2-year. **800 ppm** (equivalent to 41.93/53.5 mg/kg bw/day for ♂/♀)	[redacted] (1998) **B.6.5.1 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
rats: ♂ and ♀ No. animals: 60 rats/sex/dose Deviations from current OECD TG 453, 2018): -HCD not provided for all neoplasm incidences. -Statistical analysis not performed for all neoplastic incidences. **Study acceptable** *Guideline value for classification:* *STOT RE 2 ≤ 12.3 mg/kg bw/day* *STOT RE 1 ≤ 1.23 mg/kg bw/day* *(Haber's rule from 90-day to2-year value)*	10.17, 20.34 or 41.93 mg/kg bw/day). Females: 0, 200, 400 and 800 ppm (equivalent to 0, 13.19, 26.5 or 53.5 mg/kg bw/day). 106-week feed exposure.	Clinical signs: ▪ (↑) Absence of grasping reflex in ♂ (11.4% *vs* 2.9% in controls). ▪ (↑) Absence of traction reflex in ♂ (7.1% *vs* 1.4% in controls, ns, but significant trend test). ▪ (↑) Absence of righting reflex in ♂ (5.7% *vs* 0% in controls, ns, but significant trend test). ▪ (↑) Reduced motor activity in ♂ (17.1% *vs* 11.4% in controls, ns). ▪ (↑) Hunched posture in ♂ (10% *vs* 1.4% in controls, ns) and in ♀ (12.8% *vs* 8.6% in controls, ns). ▪ (↑) Piloerection in ♂ (14.3% *vs* 7.1% in controls, ns, ndr). Bodyweight: ▪ (↓) bw in ♂ throughout week 1-37 (5-8%) and 85-89 (7-8%). ▪ (↓) bw in ♀ throughout week 1-101 (4-16%). ▪ bwg in ♂ at week 1(↓20%), 2 (↓23%), 3 (↑16%), 5 (↓13%), 9 (↓42%), 11 (↓12%), 9 (↓42%), 12 (↓38%), 13 (↓83%), 25 (↓34%), 29 (↑60%), 41 (↑61%), 61 (↓41%). ▪ bwg in ♀ at week 1(↓25%), 3 (↓35%), 4 (↑90%), 11 (↑5444%), 25 (↓56%), 57 (↓60%), 61 (↓40%), 97 (↓156%). Food consumption: ▪ in ♂ at week 1 (↓6%), 2 (↑12%), 4 (↑7%), 9 (↓5%), 12 (↓10%), 13 (↓11%), 17 (↓5%), 21 (↓6%), 25 (↓10%), 49 (↓5%), 53 (↓8%), 61 (↓6%), 89 (↓10%), 93 (↓12%). ▪ in ♀ at week 10 (↓4%), 25 (↓7%), 37 (↓9%), 41 (↓7%), 45 (↓12%), 49 (↓8%), 69 (↓9%), 73 (↓9%), 77 (↓13%), 97 (↓16%). Clinical chemistry: ▪ (↓) alkaline phosphatase (18%) at week 26 in ♂. ▪ (↑) alkaline phosphatase (310%) at week 104 in ♀. ▪ (↓) triglyceride (22%, ndr) at week 26 in ♀. ▪ (↑) potassium (9%) at week 78 in ♀. Urinalysis: ▪ (↓) urine volume at week 25 (47%) and 51 (29%, ndr) in ♂, and in ♀ at week 51 (46%). ▪ (↑) refractive index at week 25 in ♂ (0.5%) and at week 79 in ♀ (0.3%). ▪ (↓) pH (7%) at week 26 in ♂. Haematology: ▪ (↓) WBC (24%) in ♂ at week 26. ▪ (↓) lymphocytes (26%) in ♂ at week 26. ▪ (↑) prothrombin time in ♀ at week 26 (5%) and at week 52 (8%, ncdr). Organ weight (week 105): ▪ (↑) rel brain wt in ♀ (12%, ncdr). ▪ (↓) abs heart wt in ♀ (7%, ndr, ns). ▪ (↓) rel kidney wt in ♂ (11%, ncdr, ns) and (↑) rel kidney wt in ♀ (10%, ns). ▪ (↓) rel adrenal wt in ♂ (32%, ndr, ns). ▪ (↑) abs (6%, ndr) and rel (12%) epididymis wt.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		▪ (↓) abs (50%, ncdr, ns) and rel (45%. ncdr, ns) ovary wt. ▪ (↑) rel uterus wt (32%, ndr, ns). Necropsy *(Statistical analysis not performed)* *Adrenal* ▪ (↑) enlarged in ♀ (28.6% *vs* 17.1% in controls, ncdr). ▪ (↑) white mottling in ♀ (22.9% vs 12.9% in controls, ndr). *Subcutis* ▪ (↑) preputial gland abscess in ♂ (17.1% vs 7.1% in controls, ncdr). *Thymus* ▪ (↑) small in ♀ (7.1% vs 0% in controls, ncdr). Histopathology *(Statistical analysis not performed)* *Non-neoplastic* *Ovary* ▪ (↑) granulosa/theca cell hyperplasia (14.3% vs 5.8% in controls, ncdr). *Prostate* ▪ (↑) atrophy (4.3% vs 1.5% in controls, ndr). **400 ppm** (equivalent to 20.34/26.5 mg/kg bw/day for ♂/♀) Clinical signs: ▪ (↑) Absence of grasping reflex in ♂ (8.6% *vs* 2.9% in controls, ns, but significant trend test). ▪ (↑) Absence of righting reflex in ♂ (4.3% *vs* 0% in controls, ns, but significant trend test). ▪ (↑) Reduced motor activity in ♂ (17.1% *vs* 11.4% in controls, ns). ▪ (↑) Hunched posture in ♂ (5.7% *vs* 1.4% in controls, ns) and in ♀ (12.8% *vs* 8.6% in controls, ns). ▪ (↑) Piloerection in ♂ (14.3% *vs* 7.1% in controls, ns, ndr). Bodyweight ▪ (↓) bw in ♀ at week 89 (9%) and 101 (13%). Food consumption: ▪ in ♂ at week 21 (↓8%), 25 (↓17%), 41 (↑4%) and 61 (↓5%). ▪ in ♀ at week 9 (↓8%), 13 (↓10%) and 45 (↓8%). Clinical chemistry: ▪ (↑) alkaline phosphatase (150%) at week 104 in ♀. Haematology: ▪ (↑) prothrombin time in ♀ at week 52 (6%, ncdr). Organ weight (week 105): ▪ (↑) rel brain wt in ♀ (14%, ncdr). ▪ (↓) abs heart wt in ♀ (10%, ndr). ▪ (↓) rel kidney wt in ♂ (13%, ncdr, ns). ▪ (↓) rel adrenal wt in ♂ (32%, ndr, ns). ▪ (↑) abs (11%, ndr) and rel (11%) epididymis wt. ▪ (↓) abs (53%, ncdr, ns) and rel (48%. ncdr, ns) ovary wt. Necropsy *(Statistical analysis not performed)* *Adrenal* ▪ (↑) enlarged in ♀ (24.3% vs 17.1% in controls, ncdr). *Subcutis*	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		▪ (↑) preputial gland abscess in ♂ (8.6% vs 7.1% in controls, ncdr). *Thymus* ▪ (↑) small in ♀ (1.4% vs 0% in controls, ncdr). Histopathology *(Statistical analysis not performed)* *Non-neoplastic* *Ovary* ▪ (↑) granulosa/theca cell hyperplasia (6.7% vs 5.8% in controls, ncdr). Prostate ▪ (↑) atrophy (8.2% vs 1.5% in controls, ndr). **200 ppm** (equivalent to 10.17/13.19 mg/kg bw/day for ♂/♀) Bodyweight: ▪ (↓) bw in ♀ at week 8 (6%). Food consumption: ▪ in ♂ at week 57 (↑7%). ▪ in ♀ at week 81 (↑16%). Clinical chemistry: ▪ (↑) alkaline phosphatase (73%) at week 104 in ♀. Haematology: ▪ (↑) prothrombin time in ♀ at week 52 (7%, ncdr). Organ weight (week 105): ▪ (↓) rel adrenal wt in ♂ (32%, ndr, ns). ▪ (↓) abs (45%, ncdr, ns), and rel (41%. ncdr, ns) ovary wt. ▪ (↑) rel uterus wt (22%, ndr, ns). Necropsy *(Statistical analysis not performed)* *Thymus* ▪ (↑) small in ♀ (1.4% vs 0% in controls, ncdr). Histopathology *(Statistical analysis not performed)* *Non-neoplastic* *Prostate* ▪ (↑) atrophy (3.3% vs 1.5% in controls, ndr). -**LOAEL$_{toxicity}$**= 800 ppm (~41.93/53.5 mg/kg bw/day for ♂/♀) -**NOAEL$_{toxicity}$**= 400 ppm (~20.34/26.5 mg/kg bw/day for ♂/♀) Critical effects at the LOAEL$_{toxicity}$**:** clinical signs, ↓ decreased bodyweight in ♂/♀, ↓ food consumption in ♂/♀.	
Chronic toxicity study in rats. GLP: No Method: Non-stated **Supportive information**	Test substance: Dodine Oral (diet) Dose levels: 0, 50, 200 and 800 ppm (equivalent to 0, 2.5, 10 and 40 mg/kg bw/day) 104-week exposure	*Only effects relevant for STOT RE are presented (see also section 2.6.5)* Clinical signs: No treatment-related effects. Bodyweight: (↓) at 800 ppm for ♂/♀ (9/6%). Food consumption *(data not shown in the study)*: (↓) only in males at 800 ppm at 1st year. Haematology and clinical chemistry *(data not shown in the study)*: No relevant findings reported. Organ weight *(data not shown in the study)*: No relevant findings reported. Histopathology *(data not shown in the study)*: No relevant findings reported. NOAEL not set	Levinskas. *et al.* (1961) **B.6.5.3 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
Two-generation reproductive toxicity study in rats. GLP: Yes Method: US EPA FIFRA 83-4 Rat strain: Sprague-Dawley Sex: ♂ and ♀ No. animals: P/F1: 30 rats/sex/dose. Deviations from current OECD TG 416, 2001: -Rationale for dose selection not shown. -No. of implantations, corpora lutea and pre/post-implantation loss data not shown. -Thyroid and pituitary weights not measured. -HCD not presented. - Sperm evaluation in 100 cells per male, instead of 200. **Study acceptable**	Dodine; Lot/Batch No.: 1174, Purity: 98.6% Oral (diet) Doses: 0, 200 ppm (13.14/15.6 mg/kg bw/day for ♂/♀), 400 ppm (26.2/ 31.2 mg/kg bw/day for ♂/♀) and 800 ppm (52.6/60.3 mg/kg bw/day for ♂/♀). Exposure: *Pre-mating treatment:* P/F1: 10 weeks *Mating:* 2 weeks Treatment continued in P and F_1 throughout gestation and lactation of each litter.	*Only effects relevant for STOT RE are presented (see also section 2.6.6)* **PARENTAL TOXICITY (P)** Mortality: No treatment related signs seen. Clinical signs: No treatment-related signs observed. **800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)** *Bodyweight (bw)* *Pre-mating* ▪ (↓) bw in ♂ throughout weeks 1-12 (6-9%). ▪ (↓) bw in ♀ throughout week 3 (5%), 5-8 (5-6%) and 10 (5%). *Gestation* ▪ (↓) bw in ♀ at gestation day 0 (6%), 7 (7%), 14 (6%) and 20 (6%). *Lactation* ▪ (↓) bw in ♀ at lactation day 0 (6%), 4 (8%), 7 (7%) and 14 (8%). *Accumulative bodyweight gain (bwg)* *Pre-mating* ▪ (↓) bwg in ♂ between weeks 0-12 (13%). ▪ (↓) bwg in ♀ between weeks 0-10 (17%). *Lactation* ▪ (↑) bwg in ♀ between lactation days 14-21 (20%) and 0-21 (84%). *Food consumption (g/animal/day)* *Pre-mating* ▪ (↓) in ♂ at week 1 (14%), 2 (9%) and 3 (7%). ▪ (↓) in ♀ at week 1 (15%), 2 (11%), 3 (11%), 4 (6%) and 5 (9%). *Lactation* ▪ (↓) in ♀ throughout lactation day 4-7 (10%), 7-10 (19%), and 10-14 (15%). *Organ weight* ▪ (↓) abs left kidney in ♂ (5%). ▪ (↓) abs thymus in ♂ (17%). ▪ (↓) abs brain in ♂ (3%). ▪ (↑) rel left and right adrenal in ♀ (14%). ▪ (↓) abs left and right kidney in ♀ (6%). ▪ (↑) rel brain in ♀ (7%). *Necropsy (statistical analysis not performed):* ▪ (↑) red focus area in the thymus in ♀ (19% *vs* 11% in controls, ncdr). **400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)** *Bodyweight (bw)* ▪ (↓) bw in ♀ at lactation day 4 (4%). *Food consumption* *Pre-mating* ▪ (↓) abs in ♂ at week 1 (5%). *Lactation* ▪ (↓) abs in ♀ at week 7-10 (9%). *Organ weight* ▪ (↓) abs left kidney in ♀ (6%).	[redacted] (1996) **B.6.6.1.1 (AS)**

		DEVELOPMENTAL TOXICITY (F_1) **800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)** *Bodyweight (bw)* ▪ (↓) bw in ♂/♀ at day 4 (precull) (7%/9%). ▪ (↓) bw in ♂/♀ at day 4 (postcull) (7%/9%). ▪ (↓) bw in ♂/♀ at day 7 (11%/11%). ▪ (↓) bw in ♂/♀ at day 14 (17%/17%). ▪ (↓) bw in ♂/♀ at day 21 (16%/16%). *Organ weight* ▪ (↓) abs left and right kidney in ♂ (16%). ▪ (↓) abs liver in ♂ (18%). ▪ (↑) rel brain in ♂ (12%). ▪ (↓) abs spleen in ♀ (18%, ns, ndr). ▪ (↓) rel spleen in ♀ (8%, ns, ndr). ▪ (↓) rel right kidney in ♂ (3%, ns, ndr). **400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)** *Bodyweight (bw)* ▪ (↓) bw in ♀ at day 4 (precull) (7%). ▪ (↓) bw in ♀ at day 4 (postull) (7%). ▪ (↓) bw in ♀ at day 14 (6%). ▪ (↓) bw in ♂/♀ at day 21 (7%/8%). *Organ weight* ▪ (↓) abs spleen in ♀ (25%, ndr). ▪ (↓) rel spleen in ♀ (17%, ndr). ▪ (↓) rel right kidney in ♂ (6%, ns, ndr). **200 ppm (equivalent to 13.4/15.6 mg/kg bw/day for ♂/♀)** *Organ weight* ▪ (↓) rel right kidney in ♂ (8%, ndr). **PARENTAL TOXICITY (F_1)** Mortality: No treatment related signs seen. Clinical signs: No treatment-related signs observed. **800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)** *Bodyweight (bw)* *Pre-mating* ▪ (↓) bw in ♂ throughout week 0-12 (13-19%). ▪ (↓) bw in ♀ throughout week 0-10 (12-15%). *Gestation* ▪ (↓) bw in ♀ at gestation day 0 (13%), 7(14%), 14 (14%) and 20 (12%). *Lactation* ▪ (↓) bw in ♀ at lactation day 0 (13%), 4 (15%), 7 (13%), 14 (13%) and 21 (8%). *Accumulative bodyweight gain (bwg)* *Pre-mating* ▪ (↓) bwg in ♂ between weeks 0-12 (10%). ▪ (↓) bwg in ♀ between weeks 0-10 (9%). *Gestation* ▪ (↓) bwg in ♀ between weeks 0-7 (20%), 7-14 (20%) and 0-20 (11%). *Lactation* ▪ (↑) bwg in ♀ between lactation days 14-21 (135%) and 0-21 (116%).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		Food consumption (g/animal/day) *Pre-mating* ▪ (↓) in ♂ throughout week 0-10 (6-14%). ▪ (↓) in ♀ throughout week 0-10 (9-18%). Gestation ▪ (↓) in ♀ between lactation days 0-7 (12%), 7-14 (17%) and 14-20 (12%). *Lactation* ▪ (↓) in ♀ between lactation days 4-7 (12%), 7-10 (15%) and 10-14 (20%). *Organ weight* ▪ (↑) rel left and right epididymis (13%). ▪ (↑) rel left (12%) and right (14%) testis. ▪ (↑) rel left adrenal in ♂ (21%). ▪ (↓) abs left (15%) and right (14%) kidney in ♂. ▪ (↓) abs liver in ♂ (16%). ▪ (↑) rel brain in ♂ (13%). ▪ (↑) rel left (23%) and right (20%) adrenals in ♀. ▪ (↓) abs left (11%) and right (12%) kidney in ♀. ▪ (↓) abs liver in ♀ (12%). ▪ (↓) abs brain in ♀ (4%). ▪ (↑) rel brain in ♀ (9%). ▪ (↑) rel left ovary/oviduct (11%) and right ovary/oviduct (11%, ns). *Necropsy (statistical analysis not performed):* ▪ (↑) red focus area in thymus in ♂ (13% *vs* 10% in controls, ndr) and in ♀ (17% vs 10% in controls, ndr). ▪ (↑) mottled thymus in ♀ (7% *vs* 0% in controls, ndr). **400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)** *Bodyweight (bw)* *Pre-mating* ▪ (↓) bw in ♀ throughout week 0-8 (5-6%). *Gestation* ▪ (↓) bw in ♀ at gestation days 7 (5%), 14 (5%) and 20 (5%). *Lactation* ▪ (↓) bw in ♀ at lactation days 4 (5%), 7 (4%) and 14 (5%). *Food consumption (g/animal/day)* *Pre-mating* ▪ (↓) in ♀ between weeks 3-4 (9%). *Lactation* ▪ (↓) in ♀ between weeks 7-10 (8%). *Organ weight* ▪ (↓) abs left (7%) kidney in ♂. ▪ (↑) rel left (12%) and right (9%, ns) adrenal in ♀. ▪ (↓) abs left (5%) and right (6%) kidney in ♀. *Necropsy (statistical analysis not performed):* ▪ (↑) red focus area in thymus in ♂ (31 *vs* 10% in controls, ndr) and in ♀ (22% *vs* 10% in controls, ndr). **200 ppm (equivalent to 13.4/15.6 mg/kg bw/day for**	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		♂/♀) *Bodyweight (bw)* *Pre-mating* ▪ (↓) bw in ♀ throughout week 2-3 (5%). *Food consumption (g/animal/day)* *Pre-mating* ▪ (↓) in ♀ throughout week 4-5 (8%, ndr). *Necropsy (statistical analysis not performed):* ▪ (↑) red focus area in thymus in ♂ (23 *vs* 10% in controls, ndr) and in ♀ (7% vs 10% in controls, ndr). **DEVELOPMENTAL TOXICITY (F_2)** **800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)** *Bodyweight* ▪ (↓) bw in ♂ at day 4 (precull) (9%). ▪ (↓) bw in ♂ at day 4 (postcull) (8%). ▪ (↓) bw in ♂/♀ at day 7 (9%/9%). ▪ (↓) bw in ♂/♀ at day 14 (16%/16%). ▪ (↓) bw in ♂/♀ at day 21 (17%/18%). *Organ weight* ▪ (↓) abs left and right kidney in ♂ (16%). ▪ (↓) abs spleen in ♂ (18%). ▪ (↓) abs liver in ♂ (17%). ▪ (↑) rel brain in ♂ (18%). ▪ (↓) abs left (14%) and right kidney (17%) in ♀. ▪ (↓) abs thymus in ♀ (28%). ▪ (↓) abs spleen in ♀ (22%). ▪ (↓) abs liver in ♀ (17%). ▪ (↑) rel brain in ♀ (15%). **400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)** *Bodyweight* ▪ (↓) bw in ♂ at day 14 (5%). ▪ (↓) bw in ♂/♀ at day 21 (7%/7%). *Organ weight* ▪ (↓) abs left kidney in ♂ (11%). **NOAEL developmental: 200 ppm** (equivalent to 13.14/15.6 mg/kg bw/day for ♂/♀) based on decreased male and females pup weights in F_1 and F_2 generation. **NOAEL parental toxicity: 200 ppm** (equivalent to 13.14/15.6 mg/kg bw/day for ♂/♀) based on decreased bodyweights and increased relative adrenal weight in F_1 adult ♀.	
Dose range-finding developmental toxicity study in rats. GLP: Yes Method: In house method Rat strain: Sprague-Dawley	Dodine, Lot/Batch No.:APA 92/88/2; Purity: 95% Oral (gavage) Doses: 0, 50, 70 and 100 mg/kg bw/day from day 6 to 16	*Only effects relevant for STOT RE are presented (see also section 2.6.6)* ***Maternal toxicity*** Mortality: 1 ♀ at 100 mg/kg bw/day died. Clinical signs: 1 ♀ at 100 mg/kg bw/day showed wheezing, 1 ♀ at 100 mg/kg bw/day showed piloerection, hunched posture, red/brown staining around face, fore-paws and mild ataxia. **100 mg/kg bw/day** Bodyweight and bodyweight gain: ▪ (↓) bw on day 13 (8%, ndr).	[redacted] (1989a) **B.6.6.2.1 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
10 females/dose Deviations from current OECD TG 414, 2018: -At least 20 ♀ with implantation sites at necropsy should be used. -Test chemical not administered to the day prior to scheduled caesarea. -Mating index not shown. - Sex ratio, AGD and indication of incomplete testicular descent/cryptorchidism not measured -Thyroid weight and thyroid hormones values not recorded. -Foetal alterations not examined. -Statistical analysis not performed in most of parameters. **Supportive information**	of pregnancy both included Parameters observed: *Maternal data:* Clinical signs, mortality, bw and bwg, food consumption, necropsy, histopathology. *Reproductive data:* Number (no.) of corpora lutea, no. implants, uterus wt, litter wt. *Foetal data:* Foetus wt, deaths.	▪ (↓) bwg between days 6-9 (65%, ns), 9-13 (51%, ns), 6-13 (48%), 6-17 (17%, ns). Food consumption (statistical analysis not performed): ▪ (↓) between days 6-16 (24%). Necropsy (statistical analysis not performed) ▪ (↑) ureter dilatation (20% vs 0% in controls). ▪ (↑) kidney pelvic dilatation (30% vs 0% in controls). ▪ (↑) enlarged kidney (30% vs 0% in controls). Histopathology (statistical analysis not performed) ▪ (↑) epithelial hyperplasia and chronic inflammation in the urinary bladder (10% vs 0% in controls). ▪ (↑) inflammation in the ureters (10% vs 0% in controls). ▪ (↑) pelvic dilatation, pelvic inflammation and nephritis in the kidney (10% vs 0% in controls). ▪ (↑) hyperplasia in the lumbar lymph node (10% vs 0% in controls). **70 mg/kg bw/day** Bodyweight and bodyweight gain: ▪ (↓) bw on day 13 (10%, ndr). ▪ (↓) bwg between days 6-13 (26%). Food consumption (statistical analysis not performed): ▪ (↓) between days 6-16 (15%). **50 mg/kg bw/day** Food consumption: ▪ (↓) between days 6-16 (7%). **NOAEL** developmental toxicity: 100 mg/kg bw/day based on no effects observed at high dose. **NOAEL** maternal toxicity: 50 mg/kg bw/day based on decreased bodyweight gain and food consumption.	
Developmental toxicity study in rats. GLP: Yes Method: US EPA FIFRA 83-3 Rat strain: Sprague-Dawley 25 females/dose Deviations from current OECD TG 414, 2018: -Test chemical not administered to the day prior to scheduled caesarea. -Mating index not shown.	Dodine, Lot/Batch No.:APA 92/88/2; Purity: 95% Oral (gavage) Doses: 0, 10, 45 and 90 mg/kg bw/day from day 6 to 16 of pregnancy both included Parameters observed: *Maternal data:* Clinical signs, mortality, bw and bwg, food consumption,	*Only effects relevant for STOT RE are presented (see also section 2.6.6)* ***Maternal toxicity*** Mortality: No deaths recorded. Clinical signs: 3 ♀ at 90 mg/kg bw/day showed excessive salivation after dosing for one or 2 days during the treatment period. 3 ♀ at 90 mg/kg bw/day and 1 ♀ at 45 mg/kg bw/day showed red/brown stained fur around the mouth. 1 ♀ at 45 mg/kg bw/day showed noisy breathing after dosing. **90 mg/kg bw/day** Bodyweight and bodyweight gain (Statistical analysis from [redacted] 2019a) ▪ (↓) bw on days 9 (9%), 13 (8%), and 17 (8%). ▪ (↓) bwg through days 6-9 (107%), 6-17 (20%). ▪ (↓) corrected bwg by uterus wt through days 6-17 (756%). Food consumption gain (Statistical analysis from [redacted], 2019a): ▪ (↓) at day 6 (30%), 7 (32%), 8 (37%), 9 (31%), 10	[redacted] (1989b) [redacted] (2019a) **B.6.6.2.2 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
-AGD and indication of incomplete testicular descent/cryptorchidism not measured. -Thyroid weight and thyroid hormones from dams not recorded. -Statistical analysis not performed in most of the parameters. -HCD not valid. **Acceptable**	necropsy, histopathology. *Reproductive data:* Number (no.) of corpora lutea, no. implants, uterus wt, litter wt., sex ratio. *Foetal data:* Foetus wt, deaths.	(22%), 11 (15%), 12 (13%), 13 (17%), 14 (16%), 15 (17%), 16 (19%), through days 6-10 (30%), 6-16 (22%), 3-19 (14%). Necropsy (statistical analysis not performed) *Lung* ▪ (↑) dark red areas on lung lobes (8% *vs* 0% in controls). Histopathology (statistical analysis not performed) *Lung* ▪ (↑) congestion (8% *vs* 0% in controls). ▪ (↑) haemorrhage into alveoli (4% *vs* 0% in controls). **45 mg/kg bw/day** Bodyweight and bodyweight gain (Statistical analysis from [redacted], 2019a) ▪ (↓) bwg through days 6-9 (42%). ▪ (↓) corrected bwg by uterus wt through days 6-17 (333%). Food consumption ((Statistical analysis from [redacted] 2019a): ▪ (↓) at day 6 (11%), 8 (18%), 9 (17%), 10 (12%), 11 (15%), 12 (13%), 13 (17%), 14 (16%), 15 (17%), 16 (19%), through days 6-10 (14%) and 6-16 (11%). ***Foetal toxicity:*** ***Skeletal alterations*** **90 mg/kg bw/day** ▪ 8.8% of foetuses (ndr)/ 30% of litters (ns, ncdr) with phalangeal elements, one or more ossified *vs* 2.7% of foetuses / 14% of litters in controls. **45 mg/kg bw/day** ▪ 12% of foetuses (ndr)/ 27% of litters (ns, ncdr) with phalangeal elements, one or more ossified *vs* 2.7% of foetuses / 14% of litters in controls. **10 mg/kg bw/day** ▪ 5.5% of foetuses (ns, ndr)/ 29% of litters (ns, ncdr) with phalangeal elements, one or more ossified *vs* 2.7% of foetuses / 14% of litters in controls. **NOAEL** developmental toxicity: 90 mg/kg bw/day based on no adverse effects observed at high dose tested. **NOAEL** maternal toxicity: 10 mg/kg bw/day based on reduced bw gain (6-9 GD) and reduction in food consumption.	
T-Cell dependent antibody response (TDAR) assay using sheep red blood cells (SRBC) with dodine in Sprague Dawley rats Guideline: OPPTS 870.7800. GLP: Yes Rat strain:	Dodine (batch no. 43; purity 96.62%). Vehicle: Acetone Doses: 0, 200, 500, and 1000 ppm (equivalent to 0, 18, 44, 83 mg/kg bw/day) for 28 days. Immunisation: 2 x	*Only effects relevant for STOT RE are presented (see also section 2.6.8)*	[redacted] (2013) **B.6.8.2.1 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
♀ Sprague Dawley. No. animals 10 rats/dose Deviations from US EPA TG OPPTS 870.7800 (1998): - Short acclimatization period. - Low temperature of experimental room. - No indications on frequency of water consumption. - Positive control only administered for 5 days. **Study acceptable**	10^8 SRBC/ rat, i.v., on day 24. Serum collected on day 29.	**1000 ppm** (83 mg/kg bw/day) Bodyweight: ▪ (↓) bw at day 29 (12.7%). ▪ (↓) bw gain at day 29 (34%). Organs´ weight: ▪ Thymus: (↓) abs wt (26%) and rel wt (14%, ns, ndr). ▪ Spleen: (↓) abs wt (16%, ns) and rel wt (3%, ns, ndr). **500 ppm (**44 mg/kg bw/day) Organs´ weight: ▪ Thymus: (↓) abs. wt (18%, ns) and rel. wt (16%, ns, ndr). ▪ Spleen: (↓) abs. wt (12%, ns) and rel. wt (11%, ns, ndr). **200 ppm (**18 mg/kg bw/day) Organs´ weight: ▪ Thymus: (↓) abs. wt (14%, ns) and rel. wt (18%, ns, ndr). ▪ Spleen: (↓) abs wt (18%, ns, ndr) and rel. wt (23%, ndr). **NOAEL systemic**: 500 ppm (equivalent to **44 mg/kg bw/day),** based on the decrease in bw and bw gain at 1000 ppm. **NOAEL immunotoxicity**: 1000 ppm (equivalent to **83 mg/kg bw/day),** based on the absence of immunotoxicity effects.	
Mouse toxicity studies			
8-week oral (diet) study in mice. Guideline: Not stated. GLP: Yes Mouse strain: CD-1 No. animals 5 mice/sex/dose Deviations from OECD TG 407 (2008): - Doses modified during treatment. - Only 5, instead of 10 mice/sex/dose used. - Haematological examination and clinical biochemistry determination not performed.	Dodine (batch no. APA 92/88/2 and purity of 95%). Doses: 0, 250 or 625 ppm for 8 weeks, equivalent to 0, 49.4 and 109.4 mg/kg bw/day in ♂ and 0, 61.3 and 150.4 mg/kg bw/day in ♀. 100 ppm for 3 weeks and 1250 ppm for the remaining 5 weeks, equivalent to 30.3/232.2 mg/kg bw/day in ♂ and 34/323.6 mg/kg bw/day in ♀.	**Mortality**: ♂: no deaths. ♀: 1/5 at 100/1250 ppm died after dose increase. **100/1250 ppm** (30.3/232.2♂ and 34/323.6♀ mg/kg bw/day) Bodyweight and food consumption (*no statistical analysis reported):* ▪ (↓) bw in ♀ [at week 8 (11.8%)] ▪ (↓) bw gain week 0-8 in ♂/♀ (18.2/34.3%). Organs´ weight (*no statistical analysis reported for rel wt):* ▪ Spleen: (↓) abs wt in ♂ (11.1%, ns) and in ♀ (30%); (↓) rel-to-body wt in ♂ (11.2%) and in ♀ (24.2%). Gross pathology *(no statistical analysis performed):* ▪ (↑) cystic ovary (2/5 *vs* 1/5 in controls). Histopathological findings: ▪ (↑) liver eosinophilia ♂ (5/5 *vs* 0/10 in control) and in ♀ (3/5 *vs* 0/5 in control). ▪ (↑) cellular depletion and decreased pigment deposit in spleen in ♀ (1/5 *vs* 0/5 in control). ▪ (↑) ovarian cyst (2/2 *vs* 0/1 in control). **625 ppm** (109.4♂ and 150.4♀ mg/kg bw/day) Bodyweight and food consumption (*no statistical analysis reported):* ▪ bw gain between weeks 0-8 (↑) in ♂ (11.4%, ndr)	[redacted] (1988) **B.6.3.1.5 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
- Not all the recommended organs weighed. - Histopathology not carried out in all recommended tissues and not extended to all groups when effects were observed at high dose. **Study acceptable as supportive information.** *Guideline value for classification: STOT RE 2 ≤ 167 mg/kg bw/day STOT RE 1 ≤ 16.7 mg/kg bw/day (Haber's rule from 90- to 56-day value)*		and (↓) in ♀ (2.8%, ndr). Organs´ weight (*no statistical analysis reported for rel wt):* ▪ Spleen: (↓) rel-to-body wt in ♂ (10.6%) and in ♀ (13.5%). Gross pathology *(no statistical analysis performed):* ▪ (↑) cystic ovary (2/5 *vs* 1/5 in controls). **250 ppm** (49.4♂ and 61.3♀ mg/kg bw/day) Bodyweight and food consumption (*no statistical analysis reported):* ▪ bw gain between weeks 0-8 (↑) in ♂ (22.7%, ndr) and (↓) in ♀ (14.3%, ndr). Gross pathology *(no statistical analysis performed):* ▪ (↑) cystic ovary (2/5 *vs* 1/5 in controls). **NOAEL**: 625 ppm **(109.4** and **150.4 mg/kg bw/day** in ♂/♀, respectively**).** **LOAEL**: 100/1250 ppm **(30.3/232.2**♂ and **34/323.6**♀ **mg/kg bw/day** in ♂/♀, respectively), based on reduction of the bw in ♀, reduction in the bw gain in ♂/♀, decrease in the abs. weight of spleen in ♀ and the increase in liver eosinophilia in ♂/♀.	
90-day oral (diet) study in mice. Guideline: US EPA FIFRA F-82-1 GLP: Yes Mouse strain: CR1:CD®-1(ICR)BR. No. animals 10 mice/sex/dose Deviations from OECD TG 408 (2018): - Sensory reactivity to stimuli, grip strength and motor activity not analysed. - Functional observation not performed. - Several haematological and biochemistry parameters not measured.	Dodine (batch no. APA 303/30 and purity of 94.07%). Doses: 0, 150, 300, 600, 1250 or 2500 ppm for 90 days, equivalent to 0, 24, 48, 94, 181 and 350 mg/kg bw/day in ♂ and 0, 31, 60, 116, 223 and 305 mg/kg bw/day in ♀.	**Mortality**: ♂: no deaths. ♀: 4/10 at 2500 ppm died. **2500 ppm** (350♂/305♀ mg/kg bw/day) Clinical signs *(no statistical analysis performed):* ▪ (↑) Stiffening of the tail in ♀ (4/10 *vs* 0/10 in controls). Bodyweight and food consumption: ▪ (↓) Bw in ♂ [between weeks 1-13 (between 17.3-24.3%)] and ♀ [between weeks 1-2 (between 10.7-	[redacted] (1994) **B.6.3.2.4 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
- Epididymides, adrenals, prostate + seminal vesicles with coagulating glands, uterus, thymus and thyroid not weighed. - Not all recommended tissues with histopathological examination and not extended to all dose groups when observed at high dose. - Oestrus cycle not determined. **Study acceptable** *Guideline value for classification: STOT RE 2 ≤ 100 mg/kg bw/day STOT RE 1 ≤ 10 mg/kg bw/day*		15.4%) and weeks 6-10 (between 10.3-12.6%)]. ▪ (↓) Bw gain week 0-13 in ♂/♀ (68/44%). ▪ (↓) Food consumption week 0-13 in ♂/♀ (30/46%) *(no statistical analysis reported for this period).* <u>Haematology:</u> ▪ (↑) Neutrophils in ♂ (45%, ndr). ▪ (↓) Lymphocytes in ♂ (15.5%, ns, ndr). ▪ (↓) Eosinophils in ♂ (93.8%, ns). ▪ (↓) Hb in ♂ (8.8%). ▪ (↑) RDW in ♂ (13.2%). <u>Clinical chemistry:</u> ▪ (↑) BUN in ♂ (31.1%) and in ♀ (162.8%). ▪ (↑) Total bilirubin in ♂ (30.8%, ns) and in ♀ (22.2%, ns). ▪ (↑) AST in ♂ (139.3%, ns) and in ♀ (81.1%, ns, ndr). ▪ (↑) Phosphorus in ♂ (17.3%) and in ♀ (22.3%, ns). ▪ (↑) A/G ratio in ♀ (25.9%). <u>Organs´ weight:</u> ▪ Liver: (↑) abs wt in ♀ (15.9%, ns); (↑) rel-to-body wt in ♂ (20.1%) and in ♀ (22.4%). ▪ Spleen: (↓) abs wt in ♂ (27.3%) and in ♀ (32.5%); (↓) rel-to-body wt in ♂ (7.4%, ns, ndr) and in ♀ (29.2%). ▪ Heart: (↓) abs wt in ♂ (10.4%. ns); (↑) rel-to-body wt in ♂ (15.2%). ▪ Adrenals: (↓) abs wt in ♀ (22%, ns); (↑) rel-to-body wt in ♂ (33.3%) and (↓) rel-to-body wt in ♀ (14.6%, ns); (↑) rel-to-brain wt in ♂ (15.9%, ns) and (↓) rel-to-brain wt in ♀ (14.5%, ns). ▪ Kidneys: (↓) abs wt in ♂ (18.1%) and (↑) abs wt in ♀ (16.3%); (↑) rel-to-body wt in ♀ (23.8%). ▪ Testis: (↑) rel-to-body wt (22.4%). ▪ Ovaries: (↓) abs wt (36.4%, ns); (↓) rel-to-body wt (35%, ns); (↓) rel-to-brain (34.4%, ns). ▪ Brain: (↓) abs wt in ♂ (8.8%); (↑) rel-to-body wt in ♂ (16.5%). ▪ Pituitary: (↓) abs wt in ♂ (23%, ndr) and in ♀ (19.8%, ns); (↓) rel-to-body wt in ♂ (22.2%, ns, ndr) and in ♀ (16%, ns); (↓) rel-to-brain wt in ♂ (26.9%) and in ♀ (14.9%, ns, ndr). <u>Histopathological findings:</u> ▪ (↑) Spleen lymphoid atrophy in ♀ (3/10 *vs* 0/10 in control). ▪ (↑) Thymus lymphoid necrosis in ♀ (4/10 *vs* 0/10 in control); thymus haemorrhage in ♀ (1/10 *vs* 0/10 in	

		control); thymus lymphoid atrophy in ♀ (4/10 *vs* 0/10 in control). **1250 ppm** (181♂/223♀ mg/kg bw/day) <u>Bodyweight and food consumption</u> ▪ (↓) Bw gain week 0-13 in ♂/♀ (12%/6.8%, ns, ndr). ▪ (↓) Food consumption week 0-13 in ♂/♀ (11/12%) *(no statistical analysis reported for this period).* <u>Haematology</u> ▪ (↓) Neutrophils in ♂ (26.2%, ns, ndr). ▪ (↑) Lymphocytes in ♂ (10.3%, ns, ndr). <u>Clinical chemistry:</u> ▪ (↓) BUN in ♂ (1.4%, ns, ndr) and (↑) in ♀ (43.2%, ns). ▪ (↓) Total bilirubin in ♂ (7.7%, ns, ndr) and in ♀ (11.1%, ns, ndr). ▪ (↑) AST in ♂ (1.6%, ns, ndr) and in ♀ (36.9%, ns, ndr). <u>Organs´ weight:</u> ▪ Spleen: (↓) abs wt in ♂ (15.5%, ns) and in ♀ (22.9%, ns); (↓) rel-to-body wt in ♂ (10.4%, ns, ndr) and in ♀ (20.3%, ns). ▪ Adrenals: (↓) abs wt in ♀ (11%, ns); (↓) rel-to-brain wt in ♀ (10.1%, ns). ▪ Kidneys: (↑) rel-to-body wt in ♀ (11%). ▪ Ovaries: (↓) abs wt (18.2%, ns); (↓) rel-to-body wt (15%, ns, ndr); (↓) rel-to-brain (16.1%, ns). ▪ Pituitary: (↓) abs wt in ♂ (15.4%, ndr) and in ♀ (14.8%, ns); (↓) rel-to-body wt in ♂ (22.2%, ns, ndr) and in ♀ (14%, ns); (↓) rel-to-brain wt in ♂ (19.2%, ndr) and in ♀ (15.1%, ns, ndr). **600 ppm** (94♂/116♀ mg/kg bw/day) <u>Bodyweight and food consumption</u> ▪ (↑) Bw gain week 0-13 in ♀ (16%, ns, ndr). <u>Haematology</u> ▪ (↓) Neutrophils in ♂ (41.8%, ndr). ▪ (↑) Lymphocytes in ♂ (19.6%, ndr). <u>Clinical chemistry:</u> ▪ (↓) BUN in ♂ (15.7%, ns, ndr) and in ♀ (8.1%, ns, ndr). ▪ (↑) Total bilirubin in ♂ (7.7%, ns, ndr) and (↓) in ♀ (5.6%, ns, ndr). ▪ (↓) AST in ♂ (24.4%, ns, ndr) and in ♀ (3.2%, ns, ndr). <u>Organs´ weight:</u> ▪ Spleen: (↓) rel-to-body wt in ♀ (12.2%, ns). ▪ Adrenals: (↓) rel-to-body wt in ♀ (13.3%, ns, ndr). ▪ Ovaries: (↓) abs wt (13.6%, ns); (↓) rel-to-body wt (20%, ns, ndr); (↓) rel-to-brain (15.5%, ns). ▪ Pituitary: (↓) abs wt in ♂ (23%, ndr); (↓) rel-to-body wt in ♂ (33.3%, ndr); (↓) rel-to-brain wt in ♂ (23.8%, ndr). **300 ppm** (48♂/60♀ mg/kg bw/day) <u>Bodyweight and food consumption</u> ▪ (↑) Bw gain week 0-13 in ♀ (10%, ns, ndr). <u>Haematology</u> ▪ (↑) Lymphocytes in ♂ (10%, ns, ndr). <u>Clinical chemistry:</u> ▪ (↑) BUN in ♀ (20.9%, ns, ndr). ▪ (↑) Total bilirubin in ♂ (15.4%, ns, ndr) and (↓) in ♀ (5.6%, ns, ndr).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		▪ (↑) AST in ♂ (9.5%, ns, ndr) and in ♀ (22.9%, ns, ndr). Organs´ weight: ▪ Ovaries: (↓) rel-to-brain (11%, ns). **150 ppm** (24♂/31♀ mg/kg bw/day) Clinical chemistry: ▪ (↑) BUN in ♀ (36.9%, ns, ndr). ▪ (↑) Total bilirubin in ♂ (15.4%, ns, ndr) and (↓) in ♀ (16.7%, ns, ndr). ▪ (↑) AST in ♂ (17.2%, ns, ndr) and in ♀ (155.3%, ns, ndr). **NOAEL**: 600 ppm **(94**♂ and **116**♀ **mg/kg bw/day).** **LOAEL**: 1250 ppm **(181**♂ and **223**♀ **mg/kg bw/day),** based on reduction in the bw gain in ♂, decrease in food consumption in ♂/♀ and decrease in abs spleen wt in ♂/♀ and decrease in rel-to-body spleen wt in ♀.	
78-week carcinogenicity study in mice GLP: Yes Method: US-EPA FIFRA 83-2 Mice strain: Crl:CD-1(ICR)BR mice: ♂ and ♀ No. animals: 60 mice/group Deviations from current test guideline (OECD TG 453, 2018): -HCD not provided for all neoplasm incidences, and these did not cover the 5-year recommended period on the date of the index study. - Following organs not weighed: epididymides, heart, spleen, testes, thyroid and uterus. - Haemotology and biochemistry not performed. **Study acceptable**	Test substance: Dodine technical. Purity: 98.6% Oral (diet) **Doses:** Males: 0, 200, 750 and 1500 ppm (equivalent to 0, 29.2, 109.8 or 224.8 mg/kg bw/day). Females: 0, 200, 750 and 1500 ppm (equivalent to 0, 38.3, 136.2 or 275.2 mg/kg bw/day). 78-week feed exposure.	*Only effects relevant for STOT RE are presented (see also section 2.6.5)* **Mortality**: not increased by treatment. **1500 ppm** (equivalent to 224.8/275.2 mg/kg bw/day for ♂/♀) Clinical signs: ▪ (↑) body tremors in ♂ (52.9% *vs* 40% in controls) and in ♀ (31.4% *vs* 20% in controls). ▪ (↑) maloocclusion in ♂ (18.6% *vs* 5.7% in controls). ▪ (↑) dilated pupil in ♂ (54.3% *vs* 18.6% in controls) and in ♀ (14.3% *vs* 10% in controls). ▪ (↑) excessive salivation in ♂ (51.4% *vs* 38.6% in controls) and in ♀ (20% *vs* 10% in controls). Bodyweight ▪ (↓) bw in ♂ throughout week 2-78 (3-10%). ▪ (↓) bw in ♀ at week 5 (4%), and throughout week 8-78 (4-14%). ▪ (↓) bwg in ♂ through week 1-14 (25%) and 1-78 (26%). ▪ (↓) bwg in ♀ through week 1-14 (26%), 14-54 (36%), 54-78 (63%), and 1-78 (35%). Food consumption: ▪ (↓) in ♂ at week 1-9 (8-16%), 11-12 (7%), 21-33 (6-9%), 49 (5%), 57 (5%). ▪ (↓) in ♀ at week 1-2 (13%), 4 (8%), 5 (7%), and 9-77 (8-19%). Organ weight (week 78): ▪ (↓) abs left adrenal wt in ♀ (23%, ncdr). ▪ (↑) abs/rel left (12/31%) and abs/rel right (11/30%) kidney wt in ♀. ▪ (↑) rel liver wt in ♂ (13%, ncdr). ▪ (↑) rel liver wt in ♀ (14%, ncdr). ▪ (↑) rel brain wt in ♂ (7%, ncdr). ▪ (↓) abs brain wt in ♀ (5%, ncdr). (↑) rel brain wt in ♀ (11%, ncdr). Necropsy *(Statistical analysis not performed)* *Liver* ▪ (↑) light focus area in ♂ (5.7% vs 1.4% in controls,	[redacted] (1998a) **B.6.5.2 (AS)**

Guideline value for classification: STOT RE 2 ≤ 16.7 mg/kg bw/day STOT RE 1 ≤ 1.67 mg/kg bw/day (Haber's rule from 90-day to2-year value)		ncdr). *Kidney* ▪ (↑) cyst in ♂ (7.1% vs 4.3% in controls, ncdr). *Spleen* ▪ (↑) small in ♀ (4.3% vs 0% in controls). *Testes* ▪ (↑) small (5.7% vs 1.4% in controls). <u>Histopathology</u> *Non-neoplastic (statistical analysis not performed)* *Kidney* ▪ (↑) cyst in ♂ (15% vs 5% in controls, ndr). ▪ (↑) hyperplasia tubular cell in ♂ (6.7% vs 0% in controls). *Prostate* ▪ (↑) chronic inflammation (16.7% vs 6.7% in controls, ndr). **750 ppm** (equivalent to 109.8/136.2 mg/kg bw/day for ♂/♀) <u>Clinical signs</u>: ▪ (↑) body tremors in ♂ (54.3% *vs* 40% in controls) and in ♀ (32.9% *vs* 20% in controls). ▪ (↑) dilated pupil in ♂ (41.4% *vs* 18.6% in controls) and in ♀ (17.1% *vs* 10% in controls). ▪ (↑) excessive salivation in ♂ (48.6% *vs* 38.6% in controls) and in ♀ (25.7% *vs* 10% in controls, ndr). <u>Bodyweight</u> ▪ (↓) bw in ♂ at week 7 (2%), 9-10 (3%), 18 (3%), 30-62 (4-5%). ▪ (↓) bw in ♀ at week 13-14 (4%), 22 (6%), 30-46 (5-7%) and 54-78 (7-10%). ▪ (↓) bwg in ♂ through week 1-78 (5%, ns). ▪ (↓) bwg in ♀ through week 1-78 (20%). <u>Food consumption:</u> ▪ (↓) in ♂ at week 1 (8%), 5-12 (5-8%) and 49 (7%). ▪ (↓) in ♀ at week 1-2 (10-16%), 5 (7%), 10-25 (6-11%), 33-57 (5-9%), 69 (10%) and 77 (9%). <u>Organ weight (week 78):</u> ▪ (↓) abs left adrenal wt in ♀ (18%, ncdr). ▪ (↓) abs (1%, ncdr, ns), (↑) rel (7%) left kidney wt. (↓) abs (1%, ndr, ns), (↑) rel (8%) right kidney wt in ♀. ▪ (↓) abs brain wt in ♀ (3%, ncdr). (↑) rel brain wt in ♀ (5%, ncdr, ns). <u>Necropsy *(Statistical analysis not performed)*</u> *Liver* ▪ (↑) light focus area in ♂ (2.9% vs 1.4% in controls, ncdr). *Kidney* ▪ (↑) cyst in ♂ (8.6% vs 4.3% in controls, ncdr). Uterus ▪ (↑) large (20% vs 18.6% in controls, ndr). <u>Histopathology</u> *Non-neoplastic (statistical analysis not performed)* *Kidney* ▪ (↑) cyst in ♂ (15% *vs* 5% in controls, ndr). ▪ (↑) hyperplasia tubular cell in ♂ (1.7% *vs* 0% in controls, ncdr). *Prostate* ▪ (↓) chronic inflammation (0% *vs* 6.7% in controls, ndr). **200 ppm** (equivalent to 29.2/38.3 mg/kg bw/day for ♂/♀) <u>Clinical signs</u>:	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		▪ (↑) dilated pupil in ♂ (30% *vs* 18.6% in controls) and in ♀ (25.7% *vs* 10% in controls, ndr). Bodyweight ▪ (↓) bw in ♂ at week 9-10 (3%) and 13 (4%). ▪ (↓) bw in ♀ at week 34 (3%), 58 (6%), 66 (6%) and 78 (6%). ▪ (↑) bwg in ♂ through week 54-78 (220%) and 1-78 (3%, ns). ▪ (↓) bwg in ♀ through 1-78 (11%, ns). Food consumption: ▪ (↓) in ♂ at week 5 (6%), 8-9 (5-7%), 25 (4%) and 49 (5%). Necropsy *(Statistical analysis not performed)* *Spleen* ▪ (↑) large in ♂ (15.7% vs 11.4% in controls, ndr). Histopathology: *Non-neoplastic (statistical analysis not performed)* *Kidney* ▪ (↑) cyst in ♂ (16.7% *vs* 5% in controls, ndr). *Prostate* ▪ (↑) chronic inflammation (14.3% vs 6.7% in controls, ndr). -**$LOAEL_{toxicity}$**= 750 ppm (~109.8/136.2 mg/kg bw/day for ♂/♀) -**$NOAEL_{toxicity}$**= 200 ppm (~29.2/38.3 mg/kg bw/day for ♂/♀) -Critical effects at the $LOAEL_{toxicity}$: clinical signs, ↓ bodyweight, ↓food consumption in ♂/♀.	
Dog toxicity studies			
6-week oral capsule range-finding study in dogs. Guideline: EPA Guidelines (40 CFR Part 158 and Subdivision F) OECD (C(81)30). GLP: Yes Dog breed: Beagle. No. animals 2 dogs/sex/dose **Study acceptable as supportive information.** *Guideline value for classification: STOT RE 2 ≤ 214 mg/kg bw/day STOT RE 1 ≤ 21.4 mg/kg bw/day (Haber's rule*	Dodine (batch no. APA 303/90 and purity of 94.07%). Vehicle: gelatine. Doses: 1.25 mg/kg bw/day (weeks 1-5). 6.25 mg/kg bw/day (weeks 1-3) + 60 mg/kg bw/day (weeks 4-5). 12.5 mg/kg bw/day (week 1) + 50 mg/kg bw/day (week 2-6). 25 mg/kg bw/day (week 1-6).	**Mortality**: ♂: 1/2 at 12.5/50 mg/kg bw/day died on day 36. ♀: no deaths.	[redacted] (1994) **B.6.3.1.6 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
from 90- to 56-day value)		**6.25/60 mg/kg bw/day** Bodyweight and food consumption: ▪ Mean bw loss in ♂ (0.7 kg) and in ♀ (0.6 kg). ▪ Low food consumption. Clinical chemistry: ▪ High BUN in 2/4 dogs (♂). ▪ Low albumin and globulin in 2/4 dogs. ▪ Low total protein in 3/4 dogs. Gross pathology: ▪ Dark area/discoloration in stomach/duodenum (4/4 dogs). **12.5/50 mg/kg bw/day** Clinical signs: ▪ Vomiting in ♀ [at week 1]. Bodyweight and food consumption: ▪ Mean bw loss in ♂ (0.45 kg) and in ♀ (0.15 kg). ▪ Low food consumption. Clinical chemistry: ▪ High BUN in 3/4 dogs. ▪ Low albumin and globulin in 3/4 dogs. ▪ Low total protein in 4/4 dogs. Gastric emptying time: ▪ Delayed: 4 h *vs* 2 h (normal time). Gross pathology: ▪ Dark area/discoloration in stomach/duodenum (3/4 dogs). **25 mg/kg bw/day** Clinical signs: ▪ Vomiting. ▪ Excessive salivation. Bodyweight and food consumption: ▪ Mean bw loss in ♂ (0.85 kg). ▪ Low food consumption in ♂. Clinical chemistry: ▪ High BUN in 1/4 dogs (♂). ▪ Low total protein in 1/4 dogs. Gross pathology: ▪ Dark area/discoloration in stomach/duodenum (1/4 dogs). **1.25 mg/kg bw/day** Clinical signs: ▪ Liquid faeces. **NOAEL** not derived.	
90-day oral (capsules) study in dogs. Guideline: OECD TG 409 (1998) GLP: Yes Dog strain: Beagle.	Dodine (batch no. DCH0112 and purity of 96.61/97.176%). Doses: 0, 2, 10 and 20 mg/kg bw/day for 90 days (in gelatine capsules).	**Mortality**: No deaths reported.	[redacted] (2005) **B.6.3.2.5 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
No. animals 4 dogs/sex/dose Deviations from OECD TG 409 (1998): - Ornithine decarboxylase not measured. - Bone marrow not histopathologically examined. **Study acceptable** *Guideline value for classification:* *STOT RE 2 ≤ 100 mg/kg bw/day* *STOT RE 1 ≤ 10 mg/kg bw/day*		**20 mg/kg bw/day** Clinical signs *(no statistical analysis performed):* ▪ (↑) Vomiting in ♂ (4/4 *vs* 0/4 in control) and ♀ (3/4 *vs* 0/4 in control). ▪ (↑) Blue tongue in ♂ (2/4 *vs* 0/4 in control) and ♀ (1/4 *vs* 0/4 in control). ▪ (↑) Lean appearance in ♂ (2/4 *vs* 0/4 in control) and ♀ (1/4 *vs* 0/4 in control). ▪ (↑) Calm behaviour in ♂ (1/4 *vs* 0/4 in control). Bodyweight and food consumption ▪ (↓) Bw on day 92 in ♀ (11.6%, ns). ▪ (↑) Bw loss in ♂ [between weeks 23-51] and in ♀ [between weeks 16-30]. ▪ (↓) food consumption in ♂ between days 1-29 (between 20.4-28.8%) and in ♀ between days 1-36	

		(between 26.8-51.2%); (↓) mean food consumption in ♂/♀ (12%, ns/26.8%, ns). Haematology: ▪ (↑) APTT in ♂ at week 6 (6.7%, ns) and at week 13 (2.5%). Clinical chemistry: ▪ (↑) AST at week 13 in ♂ (14.3%, ns, ndr) and (↓) at week 13 in ♀ (26.7%, ns, ndr). ▪ (↓) ALP in ♂ at pre-test (23.7%, ns), at week 6 (53%) and at week 13 (28%, ns, ndr). ▪ (↑) GLDH in ♀ at pre-test (60.4%, ns), in ♂/♀ at week 6 (191.7%, ns/87%) and in ♂/♀ at week 13 (88%, ns/109%). ▪ (↓) Cholesterol in ♀ at week 6 (31%) and at week 13 (29.3%, ns). ▪ (↑) Ca in ♀ at week 13 (1.1%, ns). ▪ (↓) Cl in ♂ at week 6 (1.8%). ▪ (↑) Albumin in ♀ at week 6 (5.1%, ns, ndr). Urinalysis: ▪ (↓) Volume in ♂ at week 6 (56.5%, ns) and at week 13 (12.2%, ns, ndr) and in ♀ at week 6 (59.3%, ns, ndr) and at week 13 (56.6%, ns, ndr). Organs´ weight: ▪ Kidneys: (↑) abs wt in ♀ (4.9%, ns, ndr) and rel-to-body wt in ♀ (24.6%). ▪ Testis: (↓) abs wt (13.2%, ns) and rel-to-body wt (9.9%, ns, ndr). ▪ Prostate: (↓) abs wt (50.8%, ns) and rel-to-body wt (51%, ns). ▪ Epididymides: (↓) abs wt (17.1%, ns, ndr) and rel-to-body wt (17.1%, ns, ndr). ▪ Uterus: (↓) abs wt (27.8%, ns, ndr) and rel-to-body wt (3.3%, ns, ndr). ▪ Ovaries: (↑) abs wt (5%, ns, ndr) and rel-to-body wt (36.4%, ns, ndr). ▪ Heart: (↓) abs wt in ♀ (10.9%, ns). ▪ Thymus: (↑) abs wt in ♂ (44.4%, ns) and (↓) abs wt in ♀ (33.6%, ns); (↑) rel-to-body wt in ♂ (47.3%, ns) and (↓) rel-to-body wt in ♀ (21.8%, ns). ▪ Thyroid: (↓) abs wt in ♀ (33.3%, ns, ncdr) and rel-to-body wt in ♀ (14.3%, ns, ndr). ▪ Spleen: (↓) abs wt in ♂ (18.9%, ns, ndr) and (↑) abs wt in ♀ (60%, ns, ndr); (↓) rel-to-body wt in ♂ (16.3%, ns, ndr) and (↑) rel-to-body wt in ♀ (85.2%, ns, ndr). ▪ Liver: (↓) abs wt in ♀ (15%). ▪ Pituitary: (↑) rel-to-body wt in ♂ (12.5%, ns) and in ♀ (25%, ns). ▪ Adrenals: (↑) rel-to-body wt in ♂ (14.3%, ns, ndr) and in ♀ (13.3%, ns, ndr). Gross pathology: ▪ (↓) Enlarged prostate (0/4 *vs* 2/4 in control). ▪ (↑) Prostate reduced in size (1/4 *vs* 0/4 in control). ▪ (↑) Thymus reduced in size in ♀ (1/4 *vs* 0/4 in control). Histopathological findings: ▪ (↑) Testes with giant cells spermatids (3/4 *vs* 1/4 in control). **10 mg/kg bw/day** Clinical signs *(no statistical analysis performed):* ▪ (↑) vomiting in ♂ (1/4 *vs* 0/4 in control) and ♀ (2/4 *vs* 0/4 in control). ▪ (↑) blue tongue in ♂ (2/4 *vs* 0/4 in control).	

		Bodyweight and food consumption: ▪ (↓) food consumption in ♀ between days 22-29 (18.8%). (↓) mean food consumption in ♀ (9.2%, ns). Clinical chemistry: ▪ (↑) AST at week 13 in ♂ (37.3%, ndr) and (↓) at week 13 in ♀ (31.4%, ns, ndr). ▪ (↓) ALP in ♂ at pre-test (11.5%, ns, ndr), at week 6 (15.7%, ns, ndr) and at week 13 (15.7%, ns, ndr). ▪ (↑) GLDH in ♀ at week 6 (19.6%, ns) and in ♀ at week 13 (14%, ns). ▪ (↑) Ca in ♀ at week 13 (4%). ▪ (↓) Cl in ♂ at week 6 (1.8%). ▪ (↑) Albumin in ♀ at week 6 (13.7%, ndr). Urinalysis: ▪ (↓) Volume in ♂ at week 6 (47.7%, ns) and (↑) at week 13 (37.4%, ns, ndr); (↓) in ♀ at week 6 (68.3%, ndr) and at week 13 (77.7%, ndr). Organs´ weight: ▪ Kidneys: (↑) abs wt in ♀ (6%, ns, ndr) and rel-to-body wt in ♀ (13.5%, ns, ndr). ▪ Testis: (↓) abs wt (11.2%, ns) and rel-to-body wt (17.1%, ns, ndr). ▪ Prostate: (↓) abs wt (41.9%, ns, ndr) and rel-to-body wt (46%, ns). ▪ Epididymides: (↑) abs wt (46.3%, ns, ndr) and rel-to-body wt (34.3%, ns, ndr). ▪ Uterus: (↑) abs wt (14.5%, ns, ndr) and rel-to-body wt (25.4%, ns, ndr). ▪ Ovaries: (↑) abs wt (63.8%, ns, ndr) and rel-to-body wt (81.8%, ns, ndr). ▪ Thymus: (↑) abs wt in ♂ (20.7%, ns) and in ♀ (1.4%, ns, ndr); (↑) rel-to-body wt in ♂ (12.2%, ns) and in ♀ (7.9%, ns). ▪ Thyroid: (↓) abs wt in ♀ (7.8%, ns, ndr). ▪ Spleen: (↑) abs wt in ♂ (83.1%, ns, ndr) and in ♀ (28.5%, ns, ndr); (↑) rel-to-body wt in ♂ (72.6%, ns, ndr) and in ♀ (38.2%, ns, ndr). ▪ Liver: (↓) abs wt in ♀ (13.4%). ▪ Pituitary: (↑) rel-to-body wt in ♀ (12.5%, ns). Histopathological findings: ▪ (↑) Spleen congestion grade 3 (2/2 *vs* 1/4 in control, ndr). **2 mg/kg bw/day** Clinical chemistry: ▪ (↓) ALP in ♂ at pre-test (22.3%, ns, ndr), at week 6 (20.9%, ns, ndr) and at week 13 (18%, ns, ndr). ▪ (↑) GLDH in ♀ at week 13 (12%, ns). Urinalysis: ▪ (↓) Volume in ♂ at week 6 (29.7%, ns) and (↑) at week 13 (68%, ns, ndr) and (↓) in ♀ at week 6 (16.7%, ns, ndr). Organs´ weight: ▪ Kidneys: (↑) abs wt in ♀ (11.6%, ns, ndr) and rel-to-body wt in ♀ (17%, ns, ndr). ▪ Prostate: (↓) abs wt (43.3%, ns, ndr) and rel-to-body wt (45%, ns). ▪ Epididymides: (↑) abs wt (30.4%, ns, ndr) and rel-to-body wt (28.6%, ns, ndr). ▪ Uterus: (↑) abs wt (44.3%, ns, ndr) and rel-to-body wt (48.4%, ns, ndr).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		▪ Ovaries: (↑) abs wt (37.5%, ns, ndr) and rel-to-body wt (45.5%, ns, ndr). ▪ Thymus: (↓) abs wt in ♀ (20.3%, ns, ndr) and rel-to-body wt in ♀ (17.8%, ns). ▪ Thyroid: (↑) abs wt in ♀ (17.6%, ns, ndr) and rel-to-body wt in ♀ (28.6%, ns, ndr) ▪ Spleen: (↑) abs wt in ♂ (18.4%, ns, ndr) and in ♀ (68.3%, ns, ndr); (↑) rel-to-body wt in ♂ (15.1%, ns, ndr) and in ♀ (85.5%, ns, ndr). ▪ Adrenals: (↑) rel-to-body wt in ♂ (14.3%, ns, ndr) and in ♀ (13.3%, ns, ndr). Histopathological findings: ▪ (↑) Spleen congestion grade 3 (1/2 *vs* 1/4 in control, ndr). **NOAEL**: **10 mg/kg bw/day.** **LOAEL**: **20 mg/kg bw/day,** based on reduction in bw in ♀, reduction in the bw gain in ♂/♀ and decrease in food consumption in ♂/♀.	
52-week oral (capsules) study in dogs. Guideline: EPA FIFRA Guideline OPP 83-1. GLP: Yes Dog strain: Beagle. No. animals 4 dogs/sex/dose Deviations from OECD TG 452 (2018): - Unknown pre-treatment bw per group. - Origin of HCD not detailed. - Prothrombin time and activated partial thromboplastin time not measured. - Only one hepatobiliary test used. - Adrenals, heart, spleen, thyroid and uterus not weighed. - Coagulating gland and lacrimal gland not	Dodine (batch no. 1174 and purity of 98.6%). Doses: 0, 2, 10 and 20 mg/kg bw/day for 52-weeks (in gelatine capsules).	**Mortality**: No deaths reported. **20 mg/kg bw/day** Clinical signs *(no statistical analysis performed):* ▪ (↑) Emesis prior to dosing in ♀ (4/4 *vs* 0/4 in control) and immediately after dosing (2/4 *vs* 0/4 in control). ▪ (↑) Diarrhoea in ♀ (4/4 *vs* 0/4 in control). ▪ (↑) Salivation from all grades of severity, prior and after dosing in ♂/♀. Bodyweight and food consumption ▪ (↑) bw loss in ♂ at week 52 and in ♀ week 44. ▪ (↓) bw gain between weeks 1-52 in ♂ (52%, ns) and in ♀ (8.3%, ns, ndr). ▪ Food consumption between weeks 1-52 (↑) in ♂ (5.6%). ▪ Supplemental feeding in ♂/♀ (1/4 *vs* 0/4 in control for ♂/♀). Haematology: ▪ (↑) Platelet in ♂ at week 52 (19%, ns). ▪ (↓) WBC in ♂ at week 52 (21.4%, ns); (↑) WBC in ♀ at week 26 (113.6%, ns) and at week 52 (54.2%). ▪ (↓) Segmented neutrophils in ♂ at week 26 (13.1%, ns, ndr) and at week 52 (15.9%, ns, ndr); (↑) segmented neutrophils in ♀ at week 26 (77.2%) and at week 52 (56.6%). ▪ (↑) Lymphocytes in ♂ at week 26 (3%, ns, ndr) and (↓) at week 52 (25%, ns, ndr); (↑) lymphocytes in ♀	[redacted] (1996) [redacted] (2008) **B.6.3.3.1 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
histopathologically examined. **Study acceptable** *Guideline value for classification: STOT RE 2 ≤ 25 mg/kg bw/day STOT RE 1 ≤ 2.5 mg/kg bw/day*		at week 26 (19.4%, ns, ndr) and at week 52 (34.8%, ns, ndr). ▪ (↓) Eosinophils in ♂ at week 26 (33.3%, ns) and at week 52 (66.7%, ns) and in ♀ at week 26 (25%, ns); (↑) eosinophils in ♀ at week 52 (300%). <u>Clinical chemistry:</u> ▪ (↓) Glucose in ♀ at week 26 (11.8%, ns) and at week 52 (1%, ns, ndr). ▪ (↑) Urea in ♂ at week 26 (28.6%, ns) and at week 52 (14.3%, ns); (↓) urea in ♀ at week 26 (5.6%, ns, ndr) and at week 52 (21.1%, ns, ndr). ▪ (↓) Creatinine in ♀ at week 26 (20%, ns). ▪ (↓) Total cholesterol in ♀ at week 26 (28.2%, ns, ndr) and at week 52 (32.9%, ns, ndr). ▪ (↑) AST in ♂ at week 26 (65.2%) and at week 52 (5.9%, ns, ndr) and in ♀ at week 52 (20.7%, ns). ▪ (↑) ALT in ♂ at week 26 (91.2%, ns) and at week 52 (89.7%, ns); (↓) ALT in ♂ at week 26 (30.3%, ns) and at week 52 (11.4%, ns, ndr). ▪ (↓) Globulin in ♂ at week 26 (15.6%, ns) and at week 52 (12.9%, ns) and in ♀ at week 26 (7.1%, ns, ndr) and at week 52 (17.9%, ns). ▪ (↑) A/G ratio in ♂ at week 26 (10.4%, ns, ndr) and in ♀ at week 26 (7%, ns, ndr) and at week 52 (30.3%, ns). <u>Organs´ weight:</u> ▪ Liver: (↑) abs wt in ♀ (13.8%, ns) and rel-to-body wt (16%, ns). ▪ Ovaries: (↑) abs wt (45.9%, ns), rel-to-body wt (53.8%, ns) and rel-to-brain wt (60%, ns). ▪ Testes/epididymides: (↑) abs wt (21.6%, ns) and rel-to-body wt (16.7%, ns, ndr). <u>Gross pathology:</u> ▪ (↑) Enlarged ovaries (3/4 *vs* 1/4 in control). ▪ (↑) Thickened mammary gland in ♀ (2/4 *vs* 1/4 in control). <u>Histopathological findings:</u> ▪ (↑) Adrenal cortex vacuolization in ♂ (1/4 *vs* 0/4 in control). ▪ (↑) Liver vacuolization in ♂ (1/4 *vs* 0/4 in control). ▪ (↑) Mandibular salivary gland chronic inflammation in ♀ (1/4 *vs* 0/4 in control). ▪ (↑) Cysts in thymus in ♂ (4/4 *vs* 3/4 in control) and in ♀ (2/4 *vs* 1/4 in control). **10 mg/kg bw/day** <u>Clinical signs</u> *(no statistical analysis performed):* ▪ (↑) Emesis immediately after dosing in ♀ (2/4 *vs* 0/4 in control). ▪ (↑) Salivation slight and moderate in ♂/♀, prior to dosing in ♂/♀ and after dosing in ♀. <u>Bodyweight and food consumption</u> ▪ (↑) bw loss in ♂ at week 52. ▪ (↓) bw gain between weeks 1-52 in ♂ (44%, ns) and	

		in ♀ (16.7%, ns, ndr). ▪ Food consumption between weeks 1-52 (↑) in ♀ (6.3%, ns, ndr). ▪ Supplemental feeding in ♀ (1/4 *vs* 0/4 in control). <u>Haematology:</u> ▪ (↑) Platelet in ♂ at week 52 (15%, ns). ▪ (↑) WBC in ♀ at week 26 (17.7%, ns) and at week 52 (33.7%, ns). ▪ (↑) Segmented neutrophils in ♂ at week 26 (14.8%, ns, ndr) and at week 52 (4.8%, ns, ndr) and in ♀ at week 26 (21.1%, ns) and at week 52 (26.4%, ns). ▪ (↓) Lymphocytes in ♂ at week 26 (12.1%, ns, ndr) and at week 52 (12.5%, ns, ndr); (↑) lymphocytes in ♀ at week 26 (25.8%, ns, ndr) and at week 52 (65.2%, ns, ndr). ▪ (↓) Eosinophils at week 26 in ♂ (33.3%, ns) and in ♀ (25%, ns); (↑) eosinophils at week 52 in ♂ (33.3%, ns, ndr) and in ♀ (200%, ns). <u>Clinical chemistry:</u> ▪ (↓) Glucose in ♀ at week 52 (12%, ns, ndr). ▪ (↑) Urea in ♂ at week 52 (14.3%, ns): (↓) urea in ♀ at week 26 (16.7%, ns, ndr) and at week 52 (15.8%, ns, ndr). ▪ (↓) Creatinine in ♀ at week 26 (10%, ns). ▪ (↓) Total cholesterol in ♀ at week 26 (32.4%, ns, ndr) and at week 52 (37.6%, ns, ndr). ▪ (↑) AST in ♂ at week 26 (26.1%, ns) and at week 52 (41.2%, ns, ndr). ▪ (↑) ALT in ♂ at week 26 (32.4%, ns) and at week 52 (23.1%, ns); (↓) ALT in ♂ at week 26 (30.3%, ns) and at week 52 (17.1%, ns, ndr). ▪ (↓) Globulin in ♂ at week 26 (12.5%, ns) and in ♀ at week 26 (7.1%, ns, ndr) and at week 52 (7.1%, ns, ndr). ▪ (↑) A/G ratio in ♂ at week 26 (7.8%, ns, ndr) and in ♀ at week 26 (10.1%, ns, ndr) and at week 52 (6.1%, ns, ndr). <u>Organs´ weight:</u> ▪ Liver: (↑) abs wt in ♀ (10.8%, ns) and rel-to-body wt (16%, ns). ▪ Testes/epididymides: (↑) abs wt (21.6%, ns) and rel-to-body wt (33.3%, ns, ndr). <u>Gross pathology:</u> ▪ (↑) Thickened mammary gland in ♀ (2/4 *vs* 1/4 in control). <u>Histopathological findings:</u> ▪ (↑) Adrenal cortex vacuolization in ♂ (1/4 *vs* 0/4 in control). **2 mg/kg bw/day** <u>Bodyweight and food consumption</u> ▪ (↓) bw gain between weeks 1-52 in ♂ (32%, ns) and in ♀ (33.3%, ns, ndr). ▪ Food consumption between weeks 1-52 (↓) in ♀ (6.3%, ndr). <u>Haematology:</u> ▪ (↑) Segmented neutrophils in ♂ at week 52 (22.2%, ns, ndr) and in ♀ at week 52 (13.2%, ns). ▪ (↑) Lymphocytes in ♂ at week 26 (15.2%, ns, ndr) and in ♀ at week 52 (25.8%, ns, ndr) and at week 52 (17.4%, ns, ndr): (↓) lymphocytes in ♂ at week 52 (40%, ns, ndr).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
		▪ (↓) Eosinophils at week 26 in ♂ (33.3%, ns) and in ♀ (25%, ns); (↑) eosinophils in ♀ (100%, ns). <u>Clinical chemistry:</u> ▪ (↓) Urea in ♂ at week 26 (14.3%, ns, ndr) and in ♀ at week 26 (16.7%, ns, ndr) and at week 52 (31.6%, ns, ndr). ▪ (↓) Creatinine in ♀ at week 26 (10%, ns). ▪ (↓) Total cholesterol in ♀ at week 26 (21.6%, ns, ndr) and at week 52 (27%, ns, ndr). ▪ (↑) AST in ♂ at week 26 (26.1%, ns). ▪ (↑) ALT in ♂ at week 26 (11.8%, ns); (↓) ALT in ♂ at week 26 (21.2%, ns, ndr) and at week 52 (20%, ns, ndr). ▪ (↓) Globulin in ♀ at week 26 (17.9%, ns, ndr) and at week 52 (10.7%, ns, ndr). ▪ (↑) A/G ratio in ♂ at week 26 (14.8%, ns, ndr) and in ♀ at week 26 (31%, ns, ndr) and at week 52 (17.4%, ns, ndr). <u>Organs´ weight:</u> ▪ Testes/epididymides: (↑) rel-to-body wt (11.1%, ns, ndr). <u>Gross pathology:</u> ▪ (↑) Enlarged ovaries (2/4 *vs* 1/4 in control). ▪ (↑) Thickened mammary gland in ♀ (3/4 *vs* 1/4 in control). **NOAEL**: **2 mg/kg bw/day.** **LOAEL**: **10 mg/kg bw/day,** based on supplemental feeding required by one ♀.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
1-year oral (diet) study in dogs. Guideline: Not stated. GLP: No Dog breed: Beagle. No. animals: 2/sex/dose. Deviations from OECD TG 452 (2018): - Test substance not characterised. - Data not reported. - Only 2 dogs/sex/dose. - Measured parameters not fully described. **Study acceptable as supportive information.**	Dodine (unknown batch no. and purity of 97%). Doses: 0, 50, 200 and 800 ppm.	**800 ppm** Bodyweight *(data not shown):* ▪ (↓) bw gain. Organs´ weight (♂/♀ pooled together): ▪ Thyroid: (↑) abs wt (25%, ns, ndr) and rel-to-body wt (35%). Gross pathology *(data not shown):* ▪ Darker thyroids in ♂. Histopathological findings *(data not shown):* ▪ Thyroid: 2 ♂ with stimulation (cuboidal and low columnar follicular epithelium, vascularity). 2 ♀ (cuboidal follicular epithelium, vascularity). **200 ppm** Bodyweight *(data not shown):* ▪ (↓) bw gain. Organs´ weight (♂/♀ pooled together): ▪ Thyroid: (↑) abs wt (39%, ns, ndr) and rel-to-body wt (35%). Histopathological findings *(data not shown):* ▪ Thyroid: 1 dog (cuboidal follicular epithelium, vascularity). **50 ppm** Bodyweight *(data not shown):* ▪ (↓) bw gain. Organs´ weight (♂/♀ pooled together): ▪ Thyroid: (↑) abs wt (44%, ndr) and rel-to-body wt (33%, ns). **NOAEL** not derived.	Levinskas, G.J., *et al* (1961) **B.6.3.3.2 (AS)**
Rabbit toxicity studies			
Dose range-finding developmental toxicity study in rabbits. GLP: Yes Method: In house method Rabbit strain: New Zealand White. 10 females/dose Deviations from current OECD TG 414, 2018: -At least 20 females with implantation sites at necropsy should be used. -Test chemical not administered to the day prior to	Dodine, Lot/Batch No.:APA 92/88/2; Purity: 95% Dodine:Oral (gavage) Doses: 0, 70, 100 mg/kg bw/day from day 6 to 18 of pregnancy both included Parameters observed: *Maternal data:* Clinical signs, mortality, bw and bwg food consumption, necropsy, histopathology *Reproductive data:*	*Only effects relevant for STOT RE are presented (see also section 2.6.6)* ***Maternal toxicity*** Mortality: 5 ♀ at 100 mg/kg bw/day and 1 ♀ at 70 mg/kg bw/day humanely killed due to morbidity signs. **100 mg/kg bw/day** Clinical signs: -1 ♀ found dead showed red staining around lower abdomen. -1 ♀ showed right eye swollen throughout GD 8-17 (humanely killed). -1 ♀ with darker irises through GD 10-12 (humanely killed). -1 ♀ showed fur wet under chin after dosing at GD 10. 2 h after dosing showed breathing difficulties and noisy slightly cyanosed, subdued (humanely killed). -1 ♀ showing few or no faeces through GD 10-17 (humanely killed). Hyperplasia of stomach mucosa, gaseous distension of caecum with softened/liquid contents and reduced faecal output found in these dead animals. Liquid faeces in 2 survival dams. Bodyweight: ▪ (↓) bw gain between days 6-19 (48%).	[redacted] (1989a) **B.6.6.2.3 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
scheduled caesarean section. -Mating index not shown. -Following developmental endpoints not measured: sex ratio and indication of incomplete testicular descent/cryptorchidism in male foetuses. -Thyroid weight and thyroid hormones not recorded. -Foetal alterations not reported. -Statistical analysis not performed in most of the tested parameters. **Supportive information**	No. of corpora lutea, no. implants, uterus wt, litter wt. *Foetal data:* Foetus wt, deaths.	Food consumption (*statistical analysis not performed*): ▪ (↓) between days 6-18 (51%). Necropsy (*statistical analysis not performed*) ▪ (↑) Liquid contents caecum/gaseous distension (50% vs 0% in controls). Histopathology (*statistical analysis not performed*) *Stomach* ▪ (↑) Cream coloured parches on mucosa. Pyloric part covered in colourness viscous fluid in stomach (30% *vs* 0% in controls). ▪ (↑) Dark point foci. Blood and sloughing of mucosa. Hyperplasia of fundic epithelium (20% *vs* 0% in controls). *Liver* ▪ (↑) Lobulation prominent. Mild chronic inflammation (periportal). Hepatocytes necrosis (20% vs 0% in controls). *Kidney* ▪ (↑) Red foci/chronic inflammation (20% *vs* 0% in controls). **70 mg/kg bw/day** Clinical signs: -1 ♀ showed few or no faeces through GD 8-17 (humanely killed). Hyperplasia in stomach mucosa Necropsy (*statistical analysis not performed*) ▪ (↑) Liquid contents caecum/gaseous distension (10% vs 0% in controls). Histopathology (*statistical analysis not performed*) *Stomach* ▪ (↑) Cream coloured parches on mucosa. Pyloric part covered in colourness viscous fluid in stomach (10% *vs* 0% in controls). **NOAEL** $_{\text{developmental}}$: 70 mg/kg bw/day based on the increase of late resorptions seen at 100 mg/kg bw/day. **NOAEL** $_{\text{maternal toxicity}}$: 70 mg/kg bw/day based on decreased bw gain and food consumption, necropsy and histopathological findings in stomach, kidney and liver.	
Developmental toxicity study in rabbits. GLP: Yes Method: US EPA FIFRA 83-3 Rabbit strain: New Zealand White. 16/20 females/dose Deviations from current OECD TG 414, 2018: -Test chemical not administered to the day prior to scheduled caesarean section.	Dodine Lot/Batch No.:APA 92/88/2; Purity: 95% Oral (gavage) Doses: 0, 10, 40 and 80 mg/kg bw/day from day 6 to 18 of pregnancy both included Parameters observed: *Maternal data:* Clinical signs, mortality, bw and	*Only effects relevant for STOT RE are presented (see also section 2.6.5)* ***Maternal toxicity*** Mortality: At 80 mg/kg bw/day: 1 ♀ died at GD15 after showing breathing difficulties, 1 ♀ humanely killed at GD11 after showing same clinical signs and 1 ♀ killed because of poor condition. At 40 mg/kg bw/day, 1 ♀ found dead due to accidental damage during dosing. **80 mg/kg bw/day** Clinical signs: - Liquid faeces in 15% *vs* 6% in controls. - No faeces in 5% *vs* 0% in controls. - Blood in cage in 5% *vs* 0% in controls. - Breathing difficulties in 15% *vs* 0% in controls. - Emaciation in 15% *vs* 0% in controls. - Pale eyes in 10% *vs* 0% in controls. - Abortion in 10% *vs* 6% in controls. Food consumption (*statistical analysis from* [redacted]	[redacted] (1989b) [redacted] (2019b) **B.6.6.2.4 (AS)**

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL [Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]	Reference
-At least 20 females with implantation sites at necropsy should be used per dose. -Mating index not shown. - Incomplete testicular descent/cryptorchidism not measured in male foetuses. -Thyroid wt and thyroid hormones from dams not recorded. -Statistical analysis not performed in most of the parameters. -HCD not valid. **Acceptable**	bwg, food consumption, necropsy, histopathology. *Reproductive data:* No. of corpora lutea, no. implants, uterus wt, litter wt., sex ratio. *Foetal data:* Foetus wt, deaths.	*2019b*): ▪ (↓) through gestation days 6 (25%), 7 (30%) and 8 (30%). Necropsy (*statistical analysis not performed*) ▪ (↑) dark lung patches (20% *vs* 0% controls) ▪ (↑) intestine distension (10% *vs* 0% controls). **40 mg/kg bw/day** Clinical signs: - Blood in cage in 5% *vs* 0% in controls (ndr). - Breathing difficulties in 6% *vs* 0% in controls. Necropsy (statistical analysis not performed) ▪ (↑) dark lung patches (12.5% vs 0% controls) **NOAEL** developmental toxicity: 10 mg/kg bw/day based on increase of post implantation loss and late resorptions from 40 mg/kg bw/day. **NOAEL** maternal toxicity: 40 mg/kg bw/day based on morality, clinical signs and reduced food consumption at 100 mg/kg bw/day.	

Table 47: Summary table of human data on repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No human data on repeated dose toxicity STOT RE available				

Table 48: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No other studies relevant for STOT RE available				

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

The above-mentioned short-term studies were already considered in the DAR and have been re-assessed according to current guidelines. Long-term, multigeneration, developmental and immunotoxicity studies have also been included in this evaluation of effects relevant for STOT RE.

Studies in rats:

In the first 28-day oral study in rats (B.6.3.1.1, AS), performed by gavage administration of dodine, mortality was reported in all animals at 200 mg/kg bw/day, in 4/10 females at 100 mg/kg bw/day and in one female at 75 mg/kg bw/day, and respiratory problems, salivation and yellow staining of the fur were observed from 75 mg/kg bw/day. A decrease in the bodyweight was observed in males from 75 mg/kg bw/day and in females from 100 mg/kg bw/day. The bodyweight gain and food consumption were reduced in both sexes from 75 mg/kg bw/day. Regarding

haematology parameters, white blood cells and segmented neutrophils levels were increased and lymphocyte percentage was reduced in both sexes at 100 mg/kg bw/day. Red cell distribution width was affected, being increased in males at 100 mg/kg bw/day. An increment in alanine aminotransferase level was observed in both sexes from 75 mg/kg bw/day, while at 100 mg/kg bw/day the relative-to-body liver weight was incremented in females. The relative-to-body lung weight was increased in males at 100 mg/kg bw/day, in which also dark, depressed and pale areas were reported. The relative-to-body adrenal weight in males and the relative-to-body and relative-to-brain adrenals weights in females were increased at 100 mg/kg bw/day. Haemorrhage was seen in adrenals from rats at 200 mg/kg bw/day (examination not extended to animals of lower dose groups). The incidence of microscopical findings was increased in stomach from 75 mg/kg bw/day in both sexes.
In this study, a NOAEL was not derived, but a **LOAEL** of **75 mg/kg bw/day** was set based on the mortality in females, the increased incidence of several clinical signs in both sexes, the reduction of bodyweight in males, the reduction in bodyweight gain in both sexes, the decrease in food consumption in both sexes and the increase in alanine aminotransferase levels in both sexes, observed at this dose which was the lower tested.
Significant effects in haematology in both sexes at 100 mg/kg bw/day are within the range for STOT RE 2 category (>30 mg/kg bw/day and ≤ 300 mg/kg bw/day, applying Haber´s rule) but the pattern of adversity is not sufficient to trigger STOT RE classification.
Significant effects in clinical biochemistry related to liver in both sexes at 75 mg/kg bw/day and effects in liver in females at 100 mg/kg bw/day are within the range for STOT RE 2 category (>30 mg/kg bw/day and ≤ 300 mg/kg bw/day, applying Haber´s rule) but the pattern of adversity is not sufficient to trigger STOT RE classification.
Effects in lungs in males at 100 mg/kg bw/day are within the range for STOT RE 2 category (>30 mg/kg bw/day and ≤ 300 mg/kg bw/day, applying Haber´s rule) but the pattern of adversity is not sufficient to trigger STOT RE classification.
Effects in adrenals in both sexes at 100 mg/kg bw/day are within the range for STOT RE 2 category (>30 mg/kg bw/day and ≤ 300 mg/kg bw/day, applying Haber´s rule) but the pattern of adversity is not sufficient to trigger STOT RE classification.

In the second 28-day oral study in rats (B.6.3.1.2, AS), performed by administration of dodine in diet, no death was reported. A decrease in the bodyweight was observed in both sexes at 1000 ppm. The bodyweight gain was reduced in males treated from 750 ppm and in females from 500 ppm, and food consumption was reduced in both sexes from 750 ppm. A decrease in the alanine aminotransferase levels was observed in females treated at 1000 ppm of dodine. The absolute and relative-to-brain weights of kidneys were reduced in both sexes at 1000 ppm. At the same dose, the incidence of mineralization of the cortico-medullary junction in females and, only slightly, the incidence of fibrosis in kidneys in both sexes were incremented. An increase in the relative-to-body lung weight in females was revealed at 1000 ppm, in which also dark areas were reported.
In this study, a NOAEL was not derived, but a **LOAEL** of 500 ppm (equivalent to **47 mg/kg bw/day**) was set based on the reduction of the bodyweight gain in females observed from the lower dose tested.
Significant effects in clinical biochemistry related to liver in females at 1000 ppm (equivalent to 92 mg/kg bw/day) are within the range for STOT RE 2 category (>30 mg/kg bw/day and ≤ 300 mg/kg bw/day, applying Haber´s rule) but the pattern of adversity is not sufficient to trigger STOT RE classification.
Effects in kidneys in both sexes at 1000 ppm (equivalent to 87 and 92 mg/kg bw/day in males and females, respectively) are within the range for STOT RE 2 category (>30 mg/kg bw/day and ≤ 300 mg/kg bw/day, applying Haber´s rule) but the pattern of adversity is not sufficient to trigger STOT RE classification.
Effects in lungs in females at 1000 ppm (equivalent to 92 mg/kg bw/day) are within the range for STOT RE 2 category (>30 mg/kg bw/day and ≤ 300 mg/kg bw/day, applying Haber´s rule) but the pattern of adversity is not sufficient to trigger STOT RE classification.

In the third 28-day oral study in rats (B.6.3.1.3, AS), performed by administration of dodine with diet, the bodyweight gain was reduced in both sexes at 800 ppm and the food consumption was reduced in males at the same dose. Moreover, decreases in the absolute and relative-to-body liver weights in females were observed at 800 ppm.
The **NOAEL** was set at 200 ppm (equivalent to **17.66 mg/kg bw/day**), based on the reduction of the bodyweight gain in both sexes, the reduction of food consumption in males and the decrease in the absolute and relative-to-body liver weight in females observed at 800 ppm.
Effects in liver in females at 800 ppm (equivalent to 76.71 mg/kg bw/day) are within the range for STOT RE 2 category (>30 mg/kg bw/day and ≤ 300 mg/kg bw/day, applying Haber´s rule) but the pattern of adversity is not sufficient to trigger STOT RE classification.

In a 7-day and 28-day, gut motility oral toxicity study in rats (B.6.3.1.4, AS), dodine administered in diet caused a reduction in the bodyweight gain in both sexes at 800 ppm.
A NOAEL was not derived. No effects relevant for STOT RE were found in this study.

In a 90-day oral dietary toxicity study in rats (B.6.3.2.1., AS), dodine administration in diet caused a reduction in the bodyweight gain in both sexes and a decrease in the food consumption in females at 800 ppm. Also at 800 ppm,

an increase in the percentage of neutrophils in males and a decrease in the levels of alanine transaminase in females were observed.
The **NOAEL** of this study was set at 200 ppm (equivalent to **14.09 mg/kg bw/day**), based on decreased bodyweight gain in both sexes, the decrease in food consumption in females, increment in neutrophils in males and the decrease in alanine transaminase in females observed at 800 ppm.
Significant effects in haematology in males at 800 ppm (equivalent to 55.84 mg/kg bw/day) are within the range for STOT RE 2 category (>10 mg/kg bw/day and ≤ 100 mg/kg bw/day) but the pattern of adversity is not sufficient to trigger STOT RE classification.
Significant effects in clinical biochemistry related to liver in females at 800 ppm (equivalent to 60.44 mg/kg bw/day) are within the range for STOT RE 2 category (>10 mg/kg bw/day and ≤ 100 mg/kg bw/day) but the pattern of adversity is not sufficient to trigger STOT RE classification.

In a 90-day oral gavage toxicity study in rats (B.6.3.2.2., AS), dodine administration caused a reduction in the leukocyte count in females and increments of bilirubin and aspartate amino transferase levels in males at 20 mg/kg bw/day.
A **NOAEL** was not set for this study because of the absence of relevant information about the methodology and because most of the data were not shown. Also for these reasons, none of the effects reported were considered relevant for the decision on STOT RE classification.

In a 100-day oral dietary toxicity study in rats (B.6.3.2.3., AS), dodine administration caused a reduction in bodyweight gain and food consumption in males at 270 mg/kg bw/day and in females at 310 mg/kg bw/day.
A **NOAEL** was not set for this study because of the absence of relevant information about the methodology and because most of the data were not shown. For these reasons also, none of the effects reported were considered relevant for the decision on STOT RE classification.

In a 28-day dermal study in rats (B.6.3.4.1.1, AS), treatment of rats with dodine caused dermal irritation on application site from 50 mg/kg bw/day. Effects on treated skin were also evident during gross pathology and histopathology examinations from 125 mg/kg bw/day. Bodyweight gain was decreased in males from 125 mg/kg bw/day.
Systemic NOAEL for this dermal study was set at 50 mg/kg bw/day dose (equivalent to **35.7 mg/kg bw/day**, considering the 5-day per week administration), based on the decreased bodyweight gain in males from 125 mg/kg bw/day. A NOAEL for local effects could not be derived and a **LOAEL for skin local effects** was set at 50 mg/kg bw/day dose (equivalent to **35.7 mg/kg bw/day**, considering the 5-day per week administration), based on dermal irritation findings found on application sites in both sexes.
In conclusion, there were not effects relevant for STOT RE in this study.

A 21-day dermal study in rats (B.6.3.4.1.2, AS) was provided. This study was not considered acceptable, because it did not test dodine and furthermore, the substance tested was reported to be CT-334-87 (1-dodecylguanidinium hydrochloride) and it was not adequately characterised.

In the 2-year oral study in rats (B.6.5.1, AS), the NOAEL for toxicity was considered to be 400 ppm (equivalent to 20.34 and 26.5 mg/kg bw/day for males and females, respectively), based on clinical signs in males, decreased bodyweight in both sexes and food consumption in both sexes at 800 ppm (equivalent to **41.93 and 53.5 mg/kg bw/day** for males and females, respectively). A decrease in white blood cells and in lymphocytes was observed in males at 41.9 mg/kg bw/day. Effects were clearly above the cut-off value for STOT RE 2 after a 106-week period (≤ 12.3 mg/kg bw/day).

A second 2-year oral study in rats (B.6.5.3, AS), was available, but in this study the level of reporting was very limited. A NOAEL was not set.

In a two-generation study in rats (B.6.6.1.1, AS), parental NOAEL was set at 200 ppm (equivalent to 13.14 and 15.6 mg/kg bw/day for males and females, respectively) based on decreased bodyweights and increased relative adrenal weight in F_1 parents observed at 400 ppm (equivalent to **26.2 and 31.2 mg/kg bw/day** for males and females, respectively). Developmental NOAEL was derived at 200 ppm (equivalent to 13.14/15.6 mg/kg bw/day for males and females, respectively) based on decreased pup weights in F1 and F2 generations observed at 400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for males and females, respectively).
Relative left adrenal weight was increased in F1 parental females from 400 ppm. Absolute left kidney weight was decreased in F2 male pups from 400 ppm. In F2 generation at 800 ppm, the absolute spleen weight was reduced at 800 ppm in male and female pups and the absolute thymus weight was decreased in female pups.
None of these effects were considered enough for STOT RE 2 classification.

In a dose range finding developmental study in rats (B.6.6.2.1, AS), the maternal toxicity NOAEL was 50 mg/kg bw/day based on decreased bodyweight gain and food consumption observed at **100 mg/kg bw/day**, and developmental toxicity NOAEL was set at 100 mg/kg bw /day based on the absence of effects. One dam died at 100 mg/kg bw/day. An increase in the kidney incidences (pelvic dilatation and enlarged; 30%) and ureters (dilatation; 20%) was observed at 100 mg/kg bw/day. Moreover, one dam at 100 mg/kg bw/day exhibited alterations consistent with a long-standing partial obstruction in the lower urinary tract (epithelial hyperplasia and chronic inflammation of the urinary bladder; ureters inflammation; pelvic dilatation and inflammation, together with nephritis in the kidney; and hyperplasia of the lumbar lymph node). These findings could have been caused by a small calculus in the bladder or urethra, which might have been voided some time before death. None of the effects observed were considered enough for STOT RE 2 classification.

In a developmental study in rats (B.6.6.2.2, AS), the maternal toxicity NOAEL was 10 mg/kg bw/day based on decreased bodyweight gain and food consumption observed at **45 mg/kg bw/day**, and developmental toxicity NOAEL was set at 90 mg/kg bw /day based on the absence of effects reported. None of the effects observed were considered enough for STOT RE 2 classification.

In the immunotoxicity study in rats (B.6.8.2.1, AS), the administration of dodine in the diet at 0, 200, 500 and 1000 ppm (equivalent to 0, 18, 44, 83 mg/kg bw/day) for 28 days to females immunized with SRBC, did not produce adverse effects in survival, clinical signs, haematology, immunological examinations (anti-SRBC IgM titers), absolute and relative spleen and thymus weights and macroscopic examinations. However, decreases in bodyweight, bodyweight gain and food consumption were seen at 1000 ppm and based on these effects the NOAEL for general toxicity was set at 500 ppm (equivalent to 44 mg/kg bw/day). None of the effects reported were considered relevant for the decision on STOT RE classification.

Studies in mice:

In the 8-week oral (diet) study in mice (B.6.3.1.5, AS), mortality was reported in 1/5 female, one day after increasing the dose level from 100 to 1250 ppm. Decreases in the bodyweight of females and in the bodyweight gain of animals from both sexes were seen at 100/1250 ppm dose groups. The absolute spleen weight was reduced in the 100/1250 ppm female group. Moreover, all the males and most of the females at 100/1250 ppm had mild eosinophilia of the liver after treatment.

The **NOAEL** of this study has been set at 625 ppm (equivalent to **109.4 mg/kg bw/day**), based on the reduction of the bodyweight in females, the reduction in the bodyweight gain in both sexes, the decrease in the absolute weight of spleen in females and the increment of eosinophilia in the liver in both sexes, observed at the 100/1250 ppm dose group.

No effects from this study can be employed for STOT RE classification, as such effects were observed only at the 100/1250 ppm dose groups, in which two different doses were tested and therefore no clear dose-response relationships can be extracted.

In a 90-day oral dietary toxicity study in mice (B.6.3.2.4, AS), mortality was reported in females at 2500 ppm during the first 2 weeks of dodine treatment. Stiffening of the tail occurred also in females at 2500 ppm. A reduction in the bodyweight was seen in males at 2500 ppm and a reduction of bodyweight gain was observed in males from 1250 ppm and in females at 2500 ppm. Food consumption was reduced in both sexes from 1250 ppm. In males at 2500 ppm, increments in the percentage of neutrophils and in red blood cells width were observed. Also at 2500 ppm, increments were reported in blood urea nitrogen in both sexes, phosphorus levels in males and A/G ratio in females. An increment in the relative-to-body liver weight was observed in both sexes at 2500 ppm. The absolute spleen weights were reduced in both sexes from 1250 ppm and the relative-to-body spleen weight in females was decreased from 1250 ppm. An increase in the incidence of splenic lymphoid atrophy was observed in females at 2500 ppm (examination of spleen not extended to animals of lower dose groups). The incidences of lymphoid necrosis and atrophy in thymus were increased in females at 2500 ppm (examination not extended to animals of lower dose groups).

The **NOAEL** of this study was set at 600 ppm (**equivalent to 94 mg/kg bw/day**), based on the decreased bodyweight gain in males, the decrement in food consumption in both sexes and the decreases in the absolute and relative-to-body spleen weights in both sexes observed from 1250 ppm (equivalent to 181 and 223 mg/kg bw/day for males and females, respectively).

Significant effects in haematology in males at 2500 ppm (equivalent to 350 mg/kg bw/day) are out of the range for STOT RE 2 category (>10 mg/kg bw/day and ≤ 100 mg/kg bw/day).

Significant effects in clinical biochemistry in both sexes at 2500 ppm (equivalent to 350 and 305 mg/kg bw/day in males and females, respectively) are out of the range for STOT RE 2 category (>10 mg/kg bw/day and ≤ 100 mg/kg bw/day).

Effects in liver in both sexes at 2500 ppm (equivalent to 350 and 305 mg/kg bw/day in males and females,

respectively) are out of the range for STOT RE 2 category (>10 mg/kg bw/day and ≤ 100 mg/kg bw/day).
Effects in spleen in both sexes at 1250 ppm (equivalent to 181 and 116 mg/kg bw/day in males and females, respectively) are out of the range for STOT RE 2 category (>10 mg/kg bw/day and ≤ 100 mg/kg bw/day).
Effects in thymus in females at 2500 ppm (equivalent to 305 mg/kg bw/day) are out of the range for STOT RE 2 category (>10 mg/kg bw/day and ≤ 100 mg/kg bw/day).

In a 78-week oral study in mice (B.6.5.2, AS), NOAEL for toxicity was considered to be 200 ppm (equivalent to 29.2 and 38.3 mg/kg bw/day for males and females, respectively), based on clinical signs in males, decreased bodyweight in both sexes and food consumption in both sexes at 750 ppm (equivalent to 109.8 and 136.2 mg/kg bw/day for males and females, respectively). Effects were clearly above the cut-off value for STOT RE 2 after a 78-week period (≤ 16.7 mg/kg bw/day).

Studies in dogs:

In the 6-week oral range-finding toxicity study in Beagle dogs (B.6.3.1.6, AS), the administration of dodine in capsules caused toxicity in animals treated at 25 mg/kg bw/day for 6 weeks. Conclusions can hardly be extracted from lower doses, as they were only applied for short times. Adverse effects as increases of salivation, emesis, liquid faeces and bodyweight loss and decrease in food consumption were reported in treated dogs. The substance also seemed to have a direct impact in the amount of undigested food found in the stomach.
No effects relevant for STOT RE were found in this study and a NOAEL was not derived due to the high complexity of doses and exposure times employed and the absence of negative controls.

In the 90-day oral toxicity study in Beagle dogs (B.6.3.2.5, AS), it was shown that the administration of dodine in capsules reduced the bodyweight in females and reduced the bodyweight gain in both sexes at 20 mg/kg bw/day. The food consumption was reduced in male and female dogs also at 20 mg/kg bw/day.
The **NOAEL** of this study was set at **10 mg/kg bw/day**, based on decreased bodyweight in females and bodyweight gain in both sexes and the decrease in food consumption in both sexes observed at 20 mg/kg bw/day.
In conclusion, there were no effects relevant for STOT RE in this study.

In the 52-week oral toxicity study in Beagle dogs (B.6.3.3.1, AS), the supplemental feeding to three dogs (a female at 10 mg/kg bw/day, a male at 20 mg/kg bw/day and a female at 20 mg/kg bw/day) was needed after the exhibition of notably marked bodyweight losses, starting during the first few weeks of dodine administration in capsules. White blood cells, segmented neutrophils and eosinophils counts were increased in females at 10 mg/kg bw/day.
The **NOAEL** of this study was set at **2 mg/kg bw/day**, based on the supplemental feeding required in one female and increased WBC count, segmented neutrophils and eosinophils in females at 10 mg/kg bw/day.
Significant effects in haematology in females at 10 mg/kg bw/day are out of the range for STOT RE 2 category (>2.5 mg/kg bw/day and ≤ 25 mg/kg bw/day, applying Haber´s rule).

In a 1-year oral dietary toxicity study in Beagle dogs (B.6.3.3.2, AS), dodine administration caused dose-response reductions in bodyweight gains, thyroid weight increments and increases in the incidence of findings in thyroid glands from 50 ppm.
A **NOAEL** was not set for this study because of the absence of relevant information about the methodology and because most of the data were not shown. For these reasons also, none of the effects reported were considered relevant for the decision on STOT RE classification.

Studies in rabbits:

In a dose-range study for a developmental study in rabbits (B.6.6.2.3, AS), the maternal toxicity NOAEL was 70 mg/kg bw/day based on decreased bodyweight gain, decreased food consumption, the increased findings in necropsy (liquid contents in caecum and gaseous distension) and increased incidence in histopathological findings in stomach, kidney and liver observed at **100 mg/kg bw/day**. Five dams at 100 mg/kg bw/day and one dam at 70 mg/kg bw/day were humanely killed due to morbidity signs. At 100 mg/kg bw/day, there was an increase in the incidence of dams with liquid contents and gaseous distension in caecum and histoathological findings in stomach, liver and kidney. None of the effects observed were considered enough for STOT RE 2 classification.

In a developmental study in rabbits (B.6.6.2.4, AS), the maternal toxicity NOAEL was 40 mg/kg bw/day based on increased mortality and clinical signs and reduced food consumption at 80 mg/kg bw/day. At 80 mg/kg bw/day one dam ♀ died at GD15 after showing breathing difficulties, one dam was humanely killed at GD11 after showing same clinical signs and one dam was killed because of poor condition. The incidence of dark patches in lung lobes was increased in dams at 40 mg/kg bw/day. None of the effects observed were considered enough for STOT RE 2 classification.

Table 49: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days [if adequate, otherwise please delete]

Study reference	Effective dose (mg/kg/day)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
[redacted] (1994a) **B.6.3.1.1 (AS)**	- 75 (mortality, ♀) - 100 (haematology, ♂/♀) - 75 (liver, ♂/♀) - 100 (lungs, ♂) - 100 (adrenals, ♂/♀)	28 days	- 23 (mortality, ♀) - 31 (haematology, ♂/♀) - 23 (liver, ♂/♀) - 31 (lungs, ♂) - 31 (adrenals, ♂/♀)	STOT RE 2
[redacted] (1994b) **B.6.3.1.2 (AS)**	- 92 (liver, ♀) - 87/92 (kidneys, ♂/♀) - 92 (lungs, ♀)	28 days	- 29 (liver, ♀) - 27/29 (kidneys, ♂/♀) - 29 (lungs, ♀)	No classification[1]
[redacted] (1997) **B.6.3.1.3 (AS)**	- 76.7 (liver, ♀)	28 days	- 24 (liver, ♀)	No classification[1]
[redacted] (1996) **B.6.3.1.4 (AS)**	No effect	7 and 28 days	-	No classification[1]
[redacted] (1998) **B.6.5.1 (AS)**	No effect	106 weeks	No effect	No classification[1]
[redacted] (1996) **B.6.6.1.1 (AS)**	- 31.2 (adrenals, F1 mothers) - 26.2 (kidney, F2 ♂ pups) - 52.6/60.3 (spleen, F2 ♂/♀ pups) - 60.3 (thymus, F2 ♀ pups)	10 weeks	- 24 (adrenals, ♀) - 20 (kidney, F2 ♂ pups) - 40/46 (spleen, F2 ♂/♀ pups) - 46 (thymus F2, ♀ pups)	No classification[1]
[redacted] (1989a) **B.6.6.2.1 (AS)**	- 100 (mortality, dams) - 100 (kidney, dams)	11 days	- 12 (mortality, dams) - 12 (kidney, dams)	STOT RE 2
[redacted] (1989b) **B.6.6.2.2 (AS)**	No effect	11 days	-	No classification[1]
[redacted] (2013) **B.6.8.2.1 (AS)**	No effect	28 days	-	No classification[1]
[redacted] (1999e) **B.6.3.3.1.1 (AS)**	No effect	28 days	-	No classification[1]
[redacted] *al* (1988) **B.6.3.1.5 (AS)**	No effect	8 weeks	-	No classification[1]
[redacted] (1998a) **B.6.5.2 (AS)**	No effect	78 weeks	No effect	No classification[1]
[redacted] (1996) **B.6.3.3.1 (AS)**	- 10 (supplemental feeding to preclude mortality) - 10 (haematology, ♀)	52 weeks	- 40 (supplemental feeding to preclude mortality) - 40 (haematology, ♀)	STOT RE 2
[redacted] (1989a) **B.6.6.2.3 (AS)**	- 70 (mortality, dams) - 100 (stomach, dams) - 100 (liver, dams) - 100 (kidney, dams)	13 days	- 10 (mortality, dams) - 14 (stomach, dams) - 14 (liver, dams) - 14 (kidney, dams)	STOT RE 1
[redacted] (1989b) **B.6.6.2.4 (AS)**	- 80 (mortality, dams) - 40 (lungs, dams)	13 days	- 12 (mortality, dams) - 6 (lungs, dams)	STOT RE 2

[1] *See section 3.9.2.8 of CLP Regulation: effects considered not to support classification for STOT RE.*

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

A substance is classified with STOT RE under CLP when it has produced or has been shown to have the potential to produce significant toxicity in humans or be harmful to human health following repeated exposure by the oral, dermal or inhalation routes. This can be on the basis of human data or evidence from studies in animals that cause such effects at or below given guidance values (≤ 10 mg/kg bw/day or ≤ 100 mg/kg bw/day in a 90 day oral study in the rat). All significant health effects that can impair function, reversible or irreversible, immediate and/or delayed are included under this classification.

In the oral 90-day study in rats ([redacted], 1982), no clear evidence of relevant effects for STOT RE classification were reported. The main adverse effect found in this study was a decrease in the bodyweight gain in at 55.84 and 60.44 mg/kg bw/day, in males and females, respectively. This effect, although having toxicological importance, does not itself indicate “significant" toxicity.

As it can be observed in the table 49 above, several effects relevant for STOT RE classification were shown in toxicity studies of greater or lesser duration than 90 days. For most of these effects, a clear pattern across the dataset was not found, being an increase in mortality associated to dodine administration the only effect repeatedly found.

Mortality was reported in the 28-day gavage study in rats, where males died from 200 mg/kg bw/day and females from 75 mg/kg bw/day. These doses were between the threshold values established in Regulation (EC) No. 1272/2008 for classification of a substance as STOT RE 2 values for oral 28-day studies (>30 mg/kg bw/day and ≤300 mg/kg bw/day). Mortality was also reported in rats in the dose range-finding developmental toxicity study, in which one of the dams at 100 mg/kg bw/day (the highest dose tested) was killed due to morbidity signs (piloerection. hunched posture, red/brown staining around face, fore-paws and mild ataxia) at gestation day 16. Extrapolating to the equivalent effective dose from a 11-day study to a 90-day study, a dose of 12 mg/kg bw/day was calculated, being this value between the threshold value of established in Regulation (EC) No. 1272/2008 for classification of a substance as STOT RE 2 for oral 90-day studies (>10 mg/kg bw/day and ≤100 mg/kg bw/day). Mortality was not reported in the rest of studies performed in rats, but it should be highlighted that in none of this studies a dose equal or above 100 mg/kg bw/day was tested.

In dogs, no deaths were reported the 90-day or the 52-week studies performed. However, in the 52-week study it was stated that supplemental feeding was required to preclude mortality from 10 mg/kg bw/day. Extrapolating to the equivalent effective dose from a 52-week study to a 90-day study, a dose of 40 mg/kg bw/day was calculated, being this value between the threshold value established in Regulation (EC) No. 1272/2008 for classification of a substance as STOT RE 2 (>10 mg/kg bw/day and ≤100 mg/kg bw/day).

In the two studies available in rabbits, mortality was reported. In the dose range-finding developmental toxicity study, 5 of 10 dams died at 100 mg/kg bw/day and 1 of 10 dams was humanely killed due to morbidity signs at 70 mg/kg bw/day. Extrapolating to the equivalent effective dose from a 13-day study to a 90-day study, a dose of 10 mg/kg bw/day was calculated, being this value ≤10 mg/kg bw/day as established in Regulation (EC) No. 1272/2008 for classification of a substance as STOT RE 1. In the developmental toxicity study in rabbits, three females died at 80 mg/kg bw/ day (1 died, 2 were killed due to poor condition): one died at gestation day 15 after showing breathing difficulties, one humanely killed at gestation day 11 after showing same clinical signs and a third female was killed because of poor condition. At 40 mg/kg bw/day, one female was found dead, however it was due to accidental damage during dosing. Therefore, extrapolating to the equivalent effective dose from a 13-day study to a 90-day study, a dose of 12 mg/kg bw/day was calculated, being this value between the threshold value established in Regulation (EC) No. 1272/2008 for classification of a substance as STOT RE 2 (>10 mg/kg bw/day and ≤100 mg/kg bw/day).

Taking all together, in the opinion of the RMS, dodine should be classified as STOT RE 2 due to mortality arising after repeated oral exposure to relatively low doses and observed in several species.

According to Regulation (EC) No. 1272/2008, an attempt should be made to determine the primary target organ of toxicity. However, the nature of the effect, together with the data available, has not allowed for further details.

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

Dodine is proposed to be classified as **STOT RE 2**: **H373**: May cause damage to organs (undetermined) through prolonged or repeated oral exposure.

2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template]

Table 50: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Bacterial gene mutation (Ames test, plate incorporation) Similar to OECD TG 471. Some deviations from OECD TG 471 (2020). - A lack of TA 102 or *E. coli* WP2 strain, no individual plate counts reported, no independent assay, no HCD. GLP: yes **Supporting information**	**Dodine** (batch 51-24-3; purity 95%)	Test system: *S. typhimurium* TA 1535, TA 1537, TA1538, TA 98 and TA 100. S9 from rat liver induced with Aroclor 1254. Dosage (µg/plate): 0.06, 0.19, 0.56, 1.67 and 5.0 (±S9) Solvent: Methanol Dose selection: Cytotoxicity pre-test at a range of 1-10000 µg/plate.	Mutagenicity: Negative Cytotoxicity: No growth inhibition observed.	[redacted] (1981) **(AS)** **B.6.4.1.1-01**
Bacterial gene mutation (Ames test, plate incorporation) OECD TG 471 A deviation from OECD TG 471 (2020): Only 1 testing strain is used (*E.coli* WP2 uvrA). GLP: yes **Acceptable**	**Dodine** (batch S01L01; purity 98.5%)	Test system: *E. coli* WP2 uvrA S9 from rat liver induced with Aroclor 1254 Dosage (µg/plate): Experiment 1: (-S9) 0.1, 0.3, 1, 3, 10, 24, 33. (+S9) 0.3, 1, 3, 10, 33, 66, 100. Experiment 2: (-S9) 0.3, 1, 3, 10, 33, 66. (+S9) 1, 3, 10, 33, 100, 200. Solvent: Ethanol Dose selection: Cytotoxicity pre-test at a range of 10-5000 µg/plate	Mutagenicity: Negative Cytotoxicity: Experiment 1: At 24, 33 µg /plate (-S9) and at 66, 100 µg /plate (+S9) Experiment 2: At 33, 66 µg /plate (-S9) and at 100, 200 µg /plate (+S9) Precipitation: Not observed.	[redacted] (2003) **(AS)** **B.6.4.1.1-02**
Bacterial gene mutation, (Ames test, plate incorporation) OECD TG 471 A deviation from OECD TG 471 (2020): A lack of TA 102 or *E. coli* WP2 strain. GLP: yes **Acceptable**	**Dodine** (batch DCH0112; purity 97.1%)	Test system: *S. typhimurium* TA98, TA100, TA1535 and TA1537. S9 from rat liver induced with Aroclor 1254 Dosage (µg/plate): Experiment 1 (-S9) 0.1, 0.3, 1, 3, 10, 20. (+S9) 0.3, 1, 3, 10, 33, 66 Experiment 2 (-S9) 0.3, 1, 3, 10, 20, 40. (+S9) 0.3, 1, 3, 10, 33, 66 Experiment 3 (-S9) 1, 3, 10, 20, 40. (only TA1537) (+S9) 3, 10, 33, 100, 200. (only TA1537 and TA98) Solvent: Ethanol Dose selection: Cytotoxicity pre-test at a range of 0.03 to 5000 µg/plate.	Mutagenicity: Negative. Cytotoxicity: In all tester strains at the highest doses Precipitation: Not observed.	[redacted] (2005) **(AS)** **B.6.4.1.1-03**
Mammalian cells gene mutation (at HGPRT locus) Similar to OECD TG 476. Some deviations from OECD TG 476 (2016): The response of positive control (+S9) was lower than	**Dodine** (batch KG 8507; purity 98%)	Test system: Chinese hamster Ovary (CHO) S9 from rat liver induced with Aroclor 1254 Dosage (µg/mL): (- S9) 2.5, 5, 10, 15, 20; (+ S9) 5, 10, 15, 20, 25, 30, 35.	Mutagenicity: Negative. Cytotoxicity: Significant (initial) toxicity from 15 µg/mL (-S9) and from 30 µg/mL (+S9) based on	[redacted] (1985) **(AS)** **B.6.4.1.2-01**

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
normal, no HCD, the recommended cytotoxicity parameter (RS) was not used. GLP: yes **Supporting information**		Solvent: Ethanol Dose selection: Cytotoxicity pre-test at a range of 2.5-20 µg/ml.	mean relative initial survival (%).	
Mammalian cells gene mutation (at TK-locus) OECD TG 476 (1997) /OECD TG 490 (2016) GLP: yes **Acceptable**	**Dodine** (batch DCH0112; purity 97.1%)	Test system: Mouse lymphoma L5178Y cells S9 from rat liver induced with Aroclor 1254 Dosage (µg/mL): 1st experiment (- S9) 24 h; 0.1, 0.2, 0.4, 0.57, 0.82, 1.1, 1.3, 1.6, 1.8. (+ S9) 4 h; 0.072, 0.14, 0.29, 0.58, 1.2, 2.4, 3.4, 4.9, 7.0. 2nd experiment (- S9) 4h; 0.47, 1.9, 2.7, 3.2, 3.8, 4.4, 5.2, 6.1. (+ S9) 4h; 0,79, 1.6, 3.2, 4.5, 6.5, 9.2, 13. Solvent: Ethanol Dose selection: Cytotoxicity pre-tests at a range of 0.16-100 µg/mL	Mutagenicity: Negative Cytotoxicity: 1st experiment: RTG 10% at 1.8 µg/mL (-S9). 2nd experiment: RTG 1% at 6.1 µg/mL (-S9). RTG 0.3% at 13 µg/mL (+S9).	[redacted] (2008) [redacted] (2020) **(AS)** **B.6.4.1.2-02**
Mammalian chromosome aberrations OECD TG 473 (2016) GLP: yes **Acceptable**	**Dodine** (batch BB-333; purity 98.6%)	Test system: Cultured peripheral human lymphocytes S9 from rat liver induced with phenobarbital and ß-naphthoflavone. Cytogenetic tests -First cytogenetic test: 0.1, 6, 8 µg /mL (- S9, 3h expos- 24 h fix. time); 0.1, 6, 10 µg /mL (+ S9, 3h expos- 24 h fix. time) -Second cytogenetic test: 0.1, 1, 2 µg /mL (- S9; 24 h expos. /fix. time); 0.01, 0.1, 1 µg /mL (-S9, 48 h expos. /fix. time) Solvent: Ethanol Dose selection: Cytotoxicity pre-tests at a range of 3.13-100 µg/mL	Mutagenicity: Negative. Cytotoxicity: MI below 50% at the highest dose level tested in both cytogenetic tests.	[redacted] (2018) **(AS)** **B.6.4.1.3-01**
Mammalian chromosome aberrations OECD TG 473 Some deviations from OECD TG 473 (2016) -A single sampling time, exposure in short term treatment (+S9) was 2 h and no short-term treatment (-S9), only 200 metaphases were analysed, the age of the donor is not stated, no HCD, polyploid cells and endoreduplicated cells no reported separately. GLP: yes	**Dodine** (batch KG 8507; purity 98%)	Test system: Cultured peripheral human lymphocytes S9 from rat liver induced with Aroclor 1254 Cytogenetic tests 0.37, 1.11, 3.33 and 10 µg/mL (-S9/24 h) 0.56, 1.67, 5.0 and 15.0 µg/mL (+S9/2 h) incubation period: 72 hours Solvent: Ethanol Dose selection: Cytotoxicity pre-tests at a range of 1.65-400 µg/mL	Mutagenicity: Negative. Cytotoxicity MI below 50% from 5 µg/mL (+S9).	[redacted] (1985) **(AS)** **B.6.4.1.3-02**

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Supporting information				

Table 51: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Mammalian chromosome aberrations in somatic cells (Micronucleus test) Similar to OECD TG 474. Some deviations from OECD TG 474 (2016): Only one dose is tested, no signs of toxicity, no HCD, no statistical analysis, just 1000 PCE per animal were scored. GLP: yes **Supporting information**	**Dodine** (batch: KG 8507; purity 98%)	Test system: ♂/♀ Albino mice (Swiss random) Dosage: 500 mg/kg bw (oral administration). Vehicle: Propylene glycol Sampling: 24, 48 and 72 h after administration. Dose selection: Acute oral toxicity test where the oral LD50 exceed 500 mg/kg bw,	Mutagenicity: Negative Toxicity: Not detected.	[redacted] [redacted] (1985) **(AS)** **B.6.4.2.1-01**
Mammalian chromosome aberrations in somatic cells (Micronucleus test) Similar to OECD TG 474. Some deviations from OECD TG 474 (2016): Negative control group is sampling only at 24 h, minimal information about HCD, just 1000 PCE per animal were scored. GLP: yes **Acceptable**	**Dodine** (batch: KG 303/90; purity 94%)	Test system: ♂/♀ Mice (ICR strain) Dosage: 100, 200 and 400 mg/kg bw (oral administration) Vehicle: Corn oil Sampling: 24, 48 and 72 h after administration. Dose selection: Toxicity test at a range of 50-500 mg/kg bw.	Mutagenicity: Negative. Toxicity: Signs of systemic toxicity at 200 and 400 mg/kg bw.	[redacted] (1992) **(AS)** **B.6.4.2.1-02**

Table 52: Summary table of human data relevant for genotoxicity / germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No human data relevant for genotoxicity/ germ cell mutagenicity were available				

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

The potential genetic toxicity of dodine has been studied by a test battery that include tests to detect gene mutations (in bacterial and mammalian cells) and structural and numerical chromosome damage in both *in vitro* and *in vivo* tests (chromosomal aberrations test in mammalian cells and micronucleus tests in mice). No studies in germ cells were submitted.

Nine studies have been submitted, eight of them were previously evaluated in the DAR (2009). One new *in vitro* chromosomal aberration study and one re-evaluation of an *in vitro* gene mutation test have been submitted. All the studies were assessed according to the current guidelines.

Under the OECD TG 471 guideline, three studies were presented with negative results. All the strains required by the current guideline were covered by the reported studies. Exposure of *Salmonella typhimurium* and *Escherichia coli* tester strains to dodine did not produce an increased number of reversions, with and without metabolic activation at the tested doses.

Two mammalian gene mutation studies were submitted according to the OECD TG 476. Dodine did not induce mutations under the conditions of any of the tests. Dodine was considered non-mutagenic in the HGPRT-locus in CHO cells under the conditions of this *in vitro* test both with and without metabolic activation. MLA was re-evaluated according to current guideline, the study was considered to be in compliance with the OECD TG 490 guideline. Under the conditions used in the study, dodine was not mutagenic at the TK-locus of mouse lymphoma L5178Y cells both with and without metabolic activation.

Dodine did not induce chromosomal aberrations in cultured human lymphocytes, in any of two assays performed both with and without metabolic activation.

Dodine did not induce the formation of micronuclei in mouse polychromatic erythrocytes following doses of up to 500 mg/kg bw. In the first study no signs of toxicity were apparent, either indication on whether the bone marrow was reached. In the second study, signs of systemic toxicity were detected at the higher doses (200 and 400 mg/kg) indicating that the test chemical could be systemically available and, thus, also the bone marrow was reached. A complete assessment in relation to the evidence of bone marrow exposure in line with EFSA recommendation was provided (see summary in Vol. 3 B6), the weight of evidence of the evaluation of toxicity data shows that bone marrow exposure in the *in vivo* micronucleus studies can be considered.

Dodine has been tested for potential genotoxic properties (gene mutation, clastogenicity and aneugenicity) in a group of *in vitro* and *in vivo* assays. In any of them dodine showed evidence *of genotoxic potential.* Therefore, with the available dataset, dodine is not considered to be of genotoxic concern.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

No human data are available for dodine, therefore a classification as Muta. 1A is not supported. There are no data from *in vivo* heritable germ cell mutagenicity tests showing mutagenic effects in germ cells of humans therefore a classification as Muta. 1B is precluded. Dodine is negative in acceptable *in vitro* tests and *in vivo* somatic cell mutagenicity tests in mammals. Therefore, classification is not warranted for germ cell mutagenicity.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

Based on the data available for dodine and according to the criteria under Regulation (EC) No 1272/2008, **no classification of genotoxicity / germ cell mutagenicity** can be derived.

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

Table 53: Summary table of animal studies on long-term toxicity and carcinogenicity

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
- NOAEL/LOAEL
- target tissue/organ
- critical effects at the LOAEL</th><th>Reference</th></tr>
<tr><td>106-week carcinogenicity study in rats
GLP: Yes
Method: OECD 453 (1981) and US-EPA FIFRA</td><td>Test substance: Dodecylguanidine acetate. Purity: 98.6%

Dodine :Oral (diet)</td><td>Survival: No significant differences were detected between treated groups and controls, only a slight decrease was observed in top dose male group after 2-year.

Survival at termination of study (106 weeks)
<table><tr><th></th><th>After 1 year chronic and</th><th>After 2 year carcinogenicity phase</th></tr></table></td><td>[redacted] (1998) (AS) B.6.5.1</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
83-5 (1984) Rat strain: Sprague-Dawley rats: ♂ and ♀ No. animals: 60 rats/group Deviations from current test guideline (OECD TG 453, 2018): -Historical control data were not provided for all neoplasm incidences. -Statistical analysis were not performed for all neoplastic incidences. **Study acceptable**	**Doses:** Males: 0, 200, 400 and 800 ppm (equivalent to 0, 10.17, 20.34 or 41.93 mg/kg bw/day). Females: 0, 200, 400 and 800 ppm (equivalent to 0, 13.19, 26.5 or 53.50 mg/kg bw/day). 106-week feed exposure.		

	carcinogenicity phase			
	male	female	male	female
Control	2/70 (2.9%)	1/70 (1.4%)	30/60 (50%)	23/60 (38.3%)
200 ppm	4/70 (5.7%)	3/70 (4.3%)	37/61 (60.7%)	28/60 (46.7%)
400 ppm	3/70 (4.3%)	2/70 (2.9%)	32/61 (52.5%)	24/60 (40%)
800 ppm	2/70 (2.9%)	1/70 (1.4%)	23/60 (38.3%)	24/60 (40%)

Clinical signs: A statistically significant increase in the absence of grasping was found in top dose male group, compared with controls; whereas a significant trend test was obtained for the absence of grasping, traction and righting reflexes incidences in dodine-male treated groups. On the other hand, a dose-related increase in the hunched posture incidence was revealed in males. Moreover, increased reduced motor activity and piloerection incidences were observed in males dodine-treated groups compared with controls.

800 ppm (equivalent to 41.93/53.5 mg/kg bw/day for ♂/♀)
Bodyweight
- (↓) bw in ♂ throughout week 1-37 (5-8%) and 85-89 (7-8%).
- (↓) bw in ♀ throughout week 1-101 (4-16%).
- bwg in ♂ at week 1(↓20%), 2 (↓23%), 3 (↑16%), 5 (↓13%), 9 (↓42%), 11 (↓12%), 9 (↓42%), 12 (↓38%), 13 (↓83%), 25 (↓34%), 29 (↑60%), 41 (↑61%), 61 (↓41%).
- bwg in ♀ at week 1(↓25%), 3 (↓35%), 4 (↑90%), 11 (↑5444%), 25 (↓56%), 57 (↓60%), 61 (↓40%), 97 (↓156%).

Food consumption:
- fc in ♂ at week 1 (↓6%), 2 (↑12%), 4 (↑7%), 9 (↓5%), 12 (↓10%), 13 (↓11%), 17 (↓5%), 21 (↓6%), 25 (↓10%), 49 (↓5%), 53 (↓8%), 61 (↓6%), 89 (↓10%), 93 (↓12%).
- fc in ♀ at week 10 (↓4%), 25 (↓7%), 37 (↓9%), 41 (↓7%), 45 (↓12%), 49 (↓8%), 69 (↓9%), 73 (↓9%), 77 (↓13%), 97 (↓16%),

Clinical chemistry:
- (↓) alkaline phosphatase activity (18%) at week 26 in ♂.
- (↓) triglyceride (22%, ndr) at week 26 in ♀.
- (↑) potassium (9%) at week 78 in ♀.
- (↑) alkaline phosphatase activity (310%) at week 104 in ♀.

Urinalysis:
- (↓) urine volume at week 25 (47%) and 51 (29%, ndr) in ♂, and in ♀ at week 51 (46%).
- (↑) refractive index (0.5%) at week 26 in ♂, and at week 79 (0.3%) in ♀.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		▪ (↓) pH (7%) at week 25 in ♂. <u>Haematology:</u> ▪ (↓) WBC (24%) in ♂ at week 26. ▪ (↓) lymphocytes (26%) in ♂ at week 26. ▪ (↑) prothrombin time in ♀ at week 26 (5%), and at week 52 (8%, ncdr). <u>Organ weight (week 105):</u> ▪ (↑) rel brain wt in ♀ (12%, ncdr). ▪ (↓) abs heart wt in ♀ (7%, ndr, ns). ▪ (↓) rel kidney wt in ♂ (11%, ncdr, ns), and (↑) rel kidney wt in ♀ (10%, ns). ▪ (↓) rel adrenal wt in ♂ (32%, ndr, ns). ▪ (↑) abs (6%, ndr) and rel (12%) epididymis wt. ▪ (↓) abs (50%, ncdr, ns), and rel (45%. ncdr, ns) ovary wt. ▪ (↑) rel uterus wt (32%, ndr, ns). <u>Necropsy *(Statistical analysis not performed)*</u> *Adrenal* ▪ (↑) enlarged in ♀ (28.6% vs 17.1% in controls, ncdr). ▪ (↑) white mottling in ♀ (22.9% vs 12.9% in controls, ndr). *Subcutis* ▪ (↑) preputial gland abscess in ♂ (17.1% vs 7.1% in controls, ncdr). *Ovary* ▪ (↓) cyst in ♀ (11.4% vs 20% in controls, ndr). *Thymus* ▪ (↑) small in ♀ (7.1% vs 0% in controls, ncdr). <u>Histopathology</u> *Non-neoplastic <u>(Statistical analysis not performed)</u>* *Ovary* ▪ (↑) granulosa/theca cell hyperplasia (14.3% vs 5.8% in controls, ncdr). *Prostate* ▪ (↑) atrophy (4.3% vs 1.5% in controls, ndr). *Kidney* ▪ (↓) pelvic mineralization in ♂ (14.5% vs 34.3% in controls, ncdr). *Liver* ▪ (↓) bile duct hyperplasia in ♀ (11.4% vs 37.7% in controls, ncdr). *Neoplastic* *Lung* ▪ (↑) metastatic-mammary gland adenocarcinoma (2.9% vs 0% in controls, ndr, ns). *Mammary gland* ▪ (↑) malignant adenocarcinoma (12.9% vs 10% in controls, ndr, ns). *Ovary* ▪ (↑) benign cystadenoma (2.9% vs 0% in controls, ndr, ns). *Uterus* ▪ (↓) benign endometrial stromal polyp (9% vs 17% in controls, ndr, ns). *Thyroid*	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		▪ (↑) benign C-cell adenomas in ♂ (42% vs 29% in controls, ncdr, ns). ▪ (↑) malignant C-cell carcinomas in ♂ (11.3% vs 6% in controls, ncdr, ns). ▪ (↑) combined C-cell adenomas and carcinomas in ♂ (53% vs 35% in controls, ns). ▪ (↓) benign C-cell adenomas in ♀ (24.6% vs 29% in controls, ndr, ns). ▪ (↓) malignant C-cell carcinomas in ♀ (6.6% vs 7.2% in controls, ndr, ns). ▪ (↓) combined C-cell adenomas and carcinomas in ♀ (31.1% vs 36.2% in controls, ndr, ns). ▪ **400 ppm** (equivalent to 20.34/26.5 mg/kg bw/day for ♂/♀) <u>Bodyweight</u> ▪ (↓) bw in ♀ at week 89 (9%) and 101 (13%). <u>Food consumption:</u> ▪ fc in ♂ at week 21 (↓8%), 25 (↓17%), 41 (↑4%) and 61 (↓5%). ▪ fc in ♀ at week 9 (↓8%), 13(↓10%) and 45(↓8%). <u>Clinical chemistry:</u> ▪ (↑) alkaline phosphatase activity (150%) at week 104 in ♀. <u>Haematology:</u> ▪ (↑) prothrombin time in ♀ at week 52 (6%, ncdr). <u>Organ weight (week 105):</u> ▪ (↑) rel brain wt in ♀ (14%, ncdr). ▪ (↓) abs heart wt in ♀ (10%, ndr). ▪ (↓) rel kidney wt in ♂ (13%, ncdr, ns), and (↑) rel kidney wt in ♀ (7%, ns). ▪ (↓) rel adrenal wt in ♂ (32%, ndr, ns). ▪ (↑) abs (11%, ndr) and rel (11%) epididymis wt. ▪ (↓) abs (53%, ncdr, ns), and rel (48%. ncdr, ns) ovary wt. ▪ (↑) rel uterus wt (2%, ndr, ns). <u>Necropsy *(Statistical analysis not performed)*</u> *Adrenal* ▪ (↑) enlarged in ♀ (24.3% vs 17.1% in controls, ncdr). *Subcutis* ▪ (↑) preputial gland abscess in ♂ (8.6% vs 7.1% in controls, ncdr). *Ovary* ▪ (↓) cyst in ♀ (2.9% vs 20% in controls, ndr). *Thymus* ▪ (↑) small in ♀ (1.4% vs 0% in controls, ncdr). <u>Histopathology</u> *Non-neoplastic* <u>*(Statistical analysis not performed)*</u> *Ovary* ▪ (↑) granulosa/theca cell hyperplasia (6.7% vs 5.8% in controls, ncdr). *Prostate* ▪ (↑) atrophy (8.2% vs 1.5% in controls, ndr). *Kidney* ▪ (↓) pelvic mineralization in ♂ (14.3% vs 34.3% in	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		controls, ncdr). *Liver* ▪ (↓) bile duct hyperplasia in ♀ (18.6% vs 37.7% in controls, ncdr). *Neoplastic* *Lung* ▪ (↑) metastatic-mammary gland adenocarcinoma (2.9% vs 0% in controls, ndr, ns). *Mammary gland* ▪ (↑) malignant adenocarcinoma (24.6% vs 10% in controls, ndr, ns). *Ovary* ▪ (↑) benign cystadenoma (8.3% vs 0% in controls, ndr, ns). *Thyroid* ▪ (↑) benign C-cell adenomas in ♂ (33.3% vs 29% in controls, ncdr, ns). ▪ (↑) malignant C-cell carcinomas in ♂ (11.7% vs 6% in controls, ncdr, ns). ▪ (↑) combined C-cell adenomas and carcinomas in ♂ (45% vs 35% in controls, ns). ▪ (↑) benign C-cell adenomas in ♀ (29.8% vs 29% in controls, ndr, ns). ▪ (↑) malignant C-cell carcinomas in ♀ (12.3% vs 7.2% in controls, ndr, ns). ▪ (↑) combined C-cell adenomas and carcinomas in ♀ (42.1% vs 36.2% in controls, ndr, ns). **200 ppm** (equivalent to 10.17/13.19 mg/kg bw/day for ♂/♀) <u>Bodyweight</u> ▪ (↓) bw in ♀ at week 8 (6%). <u>Food consumption:</u> ▪ fc in ♂ at week 57 (↑7%). ▪ fc in ♀ at week 81 (↑16%). <u>Clinical chemistry:</u> ▪ (↑) alkaline phosphatase activity (73%) at week 104 in ♀. <u>Haematology:</u> ▪ (↑) prothrombin time in ♀ at week 52 (7%, ncdr). <u>Organ weight (week 105):</u> ▪ (↑) rel brain wt in ♀ (3%, ns, ncdr). ▪ (↓) abs heart wt in ♀ (6%, ndr, ns). ▪ (↓) rel kidney wt in ♂ (6%, ncdr, ns), and (↑) rel kidney wt in ♀ (3%, ns). ▪ (↓) rel adrenal wt in ♂ (32%, ndr, ns). ▪ (↑) abs (2%, ndr, ns) and rel (4%, ns) epididymis wt. ▪ (↓) abs (45%, ncdr, ns), and rel (41%. ncdr, ns) ovary wt. ▪ (↑) rel uterus wt (22%, ndr, ns). <u>Necropsy *(Statistical analysis not performed)*</u> *Subcutis* ▪ (↓) preputial gland abscess in ♂ (5.7% vs 7.1% in controls, ncdr). *Ovary*	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		▪ (↓) cyst in ♀ (15.7% vs 20% in controls, ndr). *Thymus* ▪ (↑) small in ♀ (1.4% vs 0% in controls, ncdr). <u>Histopathology</u> *Non-neoplastic <u>(Statistical analysis not performed)</u>* *Ovary* ▪ (↓) granulosa/theca cell hyperplasia (3.3% vs 5.8% in controls, ncdr). *Prostate* ▪ (↑) atrophy (3.3% vs 1.5% in controls, ndr). *Kidney* ▪ (↓) pelvic mineralization in ♂ (22.7% vs 34.3% in controls, ncdr). *Liver* ▪ (↓) bile duct hyperplasia in ♀ (14.3% vs 37.7% in controls, ncdr). *Neoplastic* *Mammary gland* ▪ (↑) malignant adenocarcinoma (14.5% vs 10% in controls, ndr, ns). *Ovary* ▪ (↑) benign cystadenoma (5% vs 0% in controls, ndr, ns). *Thyroid* ▪ (↑) benign C-cell adenomas in ♂ (38.4% vs 29% in controls, ncdr, ns). ▪ (↓) malignant C-cell carcinomas in ♂ (2% vs 6% in controls, ncdr, ns). ▪ (↑) combined C-cell adenomas and carcinomas in ♂ (40% vs 35% in controls, ns). ▪ (↑) benign C-cell adenomas in ♀ (35.8% vs 29% in controls, ndr, ns). ▪ (↑) malignant C-cell carcinomas in ♀ (13.2% vs 7.2% in controls, ndr, ns). ▪ (↑) combined C-cell adenomas and carcinomas in ♀ (49% vs 36.2% in controls, ndr, ns). **-LOAEL$_{toxicity}$**= 800 ppm (~41.93/53.5 mg/kg bw/day for ♂/♀) **-LOAEL$_{carcinogenicity}$**= 200 ppm (~10.17/13.19 mg/kg bw/day for ♂/♀) **-NOAEL$_{toxicity}$**= 400 ppm (~20.34/26.5 mg/kg bw/day for ♂/♀) **-NOAEL$_{carcinogenicity}$** = - -Critical effects at the LOAEL$_{carcinogenicity}$: ↑ increased thyroid C-cell adenomas and carcinomas incidences in males. Critical effects at the LOAEL$_{toxicity}$**:** clinical signs, ↓decreased bodyweight, ↓food consumption. <u>Target tissue/organ</u>: Thyroid	
78-week carcinogenicity study in mice	<u>Test substance:</u> Dodine technical. Purity: 98.6%	**<u>Survival</u>:** Survival was dose-related increased in male dodine-treated groups, compared to controls displaying statistically significant result at high dose group.	[redacted] (1998a) (AS) B.6.5.2

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
- NOAEL/LOAEL
- target tissue/organ
- critical effects at the LOAEL</th><th>Reference</th></tr>
<tr>
<td>GLP: Yes
Method: US-EPA FIFRA 83-2

Mice strain: Crl:CD-1(ICR)BR mice: ♂ and ♀
No. animals: 60 mice/group

Deviations from current test guideline (OECD TG 453, 2018):
-Historical control data were not provided for all neoplasm incidences, and these didn’t cover the 5-year recommended period on the date of the index study.
- Following organs not weighed: epididymides, heart, spleen, testes, thyroid and uterus.
-Haemotology and biochemistry were not measured.

Study acceptable</td>
<td>Dodine :Oral (diet)

Doses:
Males: 0, 200, 750 and 1500 ppm (equivalent to 0, 29.2, 109.8 or 224.8 mg/kg bw/day).
Females: 0, 200, 750 and 1500 ppm (equivalent to 0, 38.3, 136.2 or 275.2 mg/kg bw/day).

78-week feed exposure.</td>
<td>Mortality rates (78-weeks)
<table><tr><th></th><th colspan="2">After 78 weeks</th></tr><tr><th></th><th>male</th><th>female</th></tr><tr><td>Control</td><td>16/70 (22.9%)##</td><td>13/70 (18.6%)</td></tr><tr><td>200 ppm</td><td>14/70 (20%)</td><td>9/70 (12.9%)</td></tr><tr><td>750 ppm</td><td>9/70 (12.9%)</td><td>11/70 (15.7%)</td></tr><tr><td>1500 ppm</td><td>3/70 (4.3%)**</td><td>15/70 (21.4%)</td></tr></table>## p≤0.01 for Cox-Tarone and Gehan-Breslow trend test
** p≤0.01 for Cox-Tarone and Gehan-Breslow test

<u>Clinical signs</u>: Increased incidence of whole body tremors was mainly noted in mid and high dose groups for both sexes (~13-14% in males and 11-13% in females compared with controls). Malocclusion occurrences was considerably increased in high male dose group (18.6% vs 5.7% in controls). On the other hand, increased dose-related incidences of dilated pupil and excessive salivation were mainly observed in the three male-dodine treated groups and in mid-top male dose groups, respectively; whereas increases, not dose related of these incidences were recorded in female dodine-treated groups.

1500 ppm (equivalent to 224.8/275.2 mg/kg bw/day for ♂/♀)
<u>Bodyweight</u>
▪ (↓) bw in ♂ throughout week 2-78 (3-10%).
▪ (↓) bw in ♀ at week 5 (4%), and throughout week 8-78 (4-14%).
▪ (↓) bwg in ♂ through week 1-14 (25%), and 1-78 (26%).
▪ (↓) bwg in ♀ through week 1-14 (26%), 14-54 (36%), 54-78 (63%), and 1-78 (35%).
<u>Food consumption:</u>
▪ (↓) fc in ♂ at week 1-9 (8-16%), 11-12 (7%), 21-33 (6-9%), 49 (5%), 57 (5%)
▪ (↓) fc in ♀ at week 1-2 (13%), 4 (8%). 5 (7%), and 9-77 (8-19%).
<u>Organ weight (week 78):</u>
▪ (↓) abs left adrenal wt in ♀ (23%, ncdr).
▪ (↑) abs/rel left (12/31%) and abs/rel right (11/30%) kidney wt in ♀.
▪ (↑) rel liver wt in ♂ (13%, ncdr).
▪ (↑) rel liver wt in ♀ (14%, ncdr).
▪ (↑) rel brain wt in ♂ (7%, ncdr).
▪ (↓) abs brain wt in ♀ (5%, ncdr). (↑) rel brain wt in ♀ (11%, ncdr).

<u>Necropsy (Statistical analysis not performed)</u>
Liver
▪ (↑) light focus area in ♂ (5.7% vs 1.4% in controls, ncdr).
Kidney
▪ (↑) cyst in ♂ (7.1% vs 4.3% in controls, ncdr).</td>
<td></td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		Spleen ▪ (↓) large in ♂ (2.9% vs 11.4% in controls, ncdr). ▪ (↓) large in ♀ (2.9% vs 17.1% in controls). ▪ (↑) small in ♀ (4.3% vs 0% in controls, ncdr). *Testes* ▪ (↑) small in ♂ (5.7% vs 1.4% in controls, ncdr). *Uterus* ▪ (↓) large in ♀ (5.7% vs 18.6% in controls, ndr). <u>Histopathology</u> *Non-neoplastic (statistical analysis not performed)* *Liver* ▪ (↓) haematopoiesis extramedullary in ♀ (3.3% vs 13.3% in controls, ncdr). ▪ (↓) infiltrate neutrophilic in ♀ (1.7% vs 11.7% in controls). ▪ (↓) infiltrate lymphohistiocystic in ♀ (46.7% vs 68.3% in controls, ncdr). ▪ (↓) hypertrophy hepatocellular in ♀ (1.7% vs 23.3% in controls, ncdr). ▪ (↓) necrosis in ♂ (10% vs 21.7% in controls, ncdr). *Kidney* ▪ (↓) infiltrate lymphohistiocystic in ♀ (10% vs 25% in controls, ncdr). ▪ (↑) cyst in ♂ (15% vs 5% in controls, ndr). ▪ (↓) cyst in ♀ (0% vs 6.7% in controls). ▪ (↓) pelvic dilatation in ♂ (1.7% vs 15% in controls, ncdr). ▪ (↓) amyloid in ♀ (5% vs 21.7% in controls, ndr). ▪ (↑) hyperplasia tubular cell in ♂ (6.7% vs 0% in controls, ncdr). *Prostate* ▪ (↑) chronic inflammation (16.7% vs 6.7% in controls, ndr). *Neoplastic* *Liver* ▪ (↑) benign adenoma in ♂ (23.3% vs 13.3% in controls, ncdr, ns). ▪ (↑) benign adenoma in ♀ (6.7% vs 0% in controls, ncdr, ns). ▪ (↓) malignant carcinoma in ♂ (1.7% vs 3.3% in controls, ndr, ns). ▪ (↑) malignant carcinoma in ♀ (1.7% vs 0% in controls, ndr, ns). ▪ (↑) combined adenomas/carcinoma in ♂ (25% vs 16.7% in controls, ncdr, ns). ▪ (↑) combined adenomas/carcinoma in ♀ (8.3% vs 0% in controls, ndr). **750 ppm** (equivalent to 109.8/136.2 mg/kg bw/day for ♂/♀) <u>Bodyweight</u> ▪ (↓) bw in ♂ at week 7 (2%), 9-10 (3%), 18 (3%), 30-62 (4-5%), ▪ (↓) bw in ♀ at week 13-14 (4%), 22 (6%), 30-46 (5-7%) and 54-78 (7-10%). ▪ (↓) bwg in ♂ through week 1-78 (5%, ns).	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		▪ (↓) bwg in ♀ through 1-78 (20%). <u>Food consumption:</u> ▪ (↓) fc in ♂ at week 1(8%), 5-12 (5-8%) and 49 (7%). ▪ (↓) fc in ♀ at week 1-2 (10-16%), 5 (7%), 10-25 (6-11%), 33-57 (5-9%), 69 (10%) and 77 (9%). <u>Organ weight (week 78):</u> ▪ (↓) abs left adrenal wt in ♀ (18%, ncdr). ▪ (↓) abs (1%, ncdr, ns), (↑) rel (7%) left kidney wt. (↓) abs (1%, ndr, ns), (↑) rel (8%) right kidney wt. ▪ (↑) rel liver wt in ♂ (4%, ncdr. ns). ▪ (↑) rel liver wt in ♀ (0.5%, ncdr, ns). ▪ (↑) rel brain wt in ♂ (2%, ncdr, ns). ▪ (↓) abs brain wt in ♀ (3%, ncdr). (↑) rel brain wt in ♀ (5%, ncdr, ns). <u>Necropsy *(Statistical analysis not performed)*</u> *Liver* ▪ (↑) light focus area in ♂ (2.9% vs 1.4% in controls, ncdr). *Kidney* ▪ (↑) cyst in ♂ (8.6% vs 4.3% in controls, ncdr). *Spleen* ▪ (↓) large in ♂ (0% vs 11.4% in controls, ncdr). ▪ (↓) large in ♀ (7.1% vs 17.1% in controls). *Uterus* ▪ (↑) large in ♀ (20% vs 18.6% in controls, ndr). <u>Histopathology</u> *Non-neoplastic (statistical analysis not performed)* *Liver* ▪ (↓) haematopoiesis extramedullary in ♀ (11.7% vs 13.3% in controls, ncdr). ▪ (↓) infiltrate neutrophilic in ♀ (5% vs 11.7% in controls). ▪ (↓) infiltrate lymphohistiocystic in ♀ (55% vs 68.3% in controls, ncdr). ▪ (↓) hypertrophy hepatocellular in ♀ (6.7% vs 23.3% in controls, ncdr). ▪ (↓) necrosis in ♂ (8.3% vs 21.7% in controls, ncdr). *Kidney* ▪ (↓) infiltrate lymphohistiocystic in ♀ (13.3% vs 25% in controls, ncdr). ▪ (↑) cyst in ♂ (15% vs 5% in controls, ndr). ▪ (↓) cyst in ♀ (1.7% vs 6.7% in controls). ▪ (↓) pelvic dilatation in ♂ (5% vs 15% in controls, ncdr). ▪ (↓) amyloid in ♀ (5% vs 21.7% in controls, ndr). ▪ (↑) hyperplasia tubular cell in ♂ (1.7% vs 0% in controls, ncdr). *Prostate* ▪ (↓) chronic inflammation (0% vs 6.7% in controls, ndr). *Neoplastic* *Liver* ▪ (↑) benign adenoma in ♂ (15% vs 13.3% in controls, ncdr, ns). ▪ (↑) benign adenoma in ♀ (1.7% vs 0% in controls, ncdr, ns). ▪ (↑) malignant carcinoma in ♂ (5% vs 3.3% in controls,	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		ndr, ns). ▪ (=) malignant carcinoma in ♀ (0% vs 0% in controls, ndr, ns). ▪ (↑) combined adenomas/carcinoma in ♂ (20% vs 16.7% in controls, ncdr, ns). ▪ (↑) combined adenomas/carcinoma in ♀ (1.7% vs 0% in controls, ndr, ns). **200 ppm** (equivalent to 29.2/38.3 mg/kg bw/day for ♂/♀) <u>Bodyweight</u> ▪ (↓) bw in ♂ at week 9-10 (3%) and 13 (4%) ▪ (↓) bw in ♀ at week 34 (3%), 58 (6%), 66 (6%) and 78 (6%). ▪ (↑) bwg in ♂ through week 54-78 (220%), and 1-78 (3%, ns). ▪ (↓) bwg in ♀ through 1-78 (11%, ns). <u>Food consumption:</u> ▪ (↓) fc in ♂ at week 5 (6%), 8-9 (5-7%), 25 (4%) and 49 (5%). <u>Organ weight (week 78):</u> ▪ (↑) abs left adrenal wt in ♀ (2%, ncdr, ns). ▪ (↓) abs (2%, ncdr, ns), (↑) rel (3%, ns) left kidney wt. (↓) abs (3%, ndr, ns), (↑) rel (4%, ns) right kidney wt. ▪ (↓) rel liver wt in ♂ (4%, ncdr. ns). ▪ (↓) rel liver wt in ♀ (0.5%, ncdr, ns). ▪ (↓) rel brain wt in ♂ (1%, ncdr, ns). ▪ (↑) abs brain wt in ♀ (0.2%, ncdr, ns). (↑) rel brain wt in ♀ (6%, ncdr, ns). <u>Necropsy *(Statistical analysis not performed)*</u> *Kidney* ▪ (↓) cyst in ♂ (2.9% vs 4.3% in controls, ncdr). *Spleen* ▪ (↑) large in ♂ (15.7% vs 11.4% in controls, ncdr). ▪ (↓) large in ♀ (12.9% vs 17.1% in controls). *Uterus* ▪ (↓) large in ♀ (14.3% vs 18.6% in controls, ndr). <u>Histopathology</u> *Non-neoplastic (statistical analysis not performed)* *Liver* ▪ (↓) haematopoiesis extramedullary in ♀ (11.7% vs 13.3% in controls, ncdr). ▪ (↓) infiltrate neutrophilic in ♀ (6.7% vs 11.7% in controls). ▪ (↑) infiltrate lymphohistiocystic in ♀ (70% vs 68.3% in controls, ncdr). ▪ (↑) hypertrophy hepatocellular in ♀ (28.3% vs 23.3% in controls, ncdr). ▪ (↓) necrosis in ♂ (16.7% vs 21.7% in controls, ncdr). *Kidney* ▪ (↓) infiltrate lymphohistiocystic in ♀ (11.7% vs 25% in controls, ncdr). ▪ (↑) cyst in ♂ (16.7% vs 5% in controls, ndr). ▪ (↓) cyst in ♀ (3.3% vs 6.7% in controls). ▪ (↓) pelvic dilatation in ♂ (5% vs 15% in controls, ncdr). *Prostate*	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		▪ (↑) chronic inflammation (14.3% vs 6.7% in controls, ndr). *Neoplastic* ▪ (↓) benign adenoma in ♂ (11.7% vs 13.3% in controls, ncdr, ns). ▪ (↑) benign adenoma in ♀ (1.7% vs 0% in controls, ncdr, ns). ▪ (↓) malignant carcinoma in ♂ (0% vs 3.3% in controls, ndr, ns). ▪ (↑) malignant carcinoma in ♀ (1.7% vs 0% in controls, ndr, ns). ▪ (↓) combined adenomas/carcinoma in ♂ (11.7% vs 16.7% in controls, ncdr, ns). ▪ (↑) combined adenomas/carcinoma in ♀ (3.3% vs 0% in controls, ndr, ns). **-LOAEL$_{toxicity}$**= 750 ppm (~109.8/136.2 mg/kg bw/day for ♂/♀) **-LOAEL$_{carcinogenicity}$**= - **-NOAEL$_{toxicity}$**= 200 ppm (~29.2/38.3 mg/kg bw/day for ♂/♀) **-NOAEL$_{carcinogenicity}$**= 1500 ppm (~224 8/275.2 mg/kg bw/day for ♂/♀) -Critical effects at the LOAEL$_{carcinogenicity}$: - -Critical effects at the LOAEL$_{toxicity}$: clinical signs, ↓ bodyweight, ↓food consumption. Target tissue/organ: Liver	
Chronic toxicity study in rats. GLP: No Method: Non-stated Rat strain: CFN rats Sex: ♂ and ♀ Deviations from current test guideline (OECD TG 453, 2018): - Test substance not fully characterised. - Only 40 animals per groups were used instead of 50 animals. - The measured parameters were not fully described.	Test substance: Dodine Dodine :Oral (diet) Dose levels: ♂/♀: 0, 50, 200 and 800 ppm (equivalent to 0, 2.5, 10 and 40 mg/kg bw/day) 104 weeks exposure	**Survival:** No treatment related effects were observed between dodine-treated groups and controls. **Clinical signs**: No treatment related effects were observed between dodine-treated groups and controls. **Bodyweight**. (↓) at top dose (800 ppm) for both sexes (9(/6% for ♂/♀). **Food consumption *(data not shown in the study)*:** Decreased food consumption was only recorded at top dose males during first year of the study. **Haematology and clinical chemistry*(data not shown in the study)***: No relevant findings were reported. **Organ weight *(data not shown in the study)***: No relevant findings were reported. **Histopathology *(data not shown in the study)*:** No relevant findings were reported. **-LOAEL= -** **-NOAEL$_{toxicity}$= -** -Critical effects at the LOAEL: -	Levinskas. *et al.* (1961) (CA) B.6.5.3

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
- Numerical results were not reported in most of the parameters. - Statistical analysis was not performed. **Supportive information**		Target tissue/organ: None	

Table 54: Summary table of human data on long-term toxicity and carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 55: Summary table of other studies relevant for long-term toxicity and carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

Three long-term toxicity and carcinogenicity studies (two conducted in rats and one in mice) have been provided to support the renewal of the active substance. Two of them were included in the original DAR (2009) in support of the inclusion of dodine in Annex I of Directive 91/414/EEC, and had been reassessed for the renewal purpose. These two studies were conducted according guidelines and presented minor deviations that did not compromise the acceptability of the study; therefore, they are deemed acceptable to assess de chronic toxicity and carcinogenicity potential of the active substance. Additionally, a new study conducted in rat has been provided, however, it showed important methodological and reporting deviations that compromise the acceptability.

-In the 106-week oncogenicity study in rats (B.6.5.1), dodine was tested at dose levels of 0, 200, 400 and 800 ppm (equivalent to 0, 10.17, 20.34 or 41.93 mg/kg bw/day for males and 0, 13.19, 26.5 or 53.50 mg/kg bw/day for females, respectively) for 106 weeks.

No important differences in mortality rates were observed between treated groups and controls for both sexes, only a slight decrease was observed in top dose male group after 2-year (38.3% vs 50% in controls).

Clinical signs related with potential neurotoxic effect on the nervous system were described. A statistically significant increase in the absence of grasping was found in top dose male group, compared with controls; whereas a significant trend was obtained for the absence of grasping, traction and righting reflexes incidences in dodine-male treated groups. On the other hand, a dose-related increase in the hunched posture incidence was revealed in males. Moreover, increased reduced motor activity and piloerection incidences were observed in males dodine-treated groups, compared with controls.

Slight statistically significant decreases in bodyweight were recorded in top dose male group throughout week 1-37 (5-8%) and weeks 85-89 (7-8%), whereas in females were noted throughout whole study (4-16%), compared with controls. On the other hand, slight reductions, not statistically significant, were recorded in female mid dose groups throughout study. Regarding bodyweight gains, a reduction was observed in most of the weeks throughout study in both sexes at high dose, whereas isolated reductions or increases, without dose relationship, were found in low and mid dose groups.

Food consumption was mostly decrease through sporadic weeks in top dose male (5-12%) and female (4-16%) dose groups without showing a clear dose relationship and consistency throughout the whole study.

Ophthalmoscopy examination was performed without showing treatment-related findings.

Regarding clinical chemistry parameters, a statistically significant lower mean alkaline phosphatase activity (18%) was seen at week 26 in top dose male group. However, this difference was mainly attributed to unusually high activities in two rats in the control group. On the other hand, at week 26, in females from high and low dose group, and at week 78 in males at mid dose, a tendency towards lower triglycerides concentrations was observed compared to controls. However, these changes were transient and no clear dose- relationship was noted. Moreover, at week 78, mean potassium concentration was higher (9%) in top dose female group, however, this difference was not considered biologically or toxicologically significant due to this parameter was not significantly altered in other weeks or dose groups. Additionally, mean alkaline phosphatase activities were higher at top and mid dose female groups (310% and 150% for top and mid dose groups, respectively) at week 104.

Urinalysis results showed a trend towards high protein content at week 25 in all male treated groups, and at week 25 and 51 in top dose female group compared with controls. Statistically significantly lower mean urinary volumes were noted at top dose male group at week 25 and 51 (47% and 29%, respectively), and in females at week 51 (46%). On the other hand, isolated statistically significant changes, without replicates at other measure time points, were recorded at week 25 in refractive index and pH in males of top dose group.

Haematology analysis revealed decreases on mean total white blood cell count (24%) in top dose male group at week 26. This variation was accompanied by a lower mean absolute lymphocyte count (26%). The same trend, but none statistically significant, was noted at week 52 in which mean total white blood cell count (22%) and mean absolute lymphocyte count (20%) were lower than controls. In females, statistically significantly higher mean prothrombin times were observed at week 26 at 800 ppm (5%) and at week 52 at low, mid and high dose groups (7%, 6% and 8% respectively). At week 104, mean corpuscular haemoglobin was statistically significantly higher (12%) at male mid dose group, compared with controls. This difference appeared to be attributable primarily to one rat, which had high mean corpuscular haemoglobin, because of a slightly lower red blood cell count and normal haemoglobin concentration; mean corpuscular volume was also higher for this animal.

Organ weight data at week 53 showed isolated statistically significant differences in different organs without a dose-relationship. On the other hand, organ weight data recorded at completion study (week 104), revealed a trend towards lower mean terminal bodyweight in top dose males (5%) and females (11%) dose groups, consistent with the lower bodyweight gain of these groups during the study. An increase, without a clear dose response, was noted in relative brain weight in females at mid and high dose (14% and 12%, respectively). On the other hand, increased absolute (11% and 6% for mid and high dose groups, respectively) and relative (11% and 12% for mid and high dose groups, respectively) epididymis weight were recorded compared with controls. A reduction, without a dose response and statistical significance, were noted in relative adrenal weight in males treated groups. An increase, without statistical significance, in the relative uterus weight was only observed in top dose female group. Additionally, a decreased trend, not statistically significant and not clear dose related, was observed in absolute and relative ovary weight in all females dodine treated groups, and in relative kidney weight in all males dodine treated groups, whereas in females, an increased trend was recorded for relative kidney weight.

Increases of enlarge (mid and top dose) and white mottling (top dose) incidences in adrenal gland were found in female-dodine treated groups, compared with controls. Moreover, increases of preputial gland abscess incidence was found in mid and top dose male groups compared with controls. On the other hand, increases small thymus occurrences (top dose), and decreases ovary cyst incidences (low, mid and high dose) were noted in female groups. No other relevant incidences were detected during necropsy examination in the female and male rats at any dose level.

Histopathology examination revealed increases in the incidences of ovary granulosa/theca cell hyperplasia, characterized by focal increase in number of granulosa/theca cells, in high dose female group (14.3% vs 5.8% in controls), showing statistically significance in prevalence tumour test. By contrast, reduction in pelvic mineralization in kidney (males) and bile duct hyperplasia in liver (females) incidences were noted in all dodine-treated groups. On the other hand, a slight not dose related increases incidences of prostate atrophy were recorded in all male dodine-treated groups.

Regarding neoplastic findings, increased benign ovary cystadenomas were noted in dodine treated-groups (0%, 9%, 14% and 6%, for control, low, mid and high dose groups, respectively). However, only one malignant cystadenocarcinomas were recorded in control and mid dose groups (1.5% and 1.7% for controls and mid dose

groups, respectively). Moreover, there was a reduction in the benign endometrial stromal polyp incidences in the uterus at top dose female group (9% vs 17% in controls). On the other hand, metastatic mammary gland adenocarcinoma in lung was detected in mid and high dose females groups, respectively. An increase, not dose related, of malignant adenocarcinomas incidences were noted in mammary gland of females from mid dose group (10, 14.5, 24.6 and 12.9% for control, low, mid and high dose groups). Remarkably, thyroid neoplasm observations were increased in males. First, non-statistically significant (prevalence tumour test), but dose related, increased incidences of combined thyroid adenomas and carcinomas were noted (35%, 40%, 45% and 53% for controls, low, mid and high dose group). The incidences in treated groups exceed the historical control range (27-38%). When incidences were analysed separately, thyroid C-cell adenoma occurrences were increased in treated groups, without a dose-response pattern and statistical significance (29%, 38%, 33% and 42% for controls, low, mid and high dose group) exceeding the HCD range for adenomas (25-32%) in all treated groups. On the other hand, thyroid C-cell carcinoma occurrences were increased in mid and top dose male group, without a dose-response pattern and statistical significance (6%, 2%, 12% and 11% for controls, low, mid and high dose group) exceeding the HCD range for carcinomas (4-10%) in mid and high dose groups.

Overall, **NOAEL for carcinogenicity** could not be stablished based on increased thyroid C-cell adenomas and carcinomas incidences in males observed at low dose (200 ppm**,** equivalent to 10.17/13.19 mg/kg bw/day for males/females). **NOAEL for toxicity** was considered to be **400 ppm** (equivalent to 20.34/26.5 mg/kg bw/day for males/females), based on clinical signs, decreased bodyweight and food consumption at high dose.

-In the 78-week oncogenicity study in mice (B.6.5.2), dodine was tested at dose levels of 0, 200, 750 and 1500 ppm (equivalent to 0, 29.2, 109.8 or 224.8 mg/kg bw/day for males and 0, 38.3, 136.2 or 275.2 mg/kg bw/day for females, respectively) for 78 weeks.

Mortality was dose-related decrease in male-dodine treated groups, compared with controls. There was a significant negative trend test in mortality ($p < 0.01$ for both Cox-Tarone and Gehan-Breslow tests) associated with a significantly decreased mortality in the high-dose group compared with controls (3/70 vs. 16/70; $p < 0.01$ for both tests). No differences were observed in female-dodine treated groups compared with controls.

Several clinical signs were described for both sexes. Increased incidence of whole body tremors was mainly noted in mid and high dose groups for males and females (~13-14% in males and 11-13% in females compared with controls). Malocclusion occurrences was considerably increased in high male dose group (18.6% vs 5.7% in controls), whereas a slight increase of irregular respiration (4.3% vs 0% in controls) and rough hair coat incidences (11.4% vs 0% in controls) were found in top dose female group. On the other hand, increased dose-related incidences of dilated pupil and excessive salivation were mainly observed in the three male-dodine treated groups and in mid-top male dose groups, respectively; whereas increases, not dose related of these incidences were recorded in female dodine-treated groups.

Statistically significantly lower bodyweights were recorded at top male (3-10%) and female (4-14%) dose groups throughout whole study, compared with controls. At mid dose groups, statistically significant reductions were mainly noted from week 30 to study termination for both sexes (2-5% for males and 4-10% for females, respectively), whereas at low dose groups, isolated reductions were recorded through few weeks for both sexes. Mean bodyweight gains of the top dose groups were significantly reduced for males and females through weeks 1-14 (26%), and for females through weeks 14 54 (36%) and 54 78 (63%), showing an overall mean bodyweight gain for males/females of 26/35%, respectively. At mid dose, overall mean bodyweight gain for females was significantly reduced compared controls (20%), whereas a slight reduction, not statistically significant, was found in males (5%). By contrast, slight increase (3%) and decrease (11%) was recorded in males and females from low dose groups, respectively.

Mean food consumption was generally reduced at top dose groups for both sexes throughout whole study (5-16% for males and 5-19% for females, respectively), compared with controls. On the other hand, at mid dose groups, statistically significant reductions were mainly noted at the first half of the study in males (5-8%), and practically through entire study in females (5-16%). In low dose groups, isolated and slight reductions were noted in males through study.

Regarding bodyweights at study termination, absolute (high dose: 12/11% for left/right) and relative (mid dose: 7/8% for left/right; and high dose: 31/30% for left/right) kidney weights were significantly increased in females. Moreover, relative liver and brain weights were statistically significant increased in top dose groups for both sexes (13/14% for males/females liver weight, and 8/11% for males/females brain weight). On the other hand, no dose-related changes, nor statistically significant results, were observed in another organ weights.

Necropsy examinations revealed slight increases of light focus area in the liver, and kidney cyst incidences in top dose male group, compared with controls. In spleen, large occurrences were reduced in mid and high dose groups for both sexes, whereas small spleen observations were slightly increased in top dose females, compared with controls. Regarding reproductive organs, small testes observations were increased in top dose male groups, whereas large uterus incidences were decreased in top dose female group. No other relevant incidences were detected during necropsy examination in male and female mice at any dose level.

Histopathology analysis did not show relevant adverse alterations in non-neoplastic findings. Most of the observations were reduced incidences noted between dodine-treated groups and controls. Firstly, in female livers, decreased incidences in haematopoiesis extra medullary (top dose), hypertrophy hepatocellular (mid and top dose), infiltrate lymphohistiocystic (mid and top dose) and neutrophilic (low, mid and top dose) were recorded. On the other hand, reductions in hepatocyte necropsy incidences were mainly noted in mid dose male group and in top dose groups for both sexes, compared with controls.

In kidney, decreases in infiltrate lymphohistiocystic incidences, as noted in liver, were also found in low, mid and top dose female group. On the other hand, cyst incidences were increased in dodine-treated males, but were decreased in dodine-treated females, compared with controls. Moreover, dilatation pelvis occurrences were reduced in dodine-treated males, whereas hyperplasia of tubular cell incidences were mainly increased in top dose male group. Amyloid decreases were noted in mid dose (female) and top dose (male and female) groups.

Moreover, an increased, not dose related, of inflammation occurrences in the prostate were recorded in low and top dose groups (6.7%, 14.3%, 0% and 16.7% for controls, low, mid and high dose groups, respectively), compared with controls.

On the other hand, neoplastic findings were mainly described in livers for both sexes. An increased incidences of hepatocellular adenomas were observed at high dose groups for both sexes (13%, 12%, 15% and 23% for controls, low, mid and high dose males groups; and 0%, 2%, 2% and 7% for controls, low, mid and high dose females groups, respectively), in which a statistically significant trend was displayed for females. All the recorded incidences in males groups (including controls) were higher than mean (6.3%) and out of the range (4.1-10%) of the accepted HCD; whereas in females only top dose group exceed both the mean (1.2%) and the range (0-3.3%) of HCD. On the other hand, no relevant increases were noted regarding hepatocellular carcinomas in dodine-treated groups (3.3%, 0, 5% and 1.7% for controls, low, mid and high dose males groups; and 0, 1.7%, 0, and 1.7% for controls, low, mid and high dose females groups, respectively). When the effects were combined, increased incidences were also observed in high dose groups (17%, 12%, 20% and 25% for controls, low, mid and high dose males groups; and 0%, 3%, 2% and 8% for controls, low, mid and high dose females groups, respectively), showing a significant trend test in females, and the only significant group comparison difference with controls was for combined adenomas and carcinomas in females given 1500 ppm dose. The combined incidences in all males groups (including controls) were higher than mean (10.2%) and out of the range of HCD, with the exception of low dose group (range: 8.3-12.2%). On the other hand, the combined incidences in female-treated groups were slightly higher than HCD mean (2.4%), except mid dose group, whereas top dose group was the only that exceed the range (2-3.3%). Furthermore, no relevant differences were noted in another organs regarding neoplasm incidences, compared with controls.

Overall**, NOAEL for carcinogenicity** is stablished in **1500 ppm** (equivalent to 224.8/275.2 mg/kg bw/day for males/females) based on no evidence of carcinogencity at high dose. **NOAEL for toxicity** is considered to be **200 ppm** (equivalent to 29.2/38.3 mg/kg bw/day for males/females), based on clinical signs (both sexes), decreased bodyweight (both sexes) and food consumption (both sexes).

Therefore, based on the available data, the **overall carcinogenicity NOAEL** could not stablished based on increased thyroid C-cell adenomas and carcinomas incidences observed at low dose (200 ppm**,** equivalent to 10.17/13.19 mg/kg bw/day for males/females) in rats (106-week study).

-A non-guideline chronic toxicity study was conducted in CFN rats (B.6.5.3). Dodine was administered *via* feed diet to 40 male and 40 female CFN rats *per* group at concentrations of 0, 50, 200 or 800 ppm (equivalent to 0, 2.5, 10 and 40 mg/kg bw/day) throughout 104 weeks. This study is a published report that presented important deviations that compromised the acceptability, so no further conclusions can be drawn. No relevant treatment effects were described after dodine administration. Only systemic toxicity effects in bodyweights and food consumption were described at top dose.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

Comparison with criteria for Category 1A classification: In accordance with the criteria in the CLP regulation,

classification for carcinogenicity Category 1A is reserved for substances known to have carcinogenic potential in humans. In the absence of human data for carcinogenicity, category 1A is not triggered.

Comparison with criteria for Category 1B classification: In accordance with the criteria in the CLP regulation, classification for carcinogenicity Category 1B is reserved for substances that are presumed to be carcinogenic in humans, and is largely based on data from animal studies where there is sufficient evidence to demonstrate animal carcinogenicity (presumed human carcinogen). This classification is not considered appropriate based on the available data in rat and mice studies.

Comparison with criteria for Category 2 classification: In accordance with the criteria in the CLP regulation, classification for carcinogenicity Category 2 is reserved for substances where there is evidence obtained from human and/or animal studies but which is not sufficiently convincing to place the substance in Category 1. The available data suggests a carcinogenicity potential of dodine in rat, but no in mice.

Rat

Thyroid C-cell adenomas: Benign C-cell adenomas were increase without showing a dose-response in all male-dodine groups (29%, 38.4%, 33.3% and 42% for controls, low, mid and high dose group). The results did no show statistically significance when dodine-treated groups were compared with controls nor a significant trend. The incidences of dodine-treated groups exceeded the HCD range for adenomas (25-32%), whereas the overall HCD mean (28.7%) was overcome in all groups, including controls.

On the other hand, the thryoid C-cell adenomas in females were increased in low dose group, but no at mid or top dose (29%, 35.8%, 29.8% and 24.6% for controls, low, mid and high dose groups). The results did no show statistically significance when dodine-treated groups were compared with controls nor a significant trend. Only the incidences of low dose group exceed the HCD range for adenomas (25-32%), whereas the overall HCD mean (28.7%) was overcome in all groups, except in the top dose female group.

Thyroid C-cell carcinomas: Malignant C-cell carcinomas were increase without showing a dose-response in mid and top male-dodine groups (6%, 2%, 11.7 and 11.3% for controls, low, mid and high dose groups). The results did no show statistically significance when dodine-treated groups were compared with controls nor a significant trend. The incidences of mid and high dodine-treated groups exceeded the HCD range (4-10%), and the overall mean (6.73%) for carcinomas.

On the other hand, the thryoid C-cell carcinomas in females were increased exlusively in low dose group, but no at mid or top dose (7.2%, 13.2%, 12.3% and 6.6% for controls, low, mid and high dose groups). The results did no show statistically significance when dodine-treated groups were compared with controls nor a significant trend. The incidences of female dodine-treated groups exceeded the HCD range (4-10%), and the overall mean (6.73%) for carcinomas.

Combined thyroid C-cell adenomas and carcinomas : Combined C-cell adenomas/carcinomas were increase in a dose related pattern in all male-dodine treated groups (35%, 40%, 45% and 53% for controls, low, mid and high dose groups). No statistical analysis were performed for the combined incidences. The incidences of all male-dodine treated groups exceeded the HCD range (27-38%), and the overall mean (32.74%) for the combined incidences.

On the other hand, combined C-cell adenomas/carcinomas in females were increased exlusively in low and mid dose groups, but no at top dose (36.2%, 49%, 42.1% and 31.1% for controls, low, mid and high dose groups). No statistical analysis were performed for the combined incidences. The incidences of female low and mid dose groups exceeded the HCD range (27-38%), and the overall mean (32.74%) for the combined incidences, but no at high dose.

Therefore, according to the criteria contained in Regulation (EC) No. 1272/2008, point 3.6.2.2.3 (b):

“Sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites”.

“Limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.”

Consequently, RMS deems that the relevance of thyroid neoplasms cannot not be excluded for human risk, based on the increased thyroid C-cell carcinomas and combined adenomas/carcinomas in male rats compared with controls, and the fact that the incidences exceed the range and mean of the HCD provided for the laboratory. Since thyroid C-cell tumours appeared only in rats and only in males, the RMS considers that according Regulation (EC) No. 1272/2008, these thyroid tumours triggers classification as carcinogenic in category 2.

Mice

Hepatocellular adenomas: Benign liver adenomas were increased without showing a clear dose-response only in top dose male group (13.3%, 11.7%, 15% and 23.3% for controls, low, mid and high dose males groups). The results did no show statistically significance when dodine-treated groups were compared with controls nor a significant trend. All the incidences recorded in males groups (including controls) were higher than mean (6.3%) and out of the range (4.1-10%) of the HCD provided.

On the other hand, the liver adenomas in females were also increased without showing a clear dose-response only in top dose female group (0%, 1.7%, 1.7% and 6.7% for controls, low, mid and high dose females groups, respectively). The results did no show statistically significance when dodine-treated groups were compared with controls, but showed a significant trend. In contrast to males, only top dose female group exceed both the mean (1.2%) and the range (0-3.3%) of HCD.

Hepatocellular carcinomas: malignant liver carcinomas were not increased after dodine administration in any sexe (3.3%, 0, 5% and 1.7% for controls, low, mid and high dose males groups; and 0, 1.7%, 0, and 1.7% for controls, low, mid and high dose females groups, respectively). Statistically significance was not obtained, and the values were all lower or close to mean and range of HCD.

Combined hepatocellular adenomas/carcinomas: Combined hepatocellular adenomas/carcinomas were slightly increased in mid and high male-dodine treated groups (16.7%, 11.7%, 20%, and 25% for controls, low, mid and high dose males groups). The results did no show statistically significance when dodine-treated groups were compared with controls nor a significant trend. The combined incidences in all males groups (including controls) were higher than mean (10.2%) and out of the range of HCD, with the exception of low dose group (range: 8.3-12.2%).

On the other hand, combined liver neoplasm incidences in females were slightly increased without showing a clear dose-response in all female-dodine treated groups, being the top dose group the group that showed the highest increase compared with controls (0%, 3.3%, 1.7% and 8.3% for controls, low, mid and high dose females groups, respectively). The results showed statistically significance only in top dose group compared with controls, and a significant trend. The combined female –treated groups incidences were slightly higher than HCD mean (2.4%), except mid dose group, whereas top dose group was the only that exceed the range (2-3.3%).

Table 56: Compilation of factors to be taken into consideration in the hazard assessment

Tumour type and background incidence	**Rat:** *Thyroid* ♂: Benign C-cell adenoma (low, mid and top dose groups). Malignant C-Cell carcinoma (mid and top dose groups). Combined C-cell adenomas/carcinomas (low, mid and top dose groups). ♀:- **Mouse:** *Liver* *♂/♀: Combined adenomas/carcinomas (high dose tested).*
Multi-site responses	No, malignant carcinomas–only appeared in thyroid.
Progression of lesions to malignancy	Likely. Benign C-cell thyroid adenomas could develop in malignant neoplasm. More information is deemed necessary.
Reduced tumour latency	No, since the tumours were observed at study termination (106 week).

Whether response single or both sexes	Thyroid C-cell adenomas and carcinomas were mainly detected in male rats.
Whether responses are in a single species or several species;	Only malignat carcinomas (thyroid C-cell carcinomas) were observed in a single specie (rats).
Routes of exposure	Only experimental studies by oral (dietary) route are available.
Possibility of a confounding effect of excessive toxicity at test doses	In rats, the increased incidences of combined thyroid C-cell adenomas and carcinomas were observed from low dose tested, in which no systemic toxicity was noted. Hepatocellular mice adenomas were recorded only at high dose, whereas systemic toxicity was observed from mid dose.
Mode of action and its relevance for humans	Dodine mode of action studies were not provided. More experimental evidences are deemed necesssary to suggest a plausible MoA.

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Based on the data available for dodine and according to the criteria under Regulation (EC) No. 1272/2008, RMS proposes the classification of this active substance as **carcinogenic in category 2 (H351).**

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

2.6.6.1 Adverse effects on sexual function and fertility – generational studies [equivalent to section 10.10.1 of the CLH report template]

Table 57: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Two-generation reproductive toxicity study in rats. GLP: Yes Method: US EPA FIFRA 83-4 Rat strain: Sprague-Dawley Sex: ♂ and ♀ No. animals: P/F1: 30 rats/sex/dose. Deviations from current test guideline (OECD TG 416, 2001):	Dodine; Lot/Batch No.: 1174, Purity: 98.6% Dodine :Oral (diet) Dose levels: ♂/♀: 0, 200 ppm (13.14/15.6 mg/kg bw/day for ♂/♀), 400 (26.2/31.2 mg/kg bw/day for ♂/♀) and 800 ppm (52.6/60.3 mg/kg bw/day for ♂/♀). Exposure: *Pre-mating treatment:* P/F1: 10 week *Mating:* 2 week Treatment continued in P and F_1 throughout	**PARENTAL TOXICITY (P)** Mortality: One female from control group and one male from low-dose group were sacrificed at week 9 and 7, respectively. The female was sacrificed after an apparent mechanical injury. Observations of hypoactive, red fluid on pan paper, dry brown material around the nasal area, and red stained hair-coat were noted before the sacrifice of the male. Clinical signs: No clinical signs related with dodine administration were observed in P animals. **800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)** *Bodyweight (bw)* *Pre-mating* ▪ (↓) bw in ♂ throughout week 1-12 (6-9%). ▪ (↓) bw in ♀ throughout week 3 (5%), 5-8 (5-6%), and 10 (5%). *Gestation* ▪ (↓) bw in ♀ at gestation day 0 (6%), 7(7%), 14 (6%) and 20 (6%). *Lactation* ▪ (↓) bw in ♀ at lactation day 0 (6%), 4 (8%), 7 (7%) and 14 (8%). *Accumulative bodyweight gain (bwg)* *Pre-mating* ▪ (↓) bwg in ♂ throughout week 0-12 (13-32%).	[redacted] (1996) (CA) B.6.6.1.1

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
- NOAEL/LOAEL (for sexual function and fertility, parents)
- target tissue/organ
- critical effects at the LOAEL</th><th>Reference</th></tr>
<tr><td>-Rationale selection was not show.
-No of implantations, corpora lutea and pre/post-implantation loss data were not showed.
-Thyroid and pituitary weights were not measured.
-Historical control data were not presented.
- Sperm evaluation in 100 cells per male, instead of 200.

Study acceptable</td><td>gestation and lactation of each litter.</td><td>▪ (↓) bwg in ♀ throughout week 0-10 (14-36%).
Lactation
▪ (↑) bwg in ♀ throughout lactation day 14-21 (20%) and 0-21 (84%).

<u>Food consumption (g/animal/day)</u>
Pre-mating
▪ (↓) in ♂ at week 1 (14%), 2 (9%) and 3 (7%).
▪ (↓) in ♀ at week 1 (15%), 2 (11%), 3 (11%), 4 (6%) and 5 (9%).
Lactation
▪ (↓) in ♀ throughout lactation day 4-7 (10%), 7-10 (19%), and 10-14 (15%).

<u>Organ weight</u>
▪ (↓) abs terminal bw in ♂ (7%).
▪ (↓) abs terminal bw in ♀ (3%).
▪ (↓) abs left kidney in ♂ (5%).
▪ (↓) abs thymus in ♂ (17%).
▪ (↓) abs brain in ♂ (3%).
▪ (↑) rel left and right adrenal in ♀ (14%).
▪ (↓) abs left and right kidney in ♀ (6%).
▪ (↑) rel brain in ♀ (7%).

<u>Necropsy</u>
▪ (↑) red focus area in the thymus in ♀ (19% vs 11% in controls, n.s. ndr).

<u>400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)</u>

<u>Bodyweight (bw)</u>
▪ (↓) bw in ♀ at lactation day 4 (4%).

<u>Food consumption</u>
Pre-mating
▪ (↓) abs in ♂ at week 1 (5%).
Lactation
▪ (↓) abs in ♀ at week 7-10 (9%).

<u>Organ weight</u>
▪ (↓) abs left kidney in ♀ (6%).

<u>SEXUAL FUNCTION AND FERTILITY (P→F1)</u>

Summary of reproductive indices (P→F1)

<table>
<tr><th colspan="2">Dose level</th><th>0 ppm</th><th>200 ppm</th><th>400 ppm</th><th>800 ppm</th></tr>
<tr><td>Number of paired females</td><td>N</td><td>29</td><td>30</td><td>30</td><td>30</td></tr>
<tr><td>Total number inseminated</td><td>N</td><td>28</td><td>30</td><td>29</td><td>30</td></tr>
<tr><td>Female mating index</td><td>%</td><td>97</td><td>100</td><td>97</td><td>100</td></tr>
<tr><td>Total number pregnant</td><td>N</td><td>28</td><td>28</td><td>26</td><td>27</td></tr>
<tr><td>Female fertility index</td><td>%</td><td>100</td><td>93</td><td>90</td><td>90</td></tr>
<tr><td>Gestation length (days)(mean±SD)</td><td></td><td>22.1 ± 0.4</td><td>22.1 ± 0.5</td><td>21.9 ± 0.5</td><td>22.1 ± 0.4</td></tr>
</table>
</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference

Sex ratio (% of males/ females at day 0	50/50	49/51	55/45	50/50

800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)

- (↓) fertility index (90% vs 100% in controls, ns., ncdr).
- (↑) live birth index (97% vs 96% in controls, ns., ndr).
- (↓) total number of pups delivered per litter (3%; ns., ndr).
- (↓) live pups/litter with live pups (1%; ns., ndr).

400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)

- (↓) fertility index (90% vs 100% in controls, ns., ncdr).
- (↑) live birth index (100% vs 96% in controls, ndr).
- (↓) total number of pups delivered per litter (8%; ns., ndr).
- (↓) live pups/litter with live pups (5%; ns., ndr).

200 ppm (equivalent to 13.4/15.6 mg/kg bw/day for ♂/♀)

- (↓) fertility index (93% vs 100% in controls, ns., ncdr).
- (↑) live birth index (98% vs 96% in controls, ndr).

DEVELOPMENTAL TOXICITY (F_1)

800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)

Bodyweight (bw)

- (↓) bw in ♂ at day 4 (precull) (7%).
- (↓) bw in ♀ at day 4 (precull) (9%)
- (↓) bw in ♂ at day 4 (postcull) (7%).
- (↓) bw in ♀ at day 4 (postull) (9%)
- (↓) bw in ♂ at day 7 (11%).
- (↓) bw in ♀ at day 7 (11%).
- (↓) bw in ♂ at day 14 (17%).
- (↓) bw in ♀ at day 14 (17%).
- (↓) bw in ♂ at day 21 (16%).
- (↓) bw in ♀ at day 21 (16%).

Organ weight

- (↓) terminal bw in ♂ (13%).
- (↓) terminal bw in ♀ (10%, ns.).
- (↓) abs left and right kidney in ♂ (16%).
- (↓) abs liver in ♂ (18%).
- (↑) rel brain in ♂ (12%).
- (↓) abs spleen in ♀ (18%, ns., ndr).
- (↓) rel spleen in ♀ (8%, ns., ndr).
- (↓) rel right kidney in ♂ (3%, ns., ndr).

400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)

Bodyweight (bw)

- (↓) bw in ♀ at day 4 (precull) (7%).
- (↓) bw in ♀ at day 4 (postull) (7%).
- (↓) bw in ♀ at day 14 (6%).
- (↓) bw in ♂ at day 21 (7%).
- (↓) bw in ♀ at day 21 (8%).

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
		Organ weight ▪ (↓) abs spleen in ♀ (25%, ndr). ▪ (↓) rel spleen in ♀ (17%, ndr). ▪ (↓) rel right kidney in ♂ (6%, ns., ndr). **200 ppm (equivalent to 13.4/15.6 mg/kg bw/day for ♂/♀)** *Organ weight* ▪ (↓) rel right kidney in ♂ (8%, ndr). **PARENTAL TOXICITY (F_1)** Mortality: One male in the mid-dose group was found dead at week 11. There were no clinical observations for this animal before death. Clinical signs: No clinical signs related with dodine administration were observed in F1 animals. **800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)** *Bodyweight (bw)* *Pre-mating* ▪ (↓) bw in ♂ throughout week 0-12 (13-19%). ▪ (↓) bw in ♀ throughout week 0-10 (12-15%). *Gestation* ▪ (↓) bw in ♀ at gestation day 0 (13%), 7(14%), 14 (14%) and 20 (12%). *Lactation* ▪ (↓) bw in ♀ at lactation day 0 (13%), 4 (15%), 7 (13%), 14 (13%) and 21 (8%). *Accumulative bodyweight gain (bwg)* *Pre-mating* ▪ (↓) bwg in ♂ throughout week 0-12 (10-15%). ▪ (↓) bwg in ♀ throughout week 0-10 (9-16%). *Gestation* ▪ (↓) bwg in ♀ throughout week 0-7 (20%), 7-14 (20%) and 0-20 (11%). *Lactation* ▪ (↑) bwg in ♀ throughout lactation day 14-21 (135%) and 0-21 (116%). *Food consumption (g/animal/day)* *Pre-mating* ▪ (↓) in ♂ throughout week 0-10 (6-14%). ▪ (↓) in ♀ throughout week 0-10 (9-18%). Gestation ▪ (↓) in ♀ throughout lactation day 0-7 (12%), 7-14 (17%), and 14-20 (12%). *Lactation* ▪ (↓) abs in ♀ throughout lactation day 4-7 (12%), 7-10 (15%), and 10-14 (20%). *Organ weight* ▪ (↓) terminal bw in ♂ (13%). ▪ (↓) terminal bw in ♀ (12%). ▪ (↑) rel left and right epididymis in ♂ (13%). ▪ (↑) rel left (12%) and right (14%) testis in ♂. ▪ (↑) rel left adrenal in ♂ (21%). ▪ (↓) abs left (15%) and right (14%) kidney in ♂.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference
		▪ (↓) abs liver in ♂ (16%). ▪ (↑) rel brain in ♂ (13%). ▪ (↑) rel left (23%) and right (20%) adrenal in ♀. ▪ (↓) abs left (11%) and right (12%) kidney in ♀. ▪ (↓) abs liver in ♀ (12%). ▪ (↓) abs brain in ♀ (4%). ▪ (↑) rel brain in ♀ (9%). ▪ (↑) rel left ovary/oviduct (11%) and right ovary/oviduct (11%, ns) n in ♀. *Necropsy* ▪ (↑) red focus area in the thymus in ♂ (13 vs 10% in controls, ns., ndr), and in ♀ (17% vs 10% in controls, ns. ndr). **400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)** *Bodyweight (bw)* *Pre-mating* ▪ (↓) bw in ♀ throughout week 0-8 (5-6%). *Gestation* ▪ (↓) bw in ♀ at gestation day 7(5%), 14 (5%) and 20 (5%). *Lactation* ▪ (↓) bw in ♀ at lactation day 4 (5%), 7 (4%) and 14 (5%). *Food consumption (g/animal/day)* *Pre-mating* ▪ (↓) in ♀ throughout week 3-4 (9%). *Lactation* ▪ (↓) in ♀ throughout week 7-10 (8%). *Organ weight* ▪ (↓) abs left (7%) kidney in ♂. ▪ (↑) rel left (12%) and right (9%, ns.) adrenal in ♀. ▪ (↓) abs left (5%) and right (6%) kidney in ♀. *Necropsy* ▪ (↑) red focus area in the thymus in ♂ (31 vs 10% in controls, ns., ndr), and in ♀ (22% vs 10% in controls, ns. ndr). **200 ppm (equivalent to 13.4/15.6 mg/kg bw/day for ♂/♀)** *Bodyweight (bw)* *Pre-mating* ▪ (↓) bw in ♀ throughout week 2-3 (5%). *Food consumption (g/animal/day)* *Pre-mating* ▪ (↓) in ♀ throughout week 4-5 (8%, ndr). *Necropsy* ▪ (↑) red focus area in the thymus in ♂ (23 vs 10% in controls, ns., ndr), and in ♀ (7% vs 10% in controls, ns. ndr). **SEXUAL FUNCTION AND FERTILITY (F1-F2)** **Summary of reproductive indices ($F_1 \rightarrow F_2$)** **Dose level** | **0 ppm** | **200 ppm** | **400 ppm** | **800 ppm**	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for sexual function and fertility, parents) - target tissue/organ - critical effects at the LOAEL	Reference

Number of paired females	**N**	30	30	30	30
Total number inseminated	**N**	30	30	29	30
Female mating index	**%**	100	100	97	100
Total number pregnant	**N**	30	29	27	30
Female fertility index	**%**	100	97	93	100
Gestation length (days)(mean±SD)		22.2 ± 0.4	22.1 ± 0.4	22.1 ± 0.4	22 ± 0.3
Sex ratio (% of males/ females at day 0)		51/49	50/50	50/50	49/51

800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)

- (↓) total number of pups delivered per litter (7%; ns., ndr).
- (↓) live pups/litter with live pups (7%; ns., ndr).

400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)

- (↓) total number of pups delivered per litter (11%; ns., ndr).
- (↓) live pups/litter with live pups (11%; ns., ndr).

200 ppm (equivalent to 13.4/15.6 mg/kg bw/day for ♂/♀)

- (↑) total number of pups delivered per litter (1%; ns., ndr).

DEVELOPMENTAL TOXICITY (F_2)

800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)

Bodyweight

- (↓) bw in ♂ at day 4 (precull) (9%).
- (↓) bw in ♂ at day 4 (postcull) (8%).
- (↓) bw in ♂ at day 7 (9%).
- (↓) bw in ♀ at day 7 (9%).
- (↓) bw in ♂ at day 14 (16%).
- (↓) bw in ♀ at day 14 (16%).
- (↓) bw in ♂ at day 21 (17%).
- (↓) bw in ♀ at day 21 (18%).

Organ weight

- (↓) terminal bw in ♂ (17%., ns.).
- (↓) terminal bw in ♀ (17%.).
- (↓) abs left and right kidney in ♂ (16%).
- (↓) abs spleen in ♂ (18%).
- (↓) abs liver in ♂ (17%).
- (↑) rel brain in ♂ (18%).
- (↓) abs left (14%) and right kidney (17%) in ♀.
- (↓) abs thymus in ♀ (28%).
- (↓) abs spleen in ♀ (22%).
- (↓) abs liver in ♀ (17%).
- (↑) rel brain in ♀ (15%).

400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
- NOAEL/LOAEL (for sexual function and fertility, parents)
- target tissue/organ
- critical effects at the LOAEL</th><th>Reference</th></tr>
<tr><td></td><td></td><td><u>Bodyweight</u>
▪ (↓) bw in ♂ at day 14 (5%).
▪ (↓) bw in ♂ at day 21 (7%).
▪ (↓) bw in ♀ at day 21 (7%).

<u>Organ weight</u>
▪ (↓) abs left kidney in ♂ (11%).

NOAEL sexual function and fertility: 800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for males/ females) based on no effects observed at high dose level.

NOAEL developmental: 200 ppm (equivalent to 13.14/15.6 mg/kg bw/day for males/ females) based on decreased male and females pup weights in F_1 and F_2 generation.

NOAEL parental toxicity: 200 ppm (equivalent to 13.14/15.6 mg/kg bw/day for males/ females) based on decreased bodyweights and increased relative adrenal weight in F_1 adult animals.</td><td></td></tr>
<tr><td>Reproductive toxicity study in rats.
<u>GLP</u>: No
<u>Method</u>: Non-stated
<u>Rat strain:</u> CFN rats

Sex: ♂ and ♀

<u>Deviations from current test guideline (OECD TG 416, 2001):</u>
- Test substance not fully characterised.
- Numerical results not reported.
- Only one dose tested.
- Measured parameters not fully described.

Supportive information</td><td>Dodine. Lot/Batch No.; Purity ≈ 97%

Dodine :Oral (diet)

Dose levels: ♂/♀: 0, 800 ppm (equivalent to 72 mg/kg bw/day).</td><td><u>Reproductive performance (statistical analysis not performed)</u>

<u>800 ppm (equivalent to 72 mg/kg bw/day for ♂/♀)</u>
▪ (↓) live pups per litter (8.6% vs 11.6 in controls).

<table>
<tr><th rowspan="3">Parameters</th><th colspan="8">Litter number</th></tr>
<tr><th colspan="2">F1</th><th colspan="2">F2a</th><th colspan="2">F2b</th><th colspan="2">F2c</th></tr>
<tr><th colspan="8">ppm</th></tr>
<tr><th>0</th><th>800</th><th>0</th><th>800</th><th>0</th><th>800</th><th>0</th><th>800</th></tr>
<tr><td>Litters born alive</td><td>10</td><td>10</td><td>14</td><td>20</td><td>11a</td><td>16b</td><td>8</td><td>17</td></tr>
<tr><td>Pups born alive</td><td>76</td><td>79</td><td>187</td><td>178</td><td>125</td><td>147</td><td>81</td><td>131</td></tr>
<tr><td>Pups weaned</td><td>64</td><td>72</td><td>167</td><td>156</td><td>103</td><td>118</td><td>74</td><td>108</td></tr>
<tr><td>Born live pups per litter</td><td>7.6</td><td>7.9</td><td>13.4</td><td>8.9</td><td>11.4</td><td>9.2</td><td>10.1</td><td>7.7</td></tr>
<tr><td>Weaned pups per litter</td><td>6.4</td><td>7.2</td><td>11.9</td><td>7.8</td><td>9.4</td><td>7.4</td><td>9.2</td><td>6.4</td></tr>
<tr><td>Mean wt of pups at weaning</td><td>40</td><td>41</td><td>29</td><td>34</td><td>35</td><td>32</td><td>36</td><td>35</td></tr>
<tr><td>Fertility index</td><td>83</td><td>83</td><td>88</td><td>100</td><td>86</td><td>85</td><td>73</td><td>100</td></tr>
<tr><td>Gestation index</td><td>100</td><td>100</td><td>100</td><td>100</td><td>92a</td><td>94b</td><td>100</td><td>100</td></tr>
<tr><td>Viability index</td><td>89</td><td>95</td><td>93</td><td>93</td><td>97</td><td>96</td><td>92</td><td>92</td></tr>
<tr><td>Lactation index</td><td>94</td><td>96</td><td>96</td><td>94</td><td>85</td><td>83</td><td>99</td><td>89</td></tr>
</table>
a) Does not include one female who died in the process of giving birth.
b) Does not include one female who gave birth to 11 stillborn pups.

NOAEL sexual function and fertility: -.

NOAEL developmental: -.

NOAEL parental toxicity: -</td><td>Levinskas. et al. (1961)
(CA)
B.6.6.1.2</td></tr>
</table>

Table 58: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 59: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

The potential effects of dodine on sexual function and fertility have been investigated in a two-generation reproductive toxicity study in Sprague Dawley rats. This study was performed according US EPA FIFRA 83-4 guideline, and it was therefore deficient in some endpoints such as number of implantations, corpora lutea and pre/post-implantation loss data were not reported, thyroid and pituitary weights were not measured, histopathology analysis was not performed in mid and low dose groups nor in pups, and HCD was not provided. However, this two-generation study is considered acceptable for the evaluation of sexual function and fertility assessment for the tested parameters.

In the two-generation reproductive study in rats, dodine was tested at 0, 200, 400 and 800 ppm dose levels (equivalent to 0, 13.4, 26.2, and 52.6 mg/kg bw/day for males and 0, 15.6, 31.2, 60.3 mg/kg bw/day for females, respectively), in which 30 pairs of males and females were mated from each test group. The rational for dose selection was not described in the study.

Neither gross mortality or morbidity signs, nor treatment related clinical signs were observed in any dose group. Few sporadic animals were found dead or humanely killed. These deaths were not considered related to treatment.

In P adult animals, mean bodyweights from the 800-ppm group were consistently lower throughout the P generation (premating, gestation, and lactation) after the initiation of treatment. Statistically significant differences were noted at high dose for the males throughout weeks 1-12 (6-9%); and for the females for weeks 3 (5%), 5-8 (5-6%), and 10 (5%) of premating, throughout all gestation period (6-7%), and on lactation days 0 (6%), 4 (8%), 7 (7%), and 14 (8%) compared with controls. Mean bodyweights were also statistically significantly lower for females given 400 ppm on lactation day 4 (4%). No other significant differences were observed in the mean bodyweight at low and mid dose tested compared with controls. Mean cumulative bodyweight changes were statistically significantly lower in the high dose males (13-32%) and females (14-36%) groups throughout the whole premating phase of P animals. On the other hand, bodyweight gain was statistically significantly higher in the high dose females group throughout lactation days 14-21 (20%) and throughout the whole lactation period (0-21 days; 84%) compared with controls. These differences in the trend were mainly caused due to dams from control, low and mid dose groups lost bodyweight during last week of lactation period.

In F1 adult animals, a statistically significant decrease in mean bodyweight was recorded at high dose male group (13-19%) during whole premating phase compared with controls. On the other hand, a slight decrease, no statistically significant was recorded in mid dose male group. In females, a statistically significant decrease was noted in the high dose group during premating (12-15%), gestation (12-14%) and lactation (8-15%) compared with controls. In the mid dose group, a reduction in bodyweight was also observed during the whole premating, gestation and lactation phases, although was only statistically significant during the premating week 0-8 (5-6%), gestation days 7 (5%), 14 (5%) and 20 (5%), and lactation days 4 (5%), 7 (4%) and 14 (5%). In the low dose female group, a slight decrease was noted in the whole premating, gestation lactation phases, although only statistically significant results were obtained during the premating week 2-3 (5%).

Absolute food consumption (g/animal/day) was statistically significant reduced in the high dose P male group from week 0 to 3 (7-14%) and in mid dose group in the first week (5%). There were no other significant differences in food consumption at high dose P male group at subsequent intervals or for males in the remaining test material-treated groups during the P generation, indicating that early decreases in food consumption were probably associated with decreased palatability. On the other hand, in the P female dose groups, mean food consumption was statistically

significant reduced throughout weeks 0-5 (6-15%) of premating phase, and during lactation days 4-7 (10%), 7-10 (19%), and 10-14 (15%) in the 800 ppm dose group, whereas in the mid dose group, mean food consumption was statistically significantly lower during lactation days 7-10 (9%).

Mean food consumption was significantly lower in the high dose male groups throughout the F1 generation compared with controls (6-14%). No relevant differences were noted in the low or mid male dose groups. In F1 females, statistically significant reduction in food consumption was recorded at high dose group throughout premating (9-18%), gestation (12-17%), and lactation (12-20%; except for days 0-4 of lactation) phases. Sporadic significant differences in mean food consumption were noted through week 4-5 (8%) at low dose group, and through weeks 3-4 (9%) and lactation days 7-10 (8%) at mid dose group.

Regarding macroscopic examinations, isolated statistically significant decreases were recorded in absolute left kidney (5%), thymus (17%), and brain (3%) weights at high dose male group of P generation, compared with controls. No statistically significant differences were noted in other P male organs from dodine-treated groups. On the other hand, P high dose females displayed increased relative adrenal weight (left and right), compared with controls (14%). Moreover, decrease in both absolute kidney weight (6%) were recorded in mid (only left kidney) and high dose P female groups, whereas relative brain weight was statistically significantly higher in high dose female group (7%).

In F1 males, statistically significant increases of relative weights were recorded at top dose level in right and left epididymis (13%), left (12%) and right testis (14%), left adrenal (21%) and brain (13%), compared with controls. On the other hand, absolute decreased weights were recorded in left (15%) and right kidney (14%), together with liver (16%) at high dose group. In the other dose groups from F1 male generation, only mid dose groups exhibited statistically significant decrease in left kidney absolute weight (7%) compared with controls. In F1 females, statistically significant increases in relative weights were recorded at top dose level in left (23%) and right adrenal (20%), brain (9%) and left ovary (11%); and in left adrenal (12%) at mid dose, compared with controls. On the other hand, absolute decreases weights were recorded in both kidneys in mid and high dose groups (5% and 11% for left kidney in mid and high dose groups, respectively; and 6% and 12% for right kidney at mid and high dose groups, respectively), together with liver (12%) and brain (4%) at high dose group.

In addition, no relevant differences were observed in necropsy analysis between P/F1 treated animals and controls. A slight increase, no statistically significant and no dose related, of lumen filled with fluid and red focus area incidences in the thymus were observed in P and F1 generation, whereas large pelvis in kidney and mottled thymus incidences were only observed in some F1 treated groups. The relevance of this findings is doubtful.

Histopathology examinations were only carried out in high dose groups and controls; in reproductive organs of females in the low and mid dose groups that failed to mate or that mated but failed to deliver a litter, and males that failed to sire progeny. Therefore, no adverse findings were recorded in P/F1 treated animals compared with controls.

Concerning estrous cycle in females and sperm parameters in males, no treatment-related differences were noted between dodine treated groups and controls in P and F1 adult animals.

Reproduction parameters such as mating, sex ratio or gestation length were not affected by treatment. Fertility indices (no. of females pregnant/no. females with positive signs of mating) for control, 200 ppm, 400 ppm and 800 ppm dose groups in the F1 generation were 100%, 93%, 90% and 90%, and 100%, 97%, 93% and 100% in the F2 generation. Therefore, the fact that trend observed during P-F1 generation was not further confirmed in the next generation, along with there was no statistical significance neither dose-response, the effect in fertility index observed in the P-F1 generation was deemed incidental and was ruled out as potential effect in sexual function and fertility. On the other hand, live birth index was statistically significant increase in the low and mid dose groups from F1 generation (96%, 98%, 100% and 97% for control, 200 ppm, 400 ppm and 800 ppm dose groups, respectively). Moreover, a decrease, not statistically significant and no dose-related, was noted in both F1 and F2 generations at mid and high dose groups in the mean total number of pups delivered per litter (8% and 3% for mid and high dose groups, respectively during F1 generation; and 11% and 7% for mid and high dose groups, respectively during F2 generation), and in the live pups/litter with live pups (5% and 1% for mid and high dose groups, respectively during F1 generation; and 11% and 7% for mid and high dose groups, respectively during F2 generation).

In the F1 pups, mean bodyweights were significantly lower for male and female pups from lactation days 4-21 in the high dose group; and on days 4 (precull and postcull, females only), 14 (females only), and 21 (males and females) for mid dose pups group. Regarding organ weights in this generation, absolute kidney (both) and liver weights were statistically significantly lower in high dose male group (16% and 18%, respectively), whereas relative brain weight was increased (12%), compared with controls. No statistically significant differences were noted in

other F1 male pups from treated-groups regarding organs weights. On the other hand, in F1 females weanling pups, statistically significantly differences were only observed in absolute and relative spleen weight at mid dose level (25% and 17%, respectively).

In F2 pups, mean bodyweights were statistically significantly lower on days 4 (pre-cull and post-cull, males only), 7, 14, and 21 for pups in the 800 ppm dose group, and on days 14 (males only) and 21 for pups in the 400 ppm dose group. Regarding organ weights, absolute kidney (both), spleen and liver weights were statistically significantly lower in high dose male group (16%, 28% and 17% for kidneys, spleen and liver, respectively), whereas relative brain weight was increased (18%), compared with controls. No statistically significant differences were noted in other F2 male pups from treated-groups regarding organs weights, except absolute left kidney weight in mid dose group. On the other hand, in F2 females weanling pups, absolute kidney (both), thymus, spleen and liver weights were statistically significantly lower in high dose group (14/17% for left/right kidney, 28% for thymus, 22% for spleen and 17% for liver, respectively), whereas relative brain weight was increased (15%), compared with controls. No statistically significant differences were noted in other organs at 800-ppm or low dose groups in F2 female pups.

Furthermore, no treatment related findings were recorded in any of F1 and F2 examined pups during necropsy evaluation.

Overall, there were no effects on sexual function and fertility at doses tested, although effects on developmental and parental were seen at mid and high dose tested.

Therefore, a **NOAEL** for **sexual function and fertility** has been established at **800 ppm (52.6/60.3 mg/kg bw/day for males/ females)** based on no effects observed at high dose level.

NOAEL for **developmental toxicity** has been established at **200 ppm (13.14/15.6 mg/kg bw/day for males/ females)** based on decreased male and females pup weights in F1 and F2 generation.

NOAEL for **parental toxicity** has been established at **200 ppm (13.14/15.6 mg/kg bw/day for males/ females)** based on decreased bodyweights in F1 adult animals, and increased relative adrenal weight in F1 adult animals.

-Information regarding sexual function and fertility parameters were also provided in an acute and chronic toxicity study in CFN rat strain (B.6.6.1.2). This study was not GLP, no guideline was followed, and presented important methodological deficiences. Animals were tested with 0 and 800 ppm (equivalent to 72 mg/kg bw/day). The various indexes calculated for the test and control animals, respectively, do not indicate any effect of feeding of 800 ppm of dodine on reproduction and lactation. The only difference of any apparent significance is a smaller mean litter size for the animals receiving dodine: 8.6 live pups per litter *vs* 11.6 live pups per litter for the controls. However, the authors of this publication stated that the litter size decrease in treated rats resulted from the prevalence of an unusually large litter size among the controls, rather than any diminution in fertility of the test animals. This conclusion could not be verified by the RMS, as no HCD was available for this laboratory, and no other reproductive studies were provided in the dossier with the CFN rat strain.

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

No human information is available on the effects of dodine on the reproductive system. Information from a reliable two-generation study in rats showed that dodine has no effects on sexual function and fertility. The available study did not include the no. of implantations, corpora lutea and pre/post-implantation losses. However, data from this study is regarded conclusive for classification since sexual function and fertility parameters available such as mating, and fertility indices, sex ratio, and gestation length were not affected by treatment. No significant nor dose related effects were seen on litters in both studies and consequently potential impairment of fertility in relation with implantation is not considered.

Consequently, classification for sexual function and fertility is not warranted.

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

Table 60: Summary table of animal studies on adverse effects on development

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
- NOAEL/LOAEL (for parent, offspring and for developmental effects)
- target tissue/organ
- critical effects at the LOAEL
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr>
<td>Dose range-finding developmental toxicity study in rats.
GLP: Yes
Method: In house method
Rat strain: Sprague-Dawley

Sex:10 mated females/group

Deviations from current test guideline (OECD TG 414, 2018):
-At least 20 female animals with implantation sites at necropsy should be used.
-The test chemical was not administered to the day prior to scheduled caesarean section.
-Mating index (number of sperm-positive females) data was not showed.
-The following developmental endpoints were not measured: sex ratio, anogenital distance, and indication of incomplete testicular descent/cryptorchidism.
-Thyroid weight and thyroid hormones (T3/T4/TSH) values from dams were not recorded.
-External, visceral and skeletal abnormalities were not examined in foetuses.
-Statistical analysis not performed in most of the tested parameters.

Supportive information</td>
<td>Dodine, Lot/Batch No.:APA 92/88/2; Purity: 95%

Dodine:Oral (gavage)

Dose levels: ♀: 0, 50, 70, 100 mg/kg bw/day from day 6 to 16 of pregnancy both included

Parameters observed:
Maternal data: Clinical signs, mortality, bw and bwg, food consumption, necropsy, histopathology.

Reproductive data: Number (no.) of corpora lutea, no. implants, uterus wt, litter wt.

Foetal data: Foetus wt, deaths.</td>
<td>Maternal toxicity

Mortality: Only one female at high dose group was killed due to morbidity signs.

Clinical signs: At high dose group, one dam showed wheezing, and another dam showed piloerection, hunched posture, red/brown staining around face, fore-paws and mild ataxia. This dam was humanely killed.

100 mg/kg bw/day
Bodyweight and bodyweight gain:
▪ (↓) bw on days 0 (2%, ns, ndr), 6 (1%, ns, ndr), 9 (4%, ns, ndr), 13 (8%, ndr), 17 (4%, ns, ndr) and 20 (2%, ns, ndr).
▪ (↓) bwg through days 6-9 (65%, ns), 9-13 (51%, ns), 6-13 (48%), 6-17 (17%, ns).

Food consumption (statistical analysis not performed):
▪ (↓) through days 6-16 (24%).

Necropsy (statistical analysis not performed)
▪ (↑) ureter dilatation (20% vs 0% in controls).
▪ (↑) kidney pelvic dilatation (30% vs 0% in controls).
▪ (↑) enlarged kidney (30% vs 0% in controls).

Histopathology (statistical analysis not performed)
▪ (↑) epithelial hyperplasia and chronic inflammation in the urinary bladder (10% vs 0% in controls).
▪ (↑) inflammation in the ureters (10% vs 0% in controls).
▪ (↑) pelvic dilatation, pelvic inflammation and nephritis in the kidney (10% vs 0% in controls).
▪ (↑) hyperplasia in the lumbar lymph node (10% vs 0% in controls).

70 mg/kg bw/day
Bodyweight and bodyweight gain:
▪ (↓) bw on days 0 (8%, ns, ndr), 6 (8%, ns, ndr), 9 (8%, ns, ndr), 13 (10%, ndr), 17 (9%, ns, ndr) and 20 (8%, ns, ndr).
▪ (↓) bwg through days 6-9 (26%, ns), 9-13 (25%, ns), 13-17 (6%, ns), 6-13 (26%), 6-17 (16%, ns).

Food consumption (statistical analysis not performed):
▪ (↓) through days 6-16 (15%).

50 mg/kg bw/day
Food consumption:
▪ (↓) through days 6-16 (7%).

Sexual function and fertility (statistical analysis not performed).
<table>
<tr><th colspan="2" rowspan="2"></th><th colspan="4">Dose (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>50</th><th>70</th><th>100</th></tr>
<tr><td>Number of paired animals</td><td>N</td><td>10</td><td>10</td><td>10</td><td>10</td></tr>
<tr><td>Total number pregnant</td><td>N</td><td>10</td><td>10</td><td>10</td><td>9</td></tr>
<tr><td>Female fertility index</td><td>%</td><td>100</td><td>100</td><td>100</td><td>90</td></tr>
</table>
</td>
<td>[redacted] (1989a) (CA) B.6.6.2.1</td>
</tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference

100 mg/kg bw/day
- (↓) fertility index (90% vs 100% in controls).

Developmental toxicity (statistical analysis not performed)

100 mg/kg bw/day
- (↑) mean dead implants (26%).
- (↑) mean early resorptions (26%).
- (↓) foetal weight (4%).

	Dose (mg/kg bw/day)			
	0	50	70	100
Corpora lutea				
Total corpora lutea	151	160	159	134
mean corpora lutea (mean±SD)	15.1±1.29	16±1.76	15.9±1.29	16.8±1.67
Implants				
Total implants	148	155	146	131
mean implants (mean±SD)	14.8±1.03	15.5±1.72	14.6±0.97	16.4±1.69
% preimplantation loss	2%	3%	8%	2%
Total live implants	143	146	142	126
mean live implants (mean±SD)	14.3±1.16	14.6±1.96	14.2±1.23	15.8±1.67
Total dead implants	5	9	4	5
mean dead implants (mean±SD)	0.5±0.55	0.9±1.29	0.4±0.52	0.63±0.92
% dead implants /litter	4.1%	1.8%	2.3%	3%
Total early death (early resorptions)	5	7	4	5
mean early deaths implants (mean±SD)	0.5±0.53	0.7±0.82 (↑40%)	0.4±0.52 (↓20%)	0.63±0.92 (↑26%)
Total late deaths (late resorptions)	0	2	0	0
mean late deaths (mean±SD)	0	0.2±0.63	0	0
Mean uterus weight (mean±SD)	80.1±8.9	83.4±9.1	78.3±5.9	87.1±10.6
Mean litter weight (mean±SD)	50.6±7	51.1±5.9	49.1±3.4	49.1±6.6
Mean foetal weight (mean±SD)	3.53±0.31	3.51±0.21	3.46±0.13 (↓2%)	3.39±0.12 (↓4%)

70 mg/kg bw/day
- (↓) mean dead implants (20%).
- (↓) mean early resorptions (20%).
- (↓) foetal weight (2%).

50 mg/kg bw/day
- (↑) mean dead implants (80%).
- (↑) mean early resorptions (40%).

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
- NOAEL/LOAEL (for parent, offspring and for developmental effects)
- target tissue/organ
- critical effects at the LOAEL
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>External, visceral or skeletal abnormalities were not measured in foetuses.

NOAEL developmental toxicity: 100 mg/kg bw/day based on no effects observed at high dose tested.

NOAEL maternal toxicity: 50 mg/kg bw/day based on decreased bodyweight gain and food consumption.</td><td></td></tr>
<tr><td>Main developmental toxicity study in rats.

GLP: Yes

Method: US EPA FIFRA 83-3

Rat strain: Sprague-Dawley

Sex:25 mated females/group

Deviations from current test guideline (OECD TG 414, 2018):

-The test chemical was not administered to the day prior to scheduled caesarean section.
-Mating index (number of sperm-positive females) data was not showed.
-Anogenital distance and indication of incomplete testicular descent/cryptorchidism were not measured.
-Thyroid weight and thyroid hormones (T3/T4/TSH) values from dams were not recorded.
-Statistical analysis not performed in most of the tested parameters.
-Historical control data were not provided.

Acceptable</td><td>Dodine, Lot/Batch No.:APA 92/88/2; Purity: 95%

Dodine:Oral (gavage)

Dose levels: ♀: 0, 10, 45, 90 mg/kg bw/day from day 6 to 16 of pregnancy both included

Parameters observed:
Maternal data: Clinical signs, mortality, bw and bwg, food consumption, necropsy, histopathology.

Reproductive data: Number (no.) of corpora lutea, no. implants, uterus wt, litter wt., sex ratio.

Foetal data: Foetus wt, deaths.</td><td>Maternal toxicity

Mortality: No deaths were recorded.

Clinical signs: Three dams at high dose group showed excessive salivation after dosing for one or 2 days during the treatment period. On the other hand, there was another three animals with red/brown stained fur around the mouth at 90 mg/kg bw/day dose groups and one in the 45 mg/kg bw/day dose group. Besides, one dam from high dose group showed noisy breathing after dosing.

90 mg/kg bw/day
Bodyweight and bodyweight gain (Statistical analysis from [redacted] 2019a)
▪ (↓) bw on days 9 (9%), 13 (8%), and 17 (8%).
▪ (↓) bwg through days 6-9 (107%), 6-17 (20%).
▪ (↓) corrected bwg by uterus wt through days 6-17 (756%).

Food consumption gain (Statistical analysis from [redacted], 2019a):
▪ (↓) at day 6 (30%), 7 (32%), 8 (37%), 9 (31%), 10 (22%), 11 (15%), 12 (13%), 13 (17%), 14 (16%), 15 (17%), 16 (19%), through days 6-10 (30%), 6-16 (22%), 3-19 (14%).

Necropsy (statistical analysis not performed)
Lung
▪ (↑) dark red areas on lung lobes (8% vs 0% in controls).

Histopathology (statistical analysis not performed)
Lung
▪ (↑) congestion (8% vs 0% in controls).
▪ (↑) haemorrhage into alveoli (4% vs 0% in controls).

45 mg/kg bw/day
Bodyweight and bodyweight gain (Statistical analysis from [redacted] 2019a)
▪ (↓) bwg through days 6-9 (42%).
▪ (↓) corrected bwg by uterus wt through days 6-17 (333%).

Food consumption ((Statistical analysis from [redacted], 2019a):
▪ (↓) at day 6 (11%), 8 (18%), 9 (17%), 10 (12%), 11 (15%), 12 (13%), 13 (17%), 14 (16%), 15 (17%), 16 (19%), through days 6-10 (14%) and 6-16 (11%).

Sexual function and fertility (statistical analysis from [redacted] 2019a)

<table>
<tr><th colspan="2" rowspan="2"></th><th colspan="4">Dose (mg/kg bw/day)</th></tr>
<tr><th>0</th><th>10</th><th>45</th><th>90</th></tr>
<tr><td>Number of paired animals</td><td>N</td><td>25</td><td>25</td><td>25</td><td>25</td></tr>
<tr><td>Total number pregnant</td><td>N</td><td>22</td><td>21</td><td>23</td><td>24</td></tr>
</table>
</td><td>[redacted]
(1989b)
(CA)
B.6.6.2.2

[redacted]
(2019a)
(CA)
B.6.6.2.2</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference

Female fertility index	**%**	88	84	92	96
Sex ratio ♂/♀	**%**	51/49	48/52	48/52	46/54

Developmental toxicity

No adverse differences were observed between treated groups and controls for the tested parameters.

	Dose (mg/kg bw/day)			
	0	**10**	**45**	**90**
Corpora lutea				
Total corpora lutea	320	295	348	361
mean corpora lutea ¥	14.5±1.7	14±2.6	15.1±2	15±1.8
Implants				
Total implants	303	254	324	347
mean implants ¥	13.8±2.7	12.1±4.9	14.1±3.3	14.5±2.3
% preimplantation loss	5%	14%	7%	4%
Total live implants	290	248	316	336
mean live implants ¥	13.2±2.6	11.8±4.8	13.7±3.3	14±2.2
% live implants	96%	98%	98%	97%
Total dead implants	13	6	8	11
mean dead implants ¥	0.6±0.7	0.3±0.7	0.3±0.6	0.5±0.6
% dead implants (post-implantation loss)	4%	2%	2%	3%
Total early death (early resorptions)	13	5	8	9
mean early deaths implants ¥	0.6±0.7	0.2±0.5	0.3±0.6	0.4±0.6
% early deaths (early resorptions)	4%	2%	2%	2%
Total late deaths (late resorptions)	0	1	0	2
Mean late deaths ¥	0	0.05±0.2	0	0.1±0.3
% late deaths (late resorptions)	0	0.4%	0	1%
% foetal deaths	0	0	0	0
Mean uterus weight (g) ¥	79.4±13.9	72±26.7	83.5±19.3	85.31±10
% Sex ratio ♂/♀ ¥	51/49	48/52	48/52	46/54
Mean foetal weight (g) ¥	3.84±0.22	3.94±0.33	3.86±0.27	3.85±0.27

¥ Statistical analysis from [redacted] 2019a. Not statistically significant

Foetal alterations:

Visceral alterations

No relevant differences in visceral findings between treated groups and controls.

Skeletal alterations

90 mg/kg bw/day

- 8.8% of foetuses (ndr)/ 30% of litters (ns, ncdr) with phalangeal elements, one or more ossified *vs* 2.7% of foetuses / 14% of litters in controls.

45 mg/kg bw/day

- 12% of foetuses (ndr)/ 27% of litters (ns, ncdr) with phalangeal elements, one or more ossified *vs* 2.7% of foetuses / 14% of litters in controls.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		10 mg/kg bw/day ▪ 5.5% of foetuses (ns)/ 29% of litters (ns, ncdr) with phalangeal elements, one or more ossified *vs* 2.7% of foetuses / 14% of litters in controls. **NOAEL** developmental toxicity: 90 mg/kg bw/day based on no adverse effects observed at high dose tested. **NOAEL** maternal toxicity: 10 mg/kg bw/day based on reduced bodyweight gain (6-9 GD) and reduction in food consumption.	
Dose range-finding developmental toxicity study in rabbits. GLP: Yes Method: In house method Rat strain: New Zealand White rabbits Sex:10 mated females/group Deviations from current test guideline (OECD TG 414, 2018): -At least 20 female animals with implantation sites at necropsy should be used. -The test chemical was not administered to the day prior to scheduled caesarean section. -Mating index (number of sperm-positive females) data was not showed. -The following developmental endpoints were not measured: sex ratio, and indication of incomplete testicular descent/cryptorchidism in male foetuses. -Thyroid weight and thyroid hormones (T3/T4/TSH) values from dams were not recorded. -External, visceral and skeletal abnormalities were not performed in foetuses.	Dodine, Lot/Batch No.:APA 92/88/2; Purity: 95% Dodine:Oral (gavage) Dose levels: ♀: 0, 70, 100 mg/kg bw/day from day 6 to 18 of pregnancy both included Parameters observed: *Maternal data:* Clinical signs, mortality, bw and bwg food consumption, necropsy, histopathology *Reproductive data:* Number (no.) of corpora lutea, no. implants, uterus wt, litter wt. *Foetal data:* Foetus wt, deaths.	***Maternal toxicity*** Mortality: Five animals at high dose group and one in low dose group were humanely killed due to morbidity signs. Clinical signs: **100 mg/kg bw/day** -One animal was found dead at GD 13 and showed red staining around lower abdomen. -One animal showed right eye swollen throughout GD 8-17 and was humanely killed due to it poor condition at GD 17. -One animal showed irises darker through GD 10-12 than and was humanely killed due to it poor condition at GD 12. -One animal showed after dosing at GD 10, fur wet under chin. 2 h after dosing showed breathing difficulties and noisy slightly cyanosed, subdued, so it was immediately killed. -One animal was humanely killed due to it poor condition at GD 17 after showing few or no faeces through GD 10-17. Hyperplasia of the stomach mucosa, gaseous distension of the caecum with softened/liquid contents, and reduced faecal output was found in these death animals. Additionally, liquid faeces were also found in another two survival dams. **70 mg/kg bw/day** One animal was humanely killed due to it poor condition at GD 17 after showing few or no faeces through GD 8-17. Histopathology analysis revealed hyperplasia of the stomach mucosa in this animal. **100 mg/kg bw/day** Bodyweight: No differences were noted between treated groups and controls. Bodyweight gain: ▪ (↓) through days 6-19 (48%). Food consumption (statistical analysis not performed): ▪ (↓) through days 6-18 (31-77%). Necropsy (statistical analysis not performed) ▪ (↑) Liquid contents caecum/gaseous distension (50% vs 0% in controls). Histopathology (statistical analysis not performed) *Stomach* ▪ (↑) Cream coloured parches on mucosa. Pyloric part covered in colourness viscous fluid in stomach (30% vs 0% in controls). ▪ (↑) Dark point foci. Blood and sloughing of mucosa. Hyperplasia of fundic epithelium (20% vs 0% in controls). *Liver* ▪ (↑) Lobulation prominent. Mild chronic inflammation (periportal). Hepatocytes necrosis (20% vs 0% in controls).	[redacted] (1989a) (CA) B.6.6.2.3

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
-Statistical analysis not performed in most of the tested parameters. **Supportive information**		*Kidney* ▪ (↑) Red foci/chronic inflammation (20% vs 0% in controls). **70 mg/kg bw/day** Necropsy (statistical analysis not performed) ▪ (↑) Liquid contents caecum/gaseous distension (10% vs 0% in controls). Histopathology (statistical analysis not performed) *Stomach* ▪ (↑) Cream coloured parches on mucosa. Pyloric part covered in colourness viscous fluid in stomach (10% vs 0% in controls). ***Sexual function and fertility toxicity (statistical analysis not performed).*** **Dose (mg/kg bw/day)** **0** **70** **100** **Number of paired animals** **N** 10 10 10 **Total number pregnant** **N** 8 9 8 **Female fertility index** **%** 80 90 80 ***Developmental toxicity (statistical analysis not performed)*** **Dose (mg/kg bw/day)** **0** **70** **100** **Corpora lutea** Total corpora lutea 87 99 84 mean corpora lutea (mean±SD) 10.9 ±1.9 12.4± 2 10.5± 2.7 **Implants** Total implants 75 90 66 mean implants (mean±SD) 9.4 ± 2.5 11.3 ± 2.4 10.5± 2.7 % preimplantation loss 14% 9% 22% Total live implants 62 76 51 Mean live implants (mean±SD) 7.8 ±2.8 9.5± 3.9 6.4± 3 Total dead implants 13 14 15 mean dead implants (mean±SD) 1.6 ± 1.2 1.8 ±2.7 1.9 ±2.2 Total early death (early resorptions) 13 12 10 Mean early deaths implants (mean±SD) 1.6 ± 1.2 1.5 ± 2.5 1.2 ± 1.4 Total late deaths (late resorptions) 0 1 5 mean late deaths (mean±SD) 0 0.1 ±0.4 0.8 ± 1.6 Total foetal death 0 1 0 Mean foetal deaths (mean±SD) 0.1 ± 0.4 **Mean uterus weight (mean±SD) (g)** 504 ± 107 551 ± 157 326.2 ± 145.3 (↓35%) **Mean foetal weight (mean±SD) (g)** 45 ± 4.7 41.4±8.9 (↓8%) 43.1 ±10.7 (↓4%) **100 mg/kg bw/day** ▪ (↑) pre-implantation loss (22% vs 14% in controls). ▪ (↓) mean live implants.	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
- NOAEL/LOAEL (for parent, offspring and for developmental effects)
- target tissue/organ
- critical effects at the LOAEL
[Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]</th><th>Reference</th></tr>
<tr><td></td><td></td><td>▪ (↑) mean dead implants.
▪ (↑) mean late resorptions.
▪ (↓) mean uterus weight (35%).
▪ (↓) foetal wt (4%, ndr).

70 mg/kg bw/day
▪ (↑) mean dead implants.
▪ (↑) mean late resorptions.
▪ (↓) foetal wt (8%, ndr).

External, visceral or skeletal abnormalities were not measured in foetuses.

NOAEL developmental: 70 mg/kg bw/day based on the increase of late resorptions seen at 100 mg/kg bw/day.

NOAEL maternal toxicity: 70 mg/kg bw/day based on decreased bodyweight gain and food consumption, necropsy and histopathological findings in stomach, kidney and liver.</td><td></td></tr>
<tr><td>Main developmental toxicity study in rabbits.

GLP: Yes

Method: US EPA FIFRA 83-3

Rat strain: New Zealand White rabbits

Sex: 16/20 mated females/group
(20 for high dose group; 16/dose for the rest)

Deviations from current test guideline (OECD TG 414, 2018):

-The test chemical was not administered to the day prior to scheduled caesarean section.
-At least 20 female animals with implantation sites at necropsy should be used at all dose groups.
-Mating index (number of sperm-positive females) data was not showed.
- Incomplete testicular descent/cryptorchidism were not measured in male foetuses.
-Thyroid weight and thyroid hormones (T3/T4/TSH) values from dams were not recorded.</td><td>Dodine Lot/Batch No.:APA 92/88/2; Purity: 95%

Dodine:Oral (gavage)

Dose levels: ♀: 0, 10, 40 and 80 mg/kg bw/day from day 6 to 18 of pregnancy both included

Parameters observed:
Maternal data: Clinical signs, mortality, bw and bwg, food consumption, necropsy, histopathology.

Reproductive data: Number (no.) of corpora lutea, no. implants, uterus wt, litter wt., sex ratio.

Foetal data: Foetus wt, deaths.</td><td>Maternal toxicity

Mortality: At 80 mg /kg bw/day, 3 mortalities were recorded (1 died, 2 were killed to poor condition): one animal died at GD 15 after showing breathing difficulties, one animal was humanely killed at GD 11 after showing same clinical signs, and another was also killed because of poor condition.

At 40 mg/kg bw/day, only one animal was found dead due to an accidental damage during dosing.

Clinical signs:

Most of clinical signs were observed in the top dose group. 15% of rabbits showed liquid faeces, breathing difficulties and emaciation. Besides, 10% showed pale eyes and two dams (10%) suffered abortions. Another clinical signs that showed low incidences were recorded in this group. Blood in cage and breathing difficulties were noted in the mid and high dose groups, whereas liquid faeces were seen in all groups. On the other hand, in control and low dose groups, an abortion in each group was also recorded.

<table>
<tr><th></th><th colspan="4">Dose (mg/kg bw/day)</th></tr>
<tr><th></th><th>0</th><th>10</th><th>40</th><th>80</th></tr>
<tr><td>Liquid faeces</td><td>1 (6%)</td><td>2 (12.5%)</td><td>1 (6%)</td><td>3 (15%)</td></tr>
<tr><td>No faeces</td><td></td><td></td><td></td><td>1 (5%)</td></tr>
<tr><td>Lip bitten during dosing</td><td>1(6%)</td><td></td><td></td><td></td></tr>
<tr><td>Slightly weeping right eye</td><td>1(6%)</td><td>1(6%)</td><td></td><td></td></tr>
<tr><td>Red eyes</td><td>1(6%)</td><td></td><td></td><td>1(5%)</td></tr>
<tr><td>Scabbing on tail</td><td></td><td></td><td>1(6%)</td><td></td></tr>
<tr><td>Blood in cage</td><td></td><td></td><td>2 (12.5%)</td><td>1(5%)</td></tr>
<tr><td>Breathing difficulties</td><td></td><td></td><td>1(6%)</td><td>3(15%)</td></tr>
<tr><td>Emaciation</td><td></td><td></td><td></td><td>3(15%)</td></tr>
<tr><td>Pale eyes</td><td></td><td></td><td></td><td>2 (10%)</td></tr>
</table></td><td>[redacted] (1989b) (CA) B.6.6.2.4

[redacted] (2019b) (CA) B.6.6.2.4</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference

-Statistical analysis not performed in most of the tested parameters.
-Historical control data were not provided.
-Visceral and skeletal incidences were not presented using the litter as the basic unit of analysis.

Acceptable

Splayed forelimbs				1(5%)
Dark ears				1(5%)
Cold ears				1(5%)
Convulsions				1(5%)
Abortion	1 (6%)	1(6%)		2(10%)

80 mg/kg bw/day
Bodyweight and bodyweight gain (Statistical analysis from [redacted], 2019b) :
No differences were noted between treated groups and controls.

Food consumption (Statistical analysis from [redacted] 2019b):
- (↓) through gestation day 6 (25%), 7 and 8 (30%).

Necropsy (statistical analysis not performed)
- (↑) dark lung patches (20% vs 0% controls)
- (↑) intestine distension (10% vs 0% controls).

Histopathology
No histopathological findings were reported in the study.

40 mg/kg bw/day
Necropsy (statistical analysis not performed)
- (↑) dark lung patches (12.5% vs 0% controls)

Sexual function and fertility (statistical analysis from [redacted] 2019b).

		Dose (mg/kg bw/day)			
		0	**10**	**40**	**80**
Number of mated animals	**N**	16	16	16	20
Total number pregnant	**N**	15	15	16	17
Abortions	**N**	1	1	0	2
Killed	**N**	0	0	0	2
Died	**N**	0	0	1	1
Total number day 29		14	14	15	12
Female fertility index	**%**	94	94	100	85

Developmental toxicity

	Dose (mg/kg bw/day) (number of litters at terminus)			
	0 (14)	**10 (14)**	**40 (15)**	**80 (12)**
Corpora lutea				
Total corpora lutea	154	167	175	145
mean corpora lutea ¥	11±2.5	11.9±3.2	11.7±1.9	12.1±1.9
Implants				
Total implants	128	139	150	118
mean implants ¥	9.1±2.5	9.9±2	10±2.3	9.8±3.5
% preimplantation loss	17%	17%	14%	19%
Total live implants	115	124	121	98
mean live implants ¥	8.2±2.2	8.9±1.9	8.1±2.3	8.2±2.9

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference

% live implants	90%	89%	81%	83%
Total dead implants	13	15	29	20
mean dead implant ¥	0.9±1.2	1.1±1.4	1.9±1.8	1.7±1.6
% dead implants (post-implantation loss)	10%	10%	19%	17%
Total early death (early resorptions)	7	10	15	10
mean early deaths¥	0.5±0.9	0.7±1.1	1±1.1	0.8±1.1
% early resorptions	5%	7%	10%	8%
Total late deaths (late resorptions)	2	1	9	5
mean late deaths ¥	0.1±0.4	0.1±0.3	0.6±1.1	0.4±0.9
% late resorptions	2%	1%	6%	4%
Total foetal dead	4	4	5	5
Mean foetal deaths ¥	0.3±0.5	0.3±0.7	0.3±0.6	0.4±0.9
% foetal deaths	3%	3%	3%	4%
Mean uterus weight (g) ¥	542±101	573±104	539±124	539±156
% Sex ratio ♂/♀	49/51	46/54	56//44	50/50
Mean foetal weight (g)	44.8±6	43.3±3.3	43.6±5	44.5±5.8

¥ Statistical analysis from [redacted] 2019b. Not statistically significant.

80 mg/kg bw/day
- (↑) total dead implants (ndr).
- (↑) mean dead implants (ndr).
- (↑) % dead implants (ndr).
- (↑) total late resorptions (ndr).
- (↑) mean late resorptions (ndr).
- (↑) % late resorptions (ndr).

40 mg/kg bw/day
- (↑) total dead implants (ndr).
- (↑) mean dead implants (ndr).
- (↑) % dead implants (ndr).
- (↑) total late resorptions (ndr).
- (↑) mean late resorptions (ndr).
- (↑) % late resorptions (ndr).

Foetal alterations

Visceral alterations

80 mg/kg bw/day
- 22% of foetuses (ns, ndr)/ 92% of litters (ns, ndr) with small accessory vessel arising from innominate artery, left carotid artery or aortic arch *vs* 17% of foetuses / 79% of litters in controls.

40 mg/kg bw/day
- 23% of foetuses (ns, ndr)/ 73% of litters (ns, ndr) with small accessory vessel arising from innominate artery, left carotid artery or aortic arch *vs* 17% of foetuses / 79% of litters in controls.

10 mg/kg bw/day
- 33% of foetuses (ndr)/ 100% of litters (ns, ndr) with small accessory vessel arising from innominate artery, left carotid artery or aortic arch *vs* 17% of foetuses / 79% of litters in controls.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL [Effects statistically significantly and dose-related unless stated otherwise as not significant (n.s.) or not dose-related (ndr)/ncdr (not clearly dose-related)]	Reference
		Skeletal alterations **80 mg/kg bw/day** ▪ 3% of foetuses (ns, ndr)/ 17% of litters (ns, ndr) with cornua(a) of hyoid bent inwards *vs* 0% of foetuses / 0% of litters in controls. ▪ 34% of foetuses (ncdr)/ 92% of litters (ncdr) with bilateral complete 13th rib *vs* 8% of foetuses / 29% of litters in controls. ▪ 3% of foetuses (ns, ndr)/ 17% of litters (ns, ndr) with hyoid retarded *vs* 1% of foetuses / 7% of litters in controls. **40 mg/kg bw/day** ▪ 8% of foetuses (ndr)/ 33% of litters (ndr) with cornua(a) of hyoid bent inwards *vs* 0% of foetuses / 0% of litters in controls. ▪ 15% of foetuses (ns, ndr)/ 40% of litters (ns, ndr) with bilateral complete 13th rib *vs* 8% of foetuses / 29% of litters in controls. ▪ 6% of foetuses (ns, ndr)/ 13% of litters (ns, ndr) with hyoid retarded *vs* 1% of foetuses / 7% of litters in controls. **10 mg/kg bw/day** ▪ 2% of foetuses (ns, ndr)/ 14% of litters (ns, ndr) with cornua(a) of hyoid bent inwards *vs* 0% of foetuses / 0% of litters in controls. ▪ 30% of foetuses (ndr)/ 71% of litters (ndr) with bilateral complete 13th rib *vs* 8% of foetuses / 29% of litters in controls. ▪ 16% of foetuses (ndr)/ 29% of litters (ns, ndr) with hyoid retarded *vs* 1% of foetuses / 7% of litters in controls. **NOAEL developmental toxicity**: 10 mg/kg bw/day based on developmental effects such as increase of post implantation losses and late resorptions seen from 40 mg/kg bw/day. **NOAEL maternal toxicity:** 40 mg/kg bw/day based on mortality, clinical signs and reduced food consumption noted at high dose tested.	

Table 61: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 62: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development

Two main developmental toxicity studies, one conducted in rats (B.6.6.2.2), and one in rabbits (B.6.6.2.4), have been presented to support the renewal assessment of the active substance dodine. Both studies were preceded by their respective pilot studies (B.6.6.2.1 in rats, and B.6.6.2.3 in rabbits), performed to estimate the suitable dose levels for the main teratogenicity studies. Additionally, the main teratogenicity studies did not present a complete statistical analysis, therefore, [redacted] (2019), carried out a further re-analysis of the data described in each developmental toxicity study, so this information has been included in the respective studies in order of the

comprehensive assessment.

The preliminary study in rats (B.6.6.2.1) was designed to select the appropriate dose levels for the main teratogenicity study (B.6.6.2.2). In this pilot study, dodine was tested at dose levels of 0, 50, 70 and 100 mg/kg bw/day, in which 10 copulated females were assigned to each test group.

Only one female at high dose group was humanely killed due to morbidity signs (piloerection. hunched posture, red/brown staining around face, fore-paws and mild ataxia). Another female from same group showed wheezing. No other mortalities or clinical signs were recorded in dodine-treated groups.

Female bodyweights were decreased in a statistically significant manner in the mid and high dose dams at gestation day 13 (10% and 8%, respectively), although not statistically significant decreases were recorded throughout gestation day 6-16 in both groups (8-10% and 1-8% for mid and high dose groups, respectively), compared with control group. Besides, reduced bodyweight gain was statistically significant reduced at gestation day 6-13 in 70 and 100 mg/kg bw/day groups (26% and 48% for 70 and 100 mg/kg bw/day dose groups, respectively), although not statistically significant decreases were recorded throughout gestation day 6-16 in both groups (6-26% and 17-65% for mid and high dose groups, respectively), compared with control group.

Moreover, mean food consumption was also decreased throughout whole gestation period in all the dodine treated groups (7%, 15% and 24% for low, mid and high dose groups, respectively).

Necropsy dam evaluation revealed a slight increase in the kidney incidences (pelvic dilatation and enlarged; 30%) and ureters (dilatation; 20%) in the top dose group, compared with controls (0%). Moreover, one dam from high dose group exhibited alterations consistent with a long standing partial obstruction in the lower urinary tract (epithelial hyperplasia and chronic inflammation of the urinary bladder; ureters inflammation; pelvic dilatation and inflammation, together with nephritis in the kidney; and hyperplasia of the lumbar lymph node). These findings could have been caused by a small calculus in the bladder or urethra, which might have been voided some time before death.

Regarding sexual function and fertility parameters, fertility index was reduced in the top dose group (90%), compared with other dose and control groups (100%). Developmental checked parameters did not display relevant differences compared with controls. An increase, not dose related, in mean deaths and early resorptions were recorded in low and high dose groups. Besides, foetal bodyweight was slightly reduced (4%) compared with controls in the high dose group.

Therefore, a **NOAEL** for **developmental toxicity** has been established at **100 mg/kg bw/day** based on no effects observed at high dose level.

NOAEL for **maternal toxicity** has been established at 5**0 mg/kg bw/day** based on decreased bodyweight gain and food consumption.

The main study in rats (B.6.6.2.2), dodine was tested *via* oral gavage at dose levels of 0, 10, 45 and 90 mg/kg bw/day throughout gestation day 6 to gestation day 16, in which 25 copulated females were assigned to each test group.

No mortalities were observed during study in any of the tested groups. Isolated clinical signs were recorded in some treated dams. Three dams at high dose group showed excessive salivation after dosing for one or 2 days during the treatment period. On the other hand, there was another three animals with red/brown stained fur around the mouth at 90 mg/kg bw/day dose groups and one in the 45 mg/kg bw/day dose group. Besides, one dam from high dose group showed noisy breathing after dosing.

Statistically significant decreases in bodyweights were recorded in gestation day 9 (9%), 13 (8%) and 17 (8%), and in bodyweight gain throughout gestation day 6-9 (107%) and 6-17 (20%) at high dose group, compared with controls. No differences were observed in low and mid dose group.

Food consumption was statistically significantly lower than controls from gestation day 6-16 (13-37%) at high dose group; and on gestation day 6 (11%) and gestation day 8-10 (12-18%) at mid dose group.

On the other hand, only two dams from high dose groups showed necropsy observations (8% showed dark red areas on lung lobes). These effects were further confirmed in the histopathology analysis (8% congestion and 4% in haemorrhage into alveoli).

Regarding reproductive data, a slight increase in fertility index were observed in dodine treated groups compared

with controls (88%, 84%, 92% and 96% for controls, low, mid and high dose groups, respectively). Gestation length and mating index were not measured.

Developmental endpoints such as corpora lutea, implantations, resorptions (early and late), foetal weight and sex ratio did not show differences between dodine-treated groups and controls. A slight increase, not dose-related, in the mean uterus weight was found in mid and high dose groups (5% and 7% for mid and high dose groups, respectively), compared with controls

On the other hand, the visceral or skeletal findings recorded in foetuses were deemed variations that did not suggest adverse developmental effects.

Therefore, a **NOAEL** for **developmental toxicity** has been established at **90 mg/kg bw/day** based on no effects observed at high dose tested.

NOAEL for **maternal toxicity** has been established at **10 mg/kg bw/day** based on decreased bodyweight gain (6-9 GD) and food consumption.

The preliminary study in rabbits (B.6.6.2.3) was designed to select the appropriate dose levels for the main teratogenicity study (B.6.6.2.4). In this pilot study, dodine was tested at dose levels of 0, 70 and 100 mg/kg bw/day, in which 10 copulated females were assigned to each test group.

Five animals at high dose group and one in low dose group were humanely killed due to morbidity signs. These animals showed few faeces previous dead. Some animals also showed eye affectations and breathing difficulties.

Bodyweight data did not revealed differences between treated groups and controls. Food consumption was reduced at top dose group throughout GD 6-18 (31-71%), compared with controls, exhibiting a mean of 51% lower than controls.

Necropsy examinations indicated that the half of animals showed liquid contents and gaseous distension in caecum (50%) at high dose group, whereas at low dose group, only one animal (10%) showed this finding, compared with controls.

Most findings of histopathology analysis revealed effects on stomach, in which 50% of animals at high dose group showed colour or dark foci areas, in some cases with epithelium hyperplasia. Additionally, 20% of rabbits from top dose group showed chronic liver or kidney inflammation. On the other hand, no relevant findings were noted at low dose group.

Sexual function and fertility parameters were not affected in treated groups compared with controls. At 100 mg/kg bw/day group there was an increase in the pre-implantation loss (22% vs 14% in controls) and in the mean live implants that cannot be attributed to test treatment. The only concerning effect was the increase in the number of late resorptions seen at the highest dose level compared to controls (5 vs 0), regarded adverse taking into account the magnitude of the variation and the absence of a statistical analysis in this study.

Therefore, a **NOAEL** for **developmental toxicity** has been established at 7**0 mg/kg bw/day** based on the increase in the number of late resorptions at the highest dose level.

NOAEL for **maternal toxicity** has been established at **70 mg/kg bw/day** based on decreased bodyweight gain and food consumption, necropsy and histopathological findings in stomach, kidney and liver.

The main study in rabbits (B.6.6.2.4), dodine was tested *via* oral gavage at dose levels of 0, 10, 40 and 80 mg/kg bw/day throughout gestation day 6 to gestation day 16, in which 16 or 20 copulated females were assigned to each test group.

Four total mortalities were recorded throughout whole study: three occurred at high dose group (one animal dies at GD 15 after showing breathing difficulties, one animal was humanely killed at GD 11 after showing same clinical signs, and another was also killed because of poor condition), and one at mid dose group (accidental damage during dosing). Most of clinical signs were observed in 80 mg/kg bw/day dose group. 15% of rabbits showed liquid faeces, breathing difficulties and emaciation. Besides, 10% showed pale eyes and two dams (10%) suffered abortions. Another clinical signs with low incidences were recorded in this group. Blood in cage and breathing difficulties were noted in the mid and high dose groups, whereas liquid faeces were seen in all groups. On the other hand, in control and low dose groups, an abortion in each group was also recorded.

No differences were observed in bodyweights and bodyweights gain between treated groups and controls. Regarding food consumption, statistically significant reduction was recorded at high dose group in gestation days 6 (25%), 7 and 8 (30%) compared with controls. Moreover, in this group, sporadic reduction, without statistical significance, were noted during mid to late treatment period.

Necropsy examinations revealed isolated and low incidence findings in liver, kidney, intestine, stomach and uterus of treated dams. Remarkably, the incidence of dark patches in lung lobes was increased in mid (12.5%) and high dose (20%) groups compared with controls, in which the half of these animals presented breathing difficulties as clinical signs.

Fertility index was slightly reduced in high dose group compared with controls, in which three dams were not pregnant (94%, 94%, 100% and 85% for controls, low, mid and high dose groups, respectively). Gestation length and mating index was not measured.

Developmental parameters indicated variations after dodine treatment from 40 mg/kg bw/d without statistical significance and not clear dose-relationship. However, the clear maternal toxicity seen at the highest tested dose level of 80 mg/kg bw/d with mortality (1 dead and 2 killed dams), clinical signs, decrease in food consumption, dark patches in the lung compromises the interpretation of the relevance of co-occurring developmental effects for classification (for instance, the abortions, the number of post implantation losses and late resorptions). These increases in abortions, post implantation losses and late resorptions can considered a direct consequence of maternal toxicity at 80 mg/kg bw/d but not at 40 mg/kg bw/d in which only dark patches in the lung were seen.

The visceral or skeletal findings recorded in foetuses did not show dose-relationship and were deemed variations that did not suggest adverse developmental effects.

Therefore, the **NOAEL** for **developmental toxicity** has been established at **10 mg/kg bw/day** based on developmental effects such as increase of post implantation losses and late resorptions seen from 40 mg/kg bw/day.

NOAEL for **maternal toxicity** has been established at **40 mg/kg bw/day** based on mortality, clinical signs and reduced food consumption noted at high dose tested.

Developmental effects observed in other studies

In the two-generation reproductive study in rats, dodine was tested at 0, 200, 400 and 800 ppm dose levels (equivalent to 0, 13.4, 26.2, and 52.6 mg/kg bw/day for males and 0, 15.6, 31.2, 60.3 mg/kg bw/day for females, respectively), in which 30 pairs of males and females were mated from each test group.

In the F1 pups, mean bodyweights were significantly lower for male and female pups from lactation days 4-21 at 800 ppm and for 400 ppm dose pups group on days 4 (precull and postcull, females only), 14 (females only), and 21 (males and females). Regarding organ weights in this generation, absolute kidney (both right and left) and liver weights were statistically significantly lower in high dose male group (16% and 18%, respectively), whereas relative brain weight was increased (12%), compared with controls. The effects at 800 ppm were seen in presence of maternal toxicity (decreases in bodweight, bodyweight gain, food consumption and significant organ weight variations) and the slight decreases in pup weights at 400 ppm with slight maternal toxicity (slight reductions in bodyweight and food consumption).

In F2 pups, mean bodyweights were statistically significantly lower on days 4 (pre-cull and post-cull, males only), 7, 14, and 21 for pups in the 800 ppm dose group, and on days 14 (males only) and 21 for pups in the 400 ppm dose group. Regarding organ weights, absolute kidney (both), spleen and liver weights were statistically significantly lower in high dose male group (16%, 28% and 17% for kidneys, spleen and liver, respectively), whereas relative brain weight was increased (18%), compared with controls. No statistically significant differences were noted in other F2 male pups from treated-groups regarding organs weights, except absolute left kidney weight in mid dose group. On the other hand, in F2 females weanling pups, absolute kidney (both), thymus, spleen and liver weights were statistically significantly lower in high dose group (14/17% for left/right kidney, 28% for thymus, 22% for spleen and 17% for liver, respectively), whereas relative brain weight was increased (15%), compared with controls. No statistically significant differences were noted in other organs at 800-ppm or low dose groups in F2 female pups. The effects at 800 ppm were seen in presence of maternal toxicity (decreases in bodyweight, bodyweight gain, food consumption and organ weight variations and the slight decreases in pup weights at 400 ppm with slight maternal toxicity (slight reductions in bodyweight and food consumption and increase in the relative weight of the adrenals).

Furthermore, no treatment related findings were recorded in any of F1 and F2 examined pups during necropsy evaluation.

NOAEL for **developmental toxicity** has been established at **200 ppm (13.14/15.6 mg/kg bw/day for males/ females)** based on decreased male and females pup weights in F1 and F2 generation.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

No human information is available on the effects of dodine on development, but there is information from two reliable developmental studies conducted in rat and rabbit.

Developmental effects in rats

Table 2.6.6.2.2/1: Main effects in rat potentially relevant for CLP

Study	Dose level	Developmental effects	Maternal toxicity
Two generation toxicity study in rats [redacted] (1996) (CA) B.6.6.1.1	**800 ppm** (52.6 and 60.3 mg/kg bw/day ♂/♀)	**P → F1** ▪ (↓) bw in ♂/♀ during lactation (>10%), ▪ (↓) terminal bw in ♂ (13%). ▪ (↓) terminal bw in ♀ (10%, ns.). ▪ (↓) abs left and right kidney in ♂ (16%). ▪ (↓) abs liver in ♂ (18%). ▪ (↑) rel brain in ♂ (12%).	**P** ▪ (↓) bw (<10%) in ♂ during premating and in ♀ during premating, gestation and lactation. ▪ (↓) bwg in ♂ throughout week 0-12 (13-32%) and in ♀ throughout week 0-10 (14-36%). ▪ (↑) bwg in ♀ throughout lactation day 14-21 (20%) and 0-21 (84%). ▪ (↓) food consumption in ♂ at week 1 (14%), 2 (9%) and 3 (7%) and in ♀ at week 1 (15%), 2 (11%), 3 (11%), 4 (6%) and 5 (9%). ▪ (↓) food consumption in ♀ throughout lactation day 4-7 (10%), 7-10 (19%), and 10-14 (15%). *Organ weights* ▪ (↓) abs thymus in ♂ (17%). ▪ (↑) rel left and right adrenal in ♀ (14%). ▪ (↑) rel brain in ♀ (7%). *Necropsy* ▪ (↑) red focus area in the thymus in ♀ (19% vs 11% in controls, n.s. ndr).
		F1→ F2 (↓) bw in ♂/♀ during lactation (>10%)	**F1** ▪ (↓) bw pre-mating in ♂ throughout week 0-12 (13-19%) and in ♀ throughout week 0-10 (12-15%). ▪ (↓) bw in ♀ at gestation day 0 (13%), 7(14%), 14 (14%) and 20 (12%). ▪ (↓) bw in ♀ at lactation day 0 (13%), 4 (15%), 7 (13%), 14 (13%) and 21 (8%). ▪ (↓) bwg pre-mating in ♂ throughout week 0-12 (10-15%) and in ♀ throughout week 0-10 (9-16%). ▪ (↓) bwg gestation in ♀ throughout week 0-7 (20%), 7-14 (20%) and 0-20 (11%). ▪ (↑) bwg lactation in ♀ throughout lactation day 14-21 (135%) and 0-21 (116%). ▪ (↓) food consumption premating in ♂ throughout week 0-10 (6-14%) and in ♀ throughout week 0-10 (9-18%). ▪ (↓) food consumption gestation in ♀ throughout lactation day 0-7 (12%), 7-14 (17%), and 14-20 (12%). ▪ (↓) food consumption lactation in ♀ throughout lactation day 4-7 (12%), 7-10 (15%), and 10-14 (20%). *Organ weight* ▪ (↓) terminal bw in ♂ (13%) and in ♀ (12%). ▪ (↑) rel left and right epididymis in ♂ (13%). ▪ (↑) rel left (12%) and right (14%) testis in ♂. ▪ (↑) rel left adrenal in ♂ (21%). ▪ (↓) abs left (15%) and right (14%) kidney in ♂. ▪ (↓) abs liver in ♂ (16%). ▪ (↑) rel brain in ♂ (13%). ▪ (↑) rel left (23%) and right (20%) adrenal in ♀. ▪ (↓) abs left (11%) and right (12%) kidney in ♀. ▪ (↓) abs liver in ♀ (12%). ▪ (↑) rel brain in ♀ (9%). ▪ (↑) rel left ovary/oviduct (11%) and right ovary/oviduct (11%,

			ns) n in ♀. *Necropsy* ▪ (↑) red focus area in the thymus in ♂ (13 vs 10% in controls, ns., ndr), and in ♀ (17% vs 10% in controls, ns. ndr).
	400 ppm (26.2 and 31.2 mg/kg bw/day ♂/♀)	**P → F1** ▪ (↓) bw in ♂/♀ during lactation (<10%)	**P** ▪ (↓) bw in ♀ at lactation day 4 (4%). ▪ (↓) food consumption in ♂ at week 1 (5%) of pre-mating and in lactation week 7-10 in (9%).
		F1→F2 ▪ (↓) bw in ♂/♀ during lactation (<10%) ▪ (↓) abs left kidney in ♂ (11%) regarded not toxicologically relevant	**F1** ▪ (↓) bw in ♀ throughout pre-mating week 0-8 (5-6%), at gestation day 7(5%), 14 (5%) and 20 (5%) and at lactation day 4 (5%), 7 (4%) and 14 (5%). ▪ (↓) food consumption in ♀ throughout pre-mating week 3-4 (9%) and throughout lactation week 7-10 (8%). *Organ weight* ▪ (↑) rel left (12%) and right (9%, ns.) adrenal in ♀.

There were no adverse developmental effects in the rat teratogenicity studies.

In the multigeneration study in rats the significant decreases in pup weights at 400 ppm in F1 and F2 were of low magnitude (5-8%) and were seen in presence of slight maternal toxicity based on decreased bodyweights and increased relative adrenal weight in F1 adult females. Even if treatment-related, the effect is not sufficiently relevant for classification.

Developmental effects in rabbits

In the range-finding developmental study in rabbits the increase in the number of late resorptions seen at the highest dose level of 100 mg/kg bw/day compared to controls (5 vs 0) is considered of doubtful toxicological relevance taking into account the presence of maternal toxicity.

In the main developmental study in rabbits, a clear maternal toxicity was observed at high dose. Three mortalities (15%) apparently related to test substance administration were recorded at top dose group. No other deaths attributed to dodine- were recorded among groups. Moreover, several clinical signs were noted at this dose, whereas at mid and low dose groups, sporadic and low clinical signs incidences were observed.

On the other hand, the non-significant increase in post-implantation losses and late resorptions from 40 mg/kg bw/day were seen in presence of maternal toxicity at 80 mg/kw bw/day and without clear maternal toxicity at 40 mg/kg bw/day. In any case, effects at this intermediate dose level are not regarded sufficient for classification.

Table 2.6.6.2.2/2: Main effects in rabbits potentially relevant for CLP

Study	Dose level	Developmental effects	Maternal toxicity
Dose range-finding developmental toxicity study in rabbits [redacted] (1989a)	**100 mg/kg bw/day**	(↑) % late resorptions (5 vs 0)	(↓) Bwg through days 6-19 (48%). (↓) food consumption through days 6-18 (31-77%). Necropsy: (↑) Liquid contents caecum/gaseous distension (50% vs 0% in controls). Histopathology: *Stomach* (↑) Cream coloured parches on mucosa. Pyloric part covered in colourness viscous fluid in stomach (30% vs 0% in controls). (↑) Dark point foci. Blood and sloughing of mucosa. Hyperplasia of fundic epithelium (20% vs 0% in controls). *Liver* (↑) Lobulation prominent. Mild chronic inflammation (periportal). Hepatocytes necrosis

			(20% vs 0% in controls). *Kidney* (↑) Red foci/chronic inflammation (20% vs 0% in controls).
Main developmental toxicity study in rabbits [redacted] (1989b)	**80 mg/kg bw/day**	(↑) abortions (10%). (↑) total dead implants (↑) mean dead implants (↑) % dead implants (↑) total late resorptions (↑) mean late resorptions (↑) % late resorptions	(↑) mortality (15%). (↑) clinical signs: : breathing difficulties (15%), liquid faeces (15%), emaciation (15%) and pale eyes (10%). Another clinical signs were observed isolated and with low incidence. (↓) food consumption in GD 6 (25%), 7 and 8 (30%). (↑) dark patches in lung (20%).
	40 mg/kg bw/day	(↑) total dead implants (↑) mean dead implants (↑) % dead implants (↑) total late resorptions (↑) mean late resorptions (↑) % late resorptions	(↑) dark patches in lung (12.5%). (↑) clinical signs: breathing difficulties (6%).

In section 3.7.1.4 of Annex I to CLP Regulation it is stated that "*Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency*".

According to the guidance on the application of the CLP criteria and based on the development effects observed in rats and rabbits, RMS proposes no classification for dodine as reproductive toxicant.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

Table 63: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
No data			

Table 64: Summary table of human data on effects on or via lactation

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data				

Table 65: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Two-generation reproductive toxicity study in rats.	Dodine	**F1 generation** **800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)** Bodyweight (bw) ▪ (↓) bw in ♂ at day 4 (precull) (7%). ▪ (↓) bw in ♀ at day 4 (precull) (9%) ▪ (↓) bw in ♂ at day 4 (postcull) (7%). ▪ (↓) bw in ♀ at day 4 (postcull) (9%) ▪ (↓) bw in ♂ at day 7 (11%). ▪ (↓) bw in ♀ at day 7 (11%). ▪ (↓) bw in ♂ at day 14 (17%). ▪ (↓) bw in ♀ at day 14 (17%). ▪ (↓) bw in ♂ at day 21 (16%). ▪ (↓) bw in ♀ at day 21 (16%). ▪ (↓) terminal bw in the 10 selected weanling ♂ animals (13%). ▪ (↓) terminal bw in the 10 selected weanling ♀ animals (10%, ns.). **400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)** Bodyweight ▪ (↓) bw in ♀ at day 4 (precull) (7%). ▪ (↓) bw in ♀ at day 4 (postcull) (7%). ▪ (↓) bw in ♀ at day 14 (6%). ▪ (↓) bw in ♂ at day 21 (7%). ▪ (↓) bw in ♀ at day 21 (8%). **F2 generation** **800 ppm (equivalent to 52.6/60.3 mg/kg bw/day for ♂/♀)** Bodyweight ▪ (↓) bw in ♂ at day 4 (precull) (9%). ▪ (↓) bw in ♂ at day 4 (postcull) (8%). ▪ (↓) bw in ♂ at day 7 (9%). ▪ (↓) bw in ♀ at day 7 (9%). ▪ (↓) bw in ♂ at day 14 (16%). ▪ (↓) bw in ♀ at day 14 (16%). ▪ (↓) bw in ♂ at day 21 (17%). ▪ (↓) bw in ♀ at day 21 (18%). ▪ (↓) terminal bw in the 10 selected weanling ♂ animals (17%., ns.). ▪ (↓) terminal bw in the 10 selected weanling ♀ animals (17%.). **400 ppm (equivalent to 26.2/31.2 mg/kg bw/day for ♂/♀)** Bodyweight ▪ (↓) bw in ♂ at day 14 (5%). ▪ (↓) bw in ♂ at day 21 (7%). ▪ (↓) bw in ♀ at day 21 (7%).	-Lower bodyweights throughout lactation period, observed in F_1 and F_2 litters, were considered to be related to reduced bodyweight and food consumption in dams. -Lactation indices were not affected by treatment.	[redacted] [redacted] (1996) (CA) B.6.6.1.1

2.6.6.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

The available information on the potential of dodine to cause adverse effects on the offspring via lactation or on lactation is contained in the generation reproductive study ([redacted] [redacted] 1996; B.6.6.1.1).

In the generational study there is no clear evidence of adverse effects in the offspring due to transfer of test substance in the milk, or of adverse effect on the quality of the milk. Only decreased offspring bodyweights were observed on lactation period, but these were considered a direct effect of the solid diet or were related to maternal toxicity.

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

No human information is available on the effects of dodine on or via lactation, but there is information reliable from a two-generation reproduction studies in rats. Based on the data available, there were no effects to warrant classification of dodine for effects on or *via* lactation.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

Based on the reproductive data available for dodine, and according to the criteria under Regulation (EC) No. 1272/2008, RMS proposes no classification for dodine.

2.6.7 Summary of neurotoxicity

Neurotoxicity studies with dodine were not available in the submitted dossier.

According to Commission regulation (EU) No 283/2013, neurotoxicity studies in rodents shall be performed for active substances with structures that are similar or related to those capable of inducing neurotoxicity, and for active substances, which induce specific indications of potential neurotoxicity, neurological signs or neuropathological lesions in toxicity studies at dose levels not associated with marked general toxicity. Performance of such studies shall also be considered for substances with a neurotoxic mode of pesticidal action.
Moreover, delayed polyneuropathy studies shall be performed for active substances of similar or related structures to those capable of inducing delayed polyneuropathy such as organophosphorus compounds.

Table 66: Summary table of animal studies on neurotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL**	**Reference**
No animal studies on neurotoxicity are available			

Therefore, a review of the existing data has been carried out.

Neurotoxicity findings in acute toxicity studies

In an acute oral study in rats ([redacted] 1999), hypoactivity was observed in all animals at 761 and 1285 mg/kg bw and impaired muscle coordination was seen in 2/5 males and 3/5 females at 1285 mg/kg bw. LD_{50} values of 830 and 817 mg/kg bw were set for males and females respectively.
In an acute oral study in female mice ([redacted], 2008), animals dosed at 1290, 1750 and 2300 mg/kg bw displayed a decreased spontaneous activity, showing reversibility in those animals which survived until the end of the study (one at 1290 and one at 1750 mg/kg bw) and in two females at 1750 and 2300, respectively. One animal at each dose of 1290, 1750 and 2300 mg/kg bw showed also decreased Preyer's reflex and righting reflex. LD_{50} value of female mice was set at 1354 mg/kg bw.

Neurotoxicity findings in short-term studies

In the 28-day oral gavage study in rats, salivation was increased in males and females from 75 mg/kg bw/day ([redacted] 1994a). No other potentially neurotoxic findings were observed in the short-term data package.

Neurotoxicity findings in genotoxicity studies

In an *in vivo* mammalian micronucleus test ([redacted] 1992), all remaining mice prior to euthanasia at the completion of the dose range finding study appeared languid in the groups in which 387.5 and 500 mg of dodine/kg bw were administered by gavage. No similar effects were reported during the main micronucleus assay in which doses up to 400 mg/kg bw were administered.

Neurotoxicity findings in developmental toxicity studies

In a dose-range finding developmental toxicity study in rats ([redacted] and [redacted], 1989a), one dam treated at 100 mg of dodine/kg bw/day showed wheezing on GD 9 and another showed piloerection, hunched posture, red/brown stain around face and fore-paws and mild ataxia on GD 15 and 16.
In the main developmental toxicity study in rats ([redacted] and [redacted], 1989b), 3 dams showed excessive salivation (after dosing for 1 or 2 days), 2 showed red/brown stained fur around the mouth (at 45 and 90 mg/kg bw/day) and 1 showed noisy breathing at 90 mg /kg bw/day.
In a dose-range finding developmental toxicity study in rabbits ([redacted], 1989a), at 100 mg/kg bw/day, 1 animal was found dead at GD 13 with red staining around lower abdomen and another showed breathing difficult and noisy, and was slightly cyanosed and subdued.

In the main developmental toxicity study in rabbits ([redacted]., 1989b), some dams showed breathing difficulties from 40 mg/kg bw/day and emaciation at 80 mg/kg bw/day.

Neurotoxicity findings in long-term studies

In the oral 106-week study in rats ([redacted] 1998), a significant increase in the absence of grasping was found in males at 800 ppm, whereas there were significant trend tests for the absence of grasping, traction and righting reflexes incidences in dodine-male treated groups. On the other hand, a non-significant but dose-related increase in hunched posture incidence was revealed in males. Moreover, increased reduced motor activity and piloerection incidences were observed in males dodine-treated groups compared with controls, although they were neither significant nor dose-response.
In the oral 78-week study in mice ([redacted] 1998a) increased incidence of whole body tremors was mainly noted in both sexes at 750 and 1500 ppm and increased incidence of dilated pupil and excessive salivation were mainly in treated males.

Neurotoxicity findings in immunotoxicity studies

In an immunotoxicity study in rats ([redacted], 2013), no signs of neurotoxicity signs were noted during the observation period.

Neurotoxicity findings in endocrine disruption studies

In the Hershberger bioassay in rats ([redacted], 2022), in which orchidoepididymectomised male rats were treated at concentrations of 5, 15 and 50 mg/kg bw of dodine, no changes in the autonomic and central nervous systems, in somatomotor activity or in behaviour were noted at examination or at 1-1.5 h post-dosing in any group.

Conclusion on neurotoxicity

The collected information indicates that mainly mild effects were observed. Moreover, the effects were mostly reported at high doses or at doses in which clear systemic toxicity was also observed, suggesting dodine has not neurotoxicity potential and, therefore, in this case the RMS does not consider further testing as necessary. A delayed polyneuropathy study is not deemed necessary because the structure of dodine is not related to those capable of inducing delayed polyneuropathy (it is neither an organophosphorus, nor a carbamate compound).

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

Table 2.6.8.1/01. Summary table of toxicity studies of metabolites

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
In Silico assessment of genotoxicity No guideline available GLP: No **Study acceptable**	Dodine (parent) Guanidine Dodecylguanidine carboxylic acid Octylguanidine carboxylic acid Hexylguanidine carboxylic acide *In silico* models: Toxtree v.3.1.0 US EPA T.E.S.T. v.4.2.1 VEGA v.1.1.4 Derek Nexus v.6.0.1 OECD (Q)SAR Toolbox v.4.4.1	No alerts were activated by any of the metabolites Chemical profiling in the OECD QSAR toolbox showed no profiler alerts activated by any of the compounds for genotoxicity endpoints. All compounds share the guanidine functional group. Three metabolites also include a carboxylic acid functional group. Overall, the metabolites did not activate predictions for genotoxicity, namely bacterial gene mutation and chromosomal damage (aneugenicity and clastogenicity).	[redacted] 2021) (CA) B.6.8.1.1
In Silico assessment of general toxicity: carcinogenicity,	Dodine (parent) Guanidine Dodecylguanidine carboxylic acid	Carcinogenicity predictions: The metabolites did not activate any alerts in any of the models.	[redacted] 2021) (CA)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
reproductive & developmental toxicity and miscellaneous endpoints No guideline available GLP: No **Study acceptable**	Octylguanidine carboxylic acid Hexylguanidine carboxylic acide *In silico* models: Toxtree v.3.1.0 US EPA T.E.S.T. v.4.2.1 VEGA v.1.1.4 Derek Nexus v.6.0.1 OECD (Q)SAR Toolbox v.4.4.1	 Developmental & Reproductive toxicity predictions: The metabolites did not activate any alerts in any of the models although most predictions were outside the applicability domain of the model (VEGA). Miscellaneous endpoints predictions: Parent and the three alkylguanidine carboxylic acids were predicted skin sensitisers in VEGA, although the compounds were outside the applicability domain of the model. No skin sensitisation alerts were activated in Derek Nexus. All metabolites active a hERG channel inhibition alert in Derek Nexus. This alert was not activated by the parent compound. Guanidine was predicted as hepatotoxicant in VEGA based on experimental data. Hexylguanidine carboxylic acid activated a hepatotoxicity alert and a nephrotoxicity rapid prototype alert. Chemical profile: No additional alerts were obtained for any metabolite compared to the parent substance. Chemical grouping: Two chemical groups are defined based on the structural features: Guanidine Guanidine carboxylic acid	B.6.8.1.2
In Silico assessment of multiple toxicity endpoints No guideline available GLP: No **Study acceptable**	Dodine (parent) Guanidine Dodecylguanidine carboxylic acid Octylguanidine carboxylic acid 6-Carbamimidamidohexanoic acid Derek Nexus v.6.0.1 (KB 2018 1.1) Species: Bacterium, dog, *E.Coli*, guinea pig, hamster, human, mammal, monkey, mouse, primate, rabbit, rat, rodent, *S. typhimurium* Endpoints: Carcinogenicity, genotoxicity (including mutagenicity and chromosome damage), irritation, miscellaneous endpoints, neurotoxicity, organ toxicity, reproductive toxicity, respiratory sensitisation, skin sensitisation.	All compounds were predicted non-mutagenic in bacteria and non-sensitisers. The system reported they do not contain misclassified or unclassified features. Dodine and the metabolites guanidine, dodecylguanidine carboxylic acid and octylguanidine carboxylic acid did not activate any toxicity alerts. The metabolite 6-carbamimidamidohexanoic acid activated a hepatotoxicity alert and a rapid prototype alert for mitochondrial dysfunction.	[redacted] (2020) (AS) B.6.8.1.3

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Mammalian cell chromosome aberration test Guideline not stated. Deviations from current OECD TG 473 (2016): No characterisation of test items, no individual results provided, treatment time exceeded the 3-6h recommended by guideline, only 100 metaphases scored, no metabolic activation, gaps not excluded from analysis, no positive control used and historical control data not available GLP: No **Supporting information**	Guanidine carbonate Guanidine hydrochloride Guanidine nitrate Guanidine phosphate Purity and batch no. not stated Chinese hamster lung fibroblasts (CHL) Solvent: DMSO or Physiological saline No metabolic activation used Negative control: Solvent Treatment time: 24h (guanidine hydrochloride and guanidine nitrate) or 48h (guanidine carbonate and guanidine phosphate) Dose: 0.50-41.3 mg/mL (guanidine carbonate), 0.50-52.3 (guanidine hydrochloride), 0.50-41.0 (guanidine nitrate), 0.46-29.3 (guanidine phosphate)	**Negative – S9**	Ishidate, M. and Shigeyoshi, O. (1977) (AS) B.6.8.1.4
Acute oral toxicity study in rats and mice Guidelines followed in the study: EPA 560/6-82-001 Federal Register 44 (No. 91): 27362 (1979) Deviations from OECD TG 420/423 (2001): Test substance not characterised (batch no. not reported), individual body weights not reported and limited reporting of results. GLP: No **Supporting information**	Guanidine hydrochloride Purity: 98%; Batch no. not stated Sprague-Dawley rats (♂,♀) ICR mice (♂,♀) Single oral dose (gavage) Dose (rat): 278, 360, 464, 600 and 775 mg/kg bw Dose (mouse): 316 (♀), 398, 495 (♂), 501, 631 and 794 mg/kg bw Vehicle: Sterile water	**Rats:** **LD_{50}: 556.5 (♂) and 474.6 mg/kg (♀)** Time of death occurred mainly at 4-26h post-dose. Central nervous system-neurological disturbance (80/86) and gastrointestinal tract symptoms (53/86) within 2-4h post-dose and disappeared after 7 days (dose-response relationship) **Mice:** **LD_{50}: 570.8 (♂) and 621 mg/kg bw (♀)** All deaths occurred within 5h post-dose. Respiratory signs (gasping 68/100) and behavioural signs (60/100). The behavioural signs included irritability, inactivity, disorientation, hyperactivity, hypotonia, jumping, tremors and twitching. All observed at all dose levels although no clear dose-response relationship of severity or duration of the symptoms In both species, the weight gains of survivors were not significantly affected by dosing.	Korte, D.W. *et al*. (1993a) (AS) B.6.8.1.5
Acute oral toxicity study in rats and mice Guidelines followed in the study: EPA 560/6-82-001 Federal Register 44 (No. 91): 27362 (1979) Deviations from	Guanidine nitrate Purity: 99.99%; Batch no. not stated Sprague-Dawley rats (♂,♀) ICR mice (♂,♀) Single oral dose (gavage) Dose (rat): 610 (♀), 683 (♂), 718 (♀), 826 (♂), 847 (♀), 1000, 1180	**Rats:** **LD_{50}: 989.6 (♂) and 729.8 mg/kg (♀)** Time of death occurred between 4-24h post-dose. Only one death occurred between 2-14 days post-dose. Clinical signs were observed within the first 48h, which included behavioural (63/99), gastrointestinal tract symptoms (37/99) and respiratory signs (26/99). **Mice:**	Korte, D.W. *et al*. (1993b) (AS) B.6.8.1.6

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
OECD TG 420/423 (2001): Test substance not characterised (batch no. not reported), individual body weights not reported and limited reporting of results. GLP: No **Supporting information**	(♀), 1210 (♂), 1390 (♀) and 1470 mg/kg bw (♂) Dose (mouse): 708, 891, 1121, 1410 and 1780 mg/kg bw Vehicle: Methylcellulose (0.2%) and Tween-80 (0.4%) in sterile water	**LD_{50}: 1105 (♂) and 1028 mg/kg bw (♀)** Most deaths occurred within 4h post-dose and the remaining within 24h post-dose. The most observed clinical signs were behavioural disturbances (51/105), hunched posture (30/105) and changes in reflex activity (26/105). Behavioural signs included irritability, inactivity, disoriented condition, hyperactivity, jumping, hypertonia, tremors, twitching or ataxia. Changes in reflex activity included decreased grasping and change in the startle reflex.	
Acute oral toxicity study in mice No guideline stated No deviations from OECD TG 420/423 (2001): None GLP: No **Study acceptable**	Guanidine hydrochloride Purity: 98%; Batch no. TP28 ICR mice (♂,♀) Single oral dose (gavage) Dose (mouse): 316 (♀), 398, 495 (♂), 501, 631 and 794 mg/kg bw Vehicle: Sterile water	**LD_{50}: 570.8 (♂) and 621.4 mg/kg bw (♀)** All deaths occurred within 5h post-dose. Respiratory signs (gasping 68/100) and behavioural signs (60/100). The behavioural signs included irritability, inactivity, disorientation, hyperactivity, hypotonia, jumping, tremors and twitching. All observed at all dose levels although no clear dose-response relationship of severity or duration of the symptoms The weight gains of survivors were not significantly affected by dosing.	[redacted] (1989) (AS) B.6.8.1.7
Acute dermal toxicity study in rabbits No guideline state Deviations from current OECD TG 402 (2017) include the use of both sexes instead of one, no individual body weights are reported and limited level of reporting GLP: No **Supporting information**	Guanidine hydrochloride Purity: 98% New Zealand White rabbits (5♂,5♀) Dose: 2000 mg/kg bw Vehicle: saline 24h exposure 14-day observation period	**LD_{50}: > 2000 mg/kg bw (♂,♀)** No mortality occurred in the study. No clinical signs. Signs of erythema (6/6/) and oedema (2/6) were observed at 0.5h after removal of the patch. These signs disappeared by 24h. No gross lesions were observed at necropsy but microscopic examination revealed epidermal ulceration covered by fibrinocellular exudate	Korte, D.W. *et al*. (1993c) (AS) B.6.8.1.8
Acute dermal toxicity study in rabbits No guideline state Deviations from current OECD TG 402 (2017) include the use of both sexes instead of one and limited level of reporting GLP: No **Supporting information**	Guanidine nitrate Purity: 99.99% New Zealand White rabbits (5♂,5♀) Dose: 2000 mg/kg bw Vehicle: saline 24h exposure 14-day observation period	**LD_{50}: > 2000 mg/kg bw (♂,♀)** No mortality occurred in the study. No clinical signs were observed during the study. Erythema and oedema were scored on days 1 and 2. Necrosis was present in one rabbit and persisted for 14 days. No effects on body weight. No gross and microscopic skin lesions observed.	Korte, D.W. *et al*. (1993d) (AS) B.6.8.1.9

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Skin irritation/corrosion study in rabbits No guideline stated Deviations from current OECD TG 404 (2015) include: Test item not fully characterised, scoring not performed at 60min, exposure was longer than the recommended by guideline, the scoring of the lesions only considered 24h and 72h and overall very limited reporting of results (no individual scoring results were provided) GLP: No **Supporting information**	Guanidine hydrochloride Purity: 98% New Zealand White rabbits (3♂,3♀) Vehicle: 0.5 mL saline 24h exposure into 2 intact and 2 abraded sites Sites scored at 1, 2, 3, 7 and 14 days after patch removal. Scoring by Draize method (averaging the scores from 1 and 3 days)	**Severe irritant to the rabbit skin** Overall score of 3.2 at 1 day and 3 days Eschar formation	Korte, D.W. *et al*. (1993c) (AS) B.6.8.1.10
Skin irritation/corrosion study in rabbits No guideline stated Deviations from current OECD TG 404 (2015) include: Test item not fully characterised, scoring not performed at 60min, exposure was longer than the recommended by guideline, the scoring of the lesions only considered 24h and 72h and overall very limited reporting of results (no individual scoring results were provided) GLP: No **Supporting information**	Guanidine nitrate Purity: 99.99% New Zealand White rabbits (4♂,4♀) Vehicle: 0.5 mL saline 24h exposure into 2 intact and 2 abraded sites Sites scored at 1, 2, 3, 7 and 14 days after patch removal. Scoring by Draize method (averaging the scores from 1 and 3 days)	**Irritant to the rabbit skin** Overall score of 2.31 on intact skin at 1 day and 3 days	Korte, D.W. *et al*. (1993d) (AS) B.6.8.1.11

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
Eye irritation/corrosion in rabbits No guideline stated Deviations from current OECD TG 404 (2015) include: test item not fully characterised, number of animals used in the study exceeds the recommended by guideline, method poorly described and the scoring appears to be performed only at 1h and no individual results reported. GLP: No **Supporting information**	Guanidine hydrochloride Purity: 98% New Zealand White rabbits (6♂) Examinations at 1h and 1, 2, 3, 4, 7 and 14 days after application Draize scoring	**Non-irritant** Slight corneal opacity (3/6), iritis (2/6), conjunctiva redness (5/6), and chemosis (4/6) at l h which had disappeared by day 1	Korte, D.W. *et al*. (1993c) (AS) B.6.8.1.12
Eye irritation/corrosion in rabbits No guideline stated Deviations from current OECD TG 404 (2015) include: test item not fully characterised, number of animals used in the study exceeds the recommended by guideline, method poorly described and the scoring appears to be performed only at 1h and no individual results reported. GLP: No **Supporting information**	Guanidine nitrate Purity: 99.99% New Zealand White rabbits (6♂) Examinations at 1h and 1, 2, 3, 4, 7 and 21 days after application Draize scoring	**Non irritant** Slight corneal opacity (5/6) which cleared in three of the rabbits in 72 h. A slight iritis was observed in four rabbits which cleared by day 14. All rabbits exhibited slight conjunctival redness and chemosis at 1 and 24 h, which had disappeared by day 14. The corneal opacity and conjunctival chemosis were present in one rabbit for the entire 21-day observation period	Korte, D.W. *et al*. (1993d) (AS) B.6.8.1.13
Skin sensitisation in guinea pig – Buehler method Deviations from OECD TG 406 (2021) include: test item not fully characterised, only 10 animals in the treatment group (a minimum of 20 recommended by guidance), method and results poorly	Guanidine hydrochloride Purity: 98% Guinea Pig Hartley (10♂) Vehicle : saline Positive control : CDNB (10 animals)	**Non-sensitiser**	Korte, D.W. *et al*. (1993c) (AS) B.6.8.1.14

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
described. GLP: No **Supporting information**	Negative control : sham and saline (10 animals/group) Induction & challenge: 0.5 mL of 10% guanidine hydrochloride in saline		
Skin sensitisation in guinea pig – Buehler method Deviations from OECD TG 406 (2021) include: test item not fully characterised, only 10 animals in the treatment group (a minimum of 20 recommended by guidance), method and results poorly described. GLP: No **Supporting information**	Guanidine nitrate Purity: 99.99% Guinea Pig Hartley (10♂) Vehicle : saline Positive control : CDNB (10 animals) Negative control : sham and saline (10 animals/group) Induction & challenge: 0.5 mL of 10% guanidine hydrochloride in saline	**Non-sensitiser**	Korte, D.W. *et al*. (1993d) (AS) B.6.8.1.15

Toxicity studies of four metabolites:

Two *in silico* studies have been submitted to address this point. Twelve additional studies have been evaluated in this section to support the toxicological evaluation of guanidine salts. These studies were provided but not evaluated by the applicant and include a range of *in vitro* chromosome aberration, acute toxicity, skin irritation, eye irritation and skin sensitisation studies on guanidine salts.

Assessment of genotoxicity

No genotoxicity data are available for any of the metabolites. Guanidinium nitrate and guanidinium hydrochloride have been reported negative in an *in vitro* chromosome aberration test (Ishidate and Shigeyoshi, 1977; B.6.8.1.4). This study is pre-guidance and several method deficiencies were identified such as longer exposure period, only 100 metaphases scored, test carried out only without metabolic activation and very limited reporting of results. For this reason, this study is only deemed as supporting information.

The applicant referred to data from the ECHA – REACH Registration dossiers of guanidinium nitrate[2] and guanidinium hydrochloride[3] (CAS No 50-01-1 and CAS No 506-93-4), which is summarised in the following Table 2.6.8.1/02.

Table 2.6.8.1/02. Summary of data extracted from ECHA on guanidinium salts

Compound	Assay type	Result	Remarks
Guanidinium chloride	Bacterial gene mutation assay	Negative ± S9	Tester strains TA98, TA100, TA1535 and TA1538. Reported GLP compliant Reliability 1 (reliable without restriction)
Guanidinium	Bacterial gene mutation assay	Negative ± S9	Tester strains TA98, TA100, TA1535 and

[2] Guanidinium nitrate, ECHA REACH Registration Dossier, available at https://echa.europa.eu/es/registration-dossier/-/registered-dossier/16017/1/1

[3] Guanidinium hydrochloride, ECHA REACH Registration Dossier, available at https://echa.europa.eu/es/registration-dossier/-/registered-dossier/13899/1/1

nitrate			TA1538. Reported GLP compliant Reliability 1 (reliable without restriction)
	In vitro chromosome aberration	Negative – S9	CHL cells Non-GLP and pre-guidance Reliability 2 (reliable with restrictions)
	Mammalian gene mutation assay	Negative ± S9	Mouse lymphoma L5178Y cells Reported GLP compliant Reliability 1 (reliable without restriction)

No experimental data are available for the metabolites dodecylguanidine-, octylguanidine- and hexylguanidine-carboxylic acids.

In Silico prognosis of the metabolites has been carried out for genotoxicity endpoints using a variety of predictive tools ([redacted], 2021; B.6.8.1.1 and [redacted], 2020; B.6.8.1.3). The integrated summary predictions and endpoint evaluation according to EFSA guidance 2020 (doi: 10.2903/sp.efsa.2019.EN-1837) are displayed in Table 2.6.8.1/04. Guanidine did not activate any alerts associated with genotoxicity. Similarly, the metabolites dodecylguanidine-, octylguanidine- and hexylguanidine-carboxylic acids did not activate any alerts with the exception of US EPA T.E.S.T *S.Typhimurium* Consensus, Hierarchical clustering and Nearest Neighbour models. These alerts were also activated by the parent compound dodine. Furthermore, the output predictions in Derek Nexus shows all compounds are predicted negative in bacterial mutation (Ames test) and they did not activate any chromosome damage (*in vitro or in vivo*) alert ([redacted] 2020; B.6.8.1.3).

The OECD (Q)SAR Toolbox was used to identify organic functional groups within the metabolites and parent compound ([redacted] 2021; B.6.8.1.1). Two main groups were identified: guanidine (both parent and metabolites share it) and guanidine carboxylic acid (only dodecylguanidine-, octylguanidine- and hexylguanidine-carboxylic acids).

Chemical profiling of the parent and metabolites using the OECD (Q)SAR Toolbox shows none of the compounds contain structural features associated with DNA/protein binding and they do not activate any endpoint-specific profiler alert ([redacted], 2021; B.6.8.1.1).

In conclusion, based on a weight of evidence approach, guanidine is not expected to be genotoxic. Both experimental data on guanidinium salts and *in silico* predictions do not indicate this compound can be reactive with DNA. Similarly, *in silico* predictions for the metabolites dodecylguanidine-, octylguanidine- and hexylguanidine-carboxylic acids do not indicate these structures have genotoxic potential. These compounds activate the same alerts as the parent compound. Furthermore, these compounds contain and additional carboxylic acid group in the molecule that it is reported not to infer further reactivity with DNA, as reported by Benigni *et al.* in the EFSA publication 2019 (doi: 10.2903/sp.efsa.2019.EN-1598).

Assessment of general toxicity

No data are available for any of the metabolites.

Guanidinium hydrochloride has a harmonised classification:
Acute Tox. 4 (H302)
Eye Irrit. 2 (H319)
Skin Irrit. 2 (H315)

The applicant provided a series of studies with experimental data on guanidinium salts (chloride and nitrate) that have been evaluated and assessed by the RMS (B.6.8.1.5 to B.6.8.1.15). The summary of these data is displayed in the following table 2.6.8.1/03.

Table 2.6.8.1/03. Summary of available data on guanidinium salts

Compound	Assay type	Result
Guanidinium chloride	Acute oral toxicity study in rats	LD_{50} (oral, rat): 556.5 (♂), 474.6 mg/kg bw (♀)
	Acute oral toxicity study in mice	LD_{50} (oral, mouse) : 570.8 (♂), 621.4 mg/kg bw (♀)
	Skin irritation study	Irritating to skin
	Eye irritation study	Irritating to the eye

Compound	Assay type	Result
	Skin sensitisation study (Buehler)	Non-sensitiser
Guanidinium nitrate	Acute oral toxicity study in rats	LD_{50} (oral, rat) : 989.6 (♂), 729.8 mg/kg bw (♀)
	Acute oral toxicity study in mice	LD_{50} (oral, mouse) : 1105 (♂) 1028 mg/kg bw (♀)
	Skin irritation study	Irritating to skin
	Eye irritation study	Irritating to the eye
	Skin sensitisation study (Buehler)	Non-sensitiser

In Silico prognosis for general toxicity has been carried out using various predictive models (2021; B.6.8.1.2 and , 2020; B.6.8.1.3). The integrated summary predictions and endpoint evaluation according to EFSA guidance 2020 (doi: 10.2903/sp.efsa.2019.EN-1837) are displayed in Table 2.6.8.1/04. Guanidine did not activate any alerts associated with carcinogenicity and reproductive and developmental toxicity or related endpoints. Guanidine activated a hepatotoxicity alert in Vega as a result of experimental data and a hERG Channel inhibition alert in Derek Nexus.
The metabolite dodecylguanidine carboxylic acid did not activate any alerts associated with carcinogenicity and reproductive and developmental toxicity (out of domain) or related endpoints. It was predicted as non-sensitiser in Derek Nexus (negative prediction) although it activated a hERG Channel inhibition alert in Derek Nexus.
The metabolite octylguanidine carboxylic acid did not activate any alerts associated with carcinogenicity and reproductive and developmental toxicity or related endpoints. It was predicted as non-sensitiser in Derek Nexus (negative prediction) although it activated a hERG Channel inhibition alert in Derek Nexus.
The metabolite hexylguanidine carboxylic acids did not activate any alerts associated with carcinogenicity and reproductive and developmental toxicity (out of domain) or related endpoints. This compound activated a hERG channel inhibition alert, a hepatotoxicity alert and a nephrotoxicity rapid prototype alert in Derek Nexus.

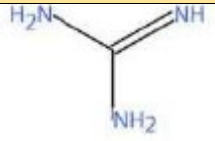
The OECD (Q)SAR Toolbox was used to identify organic functional groups within the metabolites and parent compound (2021; B.6.8.1.2). Two main groups were identified: guanidine (both parent and metabolites share it) and guanidine carboxylic acid (only dodecylguanidine-, octylguanidine- and hexylguanidine-carboxylic acids).

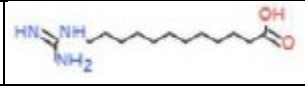
Chemical profiling of the parent and metabolites using the OECD (Q)SAR Toolbox indicates none of the compounds contain structural features associated with general toxicity other than guanidine for repeat dose toxicity (HESS profiler alert) (2021; B.6.8.1.2). No profiler alerts associated with general toxicity were activated by dodecylguanidine-, octylguanidine- and hexylguanidine-carboxylic acids.

In conclusion, based on available experimental data, guanidine has a lower LD_{50} value than dodine, which may indicate this compound is more toxic than the parent compound. Furthermore, guanidine hydrochloride is used as a pharmaceutical drug to treat muscle weakness and fatigue associated with the myasthenic complications in people suffering from Eaton-Lambert syndrome ( )[4]. Reported effects in humans include gastrointestinal and nervous system along the neuromuscular system (desired effect as a pharmaceutical drug). Despite no alerts activated for general endpoints other than hERG channel inhibition and hepatotoxicity, guanidine is expected to have different ADME properties in comparison to the parent compound based on physicochemical properties. For this reason, no conclusion can be derived on the general toxicity of this metabolite.
With regards to the remaining metabolites, they have been grouped together as alkyl guanidine carboxylic acid derivatives. *In silico* predictions for dodecylguanidine-, octylguanidine- and hexylguanidine-carboxylic acids indicate they activate a hERG channel inhibition alert in Derek Nexus and this alert is not shared by the parent compound. The metabolite hexylguanidine-carboxylic acid also activates a hepatotoxicity alert and nephrotoxicity alert in Derek Nexus. Due to uncertainties in the ADME properties together with a different toxicological profile with respect to the parent compound, no conclusion can be derived on the general toxicity of these metabolites.

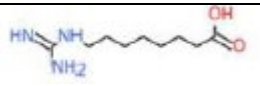
[4] (2006) A review of Toxicity and Use and Handling Considerations for Guanidine, Guanidine Hydrochloride and Urea. Report No. PNNL-15747. Pacific Northwest National Laboratory.

Table 2.6.8.1/04. Summary table integrating all available information

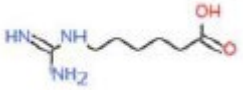
Name, code and smiles	**Structure**	**Origin (groundwater, crop, livestock, etc)**	**Group (if grouping is proposed)** Group name / Lead compound (yes/no)	**Percentage of the applied/absorbed dose in excreta, body fluids and tissues in ADME studies with parent**	**Genotoxicity conclusion and basis (endpoints).** Experimental Data/QSAR/Grouping and Read-across/Covered by parent	**General toxicity conclusion and basis (endpoints)** Experimental Data/QSAR/Grouping and Read-across/Covered by parent. Reference potency factors and/or Reference values (UF and basis)
GUANIDINE NC(N)=N		Crop (apple, strawberry, pecan)	Grouping not proposed	Not detected	**Experimental *in vitro***: Guanidinium salts (nitrate and hydrochloride) • Ames test: negative • MLA: negative • CA *in vitro*: negative ***In Silico* analysis**: • <u>Toxtree</u> Negative results on DNA binding alerts, protein binding alerts, *in vitro* mutgenicity (Ames test) alerts by ISS, Structure alerts for the *in vivo* micronucleus assay in rodents. • <u>T.E.S.T US EPA</u> Negative results on Ames mutagenicity (S. Typhimurium) – Consensus model, FDA Model, Hierarchical clustering model, Nearest Neighbour model • <u>VEGA</u> Negative results on Mutagenicity (Ames test) Consensus model, CAESAR and ISS models Possible negative on mutagenicity SarPy/IRFMN Negative (experimental) from Mutagenicity KNN/Read across model • <u>Derek Nexus</u>	**Experimental data**: Guanidinium hydrochloride has a harmonised classification : Acute Tox. 4 (H302) Eye Irrit. 2 (H319) Skin Irrit. 2 (H315) <u>Guanidine Hydrochloride</u> LD_{50} (oral, rat) 556.5 (♂), 474.6 mg/kg bw (♀) LD_{50} (oral, mouse) 570.8 (♂), 621.4 mg/kg bw (♀) LD_{50} (dermal) > 2000 mg/kg bw Irritating to skin Irritating to eye Non-sensitiser (Buehler) <u>Guanidine nitrate</u> LD_{50} (oral, rat) 989.6 (♂), 729.8 mg/kg bw (♀) LD_{50} (oral, mouse) 1105 (♂) 1028 mg/kg bw (♀) Irritating to skin Irritating to eye Non-sensitiser (Buehler) ***In Silico* analysis**: • <u>Toxtree</u> Carcinogenicity (genetox and non-genotox) : negative Skin sensitisation : negative • <u>Vega</u> Carcinogenicity IRFM/Antares : possible negative

Name, code and smiles	Structure	Origin (groundwater, crop, livestock, etc)	Group (if grouping is proposed) Group name / Lead compound (yes/no)	Percentage of the applied/absorbed dose in excreta, body fluids and tissues in ADME studies with parent	Genotoxicity conclusion and basis (endpoints). Experimental Data/QSAR/Grouping and Read-across/Covered by parent	General toxicity conclusion and basis (endpoints) Experimental Data/QSAR/Grouping and Read-across/Covered by parent. Reference potency factors and/or Reference values (UF and basis)
					Negative prediction on bacterial mutagencity (Ames test). No chromosome damage alerts were activated **Chemical profiling and grouping :** Guanidine and dodine both contain the guanidine functional group. No profiler alerts associated with genotoxicity were activated by guanidine. **Conclusion on genotoxicity:** Experimental data on guanidinium salts indicate these compounds are not mutagenic in bacteria and in mammalian cells. The outcome of the *in Silico* analysis shows no concern for bacterial mutagenicity (negative predictions) and clastogenicity/aneugenicity (no alerts activated in Derek Nexus).	Reproductive and developmental toxicity : out of AD Skin sensitisation : out of AD Hepatotoxicity IRFM : positive (experimental) • Derek Nexus Skin sensitisation : negative One alert activated : hERG channel inhibition No alerts activated for the remaining endpoints **Chemical profiling and grouping :** Guanidine and dodine both contain the guanidine functional group. No profiler alerts were activated by guanidine for the general mechanistic and endpoint-specific profilers. Guanidine was profiled for repeated dose (HESS) for mucous membrane irritation. **Conclusion on general toxicity** : Guanidine has a lower LD_{50} value than dodine, which may indicate this compound is more toxic than the parent compound. Despite no alerts activated for general endpoints, guanidine is expected to have different ADME properties in comparison with the parent compound. For this reason, no conclusion can be derived on the general toxicity of this metabolite.
Dodecylguanidine carboxylic acid		Livestock (goat)	Grouping not proposed	Not detected	**Experimental data** No data available	**Experimental data** No data available

Name, code and smiles	**Structure**	**Origin (groundwater, crop, livestock, etc)**	**Group (if grouping is proposed)** Group name / Lead compound (yes/no)	**Percentage of the applied/absorbed dose in excreta, body fluids and tissues in ADME studies with parent**	**Genotoxicity conclusion and basis (endpoints)**. Experimental Data/QSAR/Grouping and Read-across/Covered by parent	**General toxicity conclusion and basis (endpoints)** Experimental Data/QSAR/Grouping and Read-across/Covered by parent. Reference potency factors and/or Reference values (UF and basis)
NC(=N)NCCCCCCCGCGCCC(0)=0					***In Silico* analysis**: • Toxtree Negative results on DNA binding alerts, protein binding alerts, *in vitro* mutgenicity (Ames test) alerts by ISS, Structure alerts for the *in vivo* micronucleus assay in rodents. • T.E.S.T US EPA Negative results on Ames mutagenicity (S. Typhimurium) – FDA Model Positive results on Ames mutagenicity (S. Typhimurium) – Consensus model, Hierarchical clustering model and Nearest Neighbour model. Positive predictions shared with parent compound • VEGA Negative results on Mutagenicity (Ames test) Consensus model, CAESAR and ISS models Possible negative on mutagenicity SarPy/IRFMN Negative (experimental) from Mutagenicity KNN/Read across model • Derek Nexus Negative prediction on bacterial mutagencity (Ames test).	***In Silico* analysis**: • Toxtree Carcinogenicity (genetox and non-genotox) : negative Skin sensitisation : negative • Vega Carcinogenicity IRFM/Antares : possible negative Reproductive and developmental toxicity : out of AD Estrogen receptor binding (IRFM/CERAPP) : Negative Skin sensitisation : positive (could be out of AD) Hepatotoxicity IRFM : unknown • Derek Nexus Skin sensitisation : negative One alert activated : hERG channel inhibition No alerts activated for the remaining endpoints **Chemical profiling and grouping :** This compound contains an additional carboxylic acid in addition to the long-chain alkyl group. No profiler alerts were activated by this metabolite for the general mechanistic and endpoint-specific profilers. **Conclusion on general toxicity** : *In silico* predictions for dodecylguanidine carboxylic acid indicate it activates a hERG channel inhibition alert in Derek Nexus and this alert is not shared by the

Name, code and smiles	Structure	Origin (groundwater, crop, livestock, etc)	Group (if grouping is proposed) Group name / Lead compound (yes/no)	Percentage of the applied/absorbed dose in excreta, body fluids and tissues in ADME studies with parent	Genotoxicity conclusion and basis (endpoints). Experimental Data/QSAR/Grouping and Read-across/Covered by parent	General toxicity conclusion and basis (endpoints) Experimental Data/QSAR/Grouping and Read-across/Covered by parent. Reference potency factors and/or Reference values (UF and basis)
					No chromosome damage alerts were activated **Chemical profiling and grouping :** This metabolite contains a carboxylic acid group in addition to the guanidinium group. No profiler alerts were identified in the OECD QSAR Toolbox. **Conclusion on genotoxicity:** The outcome of the *in Silico* analysis shows no concern for bacterial mutagenicity (negative predictions) and clastogenicity/aneugenicity (no alerts activated in Derek Nexus). This metabolite is closely related to the parent compound and contains no further functional groups associated with genotoxicity (carboxylic acid).	parent compound. Due to uncertainties in the ADME properties, together with a different toxicological profile with respect to the parent compound, no conclusion can be derived on the general toxicity of this metabolite.
Octylguanidine carboxylic acid NC(=N)NCCCCCCCC(0)=0		Livestock (goat)	Grouping not proposed	Not detected	**Experimental data** No data available ***In Silico* analysis**: • Toxtree Negative results on DNA binding alerts, protein binding alerts, *in vitro* mutgenicity (Ames test) alerts by ISS, Structure alerts for the *in vivo* micronucleus assay in rodents. • T.E.S.T US EPA	**Experimental data** No data available ***In Silico* analysis**: • Toxtree Carcinogenicity (genetox and non-genotox) : negative Skin sensitisation : negative • Vega Carcinogenicity IRFM/Antares : possible negative Reproductive and developmental toxicity :negative

Name, code and smiles	Structure	Origin (groundwater, crop, livestock, etc)	Group (if grouping is proposed) Group name / Lead compound (yes/no)	Percentage of the applied/absorbed dose in excreta, body fluids and tissues in ADME studies with parent	Genotoxicity conclusion and basis (endpoints). Experimental Data/QSAR/Grouping and Read-across/Covered by parent	General toxicity conclusion and basis (endpoints) Experimental Data/QSAR/Grouping and Read-across/Covered by parent. Reference potency factors and/or Reference values (UF and basis)
					Negative results on Ames mutagenicity (S. Typhimurium) – FDA Model Positive results on Ames mutagenicity (S. Typhimurium) – Consensus model, Hierarchical clustering model and Nearest Neighbour model. Positive predictions shared with parent compound • VEGA Negative results on Mutagenicity (Ames test) Consensus model, CAESAR and ISS models Possible negative on mutagenicity SarPy/IRFMN Negative (experimental) from Mutagenicity KNN/Read across model • Derek Nexus Negative prediction on bacterial mutagencity (Ames test). No chromosome damage alerts were activated **Chemical profiling and grouping :** This metabolite contains a carboxylic acid group in addition to the guanidinium group. No profiler alerts were identified in the OECD QSAR Toolbox.	Estrogen receptor binding (IRFM/CERAPP) : Negative Skin sensitisation : positive (out of AD) Hepatotoxicity IRFM : unknown • Derek Nexus Skin sensitisation : negative One alert activated : hERG channel inhibition No alerts activated for the remaining endpoints **Chemical profiling and grouping :** This compound contains an additional carboxylic acid in addition to the long-chain alkyl group. No profiler alerts were activated by this metabolite for the general mechanistic and endpoint-specific profilers. **Conclusion on general toxicity** : *In silico* predictions for octylguanidine carboxylic acid indicate it activates a hERG channel inhibition alert in Derek Nexus and this alert is not shared by the parent compound. Due to uncertainties in the ADME properties together with a different toxicological profile with respect to the parent compound, no conclusion can be derived on the general toxicity of this metabolite.

Name, code and smiles	**Structure**	**Origin (groundwater, crop, livestock, etc)**	**Group (if grouping is proposed)** Group name / Lead compound (yes/no)	**Percentage of the applied/absorbed dose in excreta, body fluids and tissues in ADME studies with parent**	**Genotoxicity conclusion and basis (endpoints)**. Experimental Data/QSAR/Grouping and Read-across/Covered by parent	**General toxicity conclusion and basis (endpoints)** Experimental Data/QSAR/Grouping and Read-across/Covered by parent. Reference potency factors and/or Reference values (UF and basis)
					Conclusion on genotoxicity: The outcome of the *in Silico* analysis shows no concern for bacterial mutagenicity (negative predictions) and clastogenicity/aneugenicity (no alerts activated in Derek Nexus). This metabolite is closely related to the parent compound and contains no further functional groups associated with genotoxicity (carboxylic acid).	
6-Carbamimidamidohexanoic acid NC(=N)NCCCCCC(O)=O		Livestock (goat)	Grouping not proposed	Not detected	**Experimental data** No data available ***In Silico* analysis**: • <u>Toxtree</u> Negative results on DNA binding alerts, protein binding alerts, *in vitro* mutgenicity (Ames test) alerts by ISS, Structure alerts for the *in vivo* micronucleus assay in rodents. • <u>T.E.S.T US EPA</u> Negative results on Ames mutagenicity (S. Typhimurium) – FDA Model Positive results on Ames mutagenicity (S. Typhimurium) – Consensus model, Hierarchical clustering model and Nearest Neighbour model.	**Experimental data** No data available ***In Silico* analysis**: • <u>Toxtree</u> Carcinogenicity (genetox and non-genotox) : negative Skin sensitisation : negative • <u>Vega</u> Carcinogenicity IRFM/Antares : possible negative Carcinogenicity CAESAR model : negative (could be out of AD) Carcinogenicity ISS model : negative Reproductive and developmental toxicity : out of AD Estrogen receptor binding (IRFM/CERAPP) : Negative Skin sensitisation : positive (out of AD) Hepatotoxicity IRFM : unknown • <u>Derek Nexus</u> Skin sensitisation : negative

Name, code and smiles	Structure	Origin (groundwater, crop, livestock, etc)	Group (if grouping is proposed) Group name / Lead compound (yes/no)	Percentage of the applied/absorbed dose in excreta, body fluids and tissues in ADME studies with parent	Genotoxicity conclusion and basis (endpoints). Experimental Data/QSAR/Grouping and Read-across/Covered by parent	General toxicity conclusion and basis (endpoints) Experimental Data/QSAR/Grouping and Read-across/Covered by parent. Reference potency factors and/or Reference values (UF and basis)
					Positive predictions shared with parent compound • VEGA Negative results on Mutagenicity (Ames test) Consensus model, CAESAR and ISS models Possible negative on mutagenicity SarPy/IRFMN Negative (experimental) from Mutagenicity KNN/Read across model • Derek Nexus Negative prediction on bacterial mutagencity (Ames test). No chromosome damage alerts were activated **Chemical profiling and grouping :** This metabolite contains a carboxylic acid group in addition to the guanidinium group. No profiler alerts were identified in the OECD QSAR Toolbox. **Conclusion on genotoxicity:** The outcome of the *in Silico* analysis shows no concern for bacterial mutagenicity (negative predictions) and clastogenicity/aneugenicity (no alerts activated in Derek Nexus). This metabolite is closely related to the parent compound and contains no further functional groups	One alert activated : hERG channel inhibition One hepatotoxicity alert activated One nephrotoxicity rapid prototype alert No alerts activated for the remaining endpoints **Chemical profiling and grouping :** This compound contains an additional carboxylic acid in addition to the long-chain alkyl group. No profiler alerts were activated by this metabolite for the general mechanistic and endpoint-specific profilers. **Conclusion on general toxicity** : *In silico* predictions for hexylguanidine-carboxylic acid indicate it activates a hERG channel inhibition alert in Derek Nexus and this alert is not shared by the parent compound. This metabolite also activates a hepatotoxicity alert and nephrotoxicity alert in Derek Nexus. Due to uncertainties in the ADME properties together with a different toxicological profile with respect to the parent compound, no conclusion can be derived on the general toxicity of this metabolite.

Name, code and smiles	Structure	Origin (groundwater, crop, livestock, etc)	Group (if grouping is proposed) Group name / Lead compound (yes/no)	Percentage of the applied/absorbed dose in excreta, body fluids and tissues in ADME studies with parent	Genotoxicity conclusion and basis (endpoints). Experimental Data/QSAR/Grouping and Read-across/Covered by parent	General toxicity conclusion and basis (endpoints) Experimental Data/QSAR/Grouping and Read-across/Covered by parent. Reference potency factors and/or Reference values (UF and basis)
					associated with genotoxicity (carboxylic acid).	

2.6.8.2 Supplementary studies on the active substance

Immunotoxicity

One **immunotoxicity study in rats** was provided by the applicant for the renewal of the active substance dodine. In this study, the administration of dodine in the diet at 0, 200, 500 and 1000 ppm (equivalent to 0, 18, 44, 83 mg/kg bw/day) for 28 days to female rats immunized with SRBC, did not produce adverse effects in survival, clinical signs, haematology, immunological examinations (anti-SRBC IgM titers), absolute and relative spleen and thymus weights and macroscopic examinations. There were statistically significant decreases in bodyweight and bodyweight gain at 1000 ppm. A decrease in anti-SRBC IgM titers at the end of treatment period was seen at 200 ppm, but this was not considered of immunotoxicological relevance because statistical signification and dose-response relationship were not observed. Cyclophosphamide (positive control) produced a statistically significant decrease in the anti-SRBC IgM titers, in the white blood cell count and in absolute and relative spleen and thymus weights compared to control. The **NOAEL** for **general toxicity** was set at 500 ppm (equivalent to **44 mg/kg bw/day**) based on the decrease in bodyweight and bodyweight gain at 1000 ppm (equivalent to 83 mg/kg bw/day). Under the conditions of this study, which measured immunosuppression, dodine did not produce signs of immunotoxicity. The **NOAEL** for **immunotoxicity** was considered to be 1000 ppm (equivalent to **83 mg/kg bw/day**) based on the absence of adverse effects in immunotoxicity parameters at the highest dose tested.

With the aim of analysing the effect of dodine on the normal function of the immune system a **detailed review of the existing studies** has been carried out, in which immune-related parameters were analysed from short-term studies, chronic studies and reproductive studies. Furthermore, ADME studies and medical data were also evaluated for this purpose. The distribution of the active substance and its metabolites in tissues may be indicative of its immunotoxic potential, whilst medical data can provide useful information about effects related to inability to fight infection or excessive, poorly controlled responses (as anaphylaxis or autoimmunity).

The most relevant endpoints for this task were selected based on the *Retrospective analysis of the immunotoxic effects of plant protection products as reported in the Draft Assessment Reports for their peer review at EU level* ([redacted] 2015, EFSA supporting publication 2015: EN-782) and *Guidance for Immunotoxicity risk assessment for chemicals* (World Health Organization & International Programme on Chemical Safety (2012). Guidance for immunotoxicity risk assessment for chemicals. World Health Organization. IPCS harmonization project document; no. 10).

These endpoints were the following:
- Survival and infections, as indicators of potential immunotoxicity, considering that laboratory animals do not use to be exposed to infections.
- From the haematology parameters, total white blood cell counts (WBC) and/or differential counts, as key cell types involved in immune functioning/response.
- Globulin levels in serum, as an indicator of antibody synthesis. If globulin levels were not presented, A:G ratio or serum protein changes were used as surrogates.
- Lymph nodes, as integral parts of the immune system. At least one site of lymph nodes should be included for histopathological examination in most study protocols, but also the finding of lymph nodes with increased size from the clinical observations was included in the analysis.
- Gut associated lymphoid tissue or Peyer's patches, as important tissue in antigen presentation.
- Spleen (weight and histopathology), as organ involved in the maturation of lymphocytes. Altered weights can indicate atrophy or abnormal stimulation. Pathology can indicate altered immune function but can be secondary to other functions of the spleen.
- Thymus (weight and histopathology), as primary organ for T-lymphocyte maturation. Changes in young animals are reported to be more likely to indicate immunotoxicity, as thymus weight varies with the age of the animal, due to its normal age-associated shrinking or involution.
- Bone marrow smear, as reduced cellularity could indicate reduced potential to produce WBC (& RBC).
- Adrenal glands are organ target of cytokines and indeed, ACTH and adrenal steroids regulate the cytokine synthesis. Besides, deposits of immunoglobulins could be observed in adrenal glands. Some autoimmune syndromes as Addison´s disease are characterised by adrenal cortex damage.

Table 2.6.8.2/01: Summary table of animal studies on immunotoxicity

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, route of exposure, dose levels, duration of exposure</th><th>Results
- NOAEL/LOAEL
- target tissue/organ
- critical effects at the LOAEL
[Effects statistically significant and dose-related unless stated otherwise as not significant (ns) or not dose-related (ndr) or not clearly dose-related (ncdr)]</th><th>Reference</th></tr>
<tr>
<td>T-Cell dependent antibody response (TDAR) assay using sheep red blood cells (SRBC) with dodine in Sprague Dawley rats
<u>Guideline:</u> OPPTS 870.7800.
<u>GLP:</u> Yes
<u>Rat strain:</u>
♀ Sprague Dawley.
<u>No. animals</u>
10 rats/dose
<u>Deviations from US EPA TG OPPTS 870.7800 (1998):</u>
- Short acclimatization period.
- Low temperature of experimental room.
- No indications on frequency of water consumption.
- Positive control only administered for 5 days.
Study acceptable</td>
<td>Dodine (batch no. 43; purity 96.62%).

<u>Vehicle</u>: Acetone

<u>Doses</u>: 0, 200, 500, and 1000 ppm (equivalent to 0, 18, 44, 83 mg/kg bw/day) for 28 days.

Immunisation: 2 x 10^8 SRBC/ rat, i.v., on day 24.
Serum collected on day 29.</td>
<td>No mortality occurred.
No clinical signs noted.

Table: Antibody response (a-SRBC IgM, u/mL)
<table>
<tr><th colspan="2"></th><th>Dose (ppm)</th><th></th><th></th><th></th></tr>
<tr><th colspan="2"></th><th>0</th><th>200</th><th>500</th><th>1000</th></tr>
<tr><td rowspan="2">Day 1</td><td>Mean
SD</td><td>162
63</td><td>150
64</td><td>213
167</td><td>169
49</td></tr>
<tr><td rowspan="2">Day 29</td><td>Mean
SD</td><td>20308
22271</td><td>7123
7094</td><td>18599
24754</td><td>20546
14887</td></tr>
</table>
84.7% decrease in a-SRBC IgM in the PC, compared to control

1000 ppm (83 mg/kg bw/day)
<u>Bodyweight:</u>
▪ (↓) bw at day 29 (12.7%).
▪ (↓) bw gain at day 29 (34%).
<u>Haematology:</u>
▪ (↓) WBC (20%, ndr).
▪ (↑) Plt (11%, ns, ndr).
<u>Organs´ weight:</u>
▪ Thymus: (↓) abs wt (26%) and rel wt (14%, ns, ndr).
▪ Spleen: (↓) abs wt (16%, ns) and rel wt (3%, ns, ndr).

500 ppm (44 mg/kg bw/day)
<u>Haematology:</u>
▪ (↓) WBC (23%, ndr).
▪ (↑) Plt (31%, ndr).
<u>Organs´ weight:</u>
▪ Thymus: (↓) abs. wt (18%, ns) and rel. wt (16%, ns, ndr).
▪ Spleen: (↓) abs. wt (12%, ns) and rel. wt (11%, ns, ndr).

200 ppm (18 mg/kg bw/day)
<u>Haematology:</u>
▪ (↓) WBC (34%, ndr).
▪ (↑) Plt (13%, ns, ndr).
<u>Organs´ weight:</u>
▪ Thymus: (↓) abs. wt (14%, ns) and rel. wt (18%, ns, ndr).
▪ Spleen: (↓) abs wt (18%, ns, ndr) and rel. wt (23%, ndr).

NOAEL systemic: 500 ppm (equivalent to 44 mg/kg bw/day), based on the decrease in bw and bw gain at 1000 ppm.

NOAEL immunotoxicity: 1000 ppm (equivalent to 83 mg/kg bw/day), based on the absence of immunotoxicity effects.</td>
<td>[redacted] 2013)
B.6.8.2.1
(AS)</td>
</tr>
</table>

Table 2.6.8.2/02: Analysis of immune parameters in other studies with dodine

Study	Immune parameters analysed	Not analysed*
ADME studies		
No particular accumulation was observed in the immune system tissues.		

Study	Immune parameters analysed	Not analysed*
Short-term studies		
[redacted] (1994a) A 4-week oral (gavage) toxicity study of dodecylguanidine acetate (dodine) in the albino rat. B.6.3.1.1 (AS) Supportive study	Mortality: All rats died at 200 mg/kg bw/day; 4/10 ♀ died at 100 mg/kg bw/day; 1/10 ♀ died at 75 mg/kg bw/day Infections: not reported. Haematology: (↑) WBC and segmented neutrophils, (↓) lymphocytes, in ♂/♀ at 100 mg/kg bw/day. Biochemistry: (↓) Globulin in ♂/♀ from 75 mg/kg bw/day. (↑) A/G ratio in ♀ at 100 mg/kg bw/day. Adrenal glands: (↑) Rel-to-bw wt in ♂/♀ at 100 mg/kg bw/day. (↑, slight) Enlarged adrenals in ♂/♀ at 100 mg/kg bw/day. (↑) Haemorrhage in ♂/♀ at 200 mg/kg bw/day. Spleen: (↑) Lymphoid atrophy in ♂/♀ at 200 mg/kg bw/day. Thymus: (↑) Haemorrhage and lymphoid necrosis in ♂ at 200 mg/kg bw/day.	- Thymus and spleen weight. - Histopathology in thymus lymph nodes and bone marrow. If examined, only at 200 mg/kg bw/day (too high dose).
[redacted] (1994b) A 4-week oral (diet) toxicity study of dodecylguanidine acetate (dodine) in the albino rat. B.6.3.1.2 (AS) Acceptable study	Mortality: No treatment-related (2 ♀, at 0 and 72 mg/kg bw/day, died at blood sampling in week 4). Infections: not reported. Haematology: No effects. Biochemistry: No effects. Adrenal glands: No effects. Spleen: No effects in histopathology.	- Thymus and spleen weight. - Histopathology in thymus, lymph nodes and bone marrow.
[redacted] (1997) Dodecylguanidine acetate (dodine): 28-day toxicity study in the rat by dietary administration. B.6.3.1.3 (AS) Supportive study	Mortality: not reported. Infections: not reported.	- Haematology - Biochemistry - Adrenal glands, thymus and spleen weight. - Histopathology in spleen, adrenal glands, thymus, lymph nodes and bone marrow.
[redacted] [redacted] (1988) Dodine: 8 week dietary dose range finding study in mice. B.6.3.1.5 (AS) Supportive study	Mortality: 1/5 ♀ died after increasing the dose from 100 to 1250 ppm. Infections: not reported. Liver eosinophilia in almost all animals at 100/1250 ppm. Spleen: (↓) abs. wt in ♂/♀ at 100/1250 ppm; (↓) rel. wt in ♂/♀ from 625 ppm; cellular depletion and pigment deposit decreased in 1/5 ♀ at 100/1250 ppm.	No guideline
[redacted] [redacted] (1982) Sub-chronic (90-day) oral toxicity study with dodine in rats. B.6.3.2.1 (AS) Acceptable study	Mortality: no effect. Infections: not reported. Haematology: (↑) neutrophils, (↓, slight) lymphocytes in ♂ at 56 mg/kg bw/day. Biochemistry: no effect. Adrenal glands: no effect. Mesenteric and axillary lymph nodes: histopathology unaffected. Rectum: hyperplasia of patches of Peyer in 1/10 ♂ at 56 mg/kg bw/day. Spleen: (↓) abs. wt in ♀ at 60 mg/kg bw/day; extra medullary haematopoiesis in 2/10 ♂ at 56 mg/kg bw/day *vs* 0/10 in control. Thymus: (↓) abs./rel. wt in ♂ at 56 mg/kg bw/day; (↓) abs./rel. wt in ♀ from 15 mg/kg bw/day, histopathology: unaffected.	- Histopathology of bone marrow.
Mitjans, M., *et al*. (1999)	Mortality: not reported. Infections: not reported.	No guideline

Study	Immune parameters analysed	Not analysed*
Hematological and Biochemical Parameters in the Rat Following Subchronic Oral Administration of Dodine (n-Dodecylguanidine Acetate). B.6.3.2.2 (AS) Supportive study	Haematology: (↓) WBC in ♀ from 5 mg/kg bw/day (ns).	
[redacted] (1994) A 13-week dietary toxicity study of dodecylguanidine acetate (dodine) in the albino mouse. B.6.3.2.4 (AS) Acceptable study	Mortality: 4 ♀ at 305 mg/kg bw/day died during 1st 2 weeks (animals showing lymphoid atrophy of spleen and/or lymphoid atrophy and/or necrosis of the thymus). Infections: not reported. Haematology: (↑) segmented neutrophils in ♂ at 350 mg/kg bw/day. Biochemistry: (↑) A/G ratio in ♀ at 305 mg/kg bw/day. Adrenal glands: no effect in histopathology. Bone marrow: histopathology unaffected. Mesenteric and mandibular lymph nodes: histopathology unaffected. Spleen: (↓) abs. wt in ♂ from 181 mg/kg bw/day; (↓) abs./rel. wt in ♀ from 223 mg/kg bw/day; lymphoid atrophy in 3/10 ♀ at 305 mg/kg bw/day *vs* 0/10 in controls. Thymus: lymphoid necrosis in 4/10 ♀ at 305 mg/kg bw/day *vs* 0/10 in controls; haemorrhage in 1/10 ♀ at 305 mg/kg bw/day *vs* 0/10 in controls; lymphoid atrophy in 4/10 ♀ at 305 mg/kg bw/day *vs* 0/10 in controls.	- Adrenal glands and thymus weight.
[redacted] (2005) 90-Day repeated dose toxicity study with dodine by daily capsule administration in Beagle dogs. B.6.3.2.5 (AS) Acceptable study	Mortality: not reported. Infections: not reported. Haematology: No effects. Biochemistry: No effects. Adrenal glands: no clear effects in wt. Mesenteric and mandibular lymph nodes: histopathology unaffected. Spleen: no clear effects in wt and histopathology. Thymus: (↑) abs./rel. wt in ♂ from 10 mg/kg bw/day, ns; (↓) abs./rel. wt in ♀ at 20 mg/kg bw/day, ns; reduced thymus size in 1/4 in ♀ at 20 mg/kg bw/day; histopathology unaffected.	- Histopathology of bone marrow
[redacted] (1996) 52-Week toxicity study in dogs with dodine. B.6.3.3.1 (AS) Acceptable study	Mortality: not reported (supplemental feeding to preclude mortality). Infections: not reported. Haematology: (↑) WBC, segmented neutrophils and eosinophils in ♀ at 10 mg/kg bw/day. Biochemistry: no effects. Adrenal glands: 1/4 ♂ with vacuolization in adrenal cortex from 10 mg/kg bw/day (vs 0/4 in controls). Mesenteric and mandibular lymph nodes: histopathology unaffected. Spleen: histopathology unaffected. Thymus: 4/4 ♂ with cyst at 20 mg/kg bw/day *vs* 3/4 in controls, ncdr; 2/4 ♀ with cyst at 20 mg/kg bw/day *vs* 1/4 in controls, ncdr.	- Adrenal glands and spleen weight.
[redacted] (1999e) A 28-day dermal toxicity study of dodine technical material in rats. B.6.3.4.1.1 (AS) Acceptable study	Mortality: not reported. Infections: not reported. Haematology: no effects. Biochemistry: no effects. Adrenal glands: no effects. Bone marrow: no effects.	None

Study	Immune parameters analysed	Not analysed*
	Mesenteric lymph node: histopathology unaffected. Spleen: no effects. Thymus: (↓) abs./rel. wt in ♂/♀, ns. 1/10 ♂ with haemorrhagic thymus at 200 mg/kg bw/day *vs* 0/10 in controls.	
Long-term and carcinogenesis studies		
[redacted] (1998) Chronic toxicity and carcinogenicity study of dodecylguanidine acetate (dodine) in the Sprague-Dawley rat by dietary administration. B.6.5.1 Acceptable study	- Mortality: no treatment-related. - Infections: not reported. - Adrenals: (↓) rel. wt in ♂ (32%, ndr, ns) from 200 ppm. Enlarged in ♀: 17.1, 24.3 and 28.6% in 0, 400 and 800 ppm, respectively. White mottling ♀: 12.9 and 22.9% in 0 and 800 ppm, respectively. - Mesenteric and submaxillary lymph nodes histopathology: unaffected. - Spleen histopathology: unaffected. - Thymus gross necropsy: Small in ♀: 0, 1.4 and 7.1% in 0, 400 and 800 ppm. - Femur bone marrow: unaffected. - Haematology: (↓) WBC (24%) and lymphocytes (26%) in ♂ at 800 ppm, only at week 26.	None
[redacted] (1998a) 78-Week dietary oncongenicity study with dodine in mice B.6.5.2 Acceptable study	- Mortality: survival dose-related increased in male dodine-treated groups. - Infections: not reported. - Adrenal: (↓) left abs wt in ♀ (18%, ncdr) from 750 ppm. Histopathology, unaffected. - Mesenteric and superficial cervical: histopathology: unaffected. - Spleen: Small in ♀: 4.3% at 1500 ppm *vs* 0% in controls. - Thymus: weight and histopathology unaffected. - Femur bone marrow: unaffected.	- Haematology - Biochemistry - Spleen not weighed.
Reproduction		
[redacted] [redacted] (1996) Two-generation reproduction study with dodine in rats. B.6.6.1.1 Acceptable study	- Mortality: no treatment-related. - Infections: not reported. - Adrenal: (↑) rel left and right wt in P ♀ adults at 800 ppm (14%). (↑) rel left (12%) and right (9%) wt in F1 ♀ adults from 400 ppm. - Spleen: (↓) abs wt in F2 ♂ pups (18%) and in F2 ♀ pups (28%) at 800 ppm. - Thymus: (↓) abs wt in P ♂ adults at 800 ppm (17%). Red focus area in F1 ♂ adults: 10, 23, 31 and 13% in 0, 200, 400 and 800 ppm, respectively. Mottled in F1 ♀ adults (7% at 800 ppm *vs* 0% in controls). (↓) abs wt in F2 ♀ pups at 800 ppm (28%). - Submaxillary and mesenteric lymph nodes: unaffected histopathology.	None
[redacted] [redacted] (1989a) Dodine: Dose range finding study in rats preliminary to teratogenicity study. B.6.6.2.1 Supportive study	- Mortality: 1 ♀ at 100 mg/kg bw/day died. - Infections: not reported. - Lumbar lymph node: hyperplasia (10% at 100 mg/kg bw/day *vs* 0% in controls).	None
[redacted] [redacted] (1989b) Dodine: Teratogenicity study in rats. B.6.6.2.2 Acceptable study	- Mortality: not reported. - Infections: not reported.	None

Study	Immune parameters analysed	Not analysed*
[redacted] (1989a) Dodine: Dose range finding study in rabbits preliminary to teratogenicity study. B.6.6.2.3 Supportive study	- Mortality: 5 ♀ at 100 mg/kg bw/day and 1 ♀ at 70 mg/kg bw/day humanely killed due to morbidity signs. - Infections: not reported.	None
[redacted] (1989b) Dodine: Teratogenicity study in rabbits. B.6.6.2.4 Acceptable study	- Mortality: At 80 mg/kg bw/day, 1 ♀ died at GD15 after showing breathing difficulties, 1 ♀ humanely killed at GD11 after showing same clinical signs and 1 ♀ killed because of poor condition. At 40 mg/kg bw/day, 1 ♀ found dead due to accidental damage during dosing. - Infections: not reported.	None
Medical data		
Bokotey, S and Turi, Z. (2021) (AS) B.6.9.1	No specific changes in the health status of the workers involved in dodine production.	No guideline

** Parameters not analysed, despite they should be reported according to the respective OECD guideline.*
ns: not significant; ncdr: not clearly dose-related.

The collected data permit to build an overview on the immunotoxic potential of dodine, considering the following groups of parameters:

General health condition

The only case in which dead animals seemed to have affected immune system was in the 13-week dietary toxicity study in mice, in which four females at 305 mg/kg bw/day died during the first two weeks, showing lymphoid atrophy of spleen and/or lymphoid atrophy and/or necrosis of the thymus.
Within the limitations of the experiments performed under laboratory conditions, no particular concern about the immune system functioning arose from the analysis of mortality and infections in the rest of the dataset.

Haematology parameters

White blood cells were increased in the oral 28-day study in rats by gavage, both in males and females at 100 mg/kg bw/day. In these rats, neutrophils were increased while lymphocyte levels were decreased. No effects were observed in the oral 28-day dietary study in rats or in the dermal 28-day study. In the oral 90-day study by gavage, a non-significant decrease in white blood cells was observed in females from 5 mg/kg bw/day and in the oral 90-day dietary study increased neutrophils and slightly decreased lymphocytes were seen at 56 mg/kg bw/day. In the 106-week dietary study in rats, white blood cells and lymphocyte levels were reduced in males at 800 ppm, but only at week 26 sampling. In the T-Cell dependent antibody response (TDAR) assay, white blood cell count was reduced in all treated females, but without dose-dependency
In mice, an increase in segmented neutrophils was seen in males at 350 mg/kg bw/day tested in the 13-week dietary study, while it was not measured in the 78-week study.
In dogs, no effect were observed in the 90-day study, but increments were reported for white blood cells, segmented neutrophils and eosinophils levels in females at 10 mg/kg bw/day tested in the oral 52-week study.

Biochemical parameters

A decrease in globulin levels in both sexes from 75 mg/kg bw/day and an increase in the A/G ratio in female rats at 100 mg/kg bw/day in the 28-day study by gavage. In the T-Cell dependent antibody response (TDAR) assay, no effects were observed in the antibody response. In the 13-week study in mice, an increase in the A/G ratio was seen in females at 305 mg/kg bw/day. No other effects were observed in any of the rest of the studies available.

Organs and tissues

Lymph nodes: A slight increase in lumbar lymph node hyperplasia (10% at 100 mg/kg bw/day *vs* 0% in controls) was seen in the dose range finding study teratogenicity study in rats. No other alterations in lymph nodes were detected in the rest of the studies in which they were examined.

Peyer's patches: In the 90-day dietary study in rats, hyperplasia of patches of Peyer was seen in one female at 56 mg/kg bw/day. No more data were available.

Spleen:
In rats, an increase in the incidence of lymphoid atrophy of spleen was seen in male and female rats at 200 mg/kg bw/day in the 28-day study performed by gavage. In this study, spleen was not weighed. No histopathological effects were seen in the 28-day dietary study, nor in the 28-dermal study in rats. In the 90-day study in rats, a decrease in the absolute spleen weight was reported in females at 60 mg/kg bw/day and extra medullary haematopoiesis was incremented in males (2/10 at 56 mg/kg bw/day *vs* 0/10 in control). Spleen histopathology was unaffected in the 106-week study in rats. In the two-generation study, a decrease in the absolute spleen weight was reported only in F2 male and female pups at 800 ppm (the highest dose tested). A reduction in absolute spleen weight was observed in all treated female rats during the T-Cell dependent antibody response (TDAR) assay, but it was no statistically significant.
In the 8-week study in mice, a decrease in the absolute spleen weight was observed in both sexes at 100/1250 ppm. The relative weight was reduced in both sexes from 625 ppm. Cellular depletion and pigment deposit were decreased in one out of five females at 100/1250 ppm. In the 13-week study, reductions were reported in the absolute spleen weight of male mice from 181 mg/kg bw/day and in the absolute and relative spleen weights of females from 223 mg/kg bw/day, and the incidence of lymphoid atrophy was increased in females at 305 mg/kg bw/day (3/10 *vs* 0/10 in controls). In the 78- week study in mice, a very slight increment in the incidence of small spleens was seen in females (4.3% at 1500 ppm *vs* 0% in controls).
In dogs, no effects in spleen were observed.
Overall, no clear signs of immunotoxicity were observed from spleen data.

Thymus: Increased incidence of haemorrhage and lymphoid necrosis in thymus was reported for male rats at 200 mg/kg bw/day in the 28-day gavage study, being the dose the highest dose tested with high mortality. In the 90-day dietary study in rats, reductions in the absolute and relative thymus weight were observed, but they were non-statistically significant and histopathology was not affected. In the 106-week study in rats, the incidence of small thymus observed in the necropsy was slightly increased in females (0, 1.4 and 7.1% in 0, 400 and 800 ppm, respectively. In the two-generation study in rats, a reduction in the absolute thymus weight was reported in P male adults and in F2 female pups at 800 ppm. In this study, a slight and non dose-dependent increase in the incidence of red focus area was observed in the F1 male treated adults. The incidence of mottled thymus was slightly increased in F1 female adults at 800 ppm (7% *vs* 0% in controls). In the T-Cell dependent antibody response (TDAR) assay, a reduction in the absolute thymus weight was observed in females at 1000 ppm.
In mice, lymphoid necrosis in thymus was observed in 4/10 females at 305 mg/kg bw/day *vs* 0/10 in controls during the 13-week study, in which some females at this dose also showed haemorrhage and lymphoid atrophy. However, in the 78-week study in mice, thymus weight and histopathology were unaffected.
In dogs, an increase in the absolute and relative thymus weight of males was seen from 10 mg/kg bw/day and a decrease was observed in females at 20 mg/kg bw/day during the 90-day study. Moreover, in this last study, reduced thymus size was reported in 1/4 female at 20 mg/kg bw/day during gross necropsy examination and no histopathological effects were reported. In the 52-week study in dog, a very slightly increased incidence of thymus with cysts was observed in both sexes at 20 mg/kg bw/day (4/4 males *vs* 3/4 in controls; 2/4 females *vs* 1/4 in controls).
Overall, no clear signs of immunotoxicity were observed from thymus data.

Bone marrow: No alteration in bone marrow was detected in the short-term and long-term studies.

Adrenal gland: In the 28-day study carried out by gavage, increased relative to bodyweight was observed in rats from both sexes at 100 mg/kg bw/day. In this study, in both sexes, slight increases were seen in the incidences of enlarged adrenals at 100 mg/kg bw/day and of haemorrhage at 200 mg/kg bw/day, doses at which mortalities were reported. No effects in adrenals were reported in the 28-day and 90 -day dietary studies in rats. In the long-term study in rats, a non-statistically significant reduction in the relative adrenal weight was seen in males from 200 ppm, and very slight increments in enlarged adrenals and adrenals with white mottling in females. In the 2-generations study in rats, increased relative left and right adrenal weights were seen in P female adults at 800 ppm and in F1 female adults from 400 ppm.
In the 13-week study in mice, no effects were observed. In the 78-week study in mice, a decrease in the absolute left adrenal weight was seen in females 750 ppm, and the histopathology was unaffected.
No effects in adrenals were observed in the oral 90-day study in dogs. In the 52-week study, only a slight increase in adrenal glands of males with vacuolization in adrenal cortex was seen from 10 mg/kg bw/day (1/4 *vs* 0/4 in controls).
Overall, no clear signs of immunotoxicity were observed from adrenals data

Human data

Regarding to the available medical data, an occupational report did not show reactions or ill health in any workers.

Conclusion on immunotoxicity

Based on the available toxicology data, treatment-related changes in the immunotoxic sensitive parameters were observed, but the observations were not considered enough as to conclude that dodine is immunotoxic. In addition, dodine does not belong to a class of chemicals (e.g., the organotins, heavy metals, or halogenated aromatic hydrocarbons) that would be expected to be immunotoxic. Within the scope of this brief analysis, **it can be concluded that dodine is devoid of immunotoxic potential**.

2.6.9 Summary of medical data and information

Medical surveillance on manufacturing plant personnel and monitoring studies

A report is provided in which it is stated that there has not been any specific change in the health status of the workers working with dodine over 20 years (Vol.3, AS, B.6.9.1).

Data collected on humans

No further information was found in the literature search performed for the renewal.
The information available in the Dodine DAR reported one case of oculo-rhinitis due to sensitization to dodine (original study not assessed) and one case of fatal poisoning (but when exposed to a mixture of monocrotophos, dodine and dinocap). Furthermore, several calls to the Belgian Poisoning Center informed about weakness, dizziness and vomiting 10 h after dodine inhalation and ocular irritation (red eye, irritation and tears) after contact of dodine with eyes. Other symptoms were mentioned, but when exposed to mixtures that were not characterised.

Direct observations

There are no direct observations available in the published literature for dodine.

Epidemiological studies

There are no epidemiological studies available for dodine.

Diagnosis of poisoning and clinical tests

There have been no documented cases of human poisoning with dodine. The following signs have been mentioned mentioned by the applicant and in the DAR 2009 (no source citation): By skin contact, skin inflammation is characterised by itching, scaling, reddening, or, occasionally, blistering. Inhalation or ingestion may cause nausea, vomiting, abdominal pains. By eye contact, symptoms of irritation occur.

Proposed treatment: first aid measures, antidotes and medical treatment

No further data necessary with respect to the DAR (2009). If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a doctor. Loosen tight clothing such as collar, tie, belt or waistband. In case of skin contact, immediately flush skin with plenty of water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Wash clothing before re-use. Thoroughly clean shoes before reuse. Get medical attention. If in contact with eye, check for and remove any contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.
No specific treatment is available, treat symptomatically.

2.6.10 Toxicological end points for risk assessment (reference values)

Table 67: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Short-term toxicity						
SPF-bred, Cpb:WU, Wistar rats (10/sex/dose)	90-day oral study (diet) [redacted] (1982)	Dodine (batch no. 196.53 and purity of 95%). Doses: 0, 50, 200 and 800 ppm, equivalent to 0, 3.59, 14.09 and 55.84 mg/kg bw/day in ♂ and 0, 3.87, 14.94 and 60.44 mg/kg bw/day in ♀.	↓ Bw gain in ♂/♀ ↓ Food consumption in ♀ ↑ neutrophils in ♂ ↓ ALT in ♀.	200 ppm (equivalent to 14.09 mg/kg bw/day in ♂ and 14.94 mg/kg bw/day in ♀)	800 ppm (equivalent to 55.84 mg/kg bw/day in ♂ and 60.44 mg/kg bw/day in ♀)	**B.6.3.2.1 (AS)**
CR1:CD®-1(ICR)BR mice (10/sex/dose)	90-day oral study (diet) [redacted] (1994)	Dodine (batch no. APA 303/30 and purity of 94.07%). Doses: 0, 150, 300, 600, 1250 or 2500 ppm, equivalent to 0, 24, 48, 94, 181 and 350 mg/kg bw/day in ♂ and 0, 31, 60, 116, 223 and 305 mg/kg bw/day in ♀.	↓ Bw gain in ♂ ↓ Food consumption in ♂/♀ ↓ abs. spleen wt in ♂/♀ ↓ rel. spleen wt in ♀	600 ppm (equivalent to 94 mg/kg bw/day in ♂ and 116 mg/kg bw/day in ♀)	1250 ppm (equivalent to 181 mg/kg bw/day in ♂ and 223 mg/kg bw/day in ♀)	**B.6.3.2.4 (AS)**
Beagle dogs (4/sex/dose)	90-day oral study (capsules) [redacted] (2005)	Dodine (batch no. DCH0112). Doses: 0, 2, 10 and 20 mg/kg bw/day.	↓ Bw in ♀ ↓ Bw gain in ♂/♀ ↓ Food consumption in ♂/♀	10 mg/kg bw/day	20 mg/kg bw/day	**B.6.3.2.5 (AS)**
Beagle dogs (4/sex/dose)	52-week oral study (capsules) [redacted] (1996)	Dodine (batch no. 1174 and purity of 98.6%). Doses: 0, 2, 10 and 20 mg/kg bw/day.	Supplemental feeding required, ♀	2 mg/kg bw/day	10 mg/kg bw/day	**B.6.3.3.1 (AS)**
Long-term toxicity and carcinogenicity						
Sprague-Dawley rats (60/sex/ dose)	106-week oral study in rats [redacted] (1998)	Dodecylguanidine acetate Doses: 0, 200, 400 and 800 ppm, equivalent to 0, 10.17, 20.34 and 41.93 mg/kg bw/day in ♂ and 0, 13.19, 26.5 or 53.5 mg/kg bw/day in ♀.	↑ Thyroid c-cell adenomas and carcinomas incidences in ♂.	-	200 ppm (equivalent to 10.17 mg/kg bw/day in ♂ and 13.19 mg/kg bw/day in ♀)	**B.6.5.1 (AS)**

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Crl:CD-1(ICR)BR mice (60/sex/dose)	Carcinogenesis 78-week study (diet) [redacted] (1998a)	Dodine Doses: 0, 200, 750 and 1500 ppm, equivalent to 0, 29.2, 109.8 or 224.8 mg/kg bw/day in ♂ and 0, 38.3, 136.2 or 275.2 mg/kg bw/day in ♀.	↑ Clinical signs, ↓ bw and food consumption in ♂/♀.	200 ppm (equivalent to 29.2 mg/kg bw/day in ♂ and 38.3 mg/kg bw/day in ♀)	750 ppm (equivalent to 109.8 mg/kg bw/day in ♂ and 136.2 mg/kg bw/day in ♀)	**B.6.5.2 (AS)**
Reproductive toxicity						
Sprague-Dawley rats (30/sex/dose)	Two-generation reproduction study (diet) [redacted] 1996	Dodine (batch No.: 1174, Purity: 98.6%). Doses: 0, 200, 400 and 800 ppm, equivalent to 0, 13.14, 26.2, and 52.6 mg/kg bw/day in ♂ and 0, 15.6, 31.2 and 60.3 mg/kg bw/day in ♀.	↓ Bw in F_1 ♂/♀ parents. ↓ Bw in F_1 and F_2 pups. ↑ Rel. adrenal wt in F_1 ♀ adults.	200 ppm (equivalent to 13.14 mg/kg bw/day in ♂ and 15.6 mg/kg bw/day in ♀)	400 ppm (equivalent to 26.2 mg/kg bw/day in ♂ and 31.2 mg/kg bw/day in ♀)	**B.6.6.1.1 (AS)**
Sprague-Dawley female rats (25/dose)	Teratogenicity study in rats [redacted] 1989b	Dodine (batch No. APA 92/88/2, purity: 95%). Doses: 0, 10, 45 and 90 mg/kg bw/day.	↓ Bw gain in dams. ↓ Food consumption in dams.	10 mg/kg bw/day	45 mg/kg bw/day	**B.6.6.2.2 (AS)**
New Zealand White female rabbits (20 for high dose group; 16/dose for the rest)	Teratogenicity study in rabbits. [redacted] and [redacted] 1989b	Dodine (batch No. APA 92/88/2, purity: 95%). Doses: 0, 10, 40 and 80 mg/kg bw/day.	↑ Post implantation loss and late resorptions	10 mg/kg bw/day	40 mg/kg bw/day	**B.6.6.2.4 (AS)**

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

The acceptable daily intake (ADI) for humans is normally derived from the NOAEL in the most susceptible species in long-term toxicity studies and applying an appropriate safety factor.

The most sensitive effect in the most sensitive species was found in the one-year toxicity study in dogs ([redacted] (1996). Dodine administration to dogs at the dose of 10 mg/kg bw/day resulted in the necessity of supplemental feeding and therefore the NOAEL of the study was set at 2 mg/kg bw/day.
Therefore, NOAEL of 2 mg/kg bw/day is considered for the calculation of the acceptable daily intake. The safety factor selected was 100 (derived from 10-fold factor for inter-species variability and 10-fold factor for inter-individuals variability) and the ADI for humans was calculated as follows:

ADI = (2 mg/kg bw/day) / 100 = 0.02 mg/kg bw/day

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

The most relevant study to derive the acute reference dose (ARfD) was the developmental study in rats. The NOAEL in the developmental study in rats was 10 mg/kg bw/day, based on the bodyweight gain and food consumption reduction observed in dams from the first sampling times at 45 mg/kg bw/day.

A safety factor of 100, derived from both 10-fold factor for inter-species variability and 10-fold factor for inter-individuals variability, was considered appropriate. Thus, the ARfD for humans was calculated as follows:

ARfD = (10 mg/kg bw/day)/100= 0.1 mg/kg bw/day

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

The acceptable operator exposure level (AOEL) is defined on the basis of short-term toxicity studies in the most sensitive species and with the application of an appropriate safety factor.

The most sensitive effects in the most sensitive species were found in the one-year toxicity study in dogs ([redacted] (1996). Dodine administration to dogs at the dose of 10 mg/kg bw/day resulted in the necessity of supplemental feeding and therefore the NOAEL of the study was set at 2 mg/kg bw/day.

The RMS considers this NOAEL of 2 mg/kg bw/day as appropriate for the AOEL derivation, with a safety factor of 100 (10-fold factor for inter-species variability and 10-fold factor for inter-individuals variability) and corrected for oral absorption of 39%. Therefore, the AOEL was calculated as follows:

AOEL = (2 mg/kg bw/day) * 39 / 100 * 100= 0.008 mg/kg bw/day

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

Since an ARfD has been set, also an AAOEL is proposed in this RAR. The most relevant study to derive the ARfD was the developmental study in rats, in which the NOAEL was 10 mg/kg bw/day, based on the bodyweight gain and food consumption reduction observed in dams from the first sampling times at 45 mg/kg bw/day.

A safety factor of 100, derived from both 10-fold factor for inter-species variability and 10-fold factor for inter-individuals variability, was applied and a correction was made for oral absorption of 39%. The AAOEL was calculated as follows:

AAOEL = (10 mg/kg bw/day) * 39 / 100 * 100= 0.04 mg/kg bw/day

2.6.11 Summary of product exposure and risk assessment

RMS conclusions

- **OPERATOR**

According EFSA model, operator exposure to Dodine 544 SC (1,25-1,65 L/ha) from tractor mounted air assisted sprayer application outdoor to high crops is below the AOEL and AAOEL with the use of workwear (arms, body and legs covered) and chemical protective gloves during mixing/loading and application and closed cabin.

However, a safe use for the operator to dodine from manual application is not obtained.

In the opinion of the RMS, as according EFSA Guidance, the penetration factor of the workwear is 10 %, equivalent to a type 6 protective coverall (or the equivalent according EN-ISO 27065 :2017/A1 :2019), inconsequence, type 6 protective coverall should be worn instead workwear.

In case of tractor spraying, the specific chemical protective gloves will be used only to handle the application equipment or contaminated surfaces.

Moreover, during cleaning and handling of the equipment, the same PPE as mixing/loading should be used.

Besides, and due to the toxicological classification of the product as Eye Dam. 1; H318, facial protection is recommended during mixing/loading.

- **WORKER**

According to the results above, the exposure of workers to the active substance dodine is acceptable with the following risk mitigation measures:

- **For apples and cherries**: Protective coverall, level 1, according EN ISO 27065:2017/A1:2019 standard or workwear (arms, body and legs covered) and closed footwear.
- **For pears:** Protective coverall, level 1, according EN ISO 27065:2017/A1:2019 standard or workwear (arms, body and legs covered) and closed footwear for the first application. For the second application in addition to the above, chemical protection gloves are required for 1 day after application.
- **For peaches:** Protective coverall, level 1, according EN ISO 27065:2017/A1:2019 standard or workwear (arms, body and legs covered) and closed footwear for the first application. For the second application in addition to the above, chemical protection gloves are required for 13 days after application.

Treated crops should not be re-entered before spray deposits on leaf surfaces have completely dried.

- **RESIDENT AND BYSTANDER**

According EFSA model, resident and bystander exposure to DODINE (1,25-1,65 L/ha) from vehicle-mounted application outdoor to high crops is below the AOEL and AAOEL, with the following conditions:

- **For apples, pears and cherries:** it is necessary to use drift reduction systems, a 10m buffer strip and increase the volume of water up to 1500L/ha.
- **For peaches:** it is necessary to use drift reduction systems, a 10m buffer strip and increase the volume of water up to 1500L/ha. Furthermore, only one safe result is obtained for 1 application.

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

Three GLP freezer storage stability studies of Dodine residues were performed on the five plant matrices categories: high water, high oil, high starch, high protein and high acid content plant matrices.

The first study ([redacted] 1998), previously evaluated in the DAR, was conducted using both, incurred residues (apples/peach/apple pomace) from a magnitude of residue study, and fortified samples (apple juice). The RMS deemed not fully reliable the use of the incurred samples freezed stored for 1.5 year before being used as time 0, as the degradation of the residues, if any, could be not lineal during the storage time. Moreover, procedural recoveries were low in a few cases (apple pomace from 3 months, peach at 15-18 months), so results obtained cannot be considered conclusive. For peach and apple pomace, results were not sufficiently reliable due to the mentioned deficiencies. For apple, results were confirmed by later studies. For apple juice, the stability of dodine residues at ≤ -20°C was demonstrated for at least 18 months (see Table 2.7.1.-1 and vol. 3, B 7.1-1 for further discussion).

The second study ([redacted] 2001), also included in the DAR, was deemed acceptable and relevant for the purpose, despite a few minor deviations regarding the requirements of the current OECD guideline. Results showed no significant decline of dodine residues at -18ºC for at least 18 months in apple and cherries (see Table 2.7.1.-1).

One additional freezer stability study, investigating the storage stability of Dodine in six matrices from five commodity categories was submitted for the purpose of renewal ([redacted] 2022). An interim report was provided during the initial submission (storage up to 12 months), but the final report (up to 24 months) is now available to the RMS and included in the evaluation. The study is considered acceptable according to the OECD guideline recommendations and relevant. Results showed no significant decline of dodine residues at -20ºC for at least 24 months in the following matrices: apple fruits, beans, carrots, olives and orange (peel/pulp), representing

respectively, the five categories of high water, high protein, high starch, high oil and high acid commodities (OECD guideline 506) (see Table 2.7.1.-1).

According to the OECD guideline 506, "*If residues are shown to be stable in all commodities studied, a study on one commodity from each of the five commodity categories is acceptable. In such cases, residues in all other commodities would be assumed to be stable for the same duration of time under the same storage conditions*". Therefore, it can be assumed that dodine is stable at least for 24 months in freezer conditions (-18ºC/-20ºC) in all plant commodities.

Furthermore, a freezer storage stability study was recently performed in honey. The study followed the current guideline and demonstrated stability of residues in a 6 months storage period at ≤ -18°C. Please refer to Table 2.7.1.-2 and vol. 3, B 7.1/3.

Table 2.7.1.-1. Storage stability periods for the active substance dodine in plant matrices

CATEGORY	Commodity studied	Periods of stability (months)	Ref.
High water content	Apple	24	[redacted] 2022
	Cherry	18	[redacted] 2001
	Peach*	18	[redacted] 1998
High oil content	Olives	24	[redacted] 2022
High protein content	Beans (dry)	24	
High starch content	Carrot	24	
High acid content	Orange peel	24	
	Orange pulp	24	
Processed commodities	Apple juice	18	[redacted] 1998
	Apple pomace*	18	

* results not considered fully reliable by the RMS due to some deficiencies of the study (see vol. 3, point B.7.1)

Table 2.7.1.-2. Storage stability periods for the active substance dodine in honey

CATEGORY	Commodity studied	Periods of stability (months)	Ref.
Other	Honey	6	[redacted] 2020

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

2.7.2.1. Plants

The metabolism of Dodine was investigated in three crops, belonging to the category of fruits: apple, strawberry, pecan nuts (table 2.7.2.-1) using ^{14}C-dodine labelled in the guanidine carbon. These studies were previously evaluated in the DAR. Although some shortcomings have been identified according to the current guidelines, they are considered relevant for the purpose of renewal (for further discussion see vol. 3, point B.7.2.1.).

Table 2.7.2.-1. Summary of the metabolism studies in plants

Crop groups	Crop(s)	Application(s)	Sampling (DAT)	Comment/Source
Fruit crops	Apple	3 x 3.026 kg a.s./ha Foliar	142 DAT1 – 1st harvest 175 DAT1 (33 DAT2) – 2nd harvest 183 DAT1 (7 DAT3) – 3rd harvest	Radiolabelled active substance: [14C]dodine (guanidine carbon) ([redacted] 1992)
	Strawberry	4 x 3.026 kg a.s./ha Foliar	28 DAT1- 1st harvest 42 DAT1 (14 DAT2) - 2nd harvest 62 DAT1 (14 DAT3) - 3rd harvest 102 DAT1 (14 DAT4) - 4th harvest	Radiolabelled active substance: [14C]dodine (guanidine carbon) ([redacted] 1993)

	Pecan	3 x 5.7 kg a.s./ha Foliar	63 DAT1- 1st harvest 112 DAT1 (49 DAT2) – 2nd harvest (not analyzed) 121 DAT1 (9 DAT3) – 3rd harvest	Radiolabelled active substance: [14C]dodine (guanidine carbon) ([redacted] 1998)

Apple trees were treated 3 times with ^{14}C-Dodine at a rate of 3.026 kg a.s/ha. The foliar application covers that proposed for the representative uses (~5 N the total seasonal rate and 3.4 N each treatment, respecting the cGAP: 2 x 900 g a.i./ha). The first application was carried out at bud break (~BBCH 07), the second at immature fruit stage (~BBCH 71-79), and the last, 7 days before final harvest of mature apples (~BBCH 85-87).

Sampling at three different periods were conducted; for the first one (142 DAT1 and before the 2nd application), residue levels were so low that no characterization/identification of fractions was conducted. The 1st application was carried out at bud break, prior to the presence of fruits, so the first harvested apples assimilated radiocarbon only by translocation. Translocation experiments performed showed some radioactivity in the leaves from designated branches, but only trace amounts in the various apple matrices (rinsate, pulp, and peel).

The rinse of fruits from the 2nd and 3rd harvested fruits contained higher levels of radioactivity than the 1st sampling: 10.5% of TRR (0.153 mg/kg) for the 2nd and 12.0% of TRR (0.18 mg/kg) for the 3rd harvest, and the majority of the TRR was detected in the peel (83 and 82.3% TRR for the 2nd and 3rd harvest, respectively), as fruits were present during the applications.

In inmature apples (2nd harvest, 33 DAT2), only Dodine (78.2% of TRR) was identified. Besides Dodine, no other component was found at >10% TRR. Several unknown minor fractions (<0.05 mg/kg) were found and one not identified fraction (4.78% of TRR, 0.07 mg/kg) was detected in peel.

In mature apples (3rd harvest, 7 DAT3), only Dodine (87.2% of TRR) and tentatively ^{14}C-guanidine (1.13% TRR, 0.017 mg/kg,) in the peel were identified. Besides Dodine, no other component was found at >10% TRR or >0.01 mg/kg. More than 4 components in the pulp and 24 in the peel were detected with values <0.01 mg/kg.

Strawberries plants were treated 4 times with ^{14}C-Dodine at a rate of 3.026 kg a.s/ha. The foliar application covers that proposed for the representative uses (~8 N the total seasonal rate and 3.4 N each treatment, respecting the cGAP: 2 x 900 g a.i./ha). The first application was carried out in immature plants, the second 28 days later, the third one 20 days after the second and the last one 40 days later.

Sampling at 4 different periods were conducted, all 14 days after each treatment, except the first one (28 DALA).

Radioactivity was not readily removed from the fruits with water, because less than 6% was found in the rinses. In fruits from plants treated, most of the radioactivity was ^{14}C-Dodine (81-91%). Several fractions >0.05 mg/kg (<10%) were found; no identification of other components was performed, except urea, which was identified only in the final harvest (6.5% TRR, 0.275 mg/kg), and guanidine, tentatively identified at low levels in mature fruits in the 2nd, 3rd and 4th sampling (<1.58% TRR, <0.107 mg/kg). Numerous minor fractions (<0.05 mg/kg and <1%TRR) were observed in all sampling periods.

Translocation experiments performed showed some movement of radioactivity into growing parts (0.124, 0.412, 0.334, and 0.841 mg/kg, for the 1sst, 2nd, 3rd and 4th harvested runners, respectively). HPLC analysis of runner extracts supported the presence of urea as a metabolite in strawberries and dodine as a minor component. Translocation of radioactivity into the strawberry runners seems to occur mainly under the urea form.

Pecan trees were treated 3 times with ^{14}C-Dodine at a rate of 5.7 kg a.s/ha. The foliar application covers that proposed for the representative uses (approximately 9.5 N the total seasonal rate and 6 N each treatment, respecting the cGAP: 2 x 900 g a.i./ha). The 1st application was carried out when small nuts were present; the 2nd, 63 days after the first one, on immature but well developed nuts, and the 3rd, 49 days later, on mature nuts before hull crack. Samples were collected immediately before 2nd application (63 days after the first application), immediately before 3rd application (samples not analyzed) and 9 days after the last application.

Inmature pecans (whole nuts) from trees treated contained 2.152 mg/kg mg/kg of radioactivity. The major radiolabeled peaks from HPLC analysis of immature pecan extracts corresponded to Dodine (45.35% of TRR, 0.976 mg/kg), and guanidine (15.5% of TRR, 0.326 mg/kg). Two other unknown major peaks were detected accounted for 0.200 mg/kg (9.3% of TRR) and 0.288 mg/kg (13.38% of TRR).

In mature pecans (only nutmeat) from trees treated contained 0.114 mg/kg of radioactivity. The major components corresponded to Guanidine (36.0% TRR, 0.041 mg/kg) and Dodine (13.2% TRR, 0.015 mg/kg). Five minor unknown peaks were also detected (<0.003 mg/kg). A fraction of 4.4% of TRR (0.005 mg/kg) was characterized as non-cationic or neutral and 20% of TRR (0.023 mg/kg) was incorporated to the fatty acid fractions.

In the three studies, more applications than intended (3-4) and several intermediate samplings (2-3) were performed. The last sampling of mature fruits of apple and strawberry was made at a PHI of 7 and 14 days after last application, respectively, showing that dodine was only scarcely metabolized (87.2 and 86.7% of dodine, respectively) at these PHIs. Guanidine was only tentatively identified at low levels: 1.1% of TRR (0.017 mg/kg) in apple and 0.4-1.6 % (0.03-0.107 mg/kg) in strawberries. These results could cover the GAP of the representative use on cherry (PHI 14 days).

Longer PHIs were proposed for apple (60 days) and peach (application until BBCH 69, PHI covered by vegetation period). The intermediate samplings in the metabolism studies provide further information concerning metabolism behavior for periods longer than 14 days: for instance, apple samples from the 2nd harvest (33 DAT2 and 142 DAT1) showed that dodine is present still at high levels (78.2%) 33 days after the application, and guanidine was not identified. Strawberry samples from the 1st sampling (28 DAT1) showed dodine at levels of 81% and no guanidine identified. Therefore, at these PHIs, it could be concluded that Dodine is still present at high levels.

For pecans, the mature nutmeat corresponds to the edible part and is more representative of the use on tree nuts. However, it should be highlighted that for immature pecans, the whole fruit was analyzed, and not for mature (shell and hulls removed and not rinsed, neither analysed for radioactivity). Therefore, the results for the immature pecan could be considered more extrapolable to the representative uses. Mature nutmeat presented less radioactivity than immature pecans, likely due to absence of the shells and hulls, which could retain the radioactivity. Therefore, the majority of the radioactivity found in mature pecans likely proceeded from the 1st application (conducted 121 days before) and so, the high level of dodine degradation observed. This result suggests that, at long term, dodine could be extensively degraded in pecans, and the proportion of guanidine could be considered relevant (although at low absolute levels).

According to the Applicant: "*Dodine was only sparsely metabolized in apples/strawberries. No major metabolites were found, though urea (only in strawberry) and guanidine were tentatively identified as minor metabolites. Urea would arise from Dodine via dealkylation and oxidation. Knowing the biochemistry of urea and guanidine, the C14 in the guanidine moiety of Dodine must have been partially incorporated into natural products. The formation of either urea or guanidine from Dodine is a slow process, whereas the subsequent breakdown of urea and guanidine is fast.*

In pecans, the results indicate that a low level of applied Dodine reaches the nutmeat fraction of mature pecans. However, most of the parent compound undergoes extensive metabolism to guanidine, and the latter undergoes further metabolism to carbon dioxide and ammonia. Carbon dioxide is assimilated into the metabolic pool. The very high proportion of lipid in the nutmeat is consistent with incorporation of 14CO_2 into the fatty acid fraction.".

Qualitative differences are observed between apples/strawberry and pecans. In apples and strawberries, Dodine was only sparsely metabolized, whereas in pecans, a degradation of dodine and occurrence of guanidine as major metabolite cannot be excluded at long term. However, the results of the metabolism in mature pecans cannot be easily extrapolated to other fruits, since in mature pecans only nutmeat has been analysed.

Therefore, in order to cover the representative uses on apple and peach (PHI ≥60 days), the RMS deems advisable to submit a new study in another fruit crop (more similar to the representative uses), covering long PHI, in order to clarify the relevance of the metabolite guanidine in these fruits (see point 2.7.3).

No metabolism studies in rotational crops are submitted because the uses intended are in perennial crops.

2.7.2.2. Livestock

From the representative uses, only apple contributes to animal feed as **apple pomace**, which is only fed to **ruminants**. For the evaluation of the metabolism in livestock, a goat metabolism study was evaluated in the DAR. A goat was dosed with dodine in gelatin capsules at a dose corresponding to 0.4 mg/kg bw/day or 10 mg/kg diet (approximately 100 X the maximum dietary burden calculated for beef cattle, see point 2.7.5).

Total radioactive residues (TRR) reached a plateau of 0.014 ppm in the milk during days 3 to 5 of administration. TRRs in the tissues were highest in the liver (0.17 ppm) and kidney (0.11 ppm). Low concentrations were detected

in muscle (≤0.02 ppm) and fat (≤0.008 ppm).

Dodine was extensively metabolized in the goat (accounting at ≤5.2% of TRR). The major metabolites (>10% of TRR) in liver, kidney and muscle were identified as octylguanidine carboxylic acid, hexylguanidine carboxylic acid and urea; also dodecylguanidine carboxylic acid was identified at levels <10% of TRR. Taking into account the low levels of radioactivity found in the different tissues, all these metabolites occurred at levels <0.05 mg/kg.

Thus, regarding the metabolic pathway in livestock, it could be concluded that, after initial conversion to a carboxylic acid, the carbon chain is degraded by removal of two carbon units and that urea is produced by cleavage from the carbon chain.

Data on the metabolism of Dodine in poultry, swine or fish is not required since the representative crops are not fed to these animals.

2.7.3 Definition of the residue

The results of the plant metabolism studies showed that the parent compound dodine was identified as the major fraction of the residue (apple and strawberry), accounting for ~87% of TRR at PHI 7 and 14 days, and 78-80% of TRR at ~30 days after treatment. For pecans, dodine is still at high levels in immature ones (45% of TRR) and decreased to 13% of TRR in mature ones.

Based on the metabolism studies summarised above, the **residue definition for monitoring** purposes **for plants** is proposed as *Dodine*, as it is considered a good marker. Monitoring validated methods (LC-MS/MS) are available for matrices with high water (LOQ 0.05 mg/kg), high acid (LOQ 0.01 mg/kg), high oil content (LOQ 0.01 mg/kg) and dry commodities (LOQ 0.01 mg/kg). An ILV is also available for all four matrices.

Identified degradation products were guanidine and urea (only in strawberry):

- Guanidine, was tentatively identified in very low levels in apple (1.1% of TRR, 0.017 mg/kg), and strawberry (<1.6% of TRR, <0.107 mg/kg). On the other hand, it was detected at high levels in pecans (15% of TRR, 0.326 mg/kg and 36% of TRR, 0.041 mg/kg for immature and mature pecans, respectively).

- Urea was found at 6.4% (0.27 mg/kg) only in strawberry samples taken 14 days after the 4th application.

Regarding the toxicological evaluation of metabolite guanidine, the tox. experts concluded the following:

"*Based on a weight of evidence approach, guanidine is not expected to be genotoxic. Both experimental data on guanidinium salts and in silico predictions do not indicate this compound can be reactive with DNA*".

General toxicity: "*based on available experimental data, guanidine has a lower LD_{50} value than dodine, which may indicate this compound is more toxic than the parent compound.... Despite no alerts activated for general endpoints other than hERG channel inhibition and hepatotoxicity, guanidine is expected to have different ADME properties in comparison to the parent compound based on physicochemical properties. For this reason, no conclusion can be derived on the general toxicity of this metabolite*".

No toxicological information is available for the other metabolite identified, urea (found in strawberry only). However, as urea is already classified as a substance included in Annex IV of Reg. 396/2005 (substances for which no MRL is needed), and no toxicological reference values are established, it can be excluded from further considerations.

For **risk assessment** purposes **in plants**, the **residue definition** was proposed as *Dodine* in the first inclusion of the active substance, based on the same metabolism studies. Guanidine was found in proportions >10% only in one commodity among those studied (pecan), and corresponding to low absolute levels (0.041 mg/kg, study conducted at 9.8 N the total seasonal rate); in addition, it was only tentatively identified and at low levels in the metabolism studies in apples and strawberry. As commented above, the results for the mature pecan could be considered not easily extrapolated to the representative uses apple and cherry. Radioactive residues were low in mature pecans (nutmeat), maybe due to the fact that the residues remained retained in the hull and shell.

Four residue trials evaluated in the context of an art 10 MRL application of dodine (*EFSA-Q-2021-00390*), were also analysed for the metabolite guanidine (see point point 2.7.4.4 and vol. 3, point B.7.3.5, study 3). The RMS included this use in the dRAR as supportive information, for a more comprehensive discussion on the residue definition for risk assessment.
Results obtained supported the fact that most of residues remained retained in the hull: in all trials, residues of dodine and guanidine in hulls were far higher than in nutmeat. Levels of guanidine in nutmeat were below the LOQ (1 mg/kg). It must be highlighted that this LOQ was high. According to the study report, the high LOQ was due to the

background levels found in all control samples assayed, being guanidine a natural organic compound found widely in nature in plants and animals, and no other appropriate source of control samples could be found. Moreover, since dodine was not detected in any untreated sample, at any PHI, it is expected that guanidine is naturally present in the plan,t and not as a metabolite of Dodine. In hulls, residues of guanidine in untreated and treated plants could be considered similar. In nutmeat, however, as the LOQ was so high, there is no clear evidence that guanidine levels in untreated plants were similar as in treated. Moreover, no storage stability data of guanidine are available to validate these results.

Therefore, considering that:

- guanidine has a lower LD_{50} value than dodine, which may indicate this compound is more toxic than the parent compound; it is expected to have different ADME properties in comparison to the parent compound based on physicochemical properties, so no conclusion can be derived on its general toxicity,

- guanidine was found in high proportions in the metabolism study in pecans (15% of TRR, 0.326 mg/kg and 36% of TRR, 0.041 mg/kg for immature and mature pecans, respectively).

- the presence of guanidine in the nutmeat as a consequence of the dodine treatment cannot be excluded due to the high LOQ, in the supervised residue trials on almonds (art 10 MRL application), therefore, no data are available to compare the background levels in nutmeats with the levels in treated plants,

- no storage stability data are available to validate the guanidine results in the supervised residue trials on almonds,

the RMS would propose (as suggested with Co-RMS) a preliminary "**tentative" separate residue definition for risk assessment for guanidine**, pending the submission of general toxicological data and/or further residue data (validated with adequate storage stability data) to exclude the presence of guanidine in the edible part as a consequence of the treatment with dodine. Nevertheless, the RMS considered this proposal more relevant for tree nuts, bearing in mind the metabolism studies in apple and strawberrys and taking into account the morphological differences of that group, since the edible part in tree nuts (seed) is not the same as in other fruits.

For **livestock**: The animal dietary burden reaches the trigger value of 0.004 mg/kg bw/day only for beef cattle (see point 2.7.5). The calculated dietary burdens for dairy cattle and sheep are below the trigger value of 0.004 mg/kg bw/day. The representative crops are not fed to poultry and swine.

Regarding the main metabolites found in the livestock metabolism study (goat), the tox. expert concluded:

- "*In silico predictions for the metabolites dodecylguanidine-, octylguanidine- and hexylguanidine-carboxylic acids do not indicate these structures have genotoxic potential. These compounds activate the same alerts as the parent compound. Furthermore, these compounds contain and additional carboxylic acid group in the molecule that it is reported not to infer further reactivity with DNA"*.

General tox: "*With regards to the remaining metabolites, they have been grouped together as alkyl guanidine carboxylic acid derivatives. In silico predictions for dodecylguanidine-, octylguanidine- and hexylguanidine-carboxylic acids indicate they activate a hERG channel inhibition alert in Derek Nexus and this alert is not shared by the parent compound. The metabolite hexylguanidine-carboxylic acid also activates a hepatotoxicity alert and nephrotoxicity alert in Derek Nexus. Due to uncertainties in the ADME properties together with a different toxicological profile with respect to the parent compound, no conclusion can be derived on the general toxicity of these metabolites"*.

Considering the N rate (100 X) of the goat metabolism study and the low levels of radioactivity found in the different tissues and milk, it is expected that the levels of the metabolites identified in edible tissues would be very low at the dietary intake calculated. Furthermore, as the intake calculated was ≤ the trigger value of 0.004 mg/kg bw/day, no further data are considered necessary and the RMS would propose *Dodine* as **residue definition** by default in commodities of **animal** origin (for **monitoring** and **risk assessment**).

2.7.4 Summary of residue trials in plants and identification of critical GAP

The representative uses considered in this application are the uses in apple/pear, cherry and peach.

2.7.4.1. Apple

According to SANTE/2019/12752, a total of 8 trials in N-EU and 8 trials in S-EU are required to evaluate the residue

levels in apple and pear, and data on apple can be extrapolated to pear. A total of 23 independent supervised residue trials (12 for N-EU and 11 for S-EU) have been submitted to support the representative critical GAP of Dodine in apple/pear.

The critical GAP for the intended use relevant for consumers is 2 applications of 680 g as/ha, interval 21 days and PHI 60 day on apple in field (SEU, CEU, NEU).

All trials were done in apples with 2 two applications at a target rate of 0.68 kg a.s./ha and a PHI of 60 days. Six of these trials were already evaluated for the first EU Review of Dodine (Portugal, 2009). Within the DAR further trials were evaluated, but they were performed with a number of applications not fitting the GAPs of the representative uses for the renewal (most of them higher number of applications); therefore, they were not considered supportive anymore by the Applicant and were not submitted for the renewal purpose.

Therefore, new trials for the active substance renewal are submitted in order to complete the data set of at least 8 trials N-EU and 8 trials S-EU. All trials complied with the intended uses (±25%), however, the interval between applications (6-8 days) was more critical (>± 25%) than that proposed (minimum 21 days), so likely leading to higher residues due to the cummulative effect. However**,** it should be taken into account that the PHI is long (60 days), and therefore, the level of residues may not be so affected by the interval between applications, as with shorter PHIs. An explanation has been requested from the Applicant, and the justification provided is inline with the RMS considerations. Therefore, despite this deviation, the trials can be considered to support the representative use on apple and extrapolate to pear.

Total residues varied between 0.031 mg/kg and 0.474 mg/kg. The calculated values for STMR, HR and MRL are included in table below (see Table 2.7.4.1-1).

The Applicant proposes to combine NEU and SEU data sets, since populations were found similar according to Mann-Whitney-U test. However, the RMS does not agree with this approach, since MRL calculated from both data sets differed and, according to the technical guideline SANTE/2019/12752, "Combining NEU and SEU residue trials to derive an MRL proposal for one crop/commodity: ... *the MRL proposals derived for the individual data sets should fall into the same or a neighbouring MRL class*."

Table 2.7.4.1-1 Summary of results of apple residues trials

Matrix	Region	Residue Data (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Rounded OECD-MRL (mg/kg)	Current MRL (Reg (EU) nº 2022/1290)
Apple (extrapolated to pear)	NEU	**RD Mo/RA1:** *"dodine"* 2 x 0,06, 0.066, 0.08, 0,09, 0.115, 0.118, 0.163, 0.187, 0.192, 0.208, 0.217 **RD RA 2** (tentative) "*guanidine*": --	0.12	0.22	0.4	0.9
	SEU	**RD Mo/RA1:** *"dodine"* 0.031, 0.057, 0.07, 2x 0.125, 0.13, 0.135, 0.18, 0.28, 0.355, 0.474 **RD RA 2** (tentative) "*guanidine*": --	**0.13**	**0.47**	**0.8**	

2.7.4.2. Cherry

According to SANTE/2019/12752, a total of 8 trials in N-EU and 4 trials in S-EU are required to evaluate the residue levels in cherry. The representative critical GAP in cherry is 2 applications (minimum interval 21 days) of 0.68 kg a.s./ha from BBCH 60 till 14 days before harvest. Applications proposed after harvest would be less critical, as the consumable part is not present anymore.

In the framework of this application it is referred to a total of 14 NEU and 9 SEU supervised residue trials, the majority evaluated in the DAR:

- 9 NEU and 6 SEU trials were performed with a GAP of 2 applications (int. 6-9 days) of 0.68-0.8 kg a.s./ha and PHI 14 days;

- 5 NEU and 4 SEU trials with 3 applications of 0.8 kg a.s./ha, PHI 14 days.

The trials conducted with 2 applications complied with the intended uses (±25%) regarding application rate, number of applications, growth stage at application and type of formulation. However, the interval between applications (6-9 days) was more critical (>± 25%) than that proposed (minimum 21 days), so likely leading to higher residues due to the cumulative effect. Considering the short PHI of the intended use (14 days), there is no evidence that the first application did not contribute to the final residue levels in fruits with a shorter interval, so these trials were not fully compliant with the cGAP and were not considered relevant (for further discussion please see vol. 3, point. B.7.3.).

Trials performed with 3 applications do not correspond exactly to the intended GAP regarding the number of applications. However, since the 1st application was applied before the consumable part of the crop is present, it may be expected that the first application will not have a considerable effect on the residue level and the RMS considers these trials relevant (5 NEU and 4 SEU) (application rate within ±25% deviation, acceptable according to SANTE/2019/12752). Among these trials, only 2 NEU and 3 SEU are considered independent and valid. Therefore, 6 further NEU and 1 SEU trials would be necessary to support the representative use on cherry in both zones.

The calculated values for STMR, HR and MRL (provisional) are included in table below (see Table 2.7.4.2-1).

Table 2.7.4.2-1 Summary of results of cherry residues trials

Matrix	Region	Residue Data (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Rounded OECD-MRL (mg/kg)	Current MRL (Reg (EU) nº 2022/1290)
Cherry	NEU	**RD Mo/RA1:** *"dodine"* 0.7, 0.7 **RD RA 2** (tentative) "*guanidine*": --	-	-	-	3
	SEU	**RD Mo/RA1:** *"dodine"* 0.77, 0.46, 0.56 **RD RA 2** (tentative) "*guanidine*": --	**0.56**	**0.77**	**2**[1]	

[1] available data not sufficient to estimate a robust MRL

2.7.4.3. Peach

According to SANTE/2019/12752, a total of 4 trials in N-EU and 8 trials in S-EU are required to evaluate the residue levels in peach. The representative critical GAP is 2 applications (interval 21 days) of 0.9 kg a.s./ha up to BBCH 69 (PHI covered by the vegetation period). Applications proposed from 50% leaf falling till after leaf falling (approx. BBCH 95-97) would be less critical, as the consumable part is not present anymore.

A total of 16 new supervised trials (8 S-EU and 8 N-EU) are available, performed according to the representative critical GAP. All trials complied with the intended uses (±25%), however, the interval between applications (5-8 days) was more critical (>± 25%) than that proposed (minimum 21 days), so likely leading to higher residues due to the cummulative effect. However, bearing in mind that applications were conducted before the presence of the consumable part, that PHI are long (86-150 days), and that residues were below the LOQ in almost all cases (at normal commercial harvest), the RMS considers the trials valid and compliant with the cGAP proposed and sufficient to support the representative use on peach.

Total residues of dodine varied between <0.01 mg/kg and 0.01 mg/kg. The STMR and HR was 0.01 mg/kg. The calculated values for STMR, HR and MRL are included in table below (see Table 2.7.4.3-1).

Table 2.7.4.3-1 Summary of results of peach residues trials

Matrix	Region	Residue Data (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Rounded OECD-MRL (mg/kg)	Current MRL (Reg (EU) nº 2022/1290)
Peach	NEU	**RD Mo/RA1:** *"dodine"* 7 x <0.01, 0.01 **RD RA 2** (tentative) "*guanidine*": --	0.01	0.01	**0.02**	0.1

	SEU	**RD Mo/RA1:** *"dodine"* 7 x <0.01, 0.01 **RD RA 2** (tentative) "*guanidine*": --	0.01	0.01	**0.02**	

2.7.4.4. Additional data (almond)

The use on almond is being evaluated in the context of an art 10 MRL application of dodine (*EFSA-Q-2021-00390*). The RMS included this use in the dRAR as supportive information, for a more comprehensive discussion on the residue definition for risk assessment. The assessment performed by ES as EMS in the MRL application was as follows:

According to SANTE/2019/12752 almond is not reported as a major crop. Therefore, according to Reg 283/2013 the minimum number of trials should be 4 for almond. A total of 4 supervised outdoor residue trials on almonds were initially submitted, conducted at the intended GAP (±25%) in Southern Europe (Spain and Portugal) in 2016-2017. The intended critical GAP for the MRL application was 2 applications (interval 14-28 days) of 0.68 kg a.s./ha (PHI 30 days). Trials were considered acceptable in that framework.

According to EFSA (data requirements identified for EFSA-Q-2021-00390): "*New almonds residue trials submitted in the context of this dossier are analysed for levels of parent dodine, only. Noting that the relevance of the metabolite guanidine in tree nuts was flagged in the art 12 review ("its potential inclusion into the RD shall be evaluated in the context of future applications on tree nuts and a final decision taken", EFSA 2015), residue trials simultaneously analysed for the parent dodine and its metabolite guanidine in almonds according to the intended uses are required.... This information is required to conclude on the relevance of metabolite guanidine for the residue definition(s) for enforcement and/or risk assessment".*

After the EFSA request, 4 additional residue trials were provided. These trials were carried out in 2022 at the intended GAP (±25%) and, besides dodine, metabolite guanidine was also determined (for further assessment extracted from the MRL application, please see vol. 3, B.7.3.5).

The calculated values for STMR, HR and MRL are included in table below (see Table 2.7.4.4-1).

Table 2.7.4.4-1 Summary of results of almond residue trials

Matrix	Region	Residue Data (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Rounded OECD-MRL (mg/kg)	Current MRL (Reg (EU) nº 2022/1290)
Almond	SEU	**RD Mo/RA 1 :** "*dodine*" 2 x<0.01, 2 x 0.04, 2 x 0.05, 0.07, 0.08	0.05	0.08	**0.15**	0.01*
		RD RA 2 (tentative): "*guanidine*": 4 x <1*	<1	<1	-	-

*LOQ: 1 mg/kg
further storage stability data would be necesary to validate the data of guanidine

Results obtained supported the fact that most of residues remained retained in the hull: in all trials, residues of dodine and guanidine in hulls were far higher than in nutmeat. Levels of guanidine in nutmeat were below the LOQ (1 mg/kg). It must be highlighted that this LOQ was high. According to the study report, the high LOQ was due to the background levels found in all control samples assayed, being guanidine a natural organic compound found widely in nature in plants and animals, and no other appropriate source of control samples could be found. Moreover, since dodine was not detected in any untreated sample, at any PHI, it is expected that guanidine is naturally present in the plant, and not as a metabolite of Dodine. In hulls, residues of guanidine in untreated and treated plants could be considered similar. In nutmeat, however, as the LOQ was so high, there is no clear evidence that guanidine levels in untreated plants were similar as in treated. Moreover, no storage stability data of guanidine are available to validate these results.

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

Among the representative uses, only apple can be used to fed livestock and should be considered to estimate the dietary burden for livestock.

The dietary burden calculation was carried out according to the latest EFSA model (EFSA dietary burden calculator (Animal model 2017.xls). Estimation of animal intakes and HR, STMR and MRL calculations for products of animal origin, September 2015). Residue values used for calculation are presented in Table 2.7.5-1. The STMR value for apple of Dodine of the most critical SEU data set was used. The median processing factor of 2.6 for apple pomace was applied.

The calculated dietary burdens for dairy cattle, sheep, pig, and poultry are below the trigger value of 0.004 mg/kg bw/day. For beef cattle, the dietary burdens are calculated to be at the trigger value of 0.004 mg/kg bw/day. Results are presented in Table 2.7.5-2.

Table 2.7.5-1 Input values for dietary burden calculation

Commodity	Median dietary burden		Maximum dietary burden	
	Input value [mg/kg]	Comment	Input value [mg/kg]	Comment
Apple pomace, wet (by-products group)	0.34	STMR x PF (2.6)	0.34	STMR x PF (2.6)

Table 2.7.5-2 Results of the dietary burden calculation

Animal burden calculation

According to: "OECD Guidance Document, Series on testing and assessment No 64 and Series on pesticides No 32" and "OECD Guidance Document on Residues in livestock, Series on Pesticides No 73"

Maximum Intake	Cattle						Sheep					
	Beef	500 kg 12 kg		Dairy	650 kg 25 kg		Ram/Ewe	75 kg 2,5 kg		Lamb	40 kg 1,7 kg	
(mg/kg bw/d)	0,004	mg/kg bw/d	%	0,003	mg/kg bw/d	%	0,003	mg/kg bw/d	%	0,004	mg/kg bw/d	%
Contributor 1	Apple	pomace, wet	20	Apple	pomace, wet	10	Apple	pomace, wet	10	Apple	pomace, wet	10
Contributor 2												
Contributor 3												
Contributor 4												
Median intake	0,0041	mg/kg bw/d		0,0033	mg/kg bw/d		0,0028	mg/kg bw/d		0,0036	mg/kg bw/d	

Maximum Intake	Swine					
	Breeding	260 kg 6 kg		Finishing	100 kg 3 kg	
(mg/kg bw/d)		mg/kg bw/d	%		mg/kg bw/d	%
Contributor 1						
Contributor 2						
Contributor 3						
Contributor 4						
Median intake		mg/kg bw/d			mg/kg bw/d	

Intakes >0.004 mg/kg bw/d are highlighted

Maximum Intake	Poultry								
	Broiler	1,7 kg 0,12 kg		Layer	1,9 kg 0,13 kg		Turkey	7 kg 0,5 kg	
(mg/kg bw/d)		mg/kg bw/d	%		mg/kg bw/d	%		mg/kg bw/d	%
Contributor 1									
Contributor 2									
Contributor 3									
Contributor 4									
Median intake		mg/kg bw			mg/kg bw			mg/kg bw	

Intakes expressed on the dry mater basis (mg/kg DM)						
mg/kg DM	Cattle		Sheep		Swine	
	Beef	Dairy	Ram/Ewe	Lamb	Breeding	Finishing
Maximum	0,17	0,08	0,1	0,08		
Median	0,17	0,08	0,08	0,08		
	Poultry					
	Broiler	Layer	Turkey			
Maximum						
Median						

Intake >0.1 mg/kg DM in red characters

Relevant groups	Dietary burden expressed in				Most critical diet (a)	Most critical commodity (b)		Trigger exceeded (Yes/No)
	mg/kg bw per day		mg/kg DM					0.004
	Median	Maximum	Median	Maximum				mg/kg bw
Cattle (all diets)	0,004	0,004	0,17	0,17	Beef cattle	Apple	pomace, wet	Yes
Cattle (dairy only)	0,003	0,003	0,08	0,08	Dairy cattle	Apple	pomace, wet	No
Sheep (all diets)	0,004	0,004	0,08	0,08	Lamb	Apple	pomace, wet	No
Sheep (ewe only)	0,003	0,003	0,08	0,08	Ram/Ewe	Apple	pomace, wet	No
Swine (all diets)								No
Poultry (all diets)								No
Poultry (layer only)								No

(a): When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed as "m

(b): The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".

*No apple pomace is fed to pigs or poultry.

Very low residues were found in the goat metabolism study, which was performed with an intake of 0.4 mg Dodine/kg bw/day, which is 100-fold higher than the calculated burden for beef cattle. Total radioactive residues (TRR) in the goat reached a plateau of 0.01 mg/kg in the milk during days 3 to 5 of administration. Further total radioactive residue levels were 0.17 mg/kg in the liver, 0.11 mg/kg in the kidney, ≤0.02 mg/kg in muscle and ≤0.008 mg/kg in the fat. If these values are recalculated to an intake at the level of the expected dietary burden, the expected residues are far below 0.01 mg/kg. Thus, no relevant residues are expected in commodities of animal origin and a livestock feeding study is, therefore, not required.

2.7.6 Summary of effects of processing

One hydrolysis study was submitted, simulating the normal processing practice of pasteurisation, sterilisation and baking/brewing/boiling at 90°C and pH 4 (20 min), at 121°C and pH 6 (20 min), and at 100°C and pH 5 (60 min), using [^{14}C]Dodine in buffered solutions. The total recovered radioactivity of all test solutions after thermal processing accounted for 92.9-98.7% of the nominal applied radioactivity.

In all test solutions only [^{14}C]Dodine was detected after processing and no degradation products were found. Dodine is therefore considered hydrolytically stable at 90°C and pH 4 (20 min), 121°C and pH 6 (20 min) and 100°C and pH 5 (60 min). Thus, the same residue definitions as for primary crops would apply to processed commodities.

Three apple processing studies were performed. In these studies a total of three independent trials were performed simulating the industrial processing of apples to juice, wet pomace and canned apples. The number of trials is sufficient to derive robust processing factors:

For apple juice single processing factors of 0.09, 0.18 and 0.17 were derived (median value: 0.17). For wet pomace single processing factors of 5.1, 2.6 and 2.1 were calculated (median value: 2.6). In canned apples no residues were detected after canning (residues < LOD) – therefore processing factors were calculated using the LOQ of 0.01. The single processing factors derived are 0.13, 0.17, 0.25 (median 0.17) (See table below 2.7.6.-1).

Processing factor for apple juice may be extrapolated to other pome (pear) or stone fruits (peach).

Table 2.7.6.-1 Summary of processing factors (representative uses)

Processed commodity	Number of studies	Processing Factor (PF)	
		Individual values	Median PF
Apples, juice	3	0.09, 0.18, 0.17	0.17
Apples, wet pomace	3	5.1, 2.6, 2.1	2.6
Apples, canned	3	0.13, 0.17, 0.25	0.17

2.7.7 Summary of residues in rotational crops

Representative uses (apple, cherry, peach) are perennial crops, therefore, data on the metabolism or magnitude of residues in rotational crops are not required

2.7.8 Summary of other studies

One study for determination of residues of dodine in phacelia honey under semi-field conditions was submitted.

The representative uses (apple, cherry and peach) are considered melliferous crops. Residues in honey might occur due to the use of Dodine on these melliferous plants during flowering or from non-target plants, as the applications will be during the flowering period from April to September. According to SANTE/11956/2016, a total of 4 trials are required to evaluate the residue levels in honey. Four independent residue trials in phacelia (*Phacelia tanacetifolia*) (2 NEU and 2 SEU) have been submitted.

The critical GAP for the intended uses relevant for consumers is 2 applications of 900 g as/ha. All trials were carried out with 2 applications with a rate per treatment of 0.9 kg a.s./ha, covering the cGAP (1N).

All trials were in Phacelia under semi-field conditions (tunnel trial).

Trials were carried out according to SANTE/11956/2016, with minor deviations and they are considered relevant for the purpose of the renewal of dodine.

Honey specimens were taken for analysis 7-14 days after last application to get mature honey. Residues of Dodine were extracted and final determination was performed by HPLC-MS/MS. Total residues varied between <0.01 mg/kg and 0.12 mg/kg. The calculated values for STMR, HR and MRL are included in table below (Table 2.7.8-1).

An exceedance of the current MRL is expected. The submitted data are sufficient to derive a MRL proposal for the NEU/SEU use. An application for a modification of MRL in honey has been submitted by the applicant.

Table 2.7.8-1 Summary of results of honey residues trials

Matrix	Region	Residue Data (mg/kg)	STMR (mg/kg)	HR (mg/kg)	Rounded OECD-MRL (mg/kg)	Current MRL (Reg (EU) nº 2022/1290)
Honey	NEU/SEU	**RD Mo/RA:** *"Dodine"* *2 x < 0.01, 0.056, 0.12*	0.03	0.12	0.3	0.05*

2.7.9 Estimation of the potential and actual exposure through diet and other sources

A consumer risk assessment was conducted using the last version of the EFSA PRIMO tool, v. 3.1. The toxicological reference values used proposed by the applicant have been reviewed by the RMS and the following values included in table 2.7.9-1 were finally proposed (see vol. 1, point 2.6.10) and used for the risk estimation:

Table 2.7.9-1. ADI and ARfD for the active substance Dodine

End-Point	Value	Study	Safety factor	Reference
Acceptable Daily Intake (ADI)	0.02 mg/kg bw/day	1-year and 90-day dog studies	100	dRAR (ES, 2022)
Acute Reference	0.1 mg/kg bw	based on body weight gain and food consumption at 45	100	dRAR (ES, 2022)

Dose (ARfD)		mg/kg bw/day in the developmental toxicity study in rats (NOAEL 10 mg/kg bw/day)		

The risk assessment residue definition for plants (and animals, by default) was proposed as: "*Dodine"* and the conversion factor from monitoring to risk assessment: 1 (see point 2.7.3).

A chronic consumer risk assessment was performed using the current MRLs (Reg. (EU) 2022/1290) for the representative crops and for the animal commodities relevant for the representative uses (bovine, sheep, goat, equine) and the proposed MRL of 0.3 mg/kg for honey, for a tier one assessment. Results are included in table 2.7.9-3 and showed a TMDI of 2-72% (NL toddler, highest contributor, apples) of the ADI.

A refinement using the STMR derived from the magnitude of residue trials in the representative crops (see table 2.7.9-2) was estimated and results were 0.4-15% of ADI (DE child, highest contributor, apples) (table 2.7.9-4). It can therefore be concluded that there is no chronic risk from the consumption of apples/pears, cherries and peaches treated with Dodine according to the intended GAP or from honey.

An acute consumer risk assessment using the current MRLs showed an acute risk for the representative use on pears (1st scenario, see table 2.7.9-5). A refinement, using the Highest Residues for the intended crops according to the input values included in table 2.7.9-2, was assessed. Results showed IESTI values below the ARfD for apples, pears, cherries and peaches (51%, 65%, 37% and 1%, respectively, and 0.4% for honey). (see table 2.7.9-6).

The current MRL was used as input values for cherry, as the number of trials fitting the intended GAP is not sufficient to support the use on cherry and a data gap for further trials is identified (see point 2.7.4.2).

Hence, it can be concluded that there is no acute risk from the consumption of apples/pears, cherries and peaches treated with Dodine according to the intended GAP and from honey.

Table 2.7.9-2. Input values for the consumer risk assessment (second scenario)

Commodity	Chronic risk assessment		Acute risk assessment	
	Input (mg/kg)	**Comment**	**Input** (mg/kg)	**Comment**
apple	0.13	STMR x CF (1)	0.47	HR x CF (1)
pear	0.13	STMR x CF (1)	0.47	HR x CF (1)
apple, pear juice	0.022	STMR x PF (0.17) x CF (1)	0.022	STMR x PF (0.17) x CF (1)
cherry [a]	3	MRL [b]	3	MRL [b]
peach	0.01	STMR x CF (1)	0.01	HR x CF (1)
peach, juice	0.0017	STMR x PF (0.17) x CF (1)	0.0017	STMR x PF (0.17) x CF (1)
bovine/sheep/goat/equine commodities	0.01	MRL [b]	0.01	MRL [b]
milk	0.01	MRL[b]	0.01	MRL[b]
honey	0.03	STMR x CF (1)	0.12	HR x CF (1)

[a] current MRL used as input value for cherry, as not enough trials available to support the use (data gap)
[b] Reg. (EU) 2022/1290

For guanidine, the consumer risk assessment remained open, since no data of residue levels in the representative uses are available, nor toxicological reference values established.

Table 2.7.9-3: **Chronic consumer risk assessment of representative uses (EFSA PRIMo, rev. 3.1)**

European Food Safety Authority

EFSA PRIMo revision 3.1; 2019/03/19

dodine

LOQs (mg/kg) range from:		to:	
Toxicological reference values			
ADI (mg/kg bw/day):	**0,02**	ARfD (mg/kg bw):	**0,1**
Source of ADI:	**dRAR**	Source of ARfD:	**dRAR**
Year of evaluation:	**2022**	Year of evaluation:	**2022**

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment | Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments:

Refined calculation mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
TMDI/NEDI/IEDI calculation (based on average food consumption)	72%	NL toddler	14,42	49%	Apples	20%	Pears	3%	Milk: Cattle		72%
	66%	DE child	13,22	56%	Apples	6%	Cherries (sweet)	3%	Pears		66%
	34%	NL child	6,78	26%	Apples	5%	Pears	1%	Milk: Cattle		34%
	17%	FR toddler 2 3 yr	3,46	14%	Apples	1%	Milk: Cattle	1%	Pears		17%
	15%	DE women 14-50 yr	2,98	12%	Apples	2%	Cherries (sweet)	0,6%	Pears		15%
	15%	DK child	2,91	10%	Apples	3%	Pears	0,6%	Milk: Cattle		15%
	14%	DE general	2,75	11%	Apples	2%	Cherries (sweet)	0,6%	Milk: Cattle		14%
	12%	PL general	2,36	9%	Apples	1%	Cherries (sweet)	1%	Pears		12%
	11%	UK infant	2,26	7%	Apples	2%	Milk: Cattle	1%	Pears		11%
	11%	FR child 3 15 yr	2,18	8%	Apples	1%	Pears	1%	Milk: Cattle		11%
	10%	UK toddler	1,95	8%	Apples	1%	Milk: Cattle	0,8%	Pears		10%
	10%	LT adult	1,94	8%	Apples	0,7%	Pears	0,4%	Cherries (sweet)		10%
	10%	RO general	1,91	6%	Apples	2%	Cherries (sweet)	0,6%	Milk: Cattle		10%
	9%	ES child	1,87	5%	Apples	2%	Pears	1%	Cherries (sweet)		9%
	9%	FR infant	1,84	8%	Apples	0,8%	Milk: Cattle	0,8%	Pears		9%
	9%	GEMS/Food G11	1,72	7%	Apples	0,8%	Pears	0,4%	Milk: Cattle		9%
	8%	NL general	1,67	7%	Apples	0,8%	Pears	0,5%	Cherries (sweet)		8%
	8%	GEMS/Food G15	1,52	5%	Apples	1%	Cherries (sweet)	0,7%	Pears		8%
	8%	GEMS/Food G08	1,51	5%	Apples	1%	Cherries (sweet)	0,6%	Pears		8%
	7%	SE general	1,50	5%	Apples	2%	Pears	0,6%	Milk: Cattle		7%
	7%	IT toddler	1,41	4%	Apples	2%	Pears	1%	Cherries (sweet)		7%
	7%	PT general	1,40	5%	Apples	2%	Pears	0,6%	Cherries (sweet)		7%
	7%	GEMS/Food G06	1,30	4%	Apples	2%	Cherries (sweet)	0,4%	Pears		7%
	6%	ES adult	1,27	3%	Apples	1%	Pears	1,0%	Cherries (sweet)		6%
	6%	DK adult	1,22	4%	Apples	1%	Pears	0,3%	Milk: Cattle		6%
	6%	IE adult	1,22	3%	Apples	2%	Pears	0,5%	Cherries (sweet)		6%
	6%	GEMS/Food G07	1,22	5%	Apples	0,7%	Pears	0,4%	Cherries (sweet)		6%
	6%	IT adult	1,15	4%	Apples	1%	Pears	0,9%	Cherries (sweet)		6%
	5%	GEMS/Food G10	1,05	3%	Apples	0,7%	Cherries (sweet)	0,7%	Pears		5%
	5%	FI 3 yr	1,03	4%	Apples	0,8%	Pears	0,1%	Peaches		5%
	5%	FR adult	0,97	3%	Apples	0,7%	Pears	0,4%	Cherries (sweet)		5%
	4%	FI 6 yr	0,72	3%	Apples	0,8%	Pears	0,1%	Cherries (sweet)		4%
	3%	UK vegetarian	0,66	3%	Apples	0,3%	Pears	0,2%	Milk: Cattle		3%
	3%	FI adult	0,57	3%	Apples	0,2%	Pears	0,0%	Peaches		3%
	2%	UK adult	0,49	2%	Apples	0,3%	Pears	0,1%	Milk: Cattle		2%
	2%	IE child	0,38	1%	Apples	0,2%	Milk: Cattle	0,1%	Pears		2%

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of dodine is unlikely to present a public health concern.

Table 2.7.9-4: **Chronic consumer risk assessment of representative uses (EFSA PRIMo, rev. 3.1) (Refined)**

European Food Safety Authority

EFSA PRIMo revision 3.1; 2019/03/19

dodine

LOQs (mg/kg) range from: to:

Toxicological reference values

ADI (mg/kg bw/day):	0,02	ARfD (mg/kg bw):	0,1
Source of ADI:	dRAR	Source of ARfD:	dRAR
Year of evaluation:	2022	Year of evaluation:	2022

Input values

Details - chronic risk assessment | Supplementary results - chronic risk assessment | Details - acute risk assessment/children | Details - acute risk assessment/adults

Comments:

Refined calculation mode

Chronic risk assessment: JMPR methodology (IEDI/TMDI)

No of diets exceeding the ADI : ---

TMDI/NEDI/IEDI calculation (based on average food consumption)	Calculated exposure (% of ADI)	MS Diet	Expsoure (µg/kg bw per day)	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	Exposure resulting from: MRLs set at the LOQ (in % of ADI)	Exposure resulting from: commodities not under assessment (in % of ADI)
	15%	DE child	3,05	8%	Apples	6%	Cherries (sweet)	1,0%	Milk: Cattle		15%
	14%	NL toddler	2,74	7%	Apples	3%	Milk: Cattle	3%	Pears		14%
	7%	NL child	1,38	4%	Apples	1%	Milk: Cattle	1%	Cherries (sweet)		7%
	4%	DE women 14-50 yr	0,86	2%	Cherries (sweet)	2%	Apples	0,6%	Milk: Cattle		4%
	4%	UK infant	0,85	2%	Milk: Cattle	1%	Cherries (sweet)	1%	Apples		4%
	4%	DE general	0,77	2%	Apples	2%	Cherries (sweet)	0,6%	Milk: Cattle		4%
	4%	FR toddler 2 3 yr	0,77	2%	Apples	1%	Milk: Cattle	0,2%	Pears		4%
	4%	RO general	0,72	2%	Cherries (sweet)	0,9%	Apples	0,6%	Milk: Cattle		4%
	3%	FR child 3 15 yr	0,65	1%	Milk: Cattle	1%	Apples	0,8%	Cherries (sweet)		3%
	3%	ES child	0,63	1%	Cherries (sweet)	0,7%	Apples	0,6%	Milk: Cattle		3%
	3%	DK child	0,59	2%	Apples	0,6%	Milk: Cattle	0,4%	Pears		3%
	3%	PL general	0,57	1%	Cherries (sweet)	1%	Apples	0,2%	Pears		3%
	3%	GEMS/Food G15	0,54	1%	Cherries (sweet)	0,7%	Apples	0,4%	Milk: Cattle		3%
	3%	GEMS/Food G06	0,50	2%	Cherries (sweet)	0,6%	Apples	0,1%	Milk: Cattle		3%
	2%	UK toddler	0,49	1%	Apples	1%	Milk: Cattle	0,1%	Pears		2%
	2%	GEMS/Food G08	0,45	1%	Cherries (sweet)	0,8%	Apples	0,3%	Milk: Cattle		2%
	2%	IT toddler	0,43	1%	Cherries (sweet)	0,6%	Apples	0,2%	Pears		2%
	2%	FR infant	0,41	1%	Apples	0,8%	Milk: Cattle	0,1%	Pears		2%
	2%	SE general	0,40	0,7%	Apples	0,6%	Milk: Cattle	0,3%	Cherries (sweet)		2%
	2%	ES adult	0,40	1,0%	Cherries (sweet)	0,5%	Apples	0,2%	Milk: Cattle		2%
	2%	NL general	0,40	0,9%	Apples	0,5%	Cherries (sweet)	0,4%	Milk: Cattle		2%
	2%	GEMS/Food G11	0,39	1%	Apples	0,4%	Milk: Cattle	0,4%	Cherries (sweet)		2%
	2%	LT adult	0,38	1%	Apples	0,4%	Cherries (sweet)	0,2%	Milk: Cattle		2%
	2%	GEMS/Food G10	0,33	0,7%	Cherries (sweet)	0,5%	Apples	0,3%	Milk: Cattle		2%
	2%	IT adult	0,32	0,9%	Cherries (sweet)	0,5%	Apples	0,2%	Pears		2%
	2%	IE adult	0,31	0,5%	Cherries (sweet)	0,5%	Apples	0,3%	Pears		2%
	2%	GEMS/Food G07	0,30	0,7%	Apples	0,4%	Cherries (sweet)	0,3%	Milk: Cattle		2%
	1%	PT general	0,29	0,7%	Apples	0,6%	Cherries (sweet)	0,2%	Pears		1%
	1%	FR adult	0,25	0,5%	Apples	0,4%	Cherries (sweet)	0,2%	Milk: Cattle		1%
	1%	DK adult	0,23	0,6%	Apples	0,3%	Milk: Cattle	0,2%	Pears		1%
	0,8%	FI 3 yr	0,15	0,6%	Apples	0,1%	Pears	0,0%	Cherries (sweet)		0,8%
	0,7%	UK vegetarian	0,15	0,4%	Apples	0,2%	Milk: Cattle	0,1%	Cherries (sweet)		0,7%
	0,6%	FI 6 yr	0,13	0,4%	Apples	0,1%	Cherries (sweet)	0,1%	Pears		0,6%
	0,6%	UK adult	0,12	0,3%	Apples	0,1%	Milk: Cattle	0,1%	Cherries (sweet)		0,6%
	0,5%	IE child	0,10	0,2%	Apples	0,2%	Milk: Cattle	0,1%	Cherries (sweet)		0,5%
	0,4%	FI adult	0,08	0,4%	Apples	0,0%	Pears	0,0%	Cherries (sweet)		0,4%

Conclusion:
The estimated long-term dietary intake (TMDI/NEDI/IEDI) was below the ADI.
The long-term intake of residues of dodine is unlikely to present a public health concern.

Table 2.7.9-5: **Acute consumer risk assessment of representative uses (EFSA PRIMo, rev. 3.1)**

Show results of IESTI calculation only for crops with GAPs under assessment

Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI): 1

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
125%	Pears	0,9 / 0,9	125
97%	Apples	0,9 / 0,9	97
37%	Cherries (sweet)	3 / 3	37
10%	Peaches	0,1 / 0,1	9,5
1%	Milk: Cattle	0,01 / 0,01	1,2
1%	Honey and other apiculture	0,3 / 0,3	1,1
0,2%	Milk: Goat	0,01 / 0,01	0,24
0,08%	Bovine: Liver	0,01 / 0,01	0,08
0,07%	Bovine: Edible offals (other	0,01 / 0,01	0,07
0,07%	Bovine: Muscle/meat	0,01 / 0,01	0,07
0,06%	Equine: Muscle/meat	0,01 / 0,01	0,06
0,05%	Sheep: Muscle/meat	0,01 / 0,01	0,05
0,04%	Bovine: Kidney	0,01 / 0,01	0,04
0,04%	Milk: Sheep	0,01 / 0,01	0,04
0,02%	Bovine: Fat tissue	0,01 / 0,01	0,02

Expand/collapse list

Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation) 1

Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
30%	Cherries (sweet)	3 / 3	30
27%	Pears	0,9 / 0,9	27
25%	Apples	0,9 / 0,9	25
2%	Peaches	0,1 / 0,1	1,9
0,4%	Honey and other apiculture	0,3 / 0,3	0,41
0,4%	Milk: Cattle	0,01 / 0,01	0,39
0,2%	Milk: Goat	0,01 / 0,01	0,18
0,2%	Milk: Sheep	0,01 / 0,01	0,15
0,06%	Bovine: Muscle	0,01 / 0,01	0,06
0,05%	Equine: Muscle/meat	0,01 / 0,01	0,05
0,05%	Sheep: Muscle/meat	0,01 / 0,01	0,05
0,04%	Bovine: Liver	0,01 / 0,01	0,04
0,03%	Bovine: Edible offals (other	0,01 / 0,01	0,03
0,03%	Sheep: Liver	0,01 / 0,01	0,03
0,02%	Bovine: Kidney	0,01 / 0,01	0,02

IESTI new
Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
55%	Apples	0,9 / 0,9	55
53%	Pears	0,9 / 0,9	53
37%	Cherries (sweet)	3 / 3	37
5%	Peaches	0,1 / 0,1	5,4
1%	Milk: Cattle	0,01 / 0,01	1,2
1%	Honey and other apiculture	0,3 / 0,3	1,1
0,2%	Milk: Goat	0,01 / 0,01	0,24
0,08%	Bovine: Liver	0,01 / 0,01	0,08
0,07%	Bovine: Edible offals (other	0,01 / 0,01	0,07
0,07%	Bovine: Muscle/meat	0,01 / 0,01	0,07
0,06%	Equine: Muscle/meat	0,01 / 0,01	0,06
0,05%	Sheep: Muscle/meat	0,01 / 0,01	0,05
0,04%	Bovine: Kidney	0,01 / 0,01	0,04
0,04%	Milk: Sheep	0,01 / 0,01	0,04
0,02%	Bovine: Fat tissue	0,01 / 0,01	0,02

Total number of commodities found exceeding the ARfD/ADI in children and adult diets (IESTI new calculation)

IESTI new
Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
32%	Pears	0,9 / 0,9	32
30%	Cherries (sweet)	3 / 3	30
27%	Apples	0,9 / 0,9	27
2%	Peaches	0,1 / 0,1	2,0
0,4%	Honey and other apiculture products	0,3 / 0,3	0,41
0,4%	Milk: Cattle	0,01 / 0,01	0,39
0,2%	Milk: Goat	0,01 / 0,01	0,18
0,2%	Milk: Sheep	0,01 / 0,01	0,15
0,06%	Bovine: Muscle	0,01 / 0,01	0,06
0,05%	Equine: Muscle/meat	0,01 / 0,01	0,05
0,04%	Sheep: Muscle/meat	0,01 / 0,01	0,05
0,04%	Bovine: Liver	0,01 / 0,01	0,04
0,03%	Bovine: Edible offals (other than liver	0,01 / 0,01	0,03
0,03%	Sheep: Liver	0,01 / 0,01	0,03
0,02%	Bovine: Kidney	0,01 / 0,01	0,02

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
49%	Apples / juice	0,9 / 0,9	49
29%	Pears / juice	0,9 / 0,9	29
3%	Peaches / canned	0,1 / 0,1	2,6
2%	Peaches / juice	0,1 / 0,1	1,7

Expand/collapse list

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
30%	Apples / juice	0,9 / 0,9	30
0,8%	Peaches / canned	0,1 / 0,1	0,82
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
49%	Apples / juice	0,9 / 0,9	49
29%	Pears / juice	0,9 / 0,9	29
2%	Peaches / canned	0,1 / 0,1	1,9
2%	Peaches / juice	0,1 / 0,1	1,7

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
30%	Apples / juice	0,9 / 0,9	30
0,8%	Peaches / canned	0,1 / 0,1	0,81
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!

Conclusion:
The estimated short term intake (IESTI) exceeded the toxicological reference value for 1 commodities.

For processed commodities, no exceedance of the ARfD/ADI was identified.

Table 2.7.9-6: **Acute consumer risk assessment of representative uses (EFSA PRIMo, rev. 3.1) (Refined)**

Acute risk assessment /children	Acute risk assessment / adults / general population	Acute risk assessment /children	Acute risk assessment / adults / general population
Details - acute risk assessment /children	Details - acute risk assessment/adults	Hide IESTI new calculations	Show IESTI new calculations

The acute risk assessment is based on the ARfD.
The calculation is based on the large portion of the most critical consumer group.

IESTI new calculations:
The calculation is performed with the MRL and the peeling/processing factor (PF), taking into account the residue in the edible portion and/or the conversion factor for the residue definition (CF). For case 2a, 2b and 3 calculations a variability factor of 3 is used. Since this methodology is not based on internationally agreed principles, the results are considered as indicative only.
Since this methodology is not based on internationally agreed principles, the results are considered as indicative only.

Show results of IESTI calculation only for crops with GAPs under assessment

Unprocessed commodities

Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
65%	Pears	0,9 / 0,47	65
51%	Apples	0,9 / 0,47	51
37%	Cherries (sweet)	3 / 3	37
1%	Milk: Cattle	0,01 / 0,01	1,2
1,0%	Peaches	0,1 / 0,01	0,95
0,4%	Honey and other apiculture	0,3 / 0,12	0,43
0,2%	Milk: Goat	0,01 / 0,01	0,24
0,08%	Bovine: Liver	0,01 / 0,01	0,08
0,07%	Bovine: Edible offals (other	0,01 / 0,01	0,07
0,07%	Bovine: Muscle/meat	0,01 / 0,01	0,07
0,06%	Equine: Muscle/meat	0,01 / 0,01	0,06
0,05%	Sheep: Muscle/meat	0,01 / 0,01	0,05
0,04%	Bovine: Kidney	0,01 / 0,01	0,04
0,04%	Milk: Sheep	0,01 / 0,01	0,04
0,02%	Bovine: Fat tissue	0,01 / 0,01	0,02

Expand/collapse list

Total number of commodities exceeding the ARfD/ADI in children and adult diets (IESTI calculation)

Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
30%	Cherries (sweet)	3 / 3	30
14%	Pears	0,9 / 0,47	14
13%	Apples	0,9 / 0,47	13
0,4%	Milk: Cattle	0,01 / 0,01	0,39
0,2%	Peaches	0,1 / 0,01	0,19
0,2%	Milk: Goat	0,01 / 0,01	0,18
0,2%	Honey and other apiculture	0,3 / 0,12	0,17
0,2%	Milk: Sheep	0,01 / 0,01	0,15
0,06%	Bovine: Muscle	0,01 / 0,01	0,06
0,05%	Equine: Muscle/meat	0,01 / 0,01	0,05
0,05%	Sheep: Muscle/meat	0,01 / 0,01	0,05
0,04%	Bovine: Liver	0,01 / 0,01	0,04
0,03%	Bovine: Edible offals (other	0,01 / 0,01	0,03
0,03%	Sheep: Liver	0,01 / 0,01	0,03
0,02%	Bovine: Kidney	0,01 / 0,01	0,02

IESTI new
Results for children
No. of commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
55%	Apples	0,9 / 0,9	55
53%	Pears	0,9 / 0,9	53
37%	Cherries (sweet)	3 / 3	37
5%	Peaches	0,1 / 0,1	5,4
1%	Milk: Cattle	0,01 / 0,01	1,2
1%	Honey and other apiculture	0,3 / 0,3	1,1
0,2%	Milk: Goat	0,01 / 0,01	0,24
0,08%	Bovine: Liver	0,01 / 0,01	0,08
0,07%	Bovine: Edible offals (other	0,01 / 0,01	0,07
0,07%	Bovine: Muscle/meat	0,01 / 0,01	0,07
0,06%	Equine: Muscle/meat	0,01 / 0,01	0,06
0,05%	Sheep: Muscle/meat	0,01 / 0,01	0,05
0,04%	Bovine: Kidney	0,01 / 0,01	0,04
0,04%	Milk: Sheep	0,01 / 0,01	0,04
0,02%	Bovine: Fat tissue	0,01 / 0,01	0,02

Total number of commodities found exceeding the ARfD/ADI in children and adult diets (IESTI new calculation)

IESTI new
Results for adults
No. of commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
32%	Pears	0,9 / 0,9	32
30%	Cherries (sweet)	3 / 3	30
27%	Apples	0,9 / 0,9	27
2%	Peaches	0,1 / 0,1	2,0
0,4%	Honey and other apiculture products	0,3 / 0,3	0,41
0,4%	Milk: Cattle	0,01 / 0,01	0,39
0,2%	Milk: Goat	0,01 / 0,01	0,18
0,2%	Milk: Sheep	0,01 / 0,01	0,15
0,06%	Bovine: Muscle	0,01 / 0,01	0,06
0,05%	Equine: Muscle/meat	0,01 / 0,01	0,05
0,05%	Sheep: Muscle/meat	0,01 / 0,01	0,05
0,04%	Bovine: Liver	0,01 / 0,01	0,04
0,03%	Bovine: Edible offals (other than liver	0,01 / 0,01	0,03
0,03%	Sheep: Liver	0,01 / 0,01	0,03
0,02%	Bovine: Kidney	0,01 / 0,01	0,02

Processed commodities

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
1%	Apples / juice	0,9 / 0,02	1,2
0,7%	Pears / juice	0,9 / 0,02	0,72
0,3%	Peaches / canned	0,1 / 0,01	0,26
0,0%	Peaches / juice	0,1 / 0	0,03

Expand/collapse list

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI): ---

IESTI

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
0,7%	Apples / juice	0,9 / 0,02	0,74
0,08%	Peaches / canned	0,1 / 0,01	0,08
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!

Results for children
No of processed commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
8%	Apples / juice	0,9 / 0,15	8,3
5%	Pears / juice	0,9 / 0,15	5,0
2%	Peaches / canned	0,1 / 0,1	1,9
0,3%	Peaches / juice	0,1 / 0,02	0,28

Results for adults
No of processed commodities for which ARfD/ADI is exceeded (IESTI new): ---

IESTI new

Highest % of ARfD/ADI	Processed commodities	MRL / input for RA (mg/kg)	Exposure (µg/kg bw)
5%	Apples / juice	0,9 / 0,15	5,1
0,8%	Peaches / canned	0,1 / 0,1	0,81
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!
#¡NUM!	#¡NUM!	#¡NUM!	#¡NUM!

Conclusion:
No exceedance of the toxicological reference value was identified for any unprocessed commodity.
A short term intake of residues of dodine is unlikely to present a public health risk.
For processed commodities, no exceedance of the ARfD/ADI was identified.

2.7.10 Proposed MRLs and compliance with existing MRLs

EU MRLs for dodine are currently detailed in the Regulation (EU) 2022/1290[(a)]. According with the available data no change is proposed for enforcement residue definition, and neither for the LMR of the representative uses and for relevant animal commodities. Regarding the representative use on cherries, available data are not enough to estimate a MRL (a data gap for further trials is identified, see point 2.7.4.2).
A new MRL is proposed for honey. The submitted data are sufficient to derive a MRL proposal for honey for the NEU/SEU use. Risk for consumers unlikely. For guanidine the consumer risk assessment remained open, since no data of residue levels in the representative uses are available, nor toxicological reference values established.

Code	Commodity	Current EU MRL[(b)] (mg/kg)	Proposed EU MRL(mg/kg)
0130010	Apple	0.9	No change
0130020	Pear	0.9	No change
0140020	Cherry (sweet)	3	No change [c]
0140030	Peach	0.1	No change
1040000	Honey[a]	0.05*	0.3

[a] an application for a modification of MRL in honey has been submitted by the applicant.
[b] Reg. (EU) 2022/1290
[c] data available not sufficient to estimate a MRL

2.7.11 Proposed import tolerances and compliance with existing import tolerances

Not relevant.

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.8.1 Summary of fate and behaviour in soil

2.8.1.1 Route of degradation in soil

The aerobic and anaerobic degradation of dodine was studied under laboratory conditions. The influence of irradiation in the degradation process of dodine was also investigated. A summary is presented in the following table:

Table 2.8.1.1-1. Summary of route of degradation in soil studies

Reference	Test cond.	Compound	Soil	Radio label	CO2 [%AR]	Bound Residue [%AR]	Dodine [%AR]	Metabolites	Remarks
[redacted] (1996) KCA 7.1.1.1/02	Aerobic 25ºC 1/3 bar MHC 100 d dark	Dodine	Sandy Loam (95/18)	14C-guanidine	91.8 (100d)	2.7 (100d)	1.9 (100d)	Not relevant metabolites identified. Please, refer to position statement KCA 7.1.1.1/01.	Previously evaluated in DAR (2009). Accepted
			Sandy Loam (95/19)	14C-guanidine	91.2 (100d)	2.9 (100d)	3.8 (100d)		
[redacted] (1997) KCA 7.1.1.1/03	Aerobic 20ºC pF2.5 120 d dark	Dodine	Sandy silty loam (96/35)	14C-guanidine	98 (120d)	4.4 (120d)	95.6 (day 0) 4.3 (day 120)	Not relevant metabolites identified.	Previously evaluated in DAR (2009). Accepted
			Sand (96/45)	14C-guanidine	99.1 (120d)	1.9 (120d)	94.8 (day 0) 1.5 (day 120)		
			Clay loam (96/37)	14C-guanidine	94.7 (118d)	4.2 (118 d)	74.2 (day 0) 2.1 (day 120)		
			Clay loam (96/37)	14C-chain	81.4 (120d)	17.2 (120d)	97.1 (day 0) 2.3 (day 120)		
[redacted] (1993) KCA 7.1.1.2/01	Anerobic 25ºC Flooded conditions 12 dark	Dodine HCl	Sandy loam	14C-guanidine	<1 (12m)	11.74 (12m)	86.2 (12m)	Not relevant metabolites identified.	Previously evaluated in DAR (2009). Supplementary
[redacted] (2001) KCA 7.1.1.3/01	Aerobic 25ºC 30 d Irradiated	Dodine	Sandy loam	14C-guanidine	14.4 (30d)	5.65 (30d)	71.4 (30d)	Not relevant metabolites identified.	Previously evaluated in DAR (2009). Accepted

2.8.1.1.1 Aerobic degradation in soil

A total of 3 studies were evaluated in order to stablish the aerobic route of degradation of dodine in soil. During the first peer review of dodine, two aerobic soil degradation studies under laboratory conditions were considered and were assessed as "acceptable" (, 2006- KCA 7.1.1.1/02-; 1997 - KCA 7.1.1.1/03-). For the active substance renewal, a position paper has been submitted, (KCA 7.1.1.1/01), assessing the relevance of Cluster M1 observed in the aerobic degradation study by (1996). They have been accepted by RMS and their summaries can be found in Dodine DRAR Vol 3 B8, under point B.8.1.1.1.

(1996) investigated the metabolism of Dodine in two sandy loam soils (UK and USA soils), labelled on the guanidine carbon. Soils were incubated under aerobic conditions at 25±1ºC and aprox. 75% of their 1/3 bar moisture holding capacity in dark for up to 100 days. [14C]Dodine was applied at a rate equivalent to 4.48 kg a.s/ha, exceeding 2.5 times the maximum field rate per crop/season claimed in the GAP of 1.8 kg a.s./ha (2 x 0.9 kg a.s/ ha). According to the results, dodine degraded to <10% AR after 28 days in both soils. The major degradation products were CO2. Unextractables were less than 3% AR at the end of the study. No accumulation was observed. Volatile radioactivity consisted only of CO2 and was 91.8 and 91.2% AR at day 100 for UK and USA soils, respectively. Dodine plus a cluster of minor metabolites were detected in soil extracts. Metabolite cluster M1 was measured at maximums of 9.7 % AR and 5.3 % AR on days 0 and 1, respectively, in the UK soil, and maximums of 7.6 % AR and 5.0 % AR on day 0 and 1, respectively, in the US soil. For all other samplings, cluster M1 was always < 5 % AR in both soils. For the renewal, a position paper, assessing the relevance of Cluster M1 was provided (KCA 7.1.1.1/01).

After detailed evaluation of cluster M1 metabolite, RMS agrees with the applicant that Cluster M1 does not represent a major metabolite for the following reasons:

- It was clearly demonstrated that no relevant metabolites are observed in US soil.
- In UK soil, it is highly unlikely that on day 1, one individual component of cluster M1 reaches a 5 % AR, since it means that such component should represent ≥ 94.3 % of the total cluster. It is clear from the chromatogram that the highest peak is itself a composite peak. This was also supported by the results of (2001) where a cluster containing multiple peaks at the same RRT was observed. The HPLC profiles of the re-analysed samples in (2001) and (1996) support the argument of the RMS and the applicant that no relevant metabolites are observed in UK soil.
- It cannot be rejected that the relatively high occurrence at day 0 may be an artefact due to concentration of impurities during sample work up. Assuming that Cluster M1 are formed by impurities, it is note that the new technical specification for the renewal of dodine have a refined profile of impurities, and none of them have been classified as toxicological or ecotoxicological relevant.
- The components of cluster M1 are transient, accounting for less than 5% AR after 24 hours assuming worst-case situation (highly unlikely), and no observed in any other studied soil at relevant amounts.

In 1997), the route of degradation of dodine was determined in three soils (sand, sandy silt loam and clay loam), labelled on the guanidine carbon, and in one additional soil (clay loam), labelled in the [14C]-chain. Soils were incubated under aerobic conditions, at 20±1ºC and aprox. a moisture tension of pF2.5, in dark for up to 120 days.

In the study, the non-volatile compounds that were detected in the soil extracts were seen to comprise Dodine plus a cluster of minor metabolites ('M1 cluster'). The minor metabolite cluster M1, consisting of up to eleven compounds was measured at maximums of 9.7% AR and 5.3% AR on days 0 and 1, respectively, in the UK soil, and maximums of 7.6% AR and 5.0% AR on day 0 and 1, respectively, in the US soil. Soils were treated at a nominal application rate of 2.6325 kg a.s/ha (3 x 0.8775 kg a.s/ ha), exceeding the maximum field rate per crop/season claimed in the GAP of 1.8 kg a.s./ha (2 x 0.9 kg a.s/ ha). In the guanidine labelled experiments there was no metabolite that reached 5% AR at any time and the total of metabolites at the end of the study accounted for less than 1.5% of applied material. In the chain labelled experiment one metabolite reached a level of 5.5% of applied material after one day but thereafter the level decreased and it was not detectable at the end of the study at which time extractable metabolites accounted for only 0.3% of applied radioactivity. The results of the experiment conducted with chain labelled dodine show that the dodecyl moiety of the molecule was also ultimately degraded to carbon dioxide. Incorporation of the partially degraded chain resulted in disappearance of parent material at a similar rate to that seen in

the guanidine labelled experiments but with a slightly delayed evolution of radiolabelled carbon dioxide. Intermediate metabolites were produced in very small quantities and were themselves degraded without a build up of residues remaining associated with the soil.

All in all, it is concluded that dodine is quickly metabolised in soil and its degradation ultimately resulted in a formation of CO_2 without formation of any major metabolite or persistent unextractable residues. The location of radiolabelling in the molecule has no effect on its properties or degradation, and results from studies using either radiolabelling position are comparable.

Degradation of dodine occurred by fragmentation of the molecule in three parts: dodecane, guanidine and acetic acid. The guanidine and the dodecane chain should be rapidly used by soil microflora. The structure of dodine suggest that two mechanism of breakdown are likely. The first would involve removal of the acetate moiety and direct attack on the guanidine group and the second an oxidation mechanism, such as beta oxidation, causing successive reductions in the alkane chain which eventually allows attack on the guanidine group. Either or both these mechanisms maybe in operation in a soil environment.

A proposed metabolic pathway based on the results of both guanidine and chain experiments are presented in **Figure 2.8.1.1.1-1**.

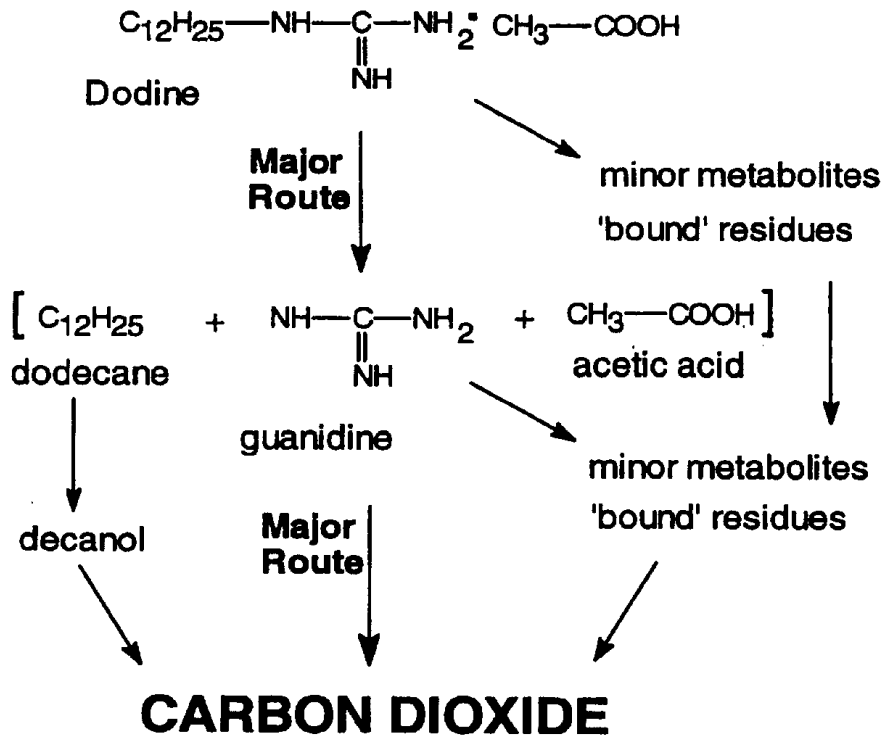


Figure 2.8.1.1.1-1 – Proposed degradation pathway of dodine in aerobic soil

2.8.1.1.2 Anaerobic degradation in soil

Applicant considers that exposure to anaerobic conditions is not expected for the representative uses applied for. Taking into consideration the low persistence of dodine in soil (modelling DT50= 5.25 d), its inmobility in soil (koc > 50000 L/kg) and the results of [redacted] 2017) relating to the irreversible binding of the active substance to soils, the occurrence of anaerobic or partial anaerobic conditions after spring-summer applications of dodine in orchards is not expected.

However, according to the GAP of the representative formulation of the renewal of the active substance, applications in autumn are claimed for. Therefore, anaerobic conditions during flooding of soils after heavy rainfalls cannot be excluded.

The anaerobic route of degradation of dodine HCl was studied by [redacted] 1993 (KCA 7.1.1.2/01). This study was already submitted and accepted in the DAR of Dodine (2009) and it is considered as supplementary information for the renewal of the active substance. No new studies are presented by the notifier. Consequently, a data gap for a new anaerobic degradation study conducted according to the current

OECD 307 guideline has been identified in order to comply with EU data requirements (please refer to Regulation (EU) No 283/2013).

[redacted] (1993) investigated the degradation of dodine HCl in a sandy loam soil from Nebraska (UK), under dark, at 25ºC, on anaerobic incubated flooded soil. It is noted that the test was conducted at an exaggerated rate of 11.6 kg/ha, adding an uncertainty to the results, since degradation of the test compound could have been affected. Results indicate that the degradation of dodine HCl under anaerobic conditions was extremely slow. The mineralisation to CO_2 was less than 0.1% AR after 12 months. The bound residues increased steadily from 2 % AR at day 0 to 12% at 12 months. Approximately 9% of the 12% where associated with the humin phase, indicating that dodine residues are strongly bound into soil matrix. No mayor transformation products were formed during the study. A polar minor metabolite, identified as a hydroxylated derivative of the parent compound, reached a concentration of 2.89% AR at the end of the study.

2.8.1.1.3 Soil photolysis

The photolysis of dodine was studied, by [redacted] 2001 (KCA 7.1.1.3/01), in a sandy loam soil (^{14}C-guanidine labelled). This soil photolysis study was already submitted and accepted in the DAR of Dodine (2009) and it is still considered acceptable for the renewal of the active substance.

Soil samples were treated at an equivalent rate of 4.5 kg dodine/ha. The soils were incubated at 25ºC and exposed to irradiation from a xenon light source using 12 hours of light and 12 hours of dark cycle for 30 days to simulate natural summer sunlight. The average intensity of light source per day (5850 w/m^2) was considered equivalent to the maximum sunlight intensity on hot summer day at latitude 50ºN. A dark control was included in the experiment.

Results show that Dodine is metabolized by soil microorganisms which ultimately results in the formation of carbon dioxide and several minor metabolites. CO2 formation accounted for 12 and 14 %AR in the irradiated soil and in the dark control, respectively. Unextractable residues were less than 6% AR. There was one fraction which accounted for aprox. 10% AR on day 1 for irradiated sample (HPLC retention time 23-24 min). This sample was isolated and re-analized with a different HPLC conditions and shown to contain multiple peaks. Therefore, no major metabolites were observed in the study.

Both irradiated and dark soils show a similar degradation pattern, indicating that degradation and metabolite formation can be considered independent of irradiation.

The half-life of Dodine was calculated and was found to be 122 and 235 days in irradiated samples and dark control samples, respectively. The slower degradation of Dodine under photolytic conditions than the one observed in aerobic soil metabolism studies could be due to the low water holding capacity of the soil used and also the design of the photolysis study.

According to the results, it can be concluded that photolysis does not play a significant role for Dodine degradation in soil.

2.8.1.2 Rate of degradation in soil

The rates of degradation of dodine were evaluated following the recommendations of the FOCUS kinetic guidance.

The rate of degradation of dodine in standard dark aerobic laboratory studies has been determined in 5 different soils at 20/25ºC. Dodine degraded rapidly in soil with persistence DT50 values at 20ºC ranging from 2.9 to 17 days (not corrected for moisture) in five soils. DT90 values ranged from 9.7 to 35.7 days. Degradation generally followed SFO kinetics, with one exception (Sandy loam soil, 95-18, FOMC kinetics) (Table 2.8.1.2-1).

Table 2.8.1.2-1: Persistence DT50 values at 20 ºC of parent compound, dodine

Dodine	Dark aerobic conditions –Persistence endpoints						
Soil type	pH[a)]	t. ºC / % MWHC	DT_{50} (d)	DT_{90} (d)	Kinetic parameters	St. (χ^2)	Method of calculation
96-35 Sandy silty loam	6.6	20/22.2	4.32	14.4		8.8	SFO
96-45 Sand	6.7	20/18.32	4.29	14.2		8.8	SFO

96-37 Clay loam	7.4	20/33.7	2.93	9.74		9.1	SFO
95-18 Sandy loam	5.3	25/16	6.91 10.8 (DT_{90}/3.32, 25º) 17 (DT_{90}/3.32, 20ºC)	35.7	**α= 2.041** **β= 17.09**	10.3	FOMC
95-19 Sandy loam	5.9	25/17	7.22 11.34 (20ºC)	24.0		12.9	SFO
Longest DT50			10.8 (25ºC) **17 (20 ºC)**		10.8 (25ºC) **17 (20 ºC)**		SFO (DT90/3.32)
pH dependence,				Yes			

[a)]Measured in water
[b)]Normalised using a Q10 of 2.58 and Walker equation coefficient of 0.7

For modelling purposes, the normalized DT_{50} values (20°C and pF2) ranged from 2.93 to 9.25 days with geomean of 5.25 days (Table 2.8.1.2-2). Details of the kinetic evaluation and temperature and moisture normalisation are provided in Vol. 3 CA B8, Point B.8.1.2, Study KCA 7.1.2.1.1/01 ([redacted] 2021). The pH dependency of the degradation of dodine was assessed by RMS using software tool pHADe (UBA). According to the results of Kendall's test and the linear fits, degradation of dodine is pH dependent, being faster under basic conditions. In the particular case of dodine, RMS considers that the pH dependence should not be included in the environmental modelling to avoid an increase in complexity of the modelling and overworking. No impact on the risk assessment conclusions from the estimated predicted concentrations in soil, groundwater and surface water is expected. For PECsoil calculation the worst-case DT50 of 17 days (20ºC, non-normalized for moisture) was used. For PECsw and PECgw calculations the geomean of 5.25 days was selected, while the normalized DT50 values were in the range of 2.93 to 9.25 days (geomean ± 2-4 days).
Under anaerobic laboratory conditions dodine does not degrade significantly according to a supplementary study conducted at an exaggerated application rate and with and experimental design that deviates from recommendations of OECD 307 guideline. No further data are available and a data gap has been identified for the representative uses where exposure to anaerobic conditions cannot be excluded (autumn applications). Moreover, photolysis does not play a significant role for Dodine degradation in soil.
Studies on field soil dissipation of Dodine are not required since laboratory aerobic soil degradation studies confirmed that the degradation of Dodine is rapid. However, the trials were conducted at locations across the USA. It should be justified that conditions of the field dissipation trials outside EU are representative of agricultural conditions in Europe. Moreover, a revision of the kinetic parameters of this study according to the recent FOCUS degradation kinetic guidance document has not been provided. All in all, the study is considered as supplementary.

Table 2.8.1.2-2: Normalization of soil modelling DT50 values to 20 ºC and pF2 for dodine

Dodine														**Modelling DT50**		
Study	Soil	Textura (USDA)	pH (water)	OC (%)	Clay (%) < 0.002 mm	Reported MWHC of soil	Reported % of MWHC during the study	Actual soil moisture during study (%)	pF2 soil moisture (%) (W10) (θref)	θ/θref	Fmoisture (θ/θref)0.7	Test temp. (ºC)	Ftemperature (Q10=2.58)	DT50 Not normalized (d)	DT50 Corrected to pF2 and 20ºC (d)	Model
[redacted] 2007	96-35 (Essex, UK)	Sandy silty loam	6.6	1.2	9.3	22.2	100	22.2	25	0.89	0.92	20	1	4.32	3.97	SFO
	96-45 (Suffolk, UK)	Sand	6.7	2.3	4.6	18.32	100	18.32	12	1.53	1.34	20	1	4.29	4.29	SFO
	96-37 (Essex, UK)	Clay loam	7.4	2.1	23.7	33.7	100	33.7	28	1.20	1.14	20	1	2.93	2.93	SFO
[redacted] 2006	95-18 (Essex, UK)	Sandy loam	5.3	2.2	8.8	16	75	12.0	19	0.63	0.72	25	1.57	8.18	9.25	SFO
	95-19 (Missisippi, USA)	Sandy loam	5.9	0.6	6.0	17	75	12.8	19	0.67	0.76	25	1.57	7.22	8.61	SFO
														Geomean	**5.25**	**n= 5**

Assessment in relation to the P-criteria
The assessment is done according to the DG SANCO Working Document on "Evidence Needed to Identify POP, PBT and vPvB Properties for Pesticides" (2012, rev. 3).
The criteria for persistence (P) in soil, as stated in Regulation (EC) 1107/2009, are DT50 >120 days for PBT and >180 days for POP and vPvB. When considering laboratory degradation rates, best-fit DT50 values at 20°C for dodine are < 120 days in the 5 available soils.
Based on all available data, it is concluded that the P-criteria in soil is not fulfilled for dodine.

2.8.1.3 Mobility

Three individual studies for adsorption and desorption of the active substance are summarised in Table 2.8.1.3-1. Two studies are experimental tests (KCA 7.1.3.1.1/01 and KCA 7.1.3.1.1/03) and one study is an expert statement (KCA 7.1.3.1.1/02).

Soil Type[a)]	**OC%**	**Soil pH**[b)]	**K_F [mL/g]**	**K_{Foc} [mL/g]**	**1/n**	**Reference**	**Acceptability**
Sand	0.05	7.6	6440	1.29x107	*	[redacted] 1991 KCA 7.1.3.1.1/03	Not accepted
Sandy loam	0.40	6.5	2202	5.51x105	*		
Clay loam	1.3	6.4	18019	2.77x106	*		
Silt loam	2.10	7.4	15228	7.25x105	*		
Sandy clay loam	2.6	7.4	1454	55905	0.938	[redacted] 2017	Accepted
Loamy sand	0.8	5.3	286	35777	0.862		
Clay	1.8	7.2	802	44574	0.887		
Loam	1.5	5.2	366	24376	0.823		
Geometric mean				38395			
Arithmetic mean					0.877		

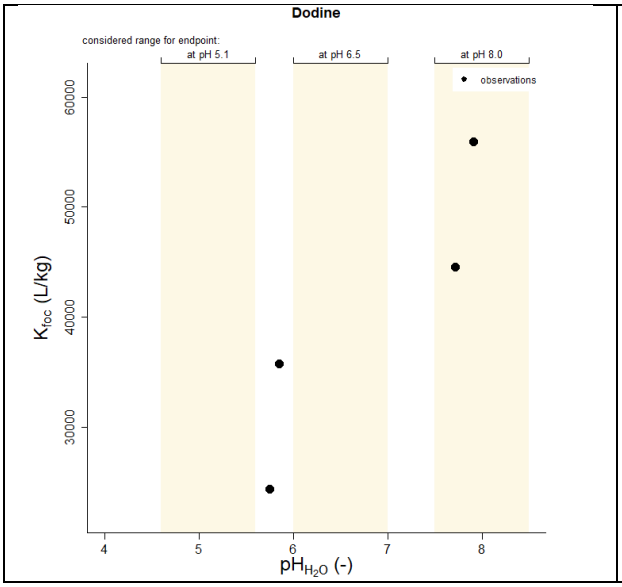
a) Spiked with guanidine-labelled Dodine
b) Measured in 0.01 M CaCl2
c) Kd and Koc were not measured

One study on absorption and desorption of Dodine hydrochloride ([redacted] 1991 - KCA 7.1.3.1.1/03-) was already submitted and accepted in the DAR of Dodine (2009). The study was performed with four different soils with UK origin (a sand, a sandy loam, a clay loam and a silt loam soil) that covered a range of organic carbon contents between 0.05% and 2.10%, a range of pH from 6.4 to 7.6 and a clay content from 4% to 38%. Due to several deficiencies identified in the study, it is considered that the adsorption has been overestimated. This is also confirmed when results of [redacted] (2017) and [redacted] and [redacted] (1991) are compared. **Therefore, RMS considers that the study is not valid to be used in the environmental risk assessment.**

The applicant has provided a new adsorption/desorption study in soil for Dodine for the purpose of renewal (KCA 7.1.3.1.1/02). In this study the advanced test was performed using four sterilised soils at a soil-to-solution ratio of 1:25 and five test concentrations covering two orders of magnitude (0.01 – 1.00 µg/mL). In addition, an evaluation of the data available from this study has been performed according to the OECD 106 Evaluator's Checklist (2017) confirmed the study was acceptable and the study meets or exceeds all established quality criteria (KCA 7.1.3.1.1./03). The calculated adsorption coefficients normalised to organic carbon content, $K_{FOC(ads)}$, range from 24376 to 55905 mL/g (geometric mean: 38395 mL/g).

The results of both batch adsorption studies ([redacted] and [redacted] 1991, and [redacted] 2017) indicate that dodine shows a strong adsorption to soil and can be considered immobile.
The pH dependency of the adsorption of dodine was assessed by RMS using software tool pHADe (UBA). According to the results of Kendall's test, adsorption of dodine seems to be pH dependent, being higher under basic conditions. However, the test was significant at 0.1 % level of significance (p-value= 0.083) and only 4 data are available. Without further data it is difficult to conclude on the pH dependence of tha active substance since its physical and chemical properties do not explain this behaviour. RMS position is that the data requirements are fulfilled and that no further information on pH dependence of adsorption is needed taking into account the high

adsorption of dodine to soil (kfoc values higher than 20000 mL/g) being considering inmobile according to [redacted]'s classification scheme.



Kendall's Tau	1
p-value	0.083
Significance	significant (level of significance: 0.1)

The leaching characteristics of dodine in aged soil were studied by [redacted] (2002) in one sandy loam soil, with organic matter content of 2.9%, pH 5.9 of and clay content of 13%. Dodine was applied at a rate of 1.2 mg/kg dry soil and aged under aerobic conditions at soil moisture content equivalent to 48% of water holding capacity, for 78 hours. No significant amounts (< 0.2% of applied) of dodine aged residues were detected in leachates under unsaturated flow conditions. Most of the activity (88-95%) was found to remain in the top of the soil columns consisting mainly of dodine. Little movement of activity down the soil occurred. In the bottom section of the column no significant amounts of activity were found (0.1%). No significant metabolites were observed after leaching. It can be concluded that dodine show low potential to leach into groundwater. Since reliable adsorption coefficients were obtained in the absorption and desorption study of Dodine, the submitted field data are regarded as supplementary information. The results of degradation and other mobility data along with the PEC_{GW} simulation results do not indicate any potential for mobility and leaching to groundwater.

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

Available environmental fate and ecotoxicology studies have been considered and summarised in the Dodine Monograph (Volume 3, Annex B8 and Annex B9, 2009) and in the renewal of approval dossier (dRAR, Volume 3, Annex B8 and Annex B9).

The key information pertinent to determine the environmental hazard classification for Dodine is presented below. Unless otherwise stated, these studies were conducted in accordance with GLP and the validity criteria of the representative test guideline, if applicable.

2.8.2.1 Rapid degradability of organic substances

All the studies considered reliable to characterize both biotic and abiotic degradation of the active substance in water and sediment are used for CLP purposes. They are reported in the following table.

Table 68: Summary of relevant information on rapid degradability

Method	Results*	Key or Supportive study[1]	Remarks	Reference
Ready biodegradability. EEC directive 92/69, C.4-C,	ThOD = 2.3 mg CO2/mg	Key study The study is considered acceptable.	Test substance: Dodine Purity: 96.2%	[redacted] (2002)

Method	Results*	Key or Supportive study[1]	Remarks	Reference
December 1992, and OECD guideline No. 301B			Not Readily Biodegradable.	
Aerobic aquatic metabolism in water/sediment systems. SETAC. Procedures for assessing the environmental fate and ecotoxicity of pesticides. Part 1, Section 8.2. Aerobic aquatic degradation. [redacted] (ed) 1995.	Whole system: DT50 = 0.1 – 0.3 days Water phase: DT50 = 0.8 – 1.7 days .	Key study	Test substance: [^{14}C]guanidine-labelled Dodine Purity: > 97.7%	[redacted] (2004b)
Aerobic aquatic metabolism in water/sediment systems. Recalculation: FOCUS 2006 and FOCUS 2014	Whole system: DT50 = 0.36 days DT90 = 3.47 days Water phase: DT50 = 0.16 days DT90 = 1.47 days Sediment phase: DT50 = 3.79 days DT90 = 12.58 days	The study is considered acceptable	Re-calcuation from [redacted] (2004)	[redacted] (2021)
Aerobic mineralisation in surface water. OECD Guideline 309	DT50 = 2.3 days	Key study. The study is considered acceptable.	Test substance: [^{14}C]guanidine-labelled Dodine] Purity: 95.4% Dodine rapidly degraded in natural surface water system and ultimately mineralized to CO_2.	[redacted] (2021)
Hydrolysis OECD Guideline 111 and EPA guideline OCSPP 835.2120	Less than 10% of hydrolysis at 50 ºC and pH 4 and pH 7. DT_{50} 1 year at 25ºC.	Key study. The study is considered acceptable.	Test substance: Dodine acetate, [guanidine-^{14}C] Purity: 100% Hydrolytically stable at every pH condition tested.	[redacted] (2017)

Method	Results*	Key or Supportive study[1]	Remarks	Reference
Photolysis in water. US-EPA Pesticide Assessment Guidelines, Subdivision N : Chemistry, Environmental Fate, section 161-2 : Photodegradation studies in water (1982). SETAC Procedures for assessing the Environmental Fate and Ecotoxicity of Pesticides. Aqueous photolysis (1995).	Dodine degraded slowly (82.1% of applied still present after 30 days), whereas in irradiated natural water Dodine degraded to 56.9% of applied after 28 days. Dodine was stable in the dark controls of buffered and natural water.	Key study The study is considered acceptable.	Test substance: [^{14}C]guanidine-labelled Dodine Purity: > 97.7%	[redacted] 2004a
Photolysis in water.	DT_{50} = 27 days in natural summer sunlight days at 40°N			[redacted] (2021)

** data on full mineralization should be reported*

2.8.2.1.1 Ready biodegradability

The readily biodegradability of the Dodine was studied by [redacted] (2002) in the DAR of the first approval (Annex I) of the active substance Dodine (DAR, 2009). The results showed that Dodine was not readily biodegradable under the conditions of the modified Sturm test performed.

[redacted] (2002).

The ready biodegradability of the active substance Dodine was studied with a test design in line with OECD guideline 301 B "CO_2 Evolution (Modified Sturm Test)". The study lasts for 28 days according to the OECD guideline. The inoculum used was an activate sludge from municipal sewage treatment plant.

The study was performed in bottles containing 2 litres of suspension. Six bottles were tested with four different treatments: Test item (duplicate), Positive control (single), Inoculum blank (duplicate), Toxicity control (single).

The study met all criteria for acceptability, therefore, this study was considered to be valid.

A ThCO_2 production of 2.30 mg CO_2/mg was found for the active substance Dodine. Therefore, it was concluded that Dodine is not readily biodegradable under the conditions of the test.

2.8.2.1.2 BOD5/COD

No data available.

2.8.2.2 Other convincing scientific evidence

No data available.

2.8.2.2.1 Aquatic simulation tests

Water-sediment studies

One water-sediment study (2004b), was already submitted and accepted during Annex I inclusion of the Dodine (DAR, 2009).

(2004b)

The aerobic degradation of Dodine in two non-contaminated water-sediment systems from Oostvaardersplassen (OVP) and Schoonrewoerdsewiel (SW) in laboratory at 20±2°C in the dark for 84 days. The study has been performed following the guideline SETAC: Procedures for assessing the environmental fate and ecotoxicity of pesticides (Part 1, Section 8.2. Aerobic aquatic degradation), and it was conducted in accordance with GLP. The study was conducted with radiolabelled dodine (radiochemical purity >97.9%) and non-radiolabelled dodine (purity 96.2%). The test substance concentration in the water layer was approximately 100 µg/L. The water used was fully characterized in terms of appearance, hardness, pH, temperature, conductivity and dissolved oxygen.

In the OVP system, mass balances were between 91-107% of AR except on days 0 and 5, when a recovery of 115 and 29% of AR were reported, respectively. However, no trend of decreasing mass balances was observed and, therefore, there were no losses of degradation products could be concluded. In the SW system, mass balances were between 93% and 104% of AR, except at days 8 and 12, when a recovery of 86 and 75%, respectively.

Non-extractable residues in the sediment layer represented a maximum of 58 and 35 % of AR after 1 day in the OVP and the SW system, respectively, and dropped to values of 33% (OVP) and 14% (SW) after 84 days. Mineralisation to CO_2 was the major degradation process (72% OVP, 89% SW after 84 days). In both systems, negligible amounts of volatile organic compounds were formed (≤0.02% of AR).

A polar fraction (M2) was detected in the water phase after extraction and acidification at concentrations higher than 10% of AR (14.5 % AR) in SW system at day 2. In addition, two other polar fractions (M1 and M3) were found at concentrations below 5% of AR. The same fraction (M2) was detected in the water phase of the OVP system, at concentration of 7.8% at day 2 and 12.4 % AR when the analysis is repeated. These fractions could not be identified in this study by TLC.

The metabolites from the polar fraction were not studied at day 5, where high polar fractions, after extraction with methyl acetate were found: 16.2% AR and 37.5% AR for OVP and SW, respectively. However, at this data point the polar fractions after acidification was 5.6 and 6.4% for OVP and SW systems, respectively.

The kinetic calculations presented in the study report are superseded by a re-evaluation (see below , 2021) performed according to recommendations of the FOCUS workgroup on degradation kinetics (2006, 2014).

The study could be considered acceptable. However, several uncertainties have been raised regarding the identity of the metabolites. Two new studies have been conducted to identify the fraction M2 in SW water/sediment system (2011 and 2020).

(2021)

This study has performed a kinetic reanalysis of the experimental data from the two water/sediment systems from the laboratory aerobic degradation study (2004b) according to recommendations of the FOCUS workgroup on degradation kinetics (2006, 2014). Persistence DT_{50} values for Dodine were calculated to be 0.4 days (0.34 OVP, FOMC kinetics and 0.38 GW, DFOP kinetics), 0.2 days (0.26 OVP, HS kinetics and 0.10 GW, DFOP kinetics) and 3.8 days (5.54 OVP, SFO kinetics and 2.59 GW, SFO kinetics) for the whole system, water compartment and sediment compartment, respectively. DT_{90} values were 3.5 days (2.80 OVP, FOMC kinetics and 4.31 GW, DFOP kinetics), 1.5 days (2.06 OVP, HS kinetics and 1.05 GW, DFOP kinetics) and 12.6 days (18.39 OVP, SFO kinetics and 8.60 GW, SFO kinetics) for the whole system, water system and sediment system, respectively.

A brief summary of the modelling endpoints to be used for the risk assessment and persistence endpoints is given in the **Table 2.8.2.2.1-1**.

Table 2.8.2.2.1-1: Persistence and modelling endpoints of Dodine in water/sediment systems

Compartment	System	Persistence endpoints			Modelling Endpoints		
		Kinetic	DT_{50}	DT_{90}	Kinetic	DT_{50}	DT_{50} appropriate for

		model	[days]	[days]	model	[days]	modelling input [a] [days]
Whole system	Silt Loam (OVP)	FOMC	0.34	2.80	FOMC	0.34	0.84
	Loamy Sand/Sand (SW)	DFOP	0.38	4.31	DFOP	0.38	1.71
	Geometric mean		0.36	3.47	-		**1.20**
Water	Silt Loam (OVP)	HS	0.26	2.06	HS	0.26	1.28
	Loamy Sand/Sand (SW)	DFOP	0.10	1.05	DFOP	0.1	1.01
	Geometric mean		0.16	1.47	-		**1.14**
Sediment	Silt Loam (OVP)	SFO	5.54	18.39	SFO	5.54	5.54
	Loamy Sand/Sand (SW)	SFO	2.59	8.60	SFO	2.59	2.59
	Geometric mean		3.79	12.58	-		**3.79**

[a] SFO, Pseudo-SFO in case of FOMC (DT_{90} / 3.32), DFOP and HS (ln(2)/ k2)

Aerobic mineralization

A new study ([redacted] 2021) to address data requirement for an investigation of aerobic mineralization in surface water with [^{14}C]Dodine was performed for the renewal of approval.

[redacted] (2021)

Aerobic mineralisation of [^{14}C]Dodine in surface water was investigated under defined laboratory conditions in the dark for 31 days. The study has been performed following the OECD 309 guideline (2004) and in accordance with GLP. A natural water were trated with [^{14}C]Dodine at two concentrations (10 and 100 µg a.i./L) and incubated under aerobic conditions at ± 20ºC in an aerobic flow-through system with attached traps for the collection of CO_2 and volatile organics, during 31 days. Sterile water was treated with both C-14 labeled test substances at a rate of 100 µg/L and incubated under identical conditions (but without air flow trapping system). System control vessels treated with 14C-benzoic acid showed that the water system used was viable. Dodine dosed samples were analysed immediately after treatment (time 0) and after 1, 2, 5, 10 and 31 days, as well as after 4 hours and 1.17 days of incubation. The water samples were analysed directly by liquid scintillation counter (LSC) and by high-performance liquid chromatography (HPLC). The chloroform phase was concentrated and analysed by HPLC.

The total mean recoveries were 100.3 ± 3.4% AR for the high dose, 102.2 ± 3.3% AR for the low dose and 100.6 ± 4.2% AR for the high dose sterile experiment.

Dodine degraded rapidly in both the high and low dose systems. After two days of incubation, Dodine mean values of 61.5% and 60.4% AR remained in the high dose and low dose system, respectively. Corresponding mean values after 31 days of incubation were 5.4% and 21.4% AR. DT_{50} values for [^{14}C]guanidine-labelled Dodine in natural surface water were calculated to be 2.3 days for the high dose and low dose, and 200 days for the high dose sterile experiment. DT_{90} values were 7.5 and 66.5 days for the high dose and low dose experiment, and 665 days for the high dose sterile experiment. In conclusion, Dodine rapidly degraded in natural surface water system and ultimately mineralized to CO_2.

One major polar fraction M1 was formed in the high dose and low dose system, indicating a maximum of 74.1% (5 DAT) and 44.8% (5 DAT), respectively. Fraction M1 was identified to consist of three components: 6-{ [amino(imino)methyl]amino} -3-hydroxyhexanoic acid, 6-{ [amino(imino)methyl]amino} hexanoic acid and (2E)-6-{ [amino(imino)methyl]amino} hex-2-enoic acid, each being a result of cleavage and oxidation to carboxylic acid of the Dodine n-dodecyl chain at the level of the C6, eventually combined with dehydrogenation on the remaining chain.

A proposed degradation pathway in surface water under aerobic conditions is described in the following figure.

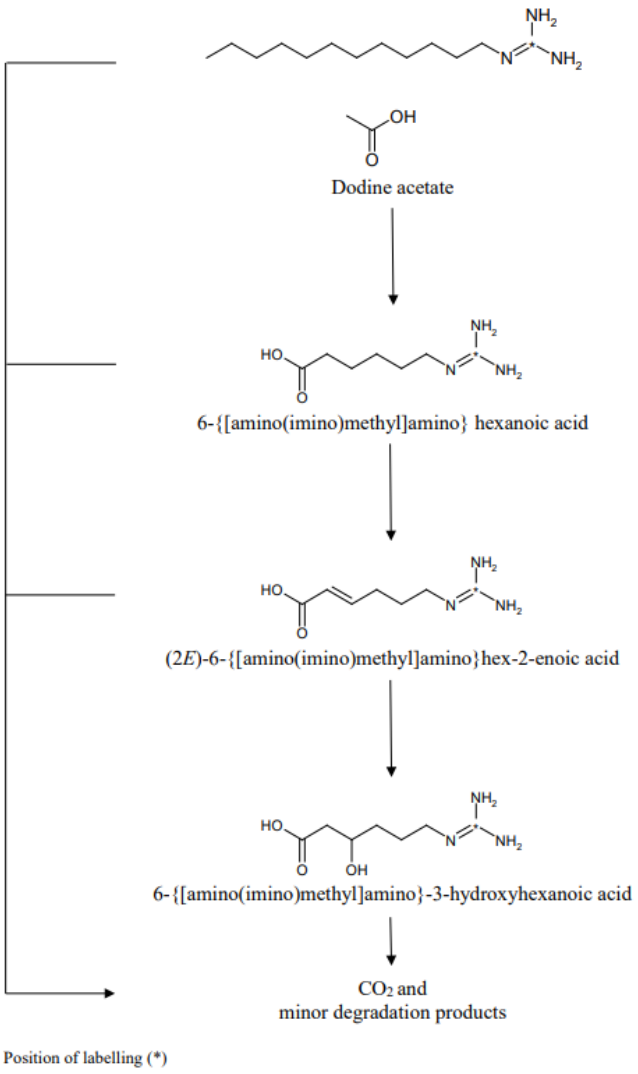


Figure 2.8.2.2.1-1. Proposed degradation route for Dodine in surface water (study duration 21 days, 20 ± 2° C)

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

Studies on field soil dissipation of Dodine are not required since laboratory aerobic soil degradation studies confirmed that the degradation of Dodine is rapid. The maximum DT_{50} value of 9.5 days and maximum DT_{90} value of 31.6 days (at 20°C and pF2) are below 60 and 200 days, respectively, that would trigger the data requirement for field soil dissipation studies.

One study on soil dissipation of Dodine (KCA 7.1.2.2.1/01) was already submitted and accepted in the DAR of Dodine (2009), and the submitted field data are regarded as supplementary information. The determination of the half-life of Dodine at each test site was based on least squares best fit exponential curves of the results of the analyses after each application. The study results indicate that Dodine has a short half-life (DT50 and DT90 range between 6 and 18 days and between 30 and 108 days respectively) and a very low potential for leaching.

No monitoring data for Dodine was available in the DAR (2009) for the Annex I approval of the active substance since it is not applicable and/or not necessary for this active substance. Therefore, no monitoring data of Dodine is submitted for the renewal of the active substance Dodine.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

Please refer to 2.8.2 and to Vol. 3 B.8.2.2.3 (AS) for sediment degradation (water/sediment systems).

2.8.2.2.4 Soil and sediment degradation data

Please refer to 2.8.2 and to Vol. 3 B.8.2.2.3 (AS) for sediment degradation (water/sediment systems).

2.8.2.2.5 Hydrolysis

One study on hydrolysis of Dodine (1991) was already submitted and accepted in the DAR of Dodine (2009), prepared in the context of the inclusion of the active substance in Annex I of the Council Directive 91/414/EEC. However, this study has not been included in the Dossier. An additional study (2017) has been submitted to evaluate the hydrolysis for the renewal of Dodine according to Regulation (EC) No 1107/2009.

(2017).

The hydrolytic behaviour of [^{14}C]guanidine-labelled Dodine was investigated at pH 4, 7 and 9 in sterile aqueous buffer solutions at 50 °C according to OECD guideline 111 and EPA guideline OCSPP 835.2120. It was conducted in accordance with GLP.

The study was performed using radio-labelled [^{14}C]guanidine-labelled Dodine over a period of 5 days. A total amount of 19.6 MBq/L was applied to the test system (corresponding to a concentration of 4.1 mg/L) and incubated at 50ºC in the dark.

In Tier 1, for all conditions and sampling intervals, the complete mass balances of applied radioactivity (AR) were obtained. The mean recoveries were between 96.4 and 109.1% of applied radioactivity in all sterilized buffer solutions.

The HPLC results indicated less than 10% of hydrolysis in pH ranges 4-9 at 50 °C, which corresponded to a half-life of approximately one year at 25 °C. Based on the results, [^{14}C]guanidine-labelled Dodine was found to be hydrolytically stable at acidic (pH 4), neutral (pH 7) and alkaline (pH 9) conditions.

This study can be considered acceptable.

2.8.2.2.6 Photochemical degradation

The photolityc degradation of Dodine in water (2004a) was already submitted and accepted in the DAR of Dodine (2009, 2014 additional report). A kinetic reevaluation of this study was performed (2021) following the recommendations in the FOCUS degradation kinetic guidance (2006, 2014) and to fulfil the requirement of EFSA Technical report (2019).

2004a.

In this study, the photolytic degradation of Dodine in water was studied in buffer pH 7. Natural water or pH 7 buffer were treated with [^{14}C]guanidine-labelled Dodine at concentrations of 1.05 and 0.98 mg/L, respectively, and incubated under continuous irradiation with sunlight-simulating light source (Xenon lamp) or under dark conditions, at ±25 °C during 28/30 (buffer pH 7/natural water) days. Only one replicate per treatment was incubated.

Mass balances were all in the range of 93.3-101.5%. In all of the systems, formation of CO_2 was negligible (2.0% of applied) and organic volatile compounds were formed in amounts ≤0.3% of applied.

Results showed that in irradiated buffer pH 7, Dodine degraded slowly (82.1% of AR after 30 days), whereas in irradiated natural water dodine degraded down to 56.9% of AR after 28 days. Dodine was stable in the dark controls of both water types.

At 40°N (draft OECD and OPPTS guidelines), only guanidine might be a relevant metabolite. Guanidine, exceeded 10% of applied radioactivity in the irradiated natural water solution (maximum 42.0% of applied after 14 days in test solution + rinsate) and in the irradiated buffer pH 7 system (max 15% in test solution + rinsate). Guanidine was not encountered in the dark test solutions but it was found in all rinsates of the natural water dark controls (max 13%) and in two rinsates of the pH 7 buffer dark controls (max 7%).

The results showed that direct photolysis is a significant degradation process for dodine in natural aquatic environment.

(2021).

A recalculation of kinetic parameters was performed for the aquatic photolysis data on Dodine ([redacted] 2004a) following the recommendations in the FOCUS guidance (2006, 2014) and to fulfil the requirement of EFSA Technical report (2019). This kinetic evaluation indicates that for irradiated conditions, SFO kinetics provided the best fit for natural water resulting in DT_{50} and DT_{90} of 11 days and 36.5 days, respectively. The equivalent half-live values in natural summer sunlight days at 40°N for Dodine was found to be 27 and 104 days for DT_{50} and DT_{90} respectively for natural water.

2.8.2.2.7 Other / Weight of evidence

No additional data available.

2.8.3 Summary of fate and behaviour in air

The vapour pressure of Dodine was determined to be < 5.49×10^{-6} Pa. Based on an evaluation of phototransformation of Dodine in air, Dodine, in the form of dodecylguanidine, has a DT_{50} = 1.18 hours following the Atkinson calculations. Due to the low half-life of Dodine in the air (1.18 h) and its very low vapour pressure (< 5.49×10^{-6} Pa), volatilization Dodine is also expected to be low.

2.8.3.1 Hazardous to the ozone layer

Table 2.8.3.1-1: Summary table of studies on hazards to the ozone layer

Method	Results	Remarks	Reference
No data	-	-	-

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

Based on an evaluation of phototransformation of Dodine in air, Dodine, in the form of dodecylguanidine, has a DT_{50} = 1.18 hours calculated with "Estimation Programs Interface (EPI) SuiteTM (Version 4.11)" using the model AOPWIN (version 1.92a) and following the Atkinson calculations. Based on these recalculations, total OH rate constant was determined at 1.08756xe-10 cm^3 molec. sec., mainly due to reactions with N, S and OH (77%) and hydrogen abstraction (23%). Other mechanisms do not contribute to hydroxyl radical estimations. The total rate of both, OH and ozone constant, is very low. This indicates that any volatilised Dodine will be extremely short-lived in the atmosphere. Therefore, global warming potential, ozone depleting potential, photochemical ozone creation potential and accumulation in the troposphere are all unlikely to occur following use of Dodine according to good agricultural practice.

There are no data provided regarding the hazard of Dodine to the ozone layer, the Ozone Depleting Potential (ODP) of Dodine has not been measured.

2.8.3.1.2 Comparison with the CLP criteria

A substance is considered hazardous to the ozone layer if the available evidence concerning its properties and its predicted or observed environmental fate and behaviour indicate that it may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

Any substances having an ODP of greater than or equal to the lowest ODP (i.e., 0.005) of the substances currently listed in Annex I to Regulation EC No 1005/2009 should be classified as hazardous to the ozone layer (category 1).

Although no specific data have been provided for this hazard, considering the chemical structure and other available information on the physicochemical properties, Dodine is not expected to be hazardous to stratospheric ozone.

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

No classification based on data conclusive but not sufficient for classification is proposed.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

No data submitted.

2.8.5 Definition of the residues in the environment requiring further assessment

For the purpose of risk assessment, the residue definition in all various compartments is limited to the parent compound Dodine. Therefore, the residue risk assessment is defined as Dodine only.

Compartment(s)	Definition of residue
Soil	Dodine
Groundwater,	Dodine
Surface water	Dodine
Sediment	Dodine
Air	Dodine

2.8.6 Summary of exposure calculations and product assessment

2.8.6.1 Predicted Environmental Concentrations in soil (PECsoil)

The formulated product Dodine 544 SC is proposed to be applied as fungicide up to 2 times per season to apples/pear, cherry and peach with minimum application intervals of 21 days. The maximum application rates (per application) range between 0.68 kg a.s./ha (apples/pear and cherry) to 0.9 kg a.s./ha (peach), equivalent to 1.25 L product/ha and 1.65 L product/ha, respectively.

Initial predicted concentrations in soil ($PEC_{S,\ act}$) values for the active substance dodine were calculated with ESCAPE 2.0 model assuming worst case conditions for application and crop scenarios : 2 applications at 0.9 kg a.s./ha in peach with a minimum interval of 21 days between applications. Plant interception was set to 50% for 1st application and 2nd application, respectively. A DT_{50} value of 17 days (the longest laboratory persistence DT50 at 20ºC) from an aerobic laboratory soil degradation study was used for Dodine. As Dodine degrades rapidly in soil with no potential for accumulation, calculation of plateau and accumulation concentrations are not required.Refer to Vol. 3 CP B8 Point B.8.2 for further details.

The calculation was performed assuming up to 2 applications of Dodine 544 SC to pome/pears, cherry and peach, where the maximum PEC_S after the last application was calculated to be 0.646 mg/kg, 0.514 mg/kg and 0.730 mg/kg, respectively.

2.8.6.2 Predicted Environmental Concentrations in groundwater (PECgw)

PEC_{GW} values for Dodine were below the trigger value of < 0.1 µg/L for all modelled scenarios and crops following modelling with FOCUS PEARL 4.4.4, FOCUS PELMO 5.5.3 and FOCUS MACRO 5.5.4. The PEC in groundwater was assessed calculating the 80th percentile concentrations of 26 years for peach only which is the worst-case scenario and covers all the representative uses (apples/pear, cherry and peach).

All calculated PECgw values for dodine were below the trigger value of 0.1 µg/L. Therefore, it is concluded that an unacceptable risk to groundwater after application of Dodine 544 according to the GAP is not expected.

2.8.6.3 Predicted Environmental Concentrations in soil (PECsw)

A FOCUS SW calculation for the product Dodine 544 SC was performed to predict the concentration of residues in surface water (PEC_{SW}) and aquatic sediment (PEC_{SED}). PEC_{SW} and PEC_{SED} values for FOCUS evaluation Steps 1 and 2 were calculated using the modelling software STEPS 1-2 (version 3.2). Within the scope of evaluation Steps 3 and 4, for every main entry route different software was used as recommended, i.e. FOCUS SWASH 5.3, Drift calculator 1.1 (spray drift), MACRO 5.5.4 (drainage) and PRZM 4.3.1 (runoff). Based on the different pesticide

inputs calculated, TOXSWA 5.5.3 was used to simulate the fate of pesticide entries in typical surface water bodies and finally to calculate maximum as well as actual and time weighted average concentrations in water layer and sediment for different dates or periods. Step 4 calculations were performed using the model-software Surface Water Assessment eNabler (SWAN) v 5.0.1 by taking mitigation options into account such as no-spray buffer zones (reduction of drift entries) and vegetated buffer zones or filter strips.

Based on predicted environmental concentrations in surface water for the parent Dodine, it was necessary to consider higher tier modelling approaches for all uses (FOCUS Step 3 and 4). For Dodine, a Tier 1-RAC of 0.18 µg/L was used for the risk assessment. Moreover, a ETO-RAC of 0.4 µg/L and a ERO-RAC of 2.5 µg/L derived from a mesocosm study (Hoomen, 2021b) have been proposed for refinement purposes Please refer to Vol. 3 CP B.9 Point B.9.4 for further details.

2.8.6.4 Predicted Environmental Concentrations in air (PECair)

The chemical properties that most affect volatilisation are vapour pressure and Henry's law constant. Dodine has a low volatility (vapour pressure < 5.49×10^{-6} Pa at 50ºC) and a Henry's law constant lower than 1.69×10^{-6} Pa m^3/mol, suggesting little potential for volatilisation in the environment.

Based on an evaluation of phototransformation of Dodine in air, Dodine, in the form of dodecylguanidine, has a DT_{50} = 1.18 hours following the Atkinson calculations.

Due to the low volatilization potential and fast degradation of dodine in air, dodine is not expected to be subject of atmospheric short- or long-range transport. Therefore, model calculations of off-site deposition (PEC) originating from volatilisation are not required. Likewise, calculations of concentrations from airborne transport are not required.

2.8.6.5 Predicted Environmental Concentrations for other routes of exposure

Atmospheric exposure resulting from other routes of exposure such as deposition of dust by drift during sowing, amenity use or indirect exposure of surface water via a sewage treatment plant (STP) after application of a plant protection product in storage rooms is not anticipated following application of the Dodine 544 SC formulation to agricultural crops as proposed. Therefore, further information is not required.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

2.9.1.1 Summary of effects on birds

Studies on the acute oral, short-term dietary and reproductive toxicity of Dodine technical to birds were already submitted for the first EU evaluation for the Annex I inclusion of the active substance. No study assessing the effects of the representative product to birds has been conducted as it is possible to extrapolate from the data available on the active substance. Although no new study relevant to address the core data requirements for either the active substance or the formulated product has been conducted for the purpose of renewal of the approval of Dodine, two new higher-tier field studies investigating the effects to blue tits and great tits following Syllit 400 SC applications under realistic use conditions are available. However, these studies were considered as supporting information by the RMS since the application of Syllit SC 400 (2x250 g a.s./ha/m crown height in approx. 1-4 weeks interval) was not a realistic worst case compared to the Dodine SC 544 as proposed in the GAP (2x680 g a.s./ha and 2x900 g a.s./ha. 21 days interval).

Considering the available, complete data set, the geometric mean of the LD_{50} on mallard duck and the LD_{50} on bobwhite quail as derived from the available short-term dietary studies, i.e., 548.1 mg a.s./kg bw, is used in the regulatory acute risk assessment and the lowest NOEL of 20 mg a.s./kg bw/d for mallard duck is used in the regulatory long-term/reproductive risk assessment for birds.

A summary of the endpoints derived from the bird studies with Dodine is presented in Table 2.9.1.1 1. The values in bold were used for risk assessment.

Table 2.9.1.1-1 Effects of Dodine to birds

Test substance	Test species	Exposure System	Endpoint	Reference	Endpoint used in the risk assessment
Acute oral toxicity to birds					
Dodine	*Colinus virginianus*	Oral Acute	LD_{50} = 981 mg a.s./kg bw	[redacted] (1990a) DAR (2009), EFSA Journal 2015;13(8):4209 Please refer to KCA 8.1.1.1/01	-
Dodine	*Anas platyrhynchos*	Oral Acute	NOEL = 200 mg a.s./kg bw[1]	[redacted] (1990b) DAR (2009), EFSA Journal 2015;13(8):4209 Please refer to KCA 8.1.1.1/02	
Short-term dietary toxicity to birds					
Dodine	*Colinus virginianus*	Dietary Short-term	LC_{50} > 5200 ppm LD_{50} > 976 mg a.s./kg bw/d	[redacted] (1990c) DAR (2009) Please refer to KCA 8.1.1.2/01	**Geomean LD_{50} = 548.1 mg a.s./kg bw/d[2]**
Dodine	*Anas platyrhynchos*	Dietary Short-term	LC_{50} = 2263 ppm LD_{50} = 307.8 mg a.s./kg bw/d	[redacted] (1990d) DAR (2009), EFSA Journal 2015;13(8):4209 Please refer to KCA 8.1.1.2/02	
Sub-chronic and reproductive toxicity to birds					
Dodine	*Colinus virginianus*	Dietary 21 weeks Reproductive toxicity	NOEC = 300 ppm NOEL = 27.1 mg a.s./kg bw/d	[redacted] (1999) DAR (2009) Please refer to KCA 8.1.1.3/03	**NOEL = 20 mg a.s./kg bw/d**
Dodine	*Anas platyrhynchos*	Dietary 20 weeks Reproductive toxicity	NOEC = 200 ppm NOEL = 20 mg a.s./kg bw/d	[redacted] (1994b) DAR (2009), EFSA Journal 2015;13(8):4209 Please refer to KCA 8.1.1.3/05	
Higher-tier effect studies					
Syllit 400 SC	Avian monitoring study investigating the potential long-term (i.e., reproductive) effects of Dodine formulated as Syllit 400 SC on insectivorous passerines, with a focus on blue tits and great tits, in commercially managed orchards in Germany; application pattern: two applications of 250 g a.s./ha/m crown height in approx. 1-4 weeks interval; application timing: April/May 2018; no negative long-term (i.e., reproductive) effects on exposed blue and great tits.			[redacted] (2018) Please refer to KCP 10.1.1.2/01	-
Syllit 400 SC	Avian monitoring study investigating the potential long-term (i.e., reproductive) effects of Dodine formulated as Syllit 400 SC on insectivorous passerines, with a focus on blue tits and great tits, in commercially managed orchards in Germany; application pattern: two applications of 250 g a.s./ha/m crown height in 8 days interval; application timing: April/May 2018 and April/May 2019 (two control orchards and three treatment orchards were already investigated in			[redacted] (2019) Please refer to KCP 10.1.1.2/02	-

Test substance	Test species	Exposure System	Endpoint	Reference	Endpoint used in the risk assessment
	2018); compared to the previous study from 2018: focus on the effects following applications over several years and data collection in two consecutive years, increased number of plots and nests; no negative long-term (i.e., reproductive) effects on exposed blue and great tits.				

a.s. active substance
[1] An LD_{50} of 857 mg a.s./kg bw is reported in EFSA Journal 2015;13(8):4209; however, this endpoint is no longer considered reliable for use in the risk assessment, the NOEL should be further considered from the acute oral toxicity. Further details are provided in the summary of the study (Vol. 3 CA B.9.1.1.1/02).

The risk assessment for effects on birds is carried out according to the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438). The acute and long-term risks of Dodine formulated as Dodine 544 SC to birds were assessed from toxicity exposure ratios between toxicity endpoints, derived from studies with Dodine technical and estimated exposure based on the maximum residues occurring on food items following applications according to the proposed use pattern.

Acute dietary risk assessment

The geometric mean of the LD_{50} on mallard duck and the LD_{50} on bobwhite quail as derived from the available short-term dietary studies, i.e., 548.1 mg a.s./kg bw, is used in the regulatory acute risk assessment for birds. All TER_A values for Dodine calculated for the relevant exposure scenarios exceed the trigger of 10 at screening step, indicating no potential acute risk for birds following the representative uses of Dodine 544 SC in apples/pear, cherry and peach.

Long-term dietary risk assessment

The lowest NOEL of 20 mg a.s./kg bw/d for mallard duck is used in the regulatory long-term/reproductive risk assessment for birds. All TER_{LT} values for Dodine calculated for the relevant exposure scenarios exceed the trigger of 5 at Tier 1 except for small granivorous birds feeding in treated apples/pear and peach fields and small insectivorous birds feeding in treated apples/pear, cherry and peach fields. For these two types of diet guild, a higher tier risk assessment is performed by considering focal species, i.e., a real species that actually occur in the crop when the pesticide is being used, and their ecological properties (i.e PT: Proportion of diet obtained in the treated area), by refining the residue decline (DT_{50}) in potential food items of the identified focal species and by incorporating in the exposure estimation the interception by the crop.

Taking into account the above refinemets high risk is still identified for small insectivorous birds for the following scenarios:

- All EU Zones: Long-term risk for small insectivorous birds, application on spring summer, for the intended use on peach (2 × 0.9 kg a.s./ha, 21-day interval)
- Souhern Zone and Northern Zone: Long-term risk for small insectivorous birds, application on spring summer, for the intended uses on Apples/pear and cherry (2 × 0.68 kg a.s./ha, 21-day interval).

The risk for granivorous birds serin has been addressed.

Consequently, low risk has been identified for the intended uses on Apples/Pear and cherry (2 × 0.68 kg a.s./ha, 21-day interval) in the Central Zone.

Drinking water risk assessment

Based on the ratios of the effective application rate to the relevant toxicity endpoints, an acceptable risk is demonstrated for birds due to exposure to Dodine via contaminated drinking water in puddles (puddle scenario). Further, considering the representative uses of Dodine 544 SC in orchards, no risk to birds is expected via exposure to contaminated drinking water in leaf whorls (leaf scenario).

Secondary poisoning

The log P_{ow} of Dodine does not exceed the trigger value of 3; thus, a risk assessment of secondary poisoning for earthworm- and fish-eating birds is not required.

2.9.1.2 Summary of effects on mammals

Studies considering the toxicity of dodine and the representative formulation to mammals were assessed for their validity to current and relevant guidelines. A more detailed summary and evaluation by the RMS are provided in Vol 3 CA B6, section 6 and Vol 3 CP B6, section 6.

2.9.1.2.1 Acute oral toxicity to mammals

Details of the acute oral studies on mammals are summarised in Volume 3 CA B6, section 6.1. A study with Dodine 544 SC on the acute oral toxicity in rat has been conducted and summarised in Volume 3 CP, section B.6.1.1.

Studies on the acute oral toxicity of Dodine technical to rats and mice were already submitted for the first EU evaluation for the Annex I inclusion of the active substance. The rat LD_{50} was calculated to be 851 mg a.s./kg bw while the mouse LD_{50} was calculated to be 1354 mg a.s./kg bw. For risk assessment purposes, the geometric mean LD_{50} of these endpoints, i.e., 1073 mg a.s./kg bw, was further considered. In addition to the already EU peer reviewed studies, an acute oral toxicity study with the formulation Dodine 544 SC is available which is newly submitted for the purpose of renewal of the EU approval of Dodine. The LD_{50} of Dodine 544 SC has been calculated to be > 300 mg/kg bw and < 2000 mg/kg bw by oral route in the rat. In accordance with the OECD guideline 423, the LD_{50} cut-off of Dodine 544 SC may be considered as 500 mg/kg bw (corresponding to 267.9 mg a.s./kg bw) by oral route in the rat. A summary of the relevant acute endpoints that are most appropriate for ecological risk assessment is provided in **Table 9.1.2.1-1**.

Table 2.9.1.2.1-1 Summary of acute effects of Dodine to terrestrial mammals

Test substance	Test species	Exposure System	Endpoint	Reference	Endpoint used in the risk assessment
Dodine	Rat	Oral Acute	LD_{50} = 851 mg a.s./kg bw	[redacted] (1999) Please refer to KCA 5.2.1/01	**Geomean LD_{50} = 1073 mg a.s./kg bw**
Dodine	Mouse	Oral Acute	LD_{50} = 1354 mg a.s./kg bw	[redacted] (2008) Please refer to KCA 5.2.1/02	
Dodine 544 SC	Rat	Oral Acute	LD_{50} = 500 mg f.p./kg bw = 267.9 mg a.s./kg bw [a]	[redacted] (2011) Please refer to KCP 7.1.1/01	-

The lower LD_{50} of 267.9 mg a.s./kg bw derived from the formulation study compared to the LD_{50} endpoints derived from the studies conducted with the active substance does not imply that Dodine is more acutely toxic to rats when formulated to Dodine 544 SC. This difference can be explained by the different methods used for testing in each study. For Dodine 544 SC, the OECD testing guideline 423 (toxic class method) was used. To reduce the number of vertebrates tested, a minimum number of animals was used following a step wise approach. As a consequence, no accurate LD_{50} but a range (300 - 2000 mg/kg bw) has been set; according to the guideline a cut-off value of 500 mg/kg has been derived based on the use of 9 female animals (which are the most sensitive gender). If more animals (with males included) were used, the real LD_{50} could have been found higher somewhere between 500 and 2000 mg/kg. For Dodine technical, the OECD testing guideline 401 was used. Thirty animals (5/5 male/female animals at 3 dose levels) were tested allowing to calculate an exact LD_{50} of 851 mg/kg (for males and females combined). The LD_{50} for females only ranged between 450 and 761 mg/kg. Finally, it can be concluded that the results obtained with both Dodine technical and Dodine 544 SC are of the same order of magnitude and that Dodine 544 SC is not expected to be more toxic than Dodine technical.

In line with the recommendations given in EFSA (2009)[5], the geometric mean of the two LD_{50}s on acute oral toxicity of Dodine technical to rats (LD_{50} = 851 mg/kg bw) and mice (LD_{50} = 1354 mg/kg bw), i.e., 1073 mg a.s./kg bw, is further used in the acute risk assessment for wild mammals. This approach was already proposed and agreed during the first EU peer review of Dodine. According to EFSA (2009), the geometric mean should be used for the acute assessment, except when the endpoint for the most sensitive species is more than a factor of 10 below the geometric mean of all the tested species. Where this is the case, the most sensitive species will be used for the risk assessment

[5] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438.

but generally without any assessment factor (unless there are specific reasons to believe that this is not appropriate). Since the lowest LD_{50} of 851 mg/kg bw is not more than a factor of 10 below the geometric mean of 1073 mg/kg bw, the later endpoint is used for the acute assessment.

In terms of studies comparability, the acute oral toxicity study with rats ([redacted] 1999) followed the EPA OPPTS Guideline 870.1100 (1998) and the OECD Guideline 401 (1987) while the acute oral toxicity study with mice ([redacted] 2008) followed the EPA Guideline 870.1000 and the OECD Guidance 425. In the former study the test material was diluted in 0.5% methylcellulose while in the later study the test material was diluted in distilled water before oral administration via gavage; both vehicles are assumed to be of low toxicity. Despite any methodological differences between the two studies (i.e., different number of animals and use of males/females or only females), endpoint setting in each study primarily depended on the dose selection and the species sensitivity. The result of rats being more sensitive than mice was also confirmed in the repeated dose studies – then it is not only for mortality but for all effects.

2.9.1.2.2 Long-term toxicity to mammals

Details, full description and RMS assessment of the toxicity studies used in this risk assessment can be found in Volume 3 CA Section B.6 and Volume 3 CA Section B.9, Point 9.1.2.2. An overview table is presented below.

Considering the available complete data set, the NOAEL of 26 mg/kg bw/d is proposed for use in the Tier 1 long-term risk assessment.

All data indicate that the critical effects of Dodine are decreased food consumption accompanied by a reduction in the body weight. Therefore, results should be interpreted with some caution. Dodine is a clear irritant and it is very plausible that irritancy in the stomach have contribute to the observed response. No effects on fertility or reproduction were observed and no other developmental effects than reduction in pup body weight were reported.

Considering all data, the lowest NOAEL for derivation of the chronic mammalian endpoint for Dodine is the lowest value seen in dogs of 10 mg/kg bw/day, in a 90-day study ([redacted] 2005) and in a one-year study ([redacted] 1996). However, RMS agrees with the applicant that the ecotoxicologically relevant NOAEL should be set to 26 mg/kg bw/day, based on the following findings:

- In the 90-d oral toxicity study with dogs ([redacted] 2005), the reduction in *body weights seen at 20 mg/kg bw/d was non- statistically significant, being the mean body weights of the female dogs at this dose in the normal range of healthy beagle females. Therefore, a* NOEAEL of 20 mg/kg bw/d was proposed by RMS (ecotoxicology).
- In the one year (52 weeks) oral toxicity study with dogs ([redacted] 1996) a *NOEAEL of 20 mg/kg bw/d was proposed by RMS (ecotoxicology), since severe effects on food consumption and body weight that require supplemental feeding of animals to prevent mortality were considered to be incidental cases and, therefore, of low population relevance. No other relevant effects were seen.*
- In the chronic oral toxicity study with rats ([redacted] 1998), the NOAEL for lifetime exposure of rats to Dodine was 400 ppm, approximately 20 and 26 mg/kg/d in males and females, respectively, based on a decrease in bw (up to 10% in males and to 15% in females) and food consumption.
- In the mouse study ([redacted] 1998), the NOAEL was set to 200 ppm (29 and 38 mg/kg bw per day in males and females, respectively) based on the same effects, decreased body weight gains and food consumption.

The above studies support the selected chronic endpoint of 26 mg/kg bw/day, derived from two generation study with rats ([redacted] 1996) as ecotoxicologically relevant based on the following findings: statistically significant reduction in bw of F1 adults (up to -15.5%) and F1 and F2 pups (up to 17.7%) at 800 ppm.

Please, refer to **Table 9.1.2.2-1** for further details on the ecotoxicological relevance assessment of the mammalian chronic endpoints.

Table 2.9.1.2.2-1 Summary of long-term and reproductive toxicity studies with dodine

Test species	Exposure System	Dosing	Results	Ecological relevance of LOAEL effects	Guideline/ GLP	Reference	Relevance
Reproductive toxicity studies							
Rat	Dietary Reproductive toxicity Two-generation study *30/sex/group*	0, 200, 400, 800 ppm equivalent to F_0: M: 0, 13.1, 26.2, 52.6; F: 0, 18.1, 35.2, 67.6 and F_1: M: 0, 14.9, 30.2, 63.0; F: 0, 19.2, 38.8, 76.6	↓ body weight and food consumption for adult F0 and F1 males and females at 800 ppm. ↓ body weight and food consumption for F1 and F2 pups at 400 ppm (statistically significant but less than 10% bw reduction) and 800 ppm (up to -17.7% bw) Fertility and reproduction not affected. **NOAEL = 26 mg a.s./kg bw/d** *(parental and offspring) - 400 ppm* LOAEL = *52.6* mg a.s./kg bw/d *(maternal and offspring)*	Potentially relevant effect on bw* Bodyweight, particularly of females, may be relevant to the ability to reproduce and to the survival of pups. Bodyweight of pups is also relevant to pup survival. Since a reduction in pups bodyweights up to 17.7% was reported, it is concluded that LOAEL effects are of ecological relevance.	*FIFRA 83-4 (*OECD 416) */ Yes*	[redacted] 1996 (KCA 5.6.1/01)	Key study
Rat	Oral Developmental toxicity *25/sex/group*	0, 10, 45, 90 mg/kg bw/d	↓ *body weight at 90 mg/kg bw/d (>10% bw reduction)* ↓ *food consumption at 45 and 90 mg/kg bw/d* *No developmental effects.* NOAEL = 10 mg/kg bw (*maternal toxicity)* LOAEL = 45 mg/kg bw (*maternal toxicity)*	*At 45 mg/kg bw/d* ↓ *food consumption* without accompanying significant overall bw reduction. Population relevance low. NOEAEL = 45 mg/kg bw	*EPA OPP 83-3* (OECD 414) */ Yes*	[redacted] 1989 (KCA 5.6.2/03 *and KCA 5.6.2/01- statistical analysis)*	Key study

Test species	Exposure System	Dosing	Results	Ecological relevance of LOAEL effects	Guideline/ GLP	Reference	Relevance
Rat	Oral Developmental toxicity *(range finding study)* *10 females/group*	*0; 50; 70; 100 mg/kg bw/d*	*↓ body weight and food consumption at 70 and 100 mg/kg bw/d* *Mortality at high dose.* *No developmental effects.* NOAEL = 50 mg/kg bw (*maternal tox)* LOAEL = 70 mg/kg bw (dev. tox)	*Not determined* *(*Supporting study)	*No (range-finding)* */Yes*	[redacted], 1989b *(KCA 5.6.2/05)*	Supporting study
Rabbit	Oral Developmental toxicity *16-20/sex/group*	0, 10, 40, 80 mg/kg bw/d	*↓ food consumption at 80 mg/kg bw/d (statistically significant). For a minority of animals the effect was severe, resulting in necessary early euthanasia or in abortion of pregnancy.* *No developmental effects* NOAEL = 40 mg/kg bw (*maternal tox)* NOAEL = 80 mg/kg bw (dev. tox) LOAEL = 80 mg/kg bw (*maternal tox*)	At 80 mg/kg/day, the food consumption was statistically significantly lower from gestation day 6 to 8, which was considered to be a transient effect in early part of the treatment period, as there was no effect on the total food consumption during the dosing period. and there were no statistically significant effects in on body weight or body weight gain compared with the control. Population relevance low. NOEAEL = 80 mg/kg bw	*EPA OPP 83-3* (OECD 414) */ Yes*	[redacted] 1989 (KCA 5.6.2/04 and KCA 5.6.2/02-statistical analysis, 2019)	Key study
Rabbit	Oral Developmental toxicity *10 females/group*	*0; 70; 100 mg/kg bw/d*	*100 mg/kg bw/d: mortality (5/10 animals), ↓ body weight and food consumption* *No developmental effects.* NOAEL = 70 mg/kg bw (*maternal tox)*	*Not determined* *(*Supporting study)	*No (range-finding)* */Yes*	[redacted] 1989b *(KCA 5.6.2/06)*	Supporting study

Test species	Exposure System	Dosing	Results	Ecological relevance of LOAEL effects	Guideline/ GLP	Reference	Relevance
			NOAEL = 100 mg/kg bw (dev. tox) LOAEL = 100 mg/kg bw (*maternal tox*)				
Subchronic and repeated dose toxicity studies							
Rats	28-day oral toxicity study Gavage *10/sex/group (range finding)*	0; 75; 100; 200 mg/kg bw/d	*Severe toxicity* *↓ body weight at lowest dose of 75 mg/kg bw Mortality, changes in clinical chemistry and in histopathology in the stomach. ↓ organ weights (thought to be related to lower bw overall).* No NOAEL could be established in the study	*Not determined* (Supporting study)	*EPA FIFRA F-82-1 (range finding)/Yes* (equivalent to OECD 407)	[redacted] 1994a *(KCA 5.3.1/01)*	Supporting study
Rats	28-day oral toxicity study *Feeding (diet)* *10/sex/group (range finding)*	0, 500, 750, 1000 ppm equivalent to M: 0, 47, 71, 87 mg/kg bw/d; F: 0, 50, 72, 92 mg/kg bw/d	*-No deaths* *-↓ body weight gains at 750 and 1000. Non statistically significant at 500 ppm (8-12% reduction of body weight)* *-↓ food consumption significantly at 750 and 1000 ppm* *- ↓ in glucose levels at 1000 ppm – related to reduced food consumption* *-changes in organ weights (testis), deemed related to variations in body weights* In the previous Dodine evaluation it was considered that since a non-significant reduction on body weight gain (-8-12%) was already seen at the lowest dose, no NOAEL could be set from this study.	*Not applicable since no NOAEL could be set from this study.*	*EPA FIFRA F-82-1 (range finding)/Yes* (equivalent to OECD 407)	[redacted] 1994b *(KCA 5.3.1/02)*	Key study
Rats	28-day oral toxicity study *Feeding (diet)* *10/sex/group (range finding)*	0, 200, 800 ppm equivalent to M: 0, 17.7, 67.7; F: 0, 19.2, 76.7 mg/kg bw/d	*800 ppm: ↓ body weight (M/F), ↓ food consumption (M), ↓ liver weight (F)* NOAEL = 17.7-19.2 mg/kg bw/d (200 ppm)	*Not determined* (Supporting study)	*No (range finding)/Yes*	[redacted] 1997 *(KCA 5.3.1/03)*	Supporting study

Test species	Exposure System	Dosing	Results	Ecological relevance of LOAEL effects	Guideline/ GLP	Reference	Relevance
Mice	8 weeks oral toxicity study *5/sex/group (range finding)*	0, 100, 250, 625, 1250 ppm equivalent to M: 0, 30.3, 49.4, 109.4, 232.2; F: 0, 34, 61.3, 150.4, 323.6 mg/kg bw/d *(after 3 weeks dosing the low dose group was increased to 232.2 (m) or 323.6 (f))*	1250 ppm: ↓ body weight and body weight gain (M/F), ↓ food consumption (F). Mild eosinophilia in the liver (M/F). NOAEL = 109.4 -150.4 mg/kg bw/d (625 ppm)	*Not determined* (Supporting study)	eq. to OECD 407 *(range finding) /Yes*	[redacted] *et al.,* 1988 *(KCA 5.3.1/05)*	Supporting study
Rats	*Gut motility assessment Oral, 7 or 28-day Feeding (diet) 10/sex/group*	*0, 200, 800 ppm equivalent to M: 0; 17.7; 67.7 F: 0; 19.2; 76.7 mg/kg bw/d*	*Normal gut motility was seen following continuous dietary administration of Dodine for 7 and 28 days in rats at dose levels of 200 or 800 ppm.*	*Not determined* (Supporting study)	*Not available/Yes*	[redacted] *1996* *(KCA 5.3.1/04)*	Supporting study
Rats	90d Sub-chronic oral toxicity study *Feeding (diet)* *10/sex/group*	*0, 50, 200, 800 ppm M: 0; 3.6; 14.1; 55.8 mg/kg bw/d F: 0; 3.9; 14.9; 60.4 mg/kg bw/d*	*800 ppm (55.8* mg/kg bw/d*): ↓ food consumption (F), ↓ body weight (M+F). Changes in heart and kidney weight.* NOAEL = 14.1-14.9 mg/kg bw/d (200 ppm) LOAEL = 55.8 mg/kg bw/d (800 ppm)	*At 800 ppm, mean body weights were slightly decreased in both sex throughtout the study. The effect on females was less than 10%, therefore, not biologically relevant. An effect of 10% reduction on bw on males was observed on days 21 and 28. However, it was transient and mainly related with a decreased*	*eq. to OECD 408/Yes*	[redacted] 1982 *(KCA 5.3.2/01)*	Key study

Test species	Exposure System	Dosing	Results	Ecological relevance of LOAEL effects	Guideline/ GLP	Reference	Relevance
				food intake at the beginning of the study. *Changes in heart and kidney weight (increased weight) are not directly linked to survival or reproduction of populations.* It is concluded that the ecological relevance of LOAEL effects is low. NOEAEL = 55.8 mg/kg bw/d			
Mice	90d Sub-chronic oral toxicity study *Feeding (diet)* *10/sex/group*	0, 150, 300, 600, 1250, 2500 ppm equivalent to M: 0, 24, 48, 94, 181, 350 mg/kg bw/d; F: 0, 31, 60, 116, 223, 305 mg/kg bw/d	1250 ppm: ↓ food consumption (11-12% lower than controls), ↓ body weight gains (non-statistically significant). ↑ liver and kidney weight without histopathological changes (not biologically relevant). 2500 ppm: Mortality (4 females died), ↓ food consumption (30-50% lower than control), ↓ body weight (17-24% lower than control). clinical signs, ↓ growth, haematological and clinical biochemistry findings. ↑ liver and kidney weight without histopathological changes. NOAEL = 94-116 mg/kg bw/d (600 ppm) LOAEL = 181 mg/kg bw/d (1250 ppm)	At 181 mg/kg bw/d (1250 ppm): - Lower mean food consumption during first weeks of treatment (transient effect) without significant effects in on body weight or body weight gain compared with the control. - Significantly higher relative (to bw) kidney weights were observed. These changes are not considered to be biologically significant since no histopatological changes were noted in any of the organs analysed. Population relevance low.	(OECD 408) *EPA OPP 82-1/Yes*	[redacted] *1994* *(KCA 5.3.2/02)*	Key study

Test species	Exposure System	Dosing	Results	Ecological relevance of LOAEL effects	Guideline/ GLP	Reference	Relevance
				NOEAEL = 181 mg/kg bw/d			
Dog	*Oral, 6-week Capsule (range-finding) 2/sex/group*	*1.25, 6.25/60, 12.5/50 and 25 mg/kg bw/d (dosing was increased in different weeks)*	*25, 50, or 60 mg/kg bw/d, lead to significant adverse effects. undigested food was found in the stomachs of some of the dogs. These doses may be not suitable for a long-term study.* *No consistent adverse effects were observed following treatment of dogs with Dodine up to 12.5 mg/kg bw/d.*	Not applicable	*No (range finding)/Yes*	[redacted] *1994* *(KCA 5.3.2/04)*	*Supporting study*
Dog	*Oral, 90-day Capsule 4/sex/group*	*0, 2, 10 and 20 mg/kg bw/d*	*2 mg/kg bw/d: no relevant findings* *10 mg/kg bw/d: ↓ slightly reduced liver weight without histopathological changes (F). ↓ slightly lower food intake (F). Incidental cases of a blue tongue (M). Considered not toxicologically relevant.* *20 mg/kg bw/d: ↓ food intake (statistically significant) (F+M); ↓ body weight (non-statistically significant, up to. -15.6 % reduction) (F+M); Vomiting of food, mucus and/or test article, with incidental cases of lean appearance, a blue tongue and calm behaviour (F+M); ↓ slightly reduced liver weight without histopathological changes (F).* NOAEL = 10 mg/kg bw/d LOAEL = 20 mg/kg bw/d	At 20 mg/kg bw/d: - Reduced body weights without statistically significant differences with control. For males, bw reduction primarily during the first eight weeks of treatment (up to -15% bw compared with control) followed by weight recovery. For females, weight remained lower throughout the treatment period (up to -15.6% bw compared with control). The observed effect is related to reduced food consumption in the first part of the study. Mean body weight of females ranged from 5.4 to 6.3 kg throughout the	*OECD 409/Yes*	[redacted], *2005* *(KCA 5.3.2/03)*	*Key study*

Test species	Exposure System	Dosing	Results	Ecological relevance of LOAEL effects	Guideline/ GLP	Reference	Relevance
				study. It is noted that body weights of two out four females belonging to group 4 (20 mg/kg bw/day treatment dose) were slightly lower compared to other groups (weights before treatment in the range of 5.2 to 7.2 kg). Taking into account that the overall mean is within the normal variation of healthy female beagle dogs and non-statistically significant differences compared with control were obtained after analysis of data, it is concluded that the observed effect on body weight is not ecologically relevant. - Relative higher kidney to body weight ratio of females *without histopathological changes. This effect* was considered to be due to slightly lower terminal body weights. Absolute mean kidney weights of females were similar to control levels. Population relevance low. NOEAEL = 20 mg/kg bw/d			

Test species	Exposure System	Dosing	Results	Ecological relevance of LOAEL effects	Guideline/ GLP	Reference	Relevance
Dog	*Oral, 52-week Capsule 4/sex/group*	*0, 2, 10 and 20 mg/kg bw/d*	*There were no differences in body weight at the end of the study. However, t*hree animals (one 10 mg/kg bw/day female, one 20 mg/kg bw/day male, and one 20 mg/kg bw/day female) exhibited notably marked body weight losses and low feed intake during the first few weeks of compound administration and they *needed diet supplementation.* *One female at the high dose group (*20 mg/kg bw/d) *needed food supplementation for the majority of the study to prevent mortality.* No definitive evidence of toxicity was seen in any of the other parameters evaluated in this study: The only clear pattern indicative of a treatment-related difference was the occurrence of dose-related salivation, which was most frequently noted in anticipation of dosing in the 10 and 20 mg/kg bw/day dogs. This finding was considered most likely to be a conditioned reflex or secondary effect, rather than a direct treatment-related effect. No subchronic toxic effects were evident in the male and female low- and mid-dose dogs and 10 mg/kg bw/day was the NOAEL. NOAEL = 10 mg/kg bw/d LOAEL = 20 mg/kg bw/d	At 20 mg/kg bw/d, only severe effect on few animals were observed: supplemental feeding regimens were instituted for the two dogs to preclude mortality; one of the two dogs (male) were successfully returned to basal diet and the other dog (the 20 mg/kg bw/day female) was maintained on supplemental feeding throughout the majority of the study, continuing through study termination However, *there were no differences in body weight at the end of the study* Population relevance low due to the low incidence of the effect. NOEAEL = 20 mg/kg bw/d	*EPA OPP 83-1/Yes*	[redacted] *1996* *(KCA 5.3.2/05 and KCA 5.3.2/06 - Notifier statement)*	*Key study*
Carcinogenecity							

Test species	Exposure System	Dosing	Results	Ecological relevance of LOAEL effects	Guideline/ GLP	Reference	Relevance
Rats	Oral, Chronic toxicity and carcinogenicity *(104 weeks)* Feeding (diet) 60+10/sex/group	0, 200, 400, 800 ppm corresponding to M: 0; 10.2; 20.3; 41.9 F: 0; 13.2; 26.5; 53.5 mg/kg bw/d	- No tretment-related clinical signs or effect on the mortality up to 800 ppm. - No meaningful changes were reported in hematology, clinical chemistry and urinalysis parameters examined, except a transient slightly lower total white blood cells counts with lower absolute lymphocytc counts in males at 800 ppm. - Necropsies and histopatological examination revealed that there was no evidence of a treatment-related effect up to 800 ppm. - At 800 ppm: ↓ bw (up to 10% in males and to 15% in females); ↓food consumption (M/F); Incidence of focal thyroid C-cell hyperplasia: controls 6/66 (9%) vs high dose 7/62 (11%). No pairwise increase in thyroid C-cell adenomas or carcinomas separately, dose-dependent increase in the combined adenomas/carcinomas (males only). Considered not relevant for humans (no statistically significant increase, tumours only in one sex and no clear dose-response for benign (adenomas) and malignant (carcinomas) tumors). **NOAEL= 20.3- 26.5 mg/kg bw/d (400 ppm)** *LOAEL*= 41.93- 53.20 mg/kg bw/d (800 ppm)	At 800 ppm (41.93- 53.20 mg/kg bw/d): - body weight evolution of the treated animals was reduced up to 10% in males and to 15% in females. Mean body weights for high dose females were statistically lower than the control values begining at week 1 and continuing throughout the study. For high dose males, statistically lower mean body weights were noted for weeks 1 to 37 and weeks 85 and 89. - Food consumption was occasionally decreased. - Transient slightly lower total white blood cells counts with lower absolute lymphocytc counts in males. Bodyweight, particularly of females, may be relevant to the ability to reproduce and to the survival of pups. Since a significant reduction in females bodyweights up to 15% was reported, it is concluded that LOAEL effects are of ecological relevance. Results are in	EPA OPP 83-5, OECD 453/Yes	[redacted] 1998 (KCA 5.5/01)	*Key study*

Test species	Exposure System	Dosing	Results	Ecological relevance of LOAEL effects	Guideline/ GLP	Reference	Relevance
				agreement with the findings of [redacted] (1996).			
Mice	Oral, Chronic and carcinogenicity toxicity (78- week) Feeding (diet) 60+10/sex/group	0, 200, 750, 1500 ppm corresponding to M: 0; 29; 110; 225 F: 0; 36; 136; 277 mg/kg bw/d	1500 ppm: ↓ bw, body weight gain and food consumption (M/F) ; Females: Incidences of combined hepatocellular adenomas and carcinomas slightly increased at 1500 ppm, but neither was statistically significant considered alone. Males: no statistical increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, or combined adenoma and carcinoma was detected. 750 ppm: ↓ bw and food consumption (F) 750 ppm is considered as the LOAEL based on the possible increased incidence of beningn hepatocellular neoplasia at 1500 ppm. **NOAEL= 29- 38 mg/kg bw/d (200 ppm)** *LOAEL*= 110- 136 mg/kg bw/d (750 ppm)	At 750 ppm (110- 136 mg/kg bw/d): - Bodyweights for both sexes tended to be lower than controls, and statistically significant differences were noted frequently for both sexes, but were consistently observed in the females, and intermittently observed for the males. - The overall mean body weight gain for females fed the 750 ppm concentration was significantly reduced compared with that of the controls (20.1% lower) LOAEL effects are of ecological relevance. Results are in agreement with the findings of [redacted] (1996).	EPA OPP 83-2/Yes	[redacted] 1998 (KCA 5.5.4 and KCA 5.5/05)	*Key study*
Neurotoxicity							
Dodine is not similar or related to active substances capable of inducing neurotoxicity, or active substances which induce specific indications of potential neurotoxicity, neurological signs or neuropathological lesions. In addition, no indications of neurotoxicity have been seen in the repeated dose studies.							

* *According to the draft GD on B&M that is not applicable to the renewal of dodine, it is considered that the use of the BMDL10 for bodyweight endpoints is ecologically relevant.*

2.9.1.3 Summary of effects on other terrestrial vertebrates

To address potential endocrine disrupting properties of dodine, an Amphibian Metamorphosis Assay according to OECD 231 was conducted. Lethal and sublethal effects as well as effects on the normal function of the hypothalamic-pituitary-thyroid (HPT) axis on tadpoles of *Xenopus laevis*, caused by the test item Dodine technical, were investigated. The tadpoles were exposed in a flow-through test during a period of 21 days to the nominal concentrations of 2.0, 10.0 and 50.0 µg/L (mean measured: 2.4, 5.8 and 29 µg/L), and to a control group consisting of aqueous test media and a solvent control group, containing the solvent dimethyl sulfoxide (DMSO). The study fulfilled the performance criteria reported in the OECD 231 guideline, except with the related to the variability of measured test concentrations over time, as CV was slightly above the limit. Considering the observed behaviour of the test item in test medium, the outcome was considered acceptable. No significant differences on the development stage were observed. A reduction of wet weight (22%) and of the SVL (11%) were observed at the middle test concentration (5.8 µg/L) at day 7, and a slight increase of both parameters at the lowest concentration at the end of the assay were found (2.4 µg/L). These effects on wet weight and SVL were considered no dose-related. The normalized Hind Limb Length (by SVL) of larvae was significantly reduced at day 7 at the lowest concentration, and in all treatments at the end of the study for larvae at NF $\leq$ 60 (reduction 10-14%), but not for tadpoles above stage NF 60. However, as no acceleration of HLL development was found, the effects observed on this parameter were not considered thyroid-related. Normal morphological development of tadpoles was reported, then, no asynchronous development was identified. Histopathological results could not be assessed by RMS (images not available, **DATA GAP**). In addition, RMS has **concerns** about the results relevance, as the selected doses did not cover the MTC (as recommended in the ED guidance and OECD 231) and could be too low to elicit any possible ED mediated effect. The results of the study should be used in the risk assessment with caution, as it only showed the ED effects up to the highest concentration tested.

2.9.1.3.1 Higher-tier effect data on mammals

In addition to the Tier 1 studies submitted to address the core data requirements for the active substance, higher-tier effect studies conducted with formulated Dodine are also presented in Volume 3 CP Point 9.1.2.3 (2009; 2018). They are used in a weight of evidence consideration by the applicant, However, **due to the lack of statistical information regarding the statistical power of the the study or MDDs in order to demostrate that the test is able to detect effects on acute or reproductive parameters, RMS has concluded that the results of higher-tier effect studies (2009; 2018) should not be used in the risk assessment of mammals pending on the submission of a re-evaluation of the statistical power of the field studies (data gap).**

2009), which was already submitted for the first Annex I inclusion of Dodine, investigated the potential long-term effects of Dodine formulated as Syllit 400 SC on free ranging, naturally occurred small herbivorous mammals (i.e., common vole). The field study was conducted in grassland as surrogate for orchards in Germany to show that the use of the active substance has no negative acute and long-term impact on common vole populations. In addition, a new study (2018) was conducted in grassland in Germany to investigate potential long-term effects of formulated Dodine as Syllit 544 SC on voles inhabiting semi-field enclosures. The later study serves as an addition to the previously conducted field study. Both studies were conducted in grassland as this type of habitat is comparable to ground vegetation in orchards.

In the first study by (2009), three study plots were treated with Syllit 400 SC and three study plots were used as control fields, where no application took place. Four applications of Syllit 400 SC were performed on each of the three treatment plots. The treatment was performed in two blocks of two applications in a 6–7-day interval, i.e., 1st application: 9 July 2008, 2nd application: 16 July 2008, 3rd application: 20-21 August 2008, 4th application: 27 August 2008. The application rate used in treatment block 1 ranged from 853.98 to 953.64 g a.s./ha. A reduced application rate ranged from 452.11 to 475.41 g a.s./ha (simulating 50% interception in orchards) was used in the second block. “Capture-Mark-Recapture” methodology was used to monitor common vole populations. Live trapping was carried out for approximately 15 weeks. One treatment and one control plot were set up in pairs on the same grassland field with a minimum distance of 100 m. Ugglan multiple-capture traps were used to live-trap voles, which were marked with passive integrated transponders and released at the site of capture. Following the initial trapping session (29 June 2008), the first treatment block took place followed by four weekly trapping sessions before treatment block 2. Six further weekly trapping sessions were carried out after the last Syllit 400 SC application. The numbers of voles captured and/or recaptured during each of the trapping sessions were used to calculate Minimum Numbers Alive (MNA). MNA values were then used to compare vole population dynamics between voles exposed to the test item on the treatment plots and those on the control plots. The persistence of voles in the study plots and information on age structure could also be derived from the live trapping data.

Abundances of common voles were generally low on all plots at begin of the study, increased during the study and then decreasing towards the end of the study on all plots The stepwise comparison of populations on treatment and control plots for each trapping session after the first treatment block showed no significant difference in population development of common voles between control and treatment plots. The common vole populations on all treatment and control plots showed analogous age structures during the study period. There was general trend of decreasing proportions of adult common voles and increasing proportions of subadults towards the later trapping sessions on all plots. The population persistence in the study plots varied greatly among the individuals on all plots. However, there was no significant difference between the mean numbers of persisting days in neither male nor female common voles between control and treatment plots. A common feature of all populations was that the cohort of individuals captured only once made up more than 50% of all marked common voles. Overall, there was no long-term effect of Syllit 400 SC applications on common vole populations in grassland detected. No statistically significant differences were found in population development and persistence of common voles known to have been exposed to the test item.

In the second study by [redacted] (2018), semi-field enclosures covered with meadow-like vegetation were used compared to the open system field effect study by [redacted] (2009). A controlled number of individuals were used as founder population, which were exposed to the treatment, and the following reproductive activity and population development was monitored on individual level. No migration or predation occurred on the study plots during the trapping period. Voles were placed in the enclosures in early spring 2017. Five enclosures were treated with Syllit 544 SC and five untreated enclosures served as controls. The first application of the test item was conducted on 20 April 2017 at a nominal application rate of 900 g a.s./ha, shortly after the release of the founding common vole populations per enclosure. The second application was carried out seven days after the first application (27 April 2017) and the third application was conducted 21 days after the first application (11 May 2017). This study design ensured that all voles in the treatment enclosures were exposed via diet (and other routes) to the test item. Live trapping was conducted using the Capture-Mark-Recapture (CMR) design with 'Ugglan' multiple capture live traps, allowing identification of individually marked animals upon recapture. The live trapping campaign was carried out between 24 April 2017 and 12 June 2017 to assess population dynamics of common voles in the treated enclosures compared to the control enclosures. Focus was on investigating development of vole densities (i.e., abundance values), Minimum Number Alive (MNA), recapture rate, longevity and survival rates of individual marked voles based on equal founder population in treated and untreated enclosures as well as further parameters such as body weight, reproductive status, sex and age.

The results of trapping success were in the same range for control and treatment. The Minimum Number Alive (MNA) increased over time for control and treatment with approximately the same slope and were slightly higher in the treatment enclosures. The MNAs in the single trapping sessions of the enclosures were similar. The proportion of reproductive active individuals fluctuated in both control and treatment, and again in the same range. The number of juveniles, the reproductive success of the founder females and the longevity of the founders was nearly identical. Exposure to the voles in treatment enclosures was verified (all diet treated). Overall, the comparison of various population parameters derived from live trapping data and related to reproduction revealed no negative effects on common voles that could be attributed to the test item. Therefore, it can be concluded that within this study no acute or long-term effects of the fungicide Dodine applied as Syllit 544 SC on common voles were detected. It is noted that the study by [redacted] (2018) serves as an addition to the previously conducted field effect study in which free-ranging voles were exposed to the same test item later in the season (i.e., early summer).

2.9.2 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

The effects of Dodine (either technical or formulated) to aquatic organisms have been investigated in various Tier 1 and higher tier studies conducted to provide reliable endpoints regarding the acute and long-term/chronic toxicity to fish and aquatic invertebrates, the potential growth inhibition of green algae and aquatic macrophytes, the long-term toxicity to sediment dwellers following water borne exposure and the effects on freshwater ecosystems under field conditions. Most of the available studies were already submitted for the first EU evaluation for the Annex I inclusion of the active substance. New studies submitted for the purpose of the renewal of approval of the active substance include two studies on the acute toxicity of Dodine technical to fish (*Cyprinus carpio*) and additional aquatic invertebrates (*Mysidopsis bahia*), respectively, two studies on the acute toxicity of Dodine 400 SC and Dodine 544 SC on aquatic invertebrates (*Daphnia magna*), three studies assessing the effects of Dodine technical, Dodine 400 SC and Dodine 544 SC on algal growth inhibition, a study on the growth inhibition of Dodine technical to aquatic macrophytes (*Lemna gibba*) and an outdoor mesocosm study in artificial freshwater ponds conducted with the current representative product, i.e., Dodine 544 SC. In addition to the later mesocosm study, a further mesocosm, already EU peer reviewed study is available which has statistically been re-evaluated for the purpose of the renewal of the approval of Dodine. Considering the available, complete data set, the assessment of potential effects and risks to aquatic organisms is based on the RAC values presented below.

Table 2.9.2-1 Acute and long-term RAC values used in the aquatic risk assessment

Taxonomic group/exposure regime	Tier I RAC [µg a.s./L]	Higher-tier RAC [µg a.s./L]
Fish/acute	3.12	Geomean RAC = 12.55
Fish/long-term	20	-
Aquatic invertebrates/acute	0.18	ETO-RAC = 0.4 ERO-RAC = 2.5[1]
Aquatic invertebrates/long-term	0.44	
Algae	0.55	
Aquatic macrophytes	6.3	
Sediment dwellers/long-term	88.3	-

[1] derived from the mesocosm study of [redacted] (2021b) by applying an assessment factor of 3 to the Effect class 3A NOEAEC = 7.5 µg a.s./L

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

Table 69: Summary of relevant information on bioaccumulation

Method	Species	Results	Key or Supportive study	Remarks	Reference
Partition co-efficient n-octanol/water EEC A8, OECD 107 (Shake-flask method)	-	log P_{ow} value for Dodine = 0.96 (at pH range 4.6 – 9.3)	The study is considered acceptable	Dodine purity 99.2% w/w	[redacted] (1999c)
Partition co-efficient n-octanol/water Estimated by calculation	-	Based on Dodine solubilities at 20ºC in water an in n-octanol_ log Pow: 1.28. (pH: 4.9) log Pow: 1.25. (pH: 6.9) log Pow: 1.32. (pH: 9.1)	The study is considered acceptable	Estimated by calculation	[redacted] 2006

2.9.2.1.1 Estimated bioaccumulation

No relevant, see 2.9.2.1.

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

The log P_{ow} value for Dodine is between 1.25 – 1.32 at 20ºC, at pH range 4.9 – 9.1

The pH value has no impact on the octanol/water partition for Dodine. In line with Annex I, Section 4.1.2.8.1 of the CLP Regulation, these log P_{ow} values are less than the CLP cut-off criteria of 4, indicating Dodine does not show potential to bioaccumulate.

2.9.2.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

The risk assessment for aquatic organisms was carried out according to the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA panel on plant protection products and their residues (PPR). European Food Safety Authority (EFSA), Parma, Italy. EFSA Journal 2013;11(7):3290. The relevant information of acute aquatic toxicity is summarized below (Table 2.9.2.2-1).
Following the recommendations of the EFSA 2015 and 2019 (supporting publication 2015:EN-924. 62 pp. and EFSA Supporting publication 2019:EN-1673) on how to express the endpoints from tier 1 studies, the endpoints from those static/semi-static tests where the measured concentrations of the test substance were not satisfactorily maintained within ± 20% of the nominal throughout the test, were re-calculated based on the geometric mean measured concentrations. For flow-through studies only, the arithmetic mean were used to calculate the mean measured concentration when test concentrations were not satisfactory maintained.

Table 2.9.2.2-1 Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
Fish						
Acute toxicity to fish OECD 203	*Oncorhynchus mykiss*	Dodine (95.3% w/w)	LC_{50} = 1.37 mg a.s./L (mm)	Key study. The study is considered acceptable.	96-hour (semi-static)	[redacted] et al. (1990) DAR (2009) Please refer to KCA 8.2.1/03
Acute toxicity to fish, Guideline EPA OPP 72-1	*Lepomis macrochirus*	Dodine (95.3 % w/w)	LC_{50} = 0.7 mg a.s./L (mm)	Key study. The study is considered acceptable.	96-hour (semi-static)	[redacted] et al. (1991) DAR (2009) Please refer to KCA 8.2.1/04
Acute toxicity to fish Guideline EPA FIFRA 72-3	*Cyprinodon variegatus*	Dodine (94.07 % w/w)	LC_{50} = 3.7 mg a.s./L (mm)	Key study. The study is considered acceptable	96-hour (flow-through)	[redacted] (1992) DAR (2009) Please refer to KCA 8.2.1/05
Acute toxicity to fish OECD 203	*Cyprinus carpio*	Dodine (96.1 % w/w)	LC_{50} = 0.598 mg a.s./L (mm)	Key study. The study is considered acceptable	96-hour (semi-static)	[redacted] (2005) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCA 8.2.1/06
Acute toxicity to fish OECD 203	*Cyprinus carpio*	Dodine (96.61 % w/w)	LC_{50} = 0.312 mg a.s./L (mm)		96-hour (flow-through)	[redacted] (2006) Please refer to KCA 8.2.1/02

Acute toxicity to fish OECD 203	*Cyprinus carpio*	Dodine 400 SC	LC_{50} = 3.4 mg f.p./L = 1.36 mg a.s./L (nom.)	The study is considered acceptable	96-hour (semi-static)	[redacted] (2008) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCP 10.2.1/05
Aquatic invertebrates						
Acute toxicity to aquatic invertebrates EPA OPP 72-2 (fulfilled criteria OECD 202)	*Daphnia magna*	Dodine (94.07 % w/w)	EC_{50} = 0.018 mg a.s./L (mm)	Key study. The study is considered acceptable	48-hour (flow-through)	[redacted] (1992) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCA 8.2.4.1/01
Acute toxicity to aquatic invertebrates EPA OPP 72-2 (fulfilled criteria OECD 202)	*Daphnia magna*	Dodine (95.3 % w/w)	EC_{50} = 0.049 mg a.s./L (mm)	Key study. The study is considered acceptable	48-hour (semi-static)	[redacted] [redacted] (1989) DAR (2009) Please refer to KCA 8.2.4.1/02
Acute toxicity to aquatic invertebrates ISO 6341 (fulfilled criteria OECD 202)	*Daphnia magna*	Dodine (98.5 % w/w)	EC_{50} = 0.089 mg a.s./L (mm)	Key study. The study is considered acceptable	48-hour (static + sediment)	[redacted] (2002) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCA 8.2.4.1/03
Acute toxicity to aquatic invertebrates EPA OPP 72-3	*Mysidopsis bahia*	Dodine (94.07 % w/w)	LC_{50} = 0.39 mg a.s./L (mm)	Key study. The study is considered acceptable	96-hour (flow-through)	[redacted] (1992) DAR (2009) Please refer to KCA 8.2.4.2/01
Acute toxicity to aquatic invertebrates EPA OPP 72-3	*Crassostrea virginica*	Dodine (94.07 % w/w)	EC_{50} = 0.098 mg a.s./L (mm)	Key study. The study is considered acceptable	96-hour (flow-through)	[redacted] (1992) DAR (2009) Please refer to KCA 8.2.4.2/02
Acute toxicity to	*Daphnia magna*	Dodine 400 SC			48-hour (semi-static)	[redacted] (2004)

aquatic invertebrates OECD 202			EC_{50} = 0.123 mg f.p./L = 0.049 mg a.s./L (mm)	The study is considered acceptable		DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCP 10.2.1/06
Acute toxicity to aquatic invertebrates OECD 202	*Daphnia magna*	Dodine 400 SC	EC_{50} = 0.0738 mg f.p./L = 0.0289 mg a.s./L (mm)	EC_{50} = 73.8 µg f.p./L = 28.9 µg a.s./L (mm) The study is considered acceptable	48-hour (static)	[redacted] (2013a) Please refer to KCP 10.2.1/01
Acute toxicity to aquatic invertebrates OECD 202	*Daphnia magna*	Dodine 544 SC	EC_{50} = 0.0458 mg f.p./L = 0.0253 mg a.s./L (mm)	The study is considered acceptable	48-hour (static)	[redacted] (2013b) Please refer to KCP 10.2.1/02
algae						
Acute toxicity to algae OECD 201	*Selenastrum capricornutum*	Dodine (98.4 % w/w)	E_yC_{50} = 0.0016 mg a.s./L E_rC_{50} = 0.0055 mg a.s./L (mm)	Key study. The study is considered acceptable.	72-hour (static)	[redacted] (2020) Please refer to KCA 8.2.6.1/01
Acute toxicity to algae OECD 201	*Selenastrum capricornutum*	Dodine 400 SC	EbC50 = 0.0142 mg f.p./L = 0.00569 mg a.s./L ErC50 = 0.0275 mg f.p./L = 0.011 mg a.s./L (mm))[e]	= The study is considered acceptable	72-hour (static)	[redacted] (2004b) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCP 10.2.1/07
Acute toxicity to algae OECD 201	*Desmodesmus subspicatus*	Dodine 400 SC	E_bC_{50} = 0.00457 mg f.p./L = 0.00179 mg a.s./L E_rC_{50} = 0.03318 mg	The study is considered acceptable	72-hour (static)	[redacted] (2013c) Please refer to KCP 10.2.1/03

			f.p./L = 0.01299 mg a.s./L (mm)			

2.9.2.2.1 Acute (short-term) toxicity to fish

Five studies with dodine and one with the formulated product Dodine 400 SC were performed to assess their acute toxicity to fish. These studies (technical and formulated) were already submitted for the first EU evaluation for the Annex I inclusion of the active substance. Nevertheless, a new study assessing the acute toxicity of Dodine technical to *Cyprinus carpio* under flow-through conditions ([redacted] 2006; KCA 8.2.1/02) is available. This study provides the lowest LC_{50} (and thus RAC) for acute toxicity of Dodine to fish, i.e., 312 µg a.s./L. All of these studies have been evaluated for the current application for the renewal and are considered acceptable by RMS. The relevant information of acute aquatic toxicity for fish is summarized below (Table 2.9.2.2.1-1).

No study was performed with the formulation Dodine 544 SC since it is possible to extrapolate data from the available study performed with the similar formulation Dodine 400 SC. The formulations Dodine 400 SC and Dodine 544 SC are fungicides based on the same active substance. The contents of the active substance are rather similar, i.e., Dodine 400 SC contains 41.49% (w/w) while Dodine 544 SC contains 55.66% (w/w) Dodine. In addition to the active substance, the two formulations share comparable compositions with regard to the type of co-formulants and their contents. Any compositional differences are not assumed to enhance the ecotoxicity profile of Dodine 544 SC compared to Dodine 400 C. Therefore, it is possible to use toxicity studies performed with Dodine 400 SC to extrapolate toxicity values to Dodine 544 SC when expressed as active substance equivalents. Please refer to Confidential Information for further details.

In addition, bridging studies under similar conditions with both Dodine 400 SC and Dodine 544 SC with aquatic non-vertebrate species (i.e., *Daphnia magna* and *Desmodesmus subspicatus*) were performed ([redacted] 2013a-d). The results of these studies confirm the equivalence of toxicity expected based on similar composition. Therefore, the formulation Dodine 400 SC could be considered as a surrogate for assessing the toxicity of Dodine 544 SC for aquatic organisms.

It is further noted that according to Regulation (EU) No 284/2013 and EFSA (2013)[6], in principle, acute exposure tests should be carried out on one species from each of the taxonomic groups fish, aquatic invertebrates, algae and/or macrophytes (in case the active substance is an herbicide or exhibits herbicidal activity); however, where the available information for the active substance permits the conclusion that one of these groups is clearly more sensitive (factor of 10 difference), only a test using a species of the relevant group needs to be performed with the formulated product. Based on the acute toxicity of Tier 1 taxonomic groups (i.e., 96-h LC_{50} 312 µg/L for fish, 48-h EC_{50} 18 µg/L for aquatic invertebrates, 72-h E_rC_{50} 5.5 µg/L for algae and 7-d E_rC_{50} 63 µg/L for aquatic macrophytes), the difference in sensitivity between fish and the most sensitive species, i.e., aquatic invertebrates and algae, is more than a factor of 10, i.e., 17 and 57, respectively. Thus, fish acute toxicity testing with the formulation is not necessary.

Considering that several studies on the acute toxicity to fish are available assessing the same effect parameter (i.e., mortality) within the same exposure duration (i.e., 96 hours) by following identical testing guidelines, it is proposed to calculate the geomean LC_{50} of all available endpoints for further use in the risk assessment. Since the endpoint derived from the available formulation study with Dodine 400 SC is within the range of calculated endpoints from the studies with Dodine technical, the formulation endpoint expressed as active substance equivalents is included in geomean LC_{50} calculation. It is noted that by excluding the formulation study the geomean LC_{50} will be slightly affected, i.e., it will be decreased from 1255 to 1141 µg a.s./L. In any case the geomean RAC (12.55 or 11.41 µg a.s./L) for acute toxicity to fish would be lower than the lowest LC_{50} for the most sensitive species, i.e., 312 µg/L, thus the geomean approach is applicable according to EFSA (2013)[1].

Table 2.9.2.2.1-1. Summary of acute toxicity of dodine to fish and derivation of RAC for the risk assessment

[6] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290.

Endpoint	Test substance	Toxicity	Value selected	Geomean
Oncorhynchus mykiss – 96-h LC_{50}	Dodine	1370 µg a.s./L	1509 µg a.s./L[a]	1255 µg a.s./L[b]
Lepomis macrochirus – 96-h LC_{50}	Dodine	702 µg a.s./L	702 µg a.s./L	
Cyprinodon variegatus – 96-h LC_{50}	Dodine	3700 µg a.s./L	3700 µg a.s./L[a]	
Cyprinus carpio – 96-h LC_{50}	Dodine	598 µg a.s./L[a]	Geomean: 633 µg a.s./L	
Cyprinus carpio – 96-h LC_{50}	Dodine	312 µg a.s./L		
Cyprinus carpio – 96-h LC_{50}	Dodine 400 SC	1360 µg a.s./L		

a Pending on the submission of the statistical robustness by the applicant.
b Geometric mean calculation is based on the results from all available acute toxicity studies with fish including the formulation study. Preliminary endpoint, pending on the submission of the statistical robustness of some LC_{50} by the applicant.

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

Five studies with dodine and three with the formulated products Dodine 400 SC and Dodine 544 SC were performed to assess their toxicity on aquatic invertebrates (i.e., *Daphnia magna, Mysidopsis bahia, Crassostrea virginica*). They were already submitted for the first EU evaluation for the Annex I inclusion of the active substance, except two new studies with formulated Dodine as Dodine 400 SC (48-h EC_{50} = 28.9 µg a.s./L) and Dodine 544 SC (48-h EC_{50} = 25.3 µg a.s./L). The new studies were conducted for bridging purposes.

All of these studies have been evaluated for the current application for the renewal and are considered acceptable by RMS. The relevant information of acute aquatic toxicity to aquatic invertebrates is summarized below (Table 2.9.2.2.2-1).

The new studies not only confirm the similar toxicity of the two formulations to aquatic organisms but also indicate that the toxicity of technical Dodine (48-h EC_{50} = 18 µg a.s./L) is not significantly enhanced when formulated to either product. The worst-case EC_{50} of 18 µg a.s./L is used for Tier 1 RAC setting while any unresolved acute risk to aquatic invertebrates at Tier 1 level is addressed on the basis of the results of the available mesocosm studies.

Table 2.9.2.2.2-1. Summary of acute toxicity of dodine to aquatic invertebrates and derivation of RAC for the risk assessment

Endpoint	Test substance	Toxicity	Value selected (RAC)
Daphnia magna	Dodine	EC_{50} = 18 µg a.s./L (mm)	EC_{50} = 18 µg a.s./L
Daphnia magna	Dodine	-[a]	
Daphnia magna	Dodine	EC_{50} = 89 µg a.s./L (mm)[b]	
Mysidopsis bahia	Dodine	LC_{50} = 390 µg a.s./L (mm)	
Crassostrea virginica	Dodine	EC_{50} >98 µg a.s./L (mm)	
Daphnia magna	Dodine 400 SC	EC_{50} = 123 µg f.p./L = 49 µg a.s./L (mm)	
Daphnia magna	Dodine 400 SC	EC_{50} = 73.8 µg f.p./L = 28.9 µg a.s./L (mm)	
Daphnia magna	Dodine 544 SC	EC_{50} = 45.8 µg f.p./L = 25.3 µg a.s./L (mm)	

a new calculations should be submitted by the applicant following the recommendations of OECD 23 (2019)
b In the previous LoEP, the numerical value of the LC_{50} endpoint expressed as nominal concentrations is reported although it is stated that it is expressed based on mean measured concentrations. As the measured concentration by end of the study decreased by approx. 60% of the initial concentration, the endpoint should be expressed on the basis of the mean measured concentration, i.e., 89 µg/L.

2.9.2.2.3 Acute (short-term) toxicity to algae or aquatic plants

Studies investigating the toxicity of Dodine (technical and formulated) to green algae (i.e., *Selenastrum capricornutum*) were already submitted for the first EU evaluation for the Annex I inclusion of the active substance. Nevertheless, study re-evaluation indicated that the DAR studies conducted with Dodine technical ([redacted] 1993; KCA 8.2.6.1/03) does not meet the validity criteria of the recent OECD testing guideline 201 (2011). In order to address the core data requirement on toxicity to green algae, a new study with *Raphidocelis subcapitata* (*Selenastrum capricornutum)* was conducted with Dodine technical ([redacted] 2020; KCA 8.2.6.1/01) generating the critical endpoint for further use in the aquatic risk assessment (i.e., E_rC_{50} = 5.5 µg a.s./L).

Although the DAR study conducted with Dodine formulated as Dodine 400 SC meets the validity criteria of OECD 201 (2011), two new studies investigating the toxicity of Dodine 400 SC (72-h E_rC_{50} = 12.99 µg a.s./L) and Dodine 544 SC (72-h E_rC_{50} = 10.78 µg a.s./L) to *Desmodesmus subspicatus* have been conducted for bridging purposes. The new studies not only confirm the similar toxicity of the two formulations to aquatic organisms but also indicate that the toxicity of technical Dodine to green algae (72-h E_rC_{50} = 5.5 µg a.s./L) is not significantly enhanced when formulated to either product. It is noted that a new statistical analysis of the biological data obtained in the DAR study with Dodine 400 SC was conducted to address the current data requirements according to Commission Regulation (EU) No 283/2013 with respect to $EC_{10/20}$ endpoints.

As for aquatic invertebrates, any potential Tier 1 risk to algae is addressed at higher-tier level on the basis of the results of the available mesocosm studies.

Table 2.9.2.2.3-1. Summary of acute toxicity of dodine to algae and derivation of RAC for the risk assessment

Endpoint	Test substance	Toxicity	Value selected (RAC)
Selenastrum capricornutum	Dodine	E_yC_{50} = 1.6 µg a.s./L E_rC_{50} = 5.5 µg a.s./L (mm)	E_rC_{50} = 5.5 µg a.s./L
Selenastrum capricornutum	Dodine 400 SC	E_bC_{50} = 14.23 µg f.p./L = 5.69 µg a.s./L E_rC_{50} = 27.48 µg f.p./L = 10.99 µg a.s./L (mm) [a]	
Desmodesmus subspicatus	Dodine 400 SC	E_bC_{50} = 4.57 µg f.p./L = 1.79 µg a.s./L E_rC_{50} = 33.18 µg f.p./L = 12.99 µg a.s./L (mm)	
Desmodesmus subspicatus	Dodine 544 SC	E_bC_{50} = 5.70 µg f.p./L = 3.14 µg a.s./L E_rC_{50} = 19.56 µg f.p./L = 10.78 µg a.s./L (mm)	

a A statistical re-analysis of study results has been performed to address the Regulations 283/2013 and 284/2013 requirements regarding $EC_{10}/EC_{20}/EC_{50}$ (together with NOEC) endpoints derivation. The reported endpoints in the table above have been derived from the new statistical analysis.

2.9.2.2.4 Acute (short-term) toxicity to other aquatic organisms

No studies available.

2.9.2.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

The relevant information of chronic aquatic toxicity is summarized below (Table 2.9.2.3-1).

Table 2.9.2.3-1: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Relevant study	Remarks	Reference
fish						
Chronic toxicity to fish. EPA OPP 72-4 (fulfilled criteria OECD 210)	*Pimephales promelas*	Dodine (98.6 % w/w)	NOEC = ~~170 µg~~ 0.170 mg a.s./L EC_{10} > ~~170 µg~~ 0.170 mg a.s./L (nm)	Key study. The study is considered acceptable	30 d (flow-through)	[redacted] 1995) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCA 8.2.2.1/01
Aquatic invertebrates						
Chronic toxicity to aquatic invertebrates	*Daphnia magna*	Dodine (98.6 % w/w)	NOEC = 0.0044mg a.s./L EC_{10} = 0.007	Key study. The study is considered acceptable	21 d (flow-through)	[redacted] (1995) DAR (2009), EFSA Journal 2010; 8(6):1631

EPA OPP 72-4 (fulfilled criteria OECD 211)			mg a.s./L (mm)			Please refer to KCA 8.2.5.1/01
Chronic toxicity to aquatic invertebrates EPA OPPTS 850.1350	*Mysidopsis bahia*	Dodine (95.6 % w/w)	-	not acceptable	28 d (flow-through)	[redacted] (2008) Please refer to KCA 8.2.5.2/01
Chronic toxicity to aquatic invertebrates OECD 219	*Chironomus riparius*	Dodine (96.2 % w/w)	NOEC = 0.883 mg a.s./L (mm)	Key study. The study is considered acceptable	28 d (static, water-spiked)	[redacted] (2002) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCA 8.2.5.3/01
Algae and macrophytes						
Chronic toxicity to algae OECD 201	*Selenastrum capricornutum*	Dodine (98.4% w/w)	~~Acceptable~~ E_rC_{10} = 0.0019 mg a.s./L (mm) NOErC = 0.00015 mg a.s./L	Key study. The study is considered acceptable.	72-hour (static)	[redacted] (2020) Please refer to KCA 8.2.6.1/01
Chronic toxicity to algae OECD 201	*Selenastrum capricornutum*	Dodine (98.4 % w/w)	5-day NOErC = 0.0003 mg as/L 10-day NOErC = 0.0012 mg as/L 15-day NOErC = 0.00015 mg as/L	The study is considered as supplemental information	15-d (static; partial renewal test)	[redacted] (1995) [d] DAR (2009) Please refer to KCA 8.2.6.1/04
Chronic toxicity to aquatic macrophytes. EPA OPPTS 850.4400	*Lemna gibba*	Dodine (95.06 % w/w)	Frond number: ErC_{10} = 0.0167 mg a.s./L	Key study. The study is considered acceptable	7-d (static)	[redacted] (2008) Please refer to KCA 8.2.7/01

(fulfilled criteria OECD 221)			NOErC = 0.0046 mg a.s./L			
Mesocosms						
SETAC, 1999; Giddings *et al,* 2002; EFSA PPR 2013; Brock et al., 2015	Dodine 400 SC	Outdoor mesososm study in artificial freshwater ponds Range of treatments (actual applied levels): 3, 6, 16, 41, 109 µg a.s./L; 2 applications with 5 days interval; observation of effects for 77 days (=11 weeks) after the second test item application Effect class 1 NOEC = 3 µg a.s./L Effect class 2 NOEC = 6 µg a.s./L Effect class 3A NOEC = 16 µg a.s./L				[redacted] (2007) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCP 10.2.3/04 Re-evaluation (MDD & effect classification) by [redacted] (2021a) Please refer to KCP 10.2.3/01
SETAC, 1999; Giddings *et al,* 2002; EFSA PPR 2013; Brock et al., 2015	Dodine 544 SC	Outdoor mesososm study in artificial freshwater ponds Range of treatments (nominal levels): 0.8, 1.6, 3.0, 7.5, 25 µg a.s./L; 2 applications with 7 days interval; observation of effects for 63 days (= 8 weeks) after the second test item application Effect class 2 NOEC = 0.8 µg a.s./L Effect class 3A NOEC = 1.6 µg a.s./L (population-level) Effect class 3A NOEC = 7.5 µg a.s./L(community-level)				[redacted] (2021b) Please refer to KCP 10.2.3/02

* Endpoints re-calculated by RMS to fulfil the current recommendations reported in EFSA opinions 2015 and 2019 (EFSA supporting publication 2015:EN-924. 62 pp. and EFSA Supporting publication 2019:EN-1673).

a In the previous LoEP, a NOEC of 99 µg/L is reported for the long-term toxicity of Dodine to fish. However, effect endpoints from the available study were re-calculated to fulfil the Regulation 283/2013 requirements with regard to $EC_{10}/EC_{20}/EC_{50}$ (together with NOEC) derivation. Regarding fish lengths and weights, the test chamber and not the individual fish, was considered the unit of the new analysis. Further, for these two parameters the results related to the 400 µg a.s./L treatment level were excluded from the new analysis due to the high mortality observed.

b The EC_{10} of 7 µg/L is not reported in the original study report but calculated for the purpose of the renewal of the active substance via statistical re-analysis of the biological data (ToxRat Professional 3.2) to fulfil the Regulation 283/2013 requirements regarding $EC_{10}/EC_{20}/EC_{50}$ endpoints (together with NOEC) derivation.

c In the previous LoEP, a NOEC of 3200 µg/L based on nominal concentration is reported for *Chironomus riparius* as a result of the long-term water-borne exposure to Dodine. However, in the current evaluation the relevant NOEC has been re-calculated on the basis of the geometric mean measured concentration.

d The study is considered of providing only supplemental information with regard to the risk assessment

2.9.2.3.1 Chronic toxicity to fish

An ELS (Early-Life Stage) study to assess the chronic toxicity of Dodine technical to fish was already submitted for the first EU evaluation for the Annex I inclusion of the active substance ([redacted] 1995; KCA 8.2.2.1/01). Nevertheless, a new statistical analysis of the biological data obtained in the study was conducted to address the current data requirements according to Commission Regulation (EU) No 283/2013 with respect to $EC_{10/20}$ endpoints. The new statistical analysis was performed by using the ToxRat Professional 3.2 software and included NOEC, EC_{10}, EC_{20} and EC_{50} estimations. The worst-case NOEC was re-estimated to be 200 µg a.s./L (based on larval survival, length and wet weight) which is higher than the previous EU agreed NOEC of 99 µg a.s./L. The worst-case EC_{10}/EC_{20} endpoints were estimated to be > 200 µg a.s./L (based on larval survival, length and wet weight), therefore the NOEC of 200 µg a.s./L is further used in the risk assessment. Further details on the model assumptions used in the statistical re-analysis are provided in the study summary.

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

A study assessing the chronic toxicity of Dodine technical to *Daphnia magna* was already submitted for the first EU evaluation for the Annex I inclusion of the active substance. Nevertheless, a new statistical analysis of the biological data obtained in the study was conducted to address the current data requirements according to Commission Regulation (EU) No 283/2013 with respect to $EC_{10/20}$ endpoints. The new statistical analysis was performed by using the ToxRat Professional 3.2 software and included NOEC, EC_{10}, EC_{20} and EC_{50} estimations. The worst-case NOEC re-estimated is in agreement with the current EU agreed NOEC of 4.4 µg a.s./L (based on the number of offspring produced per survived and introduced parent). The worst-case EC_{10} estimated, i.e., 7.0 µg a.s./L (based on the number of offspring produced per introduced parent), is higher than the respective NOEC thus it will not be further considered in the risk assessment.

In addition to the EU peer reviewed study on the chronic toxicity of Dodine to *Daphnia magna*, a new study assessing the chronic toxicity of Dodine technical to *Americamycis bahia* (formerly *Mysidopsis bahia*) under flow-through conditions (i.e., [redacted] 2008; KCA 8.2.5.2/01) is available. However, the study does not fulfil the acceptability criteria of the relevant testing guideline [i.e., OPPTS 850.1350 (1996)] and thus it will not be further considered in the effects and risk assessment for aquatic organisms.

As for the acute toxicity, any potential Tier 1 risk of chronic toxicity to aquatic invertebrates is addressed at higher-tier level on the basis of the results of the available mesocosm studies.

A water-sediment study assessing the chronic toxicity of Dodine technical to *Chironomus riparius* was already submitted for the first EU evaluation for the Annex I inclusion of the active substance. Nevertheless, considering the recommendations of EFSA (2019) with regard to the expression of endpoints on the basis of the mass balance calculation, the NOEC for *Chironomus riparius* was re-calculated based on geometric mean concentrations. As no significant effects were determined at either test concentration, EC_{10}, EC_{20} and EC_{50} endpoints are not statistically determined but estimated to be above the highest concentration tested.

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

The DAR study by [redacted] (1995; KCA 8.2.6.1/04) included multiple treatments (achieved with partial renewal of test medium at 5-day intervals with maintenance of initial cell density) to simulate the potential build-up of Dodine concentration followed by a 9-day recovery phase (constituted by a 4-day recovery phase in the aged treated medium and a 5-day recovery phase in fresh untreated medium). The validity criterion of mean coefficient of variation for section-by-section growth (OECD 201, 2011) was not met for the exposure period 0-5 d whereas it is not applicable for the exposure periods 5-10 and 10-15 d; however, considering the test design, study findings can still provide valuable information on the toxicity profile of Dodine to green algae.

No study assessing the toxicity of Dodine (technical or formulated) to aquatic macrophytes was submitted for the first EU evaluation for the Annex I inclusion of the active substance. A new study on the effects of Dodine technical to *Lemna gibba* is available resulting in a worst-case E_rC_{50} of 63.2 µg a.s./L based on dry weight for further use in the risk assessment. As for aquatic invertebrates and algae, any potential Tier 1 risk to aquatic macrophytes is addressed at higher-tier level on the basis of the results of the available mesocosm studies.

2.9.2.3.4 Chronic toxicity to other aquatic organisms

Two outdoor mesocosm studies were performed to investigate the effects of multiple applications of Dodine formulated as Dodine 400 SC ([redacted] 2007; KCP 10.2.3/04) and Dodine 544 SC ([redacted] 2021b; KCP 10.2.3/02), respectively, on freshwater ecosystems (zooplankton, macroinvertebrates, phytoplankton, periphyton and macrophytes excluding vertebrates) as well as to determine the fate of the test substance in the mesocosm systems. The former study by [redacted] (2007; KCP 10.2.3/04) was already submitted for the first EU evaluation for the Annex I inclusion of the active substance and considered to be acceptable for use in the higher-tier risk assessment for aquatic organisms. However, considering the recent recommendations of EFSA (2013)[1] and Brock et al. (2015)[7] on the statistical (i.e., MDD) evaluation and effects classification of micro-/mesocosm studies, the study of [redacted] (2007; KCP 10.2.3/04) was re-evaluated by [redacted] (2021a; KCP 10.2.3/01). The later mesocosm study by [redacted] (2021b; KCP 10.2.3/02) has been conducted for the purpose of renewal of the EU approval of Dodine.

Both studies were conducted in Central Europe, i.e., the Netherlands (Wageningen IMARES location Den Helder) and Germany [Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) in cooperation with the test site MESOCOSM GmbH in Homberg-Ohm], respectively. In the former study by [redacted] (2007; KCP 10.2.3/04), five levels of the test item, i.e., 3, 6, 16, 41 and 109 µg a.s./L (actual), were tested which were applied

[7] Brock TCM; Hammers-Wirtz M, Hommen U, Preuss TG, Ratte HT, Roessink I, Strauss T, Van den Brink PJ. (2015): The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. Environ Sci Pollut Res. 22:1160–1174.

twice in a 5-day interval. In the later study by [redacted] (2021b; KCP 10.2.3/02), five levels of the test item, i.e., 0.8, 1.6, 3.0, 7.5, 25 µg a.s./L (nominal), were tested which were applied twice in a 7-day interval.

A comparison of the key elements between the two mesocosm studies is provided in Table 2.9.2.3.4-1. Both studies were considered acceptable, however, the endpoints selected by RMS for the risk assessment were only derived from the mesocosms study performed by Homment et al., 2021, due to its higher quality. The following aspects were taken into account for this selection:

- *Differences on establishment phase*. The stabilization phase of [redacted] (2007) was considerably smaller resect to that of [redacted] (2021b), it could be considered limited (37 days versus >5 years). Therefore, the freshwater community will be significantly more stable in [redacted] (2021b). The stress of adaptation of some populations to the mesocosms in [redacted] (2007) could camouflage some effect as could see for rotifers (see Vol 3 CP B9.3.3/01 for details). Therefore, sensitivity of [redacted] (2007) study could be compromised, the treatment effect camouflaged, leading uncertainties to the results.
- *Better identification of the most sensitive taxa (flagellates) in [redacted] (2021b)*. In the assessment of [redacted] (2007) reported in the Final addendum to the DAR and Additional Report (2010), it was noted that the effect on phytoplankton was not easy to explain, but flagellates were noted to be the most sensitive. The sensitivity of flagellates (and by extension phytoplankton) was confirmed in [redacted] (2021b), but with this new study it is possible to evaluate the effect on both phytoplankton and flagellates with much more detail, since the taxonomic identification carried out is better (species level versus flagellate grouping by size)
- *Better representativeness of the most sensitive taxa (flagellates) in [redacted] (2021b).* In [redacted] (2021b) study, the abundance of sensitive flagellates in phytoplankton is important (good representativeness), being the dominant algae, which makes it possible to identify effects on the structure of the phytoplankton community (for example, diatom growth (indirect effect)).
- *Absence of EPT (Ephemeroptera, Plecoptera and Trichoptera) in [redacted] (2007), versus presence of E and T (not P) in [redacted] (2021b).* According to the Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EN-924. 62 pp. and EFSA Supporting publication 2019:EN-1673; EFSA 2019), the representation of EPT is important because they tend to be more sensitive and vulnerable due to their cycles reproductive, although the absence does not invalidate a study, especially if it is shown that they are not the most sensitive groups. [redacted] (2021b) mesocosms would show that these taxonomic groups are no more sensitive than plankton (although there are no data for Plecoptera). Nevertheless, it would be an aspect to take into account in terms of the quality of the study.
- *Higher number of replicates in [redacted] (2021b) mesocosms*. In [redacted] (2007), the number of replicates was the minimum necessary according to the EFSA 2013 guidance.

Therefore, the fully established and more complex community of [redacted] (2021b) allowed differentiating toxic effects in more sensitive organisms at lower concentrations and in greater detail than [redacted] (2007) study (better representativeness of most sensitive taxa, better taxonomic identification, higher number of experimental replicates, etc.).

Taking into account these differences between both mesocosms studies and concerns, RMS considered that the use in the risk assessment of endpoints obtained from [redacted] (2021b) was the most conservative approach. In this sense, an assessment factor of 2 was proposed for ETO-RAC derivation. It is noted that the ETO-RAC of 0.4 µg/L (i.e., Effect class 2 NOEC/2 = 0.8/2 µg/L) it is protective for all model ecosystems tested as it is below the Tier 1 NOEC endpoints (lowest NOEC = 4.4 µg/L for *Daphnia magna*).

The ERO-RAC was based on the effect class 3A concentration at the community-level derived from the mesocosm study of [redacted] (2021b) and an assessment factor of 3. It could be considered sufficiently protective taking into account the results obtained in the mesocosm study of [redacted] (2007).

Moreover, RMS noted two aspects that could limit the use of ERO-RAC in the risk assessment:

- In the mesocosms study of [redacted] (2021b), the formulated product Dodine 544 SC was applied in early summer dates (29/05/2019 and 05/06/2019) and the same occurred for mesocosms study of [redacted] (2007). Therefore, **post-harvest applications would not be properly covered by the mesocosms studies.** Considering that the timing of application could influence on recovery of species, **recovery option is considered not acceptable to refine the risk in post-harvest applications.** This aspect would not affect to the use of ETO-RAC, as according EFSA 2019, this enpoint can be considered as independent of the experimental conditions.

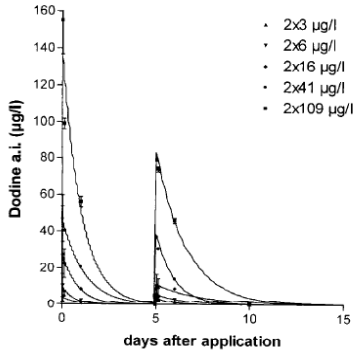
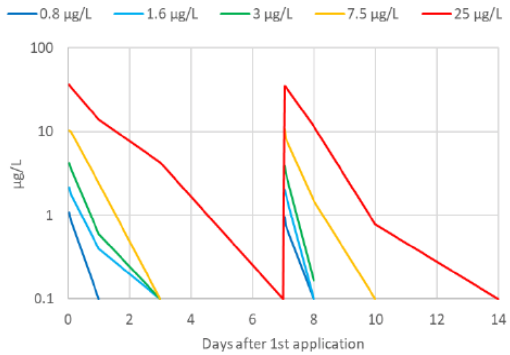
- According to the recommendations of EFSA 2019 (EN-924. 62 pp. and EFSA Supporting publication 2019:EN-1673), when an ERO-RAC is derived, the extrapolation between zones should be considered carefully taking into account the fact that the ability for recovery may vary pending on the agroclimatic conditions. In this case, the mesocosms studies were performed under representative conditions of the Central Zone. Consequently, the suitability of the use of ERO-RAC in the risk assessment or the requeriment of a justification for the extrapolation between EU regulatory zones, should be assessed at Member State-level. This aspect would not affect to the use of ETO-RAC, as according EFSA 2019, this enpoint can be considered as independent of the experimental conditions (e.g. the climatic zone).

Table 2.9.2.3.4-1 Comparison between the two mesocosm studies

	[redacted] 2007 (KCP 10.2.3/04), [redacted] 2021a (KCP 10.2.3/01)	[redacted] 2021b (KCP 10.2.3/02)	Remarks
Test item	Dodine 400 SC	Dodine 544 SC	Bridging studies conducted under similar conditions with Dodine 400 SC and Dodine 544 SC with aquatic non-vertebrate species confirm that the two products are equivalent
Test site	The Netherlands - CEU	Germany – CEU	-
In-life dates	24.05.2007 – 26.07.2007	29.05.2019 – 23.08.2019	-
Application pattern	The test item was applied twice in a 5-day interval; 1st application: 24.05.2007, 2nd application: 29.05.2007	The test item was applied twice in a 7-day interval; 1st application: 29.05.2019, 2nd application: 05.06.2019	Representative GAP: 2 applications in a 21-day interval; Application regime is overlapping in the two studies.
Application method	The application solution was applied evenly on the water surface of the mesocosm by means of a polyethylene watering can with roses/nozzle.	The application solution was introduced directly into the water column (approximately 15 to 25 cm below the water surface) by means of separating funnels (toxicological approach).	-
Test duration	63 days after the first application, i.e., 8 weeks after the second application	84 days (=12 weeks) after the first application, 77 days (=11 weeks) after the second application	EFSA (2013): Test duration should be at least 8 weeks after the first application to monitor recovery
Replicate mesocosms	2/treatment; 3/control	3/treatment; 5/control	EFSA (2013): At least 2 replicates per treatment; more replicates for the controls (often double the amount) than for treatments
No of test concentrations	5 Intended (nominal) application levels: 3, 7, 18, 45 and 110 µg a.s./L Actual applied levels: 3, 6, 16, 41 and 109 µg a.s./L (results are based on actual applied levels)	5 Intended (nominal) application levels: 0.8, 1.6, 3.0, 7.5, 25 µg a.s./L (results are based on nominal concentrations as analysis of the application solutions confirmed the intended concentrations	EFSA (2013): Preferably five or more concentrations
Establishment time	37 days for the enclosures	26 days for the enclosures **(> 5 years** for the artificial pond)	EFSA (2013): Artificially constructed model ecosystems require a pre-treatment period of at least several weeks (plankton-dominated systems) to several months or longer (structurally more complex systems dominated by long-living macro-invertebrates and macrophytes) in order to allow the establishment of a community that has recovered from the "construction stress", adapted to the conditions in the test system and characterised by realistic food web interactions. EFSA (2019): The pre-treatment period should be sufficient to allow the populations and

	[redacted] 2007 (KCP 10.2.3/04), [redacted] 2021a (KCP 10.2.3/01)	[redacted] 2021b (KCP 10.2.3/02)	Remarks
			communities to be well-established in the system before the first treatment. If this period is too limited, it can lead to low abundance of some (sensitive or vulnerable) populations which will make any effects more difficult to detect. The stabilization phase of [redacted] (2007) was considerably smaller resect to that of [redacted] (2021b), it could be considered **limited** (37 days versus >5 years). Therefore, the freshwater community will be significantly more stable in [redacted] (2021b). The stress of adaptation of some populations to the mesocosms in [redacted] (2007) could camouflage some effect as could see for rotifers (see Vol 3 CP B9.3.3/01 for details).
Test units	Circular glass-fibre tanks with a height of 110 cm and an internal diameter between ca. 200 cm (top) and ca. 190 cm (bottom). The surface area at the sediment-water interface was ca. 3 m^2. A ca. 10 cm sediment layer was covered by ca. 90 cm deep, water column.	Stainless-steel enclosures with a diameter of approximately 130 cm (surface approximately 1.3 m^2) and a depth of approximately 150 cm. A sediment layer of about 10 cm was covered by a water body of a depth of about 115 cm ± 20 %.	-
Taxonomic groups tested	Phytoplankton, periphyton, macrophytes, zooplankton, macroinvertebrates. Zooplankton and phytoplankton were introduced into the mesocosms with the natural sediment and water. Macrophytes and macroinvertebrates (gastropods, crustaceans) were introduced. Small or medium direct effects could be assessed for 11 plankton taxa, i.e. flagellates (3 – 10 µm), the green algae *Scenedesmus* sp., unidentified microalgae, and the diatom *Stephanodiscus hantzschii* in the phytoplankton, *Bosmina* sp., *Ceriodaphnia* sp., *Daphnia longispina*, *Simocephalus vetulus*, cyclopoid and calanoid copepods, and *Filinia longiseta* in the zooplankton, as well as for *Asellus aquaticus* in the macroinvertebrate data set and for cumulative emergence of the chironomid *Glyptotendipes pallens* and 'other insects'. Thus, direct small or medium effects could be assessed for 14 taxa. If an assessment of medium to large effects (MDDs < 90 %) is considered acceptable, three additional algae, three rotifer taxa, *Gammarus* sp. and cumulative emergence of *Chaoborus* sp., *Micropsectra* sp.	Phytoplankton, periphyton, macrophytes, zooplankton, macroinvertebrates. All species contained naturally in pond water/sediment. Twenty-two diverse taxa showed MDDs < 70 % during or shortly after the applications which is sufficient to detect medium effects according to EFSA (2013): Cryptophyceae (2 species), Chrysophyceae (2 species), Chlorophyceae (in total), Bacillariophyceae (1 diatom species) Cyanophyceae (chlorophyll a), 2 macrophytes, 4 cladocerans, copepods (Cyclopidae), rotifers (2 species), insects (4 species for 3 orders), and snails (2 families). If also the assessment of direct medium to large effects indicated by MDDs < 90 % directly after applications is considered, several other taxa can be included (6 phytoplankton, 2 zooplankton, and 4 macroinvertebrate taxa).	EFSA (2013): The sensitive taxonomic group for fungicides may comprise a wider array of non-vertebrate taxa. Based on the results from Tier 1 laboratory testing algae and aquatic invertebrates are assumed to be the most sensitive taxonomic groups towards Dodine. Both studies provide data for a sufficient number of potentially sensitive populations. Absence of EPT (Ephemeroptera, Plecoptera and Trichoptera) in [redacted] (2007), versus presence of E and T (not P) in [redacted] (2021b). According to the Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EN-924. 62 pp. and EFSA Supporting publication 2019:EN-1673; EFSA 2019), the representation of EPT is important because they tend to be more sensitive and vulnerable due to their cycles reproductive, although the absence does not invalidate a study,

	[redacted] 2007 (KCP 10.2.3/04), [redacted] 2021a (KCP 10.2.3/01)	[redacted] 2021b (KCP 10.2.3/02)	Remarks
	and *Tanytarsus* sp. and *Glyptotendipes pallens* allowed to assess direct effects.		especially if it is shown that they are not the most sensitive groups. [redacted] (2021b) mesocosms would show that these taxonomic groups are no more sensitive than plankton (although there are no data for Plecoptera). Nevertheless, it would be an aspect to take into account in terms of the quality of the studies.
Climatic conditions	Air temperature: 9 – 25ºC Sunshine: 0.0 – 15.2 h/day Precipitation: 0.0 – 28.9 mm/day; no precipitation on application days Water temperature: 14.0 – 21.5ºC; Day 0 (at first application): 18ºC; Day 5 (at second application): 14ºC	Air temperature: 10.4 – 19.1°C Sunshine: 11381 – 19091 minutes Precipitation: May 2019: 98.3 mm, June 2019: 25.9 mm, July 2019: 42.2 mm, August 2019: 60.5 mm; no precipitation on application days Water temperature: 14.7°C – 22.6°C	-
Biological sampling	Zooplankton: on days -17, -10, -3, 4, 11, 18, 25, 33, 39, 46, 53, 60 Phytoplankton biomass (as chlorophyll-a concentration): on days -28, -23, -21, -17, -14, -10, -7, -3, 0 (before first application), 1, 4, 5, 6, 7, 11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46, 49, 53, 56, 60 Phytoplankton species composition: on days -17, -10, -3, 4, 11, 18, 25, 33, 39, 46, 53, 60 Periphyton biomass (as chlorophyll-a concentration): on days -3, 11, 25, 39, 60 Macroinvertebrates: on days -2, 5, 11, 18, 25, 35, 40, 47, 54, 61 by means of litter traps. Emerging insects: on days -3, 5, 11, 18, 25, 35, 39, 46, 53, 60 with floating insect traps Macrophyte biomass: on day 63 by retrieving all plants by hand to calculate dry weight	Zooplankton: on days -8, -2, 2, 5, 9, 13, 19, 26, 33, 40, 47, 54, 61, 68, 75, 82 (samples taken on 19, 33, 47, 61 and 75 were not further analyzed) Phytoplankton: on days -9, -2, 2, 5, 9, 14, 19, 27, 34, 41, 48, 55, 62, 69, 76, 83 Periphyton: on days -2, 13, 26, 40, 54, 68, 82 Macroinvertebrates: on days -12, -1, 6, 16, 29, 42, 56, 71, 85 with macroinvertebrate artificial substrate samplers (MASS), macrophyte samplers and netting. Emerging insects: sampling by means of emergence traps was done but analysis was not conducted since the macroinvertebrate data set was sufficient to assess the effects on insects. Macrophytes: on days -2, 16, 30, 54 and 86 to calculate surface coverage in percent	-
Analytical method validation	Method validated according to the SANCO 825/00 rev. 7 (17/03/2004) guidance document; Criteria of SANCO/3029/99 rev.4 (11/07/00) guidance document are also met.	Method validation according to SANCO/3029/99 rev.4 (11/07/00) guidance document.	-
Exposure profile, dissipation dynamics	The Dodine concentration in the mesocosm water showed a rapid dissipation and just before the second application the concentrations were below 1 µg/L in all treatments except the highest (109 µg/L); here around 5 µg/L Dodine was measured. Five days after the second application (Day 10), the Dodine concentration in the water of all mesocosms was	Dodine dissipated quickly in the water column. After Day 10 only one sample in the enclosures treated with 25 µg a.s./L was above the LOQ of 0.1 µg a.s./L. The DT_{50} based on the mean % of nominal concentration per sampling data calculated by fitting a first order kinetics was about 1 day (1 d after the 1st and 0.56 d after the 2nd application).	

	[redacted] 2007 (KCP 10.2.3/04), [redacted] 2021a (KCP 10.2.3/01)	[redacted] 2021b (KCP 10.2.3/02)	Remarks
	below 1 µg/L again, while on day 28, thus 23 days after the second application, they were all below the limit of detection of 0.1 µg/L. The calculated average dissipation rate (DT_{50}) in the water phase was 0.83 ± 0.39 days (average for both applications for all treatment levels).  **Figure 10.2-1: Measured concentration of Dodine in water samples (average with SD) and calculated dissipation curves.**	 **Figure 10.2-2: Dissipation of Dodine in the enclosure water: Mean measured concentrations per treatment level over time (values below the LOQ of 0.1 µg a.s./L were plotted as 0.1 µg a.s./L).**	
Statistical analysis	The program Community Analysis V4 (CA) was used for NOEC [multiple t-test by Williams (1971, 1972)] and MDD calculations. Calculations of the CA program have been validated by means of example data and of calculations using MS-Excel™ (Microsoft® Corp.). The PRC analysis was performed with CANOCO™ 4.5 (DLO, Wageningen, The Netherlands).	The program Community Analysis V4.3 (CA) was used for NOEC [multiple t-test by Williams (1971, 1972)], MDD and diversity calculations. Calculations of the CA program have been validated by means of example data and of calculations using MS-Excel™ (Microsoft® Corp.) and ToxRat® (Vers. 2.09). The PRC analysis was performed with CANOCO™ 4.5 (DLO, Wageningen, The Netherlands).	Statistical analysis was conducted by [redacted] (2021a, b) for both studies.
Effect classification and MDD evaluation	Update by [redacted] (2021a) in line with EFSA (2013) and Brock et al. (2015).	In line with EFSA (2013) and Brock et al. (2015).	Identical approach used.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 70: Summary of information on acute aquatic toxicity relevant for classification

Method	Species	Test material	Results	Remarks	Reference
Acute toxicity to fish OECD 203	*Cyprinus carpio*	Dodine	96h-LC50 = 0.312 mg a.s./L (mm)	Accepted	[redacted] (2006) Please refer to KCA 8.2.1/02
Acute toxicity to aquatic invertebrates EPA OPP 72-2 (fulfilled criteria OECD 202)	*Daphnia magna*	Dodine	48h-EC50 = 0.018 mg a.s./L (mm)	Accepted	[redacted] (1992) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCA 8.2.4.1/01
Acute toxicity to algae OECD 201	*Selenastrum capricornutum*	Dodine	E_rC_{50} = 0.0055 mg a.s./L (mm)	Accepted	[redacted] (2020) Please refer to KCA 8.2.6.1/01

Full acute set was available for odine as there were acute studies on fish, aquatic invertebrates and algae and aquatic plants, covering the three trophic levels (see Table 70). The acute toxicity (LC_{50} / EC_{50}) values for all three trophic levels are < 1 mg dodine/L, and algae is the most sensitive trophic level with the 72h-ErC_{50} of 0.0055 mg/L.

For classification of a substance in relation to acute aquatic hazard, table 4.1.0 (a) of Annex I of Regulation (EC) No. 1272/2008 should be used. The acute endpoint selected has to be compared with the cut-off value (acute toxicity values ≤ 1 mg/l).

For setting the M factor in relation to aquatic hazard, table 4.1.3 of Annex I of Regulation (EC) No. 1272/2008 should be used. The acute endpoint selected has to be compared with the cut-off values indicated in the mentioned table.

Based on the available data, the lowest acute endpoint is 72h- ErC50 (*Selenastrum capricornutum)* of **0.0055 mg/L**. This endpoint will establish the M factor needed for the CLP environmental classification.

It is concluded that Dodine does fulfil the criteria for classification and it should be classified according to Regulation (EC) No. 1272/2008 as:

Aquatic Acute 1 with M factor of 100.

CLP criteria:
- for EC_{50} acute toxicity values below or equal to 1 mg/l [E_rC_{50} = 0.0055 mg/L ≤ 1 mg/L] and
- for M factor, acute toxicity value in the range 0.001 < L(E)C50 ≤ 0.01 mg/L.

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 71: Summary of information on long-term aquatic toxicity relevant for classification

Method	Species	Test material	Results	Remarks	Reference
Chronic toxicity to fish EPA OPP 72-4 (fulfilled criteria OECD 210)	*Pimephales promelas*	Dodine	NOEC = 0.170 mg/L EC_{10} > 0.170 mg/L	Accepted	[redacted] (1995) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCA 8.2.2.1/01
Chronic toxicity to aquatic invertebrates EPA OPP 72-4 (fulfilled criteria OECD 211)	*Daphnia magna*	Dodine	EC_{10} = 0.007 mg a.s./L (mm)	Accepted	[redacted] (1995) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCA 8.2.5.1/01
Chronic toxicity to algae OECD 2201	*Selenastrum capricornutum*	Dodine	~~Acceptable~~ E_rC_{10} = 0.0019 mg a.s./L (mm) NOErC = 0.00015 mg a.s./L	Accepted	[redacted] (2020) Please refer to KCA 8.2.6.1/01
Chronic toxicity to aquatic macrophytes EPA OPPTS 850.4400 (fulfilled criteria OECD 221)	*Lemna gibba*	Dodine	Frond number: ErC_{10} = 0.0167 mg a.s./L NOErC = 0.0046 mg a.s./L		[redacted] (2008) Please refer to KCA 8.2.7/01

Bioaccumulation

The log Kow values for Dodine are between 1.25 – 1.32 at 20ºC, at pH range 4.9 – 9.1, which are less than the CLP log Kow trigger value of > 4 intended to identify substances with a potential to bioaccumulate under CLP criteria. According to CLP guidance, measured estimates should be used in preference when available to conclude on the bioaccumulation potential of a substance (BCF ≥ 500 indicates bioaccumulation potential). Since, no studies are available to establish measured BCF estimates, log Kow data have been used to conclude on the potential for bioaccumulation of Dodine. log Kow estimates is lower than the CLP trigger value of 4, Dodine is considered to have low potential to bioaccumulate.

Degradation

A ready biodegradability test (OECD test guideline 301B) shows Dodine being not readily biodegradable for purposes of classification as the pass level criteria of ready biodegradation test (70% of DOC removal or 60% of theoretical oxygen demand) within 28 days was not reached.

Dodine is hydrolytically stable at pH values of 4, 7 and 9 at 50ºC in the dark over a period of 5 days, with half live 1 year at 25ºC.

Regarding photodegradation, degradation of Dodine was slowly (82.1% of AR after 30 days), whereas in irradiated natural water Dodine degraded down to 56.9% of AR after 28 days. Dodine was stable in the dark controls of both water types. The half-live values in natural summer sunlight days at 40°N for Dodine was found to be 27 and 104 days for DT_{50} and DT_{90} respectively for natural water.

In an aerobic mineralization study Dodine degraded rapidly and ultimately mineralized to CO2, with DT_{50} values of 2.3 days. In the water-sediment study, the whole system half live in a natural water/sediment study is 0.36 days of Dodine and mineralisation to CO_2 is the major degradation process. Dodine can be considered as rapidly degradable in the aquatic environment from the aerobic mineralization and water/sediment system studies carried out.

Overall, degradation information does provide data to show that Dodine is ultimately degraded to > 70% within 28 days (equivalent to a half-life of less than 16 days) or being transformed to non-classifiable products. Therefore, Dodine is considered to be **Rapidly degradable** according to the CLP criteria.

Toxicity

Long-term aquatic toxicity data regarding technical Dodine are available for fish, aquatic invertebrates including sediment dwelling organisms, algae and other aquatic plants (i.e., there is appropriate data for all three trophic levels that need to be assessed for CLP classification).

For classification of a substance in relation to chronic aquatic hazard, table 4.1.0 (b) of Annex I of Regulation (EC) No. 1272/2008 should be used. The acute endpoint selected has to be compared with the cut-off value setting in the mentioned table above.

For setting the M factor in relation to aquatic hazard, table 4.1.3 of Annex I of Regulation (EC) No. 1272/2008 should be used. The chronic endpoint selected has to be compared with the cut-off values indicated in the mentioned table, taking into account the degradability of the substance.

The lowest ErC_{10} value is the measured **72h-ErC_{10} of 0.0019 mg a.s./L** for algae *(Selenastrum capricornutum)*. This is > 0.001 mg/L but ≤ 0.01 mg/L, and since Dodine is considered to be 'rapidly degradable' as well as not potentially bioaccumulative, it should be classified according to Regulation (EC) No. 1272/2008 as:

Aquatic Chronic 1 with a chronic M-factor of 1.

CLP criteria:

- for EC_{50} chronic toxicity values below or equal to 0.1 mg/l [E_rC_{10}(72h) = 0.0019 mg/L ≤ 0.1 mg/L] and

- for M factor, Dodine is considered rapidly degradable substance and the chronic toxicity value is in the range 0.001 < L(E)C10 ≤ 0.01 mg/L [ErC10 (72h) = 0.0019 mg a.s./L].

2.9.2.5 Conclusion on classification and labelling for environmental hazards

Taking into account all the information and the assessment summarized in the previous sections 2.9.2.4.1 and 2.9.2.4.2, the following classification class and category can be concluded for this active substance dodine, in accordance with Regulation (EC) 1272/2008:

CLP Annex ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification[1]	Reason for no clasification[2]
4.1	Hazardous to the aquatic environment	Aquatic Acute 1 H400 Aquatic Chronic 1 H410	M-factor = 100 M-factor = 1	Aquatic Acute 1 Aquatic Chronic 1	-
5.1	Hazardous to the ozone layer	-	-	-	Data conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Warning

Hazard statements: Very toxic to aquatic life with long lasting effects (H410)

Precautionary statements:

P273: Avoid release to the environment
P391: Collect spillage
P501: Dispose of contents/container in accordance with national hazardous waste regulations

Pictogram: GSH09

The following additional statements are recommended.

- EUH401: To avoid risks to human health and the environment, comply with the instructions for use.

2.9.3 Summary of effects on arthropods

2.9.3.1 Effects on bees

Studies investigating the acute oral and contact toxicity of Dodine (technical and formulated as Dodine 400 SC) to bees were already submitted for the first EU evaluation for the Annex I inclusion of the active substance. Nevertheless, new studies to address the acute toxicity of the current representative formulation, i.e., Dodine 544 SC, to bees as well as the requirements of Regulations (EU) No 283/2013 and 284/2013 regarding data on the chronic oral toxicity to adult honeybees and honeybee larvae have been conducted and submitted for the purpose of the renewal of the approval of the active substance. In addition to the new Tier 1 studies, two higher-tier effect studies (i.e., semi-field tunnel tests) conducted with the formulation Dodine 544 SC were also submitted. Considering the available, complete data set, the acute oral LD_{50} > 55.7 µg a.s./bee, the contact LD_{50} > 100 µg a.s./bee, the LDD_{50} of 1.86 µg a.s./bee/d (relevant for the chronic toxicity to adult bees) and the ED_{10} of 5.7 µg a.s./larva (relevant for the chronic toxicity to honeybee larva) were used in the Tier 1 bee risk assessment.

Table 2.9.3.1-1: Effects of Dodine and the representative formulations on honeybees.

Test substance	Test species	Exposure system	Endpoint	Reference	Endpoint used in the risk assessment
Acute toxicity to bees					
Dodine	*Apis mellifera*	Oral	LD_{50} (48 h) > 200 µg a.s./bee	[redacted] (2004) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCA 8.3.1.1.1/02, 8.3.1.1.2/02	-
		Contact	LD_{50} (48 h) > 100 µg a.s./bee		**LD_{50} (48 h, contact) > 100 µg a.s./bee**
Dodine		Oral	LD_{50} (24 h) > 200 µg a.s./bee	[redacted] (1984) [a] Please refer to KCA 8.3.1.1.1/01, 8.3.1.1.2/01	-
		Contact	LD_{50} (24 h) > 200 µg a.s./bee		
Syllit 400 SC		Oral	LD_{50} (48 h) = 153 µg f.p./bee corresponding to 61.2 µg a.s./bee	[redacted] (2004) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCP 10.3.1.1.1/01, 10.3.1.1.2/01	-
		Contact	LD_{50} (48 h) > 100 µg f.p./bee corresponding to 40.0 µg a.s./bee		

Test substance	Test species	Exposure system	Endpoint	Reference	Endpoint used in the risk assessment
Dodine 544 SC		Oral	LD_{50} (48 h) > 102 µg f.p./bee corresponding to > 55.7 µg a.s./bee	[redacted] (2017a) Please refer to KCP 10.3.1.1.1/02, 10.3.1.1.2/02	**LD_{50} (48 h, oral) > 55.7 µg a.s./bee**
		Contact	LD_{50} (48 h) > 189 **µg f.p./bee corresponding to > 102.4 µg a.s./bee**		-
Chronic toxicity to bees					
Dodine	*Apis mellifera*	Oral	LDD_{50} = 1.86 µg a.s./bee/d NOEDD = 0.40 µg a.s./bee/d	[redacted] (2016) Please refer to KCA 8.3.1.2/01	**LDD_{50} = 1.86 µg a.s./bee/d**
Effects on honeybee development and other honeybee life stages					
Dodine	*Apis mellifera*	Oral	ED_{10} = 5.7 µg a.s./larva	[redacted] (2017) Please refer to KCA 8.3.1.3/01	**ED_{10} = 5.7 µg a.s./larva**
Cage and tunnel tests					
Dodine 544 SC	Semi-field (tunnel) test in Southern Europe (Spain), assessment of effects on honeybee *Apis mellifera* after two foliar applications of Dodine 544 SC with each at 900 g a.s./ha in a 7-day interval (±1 day) on flowering Phacelia (*Phacelia tanacetifolia*) under semi-field conditions; No adverse effects were assessed on honeybees including their brood nor the survival of the honeybee colony.		[redacted] (2020) Please refer to KCP 10.3.1.5/01	No adverse effects at 2 × 0.9 g a.s./ha, 7-day interval (±1 day)
Dodine 544 SC	Semi-field (tunnel) test in Central Europe (Germany), assessment of effects on honeybee *Apis mellifera* after two foliar applications of Dodine 544 SC with each at 900 g a.s./ha in a 7-day interval (±1 day) on flowering Phacelia (*Phacelia tanacetifolia*) under semi-field conditions; Effects on mortality were observed in the Germany trial for the exposure period when the application was done during the bee flight, however for the overall exposure there were not any significant effect between test items I, II and control. Mitigation measures could be proposed to avoid the effects on mortality observed during the exposure period. i.e *to protect bees and other pollinating insects do not use where bees are actively foraging.* Overall, no adverse effects on honeybee brood or honeybee colony survival were observed.		[redacted] (2021) Please refer to KCP 10.3.1.5/02	Effects on mortality were observed in one trial for the exposure period when the application was done during the bee flight, however for the overall exposure there were not any significant effect between test items I, II and control. at 2 × 0.9 g a.s./ha, 7-day interval (±1 day)

2.9.3.2 Effects on non-target arthropods other than bees

Studies assessing the effects to non-target arthropods other than bees were available with Dodine formulated as Dodine 400 SC and Dodine 544 SC. All studies, standard and extended laboratory tests, conducted with the formulation Dodine 400 SC were already submitted for the first EU evaluation for the Annex I inclusion of the active substance. In addition to the already EU peer reviewed studies, two new laboratory tests with the standard species *Aphidius rhopalosiphi* and *Typhlodromus pyri* have been conducted with the current representative formulation, i.e., Dodine 544 SC. The endpoints derived from the later studies, i.e., LR_{50}/ER_{50} > 1530 g a.s./ha for

Aphidius rhopalosiphi and the endpoint derived for the previous study LR_{50} > 900 g a.s./ha for *Typhlodromus pyri*, were retained in the risk assessment for non-target arthropods.

Table 2.9.3.2-1: Effects of Dodine and the representative formulations on non-target arthropods

Test substance	Test species	Exposure system	Endpoint	Reference	Endpoint used in the risk assessment
Laboratory tests – glass plates (2D)					
Dodine 544 SC	*Aphidius rhopalosiphi*	Glass plates	LR_{50}/ER_{50} > 2760 mL f.p./ha corresponding to LR_{50}/ER_{50} > 1530 g a.s./ha	[redacted] (2020a) Please refer to KCP 10.3.2.1/01	**LR_{50}/ER_{50} > 1530 g a.s./ha**
Dodine 544 SC	*Typhlodromus pyri*	Glass plates	LR_{50}/ER_{50} > 3247 mL f.p./ha corresponding to LR_{50}/ER_{50} > 1800 g a.s./ha	[redacted] (2020b) Please refer to KCP 10.3.2.1/02	-
Dodine 400 SC	*Aphidius rhopalosiphi*	Glass plates	LR_{50} > 4.46 kg f.p./ha corresponding to LR_{50} > 1800 g a.s./ha	[redacted] (1997a) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCP 10.3.2.1/03	-
Dodine 400 SC	*Typhlodromus pyri*	Glass plates	2.23 kg f.p./ha < LR_{50} < 4.46 kg f.p./ha ER_{50} < 2.23 kg f.p./ha corresponding to 900 < LR_{50} < 1800 g a.s./ha ER_{50} < 900 g a.s./ha	[redacted] (1997b) DAR (2009) Please refer to KCP 10.3.2.1/04	**LR_{50} > 900 g a.s./ha**
Extended laboratory tests (2D/3D)					
Dodine 400 SC	*Typhlodromus pyri*	Detached bean leaves	LR_{50} = 18.78 L f.p./ha corresponding to **LR_{50} = 7512 g a.s./ha** ER_{50} > 20 L f.p./ha corresponding to **ER_{50} > 8000 g a.s./ha**	[redacted] (2007) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCP 10.3.2.2/02	Based on the outcome of the first-tier risk assessment, no higher-tier risk assessment was necessary. Nevertheless, the Tier 2 has been included for completeness using the worst application pattern with the lowest endpoint for the 50% effects.
Dodine 400 SC	*Coccinella septempunctata*	Detached bean leaves	LR_{50}/ER_{50} > 4.5 L f.p./ha corresponding to LR_{50}/ER_{50} > 1800 g a.s./ha	[redacted] (2001b) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCP 10.3.2.2/03	
Dodine 400 SC	*Orius insidiosus*	Detached bean leaves	LR_{50} > 4.5 L f.p./ha corresponding to	[redacted] (2002)	

Test substance	Test species	Exposure system	Endpoint	Reference	Endpoint used in the risk assessment
			LR_{50} > 1800 g a.s./ha	DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCP 10.3.2.2/04	
Dodine 400 SC	*Chrysoperla carnea*	Detached bean leaves	LR_{50} > 4.5 L f.p./ha corresponding to LR_{50} > 1800 g a.s./ha	[redacted] (2001c) DAR (2009), EFSA Journal 2010; 8(6):1631 Please refer to KCP 10.3.2.2/05	

2.9.4 Summary of effects on non-target soil meso- and macrofauna

2.9.4.1 Effects on earthworms

Studies on the acute toxicity of Dodine technical and the chronic toxicity of Dodine formulated as 400 g/L SC to earthworms (*Eisenia fetida*) were already submitted for the first EU evaluation for the Annex I inclusion of Dodine. No new studies on earthworms have been conducted for the purpose of renewal of the approval of the active substance.

A new statistical analysis of the biological data obtained in the available chronic toxicity study was conducted to address the current data requirements according to Commission Regulations (EU) No 283/2013 and 284/2013 with respect to $EC_{10/20}$ endpoints. The new statistical analysis was performed by using the ToxRat Professional 3.2 software and included NOEC, EC_{10}, EC_{20} and EC_{50} estimations. The worst-case NOEC was re-estimated to be 66.1 mg a.s./kg dw (based on reproductive performance) while the worst-case EC_{10} endpoint was calculated to be 62.4 mg a.s./kg dw (based on reproductive performance). Thus, the latter endpoint was selected to be used in the risk assessment. However, **details of statistiscal re-evaluation was not submitted to RMS, therefore, a data gap is set. The EC_{10} = 62.4 mg a.s./kg dw is considered provisional pending on the submission of the statistiscal re-evaluation of [redacted] 2007 (KCP 10.4.1.1/01).**

A summary of the available studies is presented in **Table 2.9.4.1-1**.

Table 2.9.4.1-1 Effects of the representative formulation on earthworms

Test substance	Test species	Exposure system	Endpoint	Reference	Endpoint used in the risk assessment
Acute toxicity to earthworms					
Dodine technical	*Eisenia fetida*	Mixed into substrate 14 d, acute 10.1% w/w sphagnum moss content	LC_{50} = 547 mg a.s./kg dw	[redacted] (1995) KCA 8.4.1/01	-
Chronic toxicity to earthworms					
Dodine 400 SC	*Eisenia fetida*	Mixed into substrate 56 d, chronic 10% w/w sphagnum peat content	NOEC = 172 mg f.p./kg dw corresponding to 66.1 mg a.s./kg dw EC_{10} = 62.4 mg a.s./kg dw	[redacted] (2007) KCP 10.4.1.1/01	EC_{10} = 62.4 mg a.s./kg dw*

a.s. active substance
f.p. formulated product
* The EC10 = 62.4 mg a.s./kg dw is considered provisional pending on the submission of the statistiscal re-evaluation of [redacted] 2007 (KCP 10.4.1.1/01).

Although no specific toxicity data on the current representative formulation, i.e., Dodine 544 SC, have been generated, further toxicity testing with earthworms is not required as it is possible to extrapolate from the available study with the formulation Dodine 400 SC provided that the respective endpoint is expressed as active substance equivalents. Please refer to Volume 4 "Confidential Information" for further details. Moreover, the ecotoxicological equivalence between the two formulations is confirmed based on the results of bridging studies conducted with aquatic non-vertebrate species (i.e., *Daphnia magna* and *Desmodesmus subspicatus*). Considering all available information, RMS agrees that the formulation Dodine 400 SC could be considered as a surrogate for assessing the toxicity of Dodine 544 SC.

2.9.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

No studies on the effects of Dodine (technical or formulated) on non-target soil meso- and macrofauna other than earthworms were conducted for the first EU evaluation for the Annex I inclusion of Dodine. According to Commission Regulation (EU) No 284/2013, testing shall be carried out on both *Folsomia candida* and *Hypoaspis aculeifer* if either of the following conditions are met: (i) the plant protection product under concern is applied directly to soil as soil treatment either as a spray or as a solid formulation, (ii) concern is raised regarding potential unacceptable effects to the standard non-target arthropod species *Aphidius rhopalosiphi* and *Typhlodromus pyri*. Given that Dodine 544 SC is intended to be applied as a foliar spray and no unacceptable risk to non-target arthropods is concluded on the basis of the Tier 1 risk assessment conducted, typically, no further testing with either *Folsomia candida* or *Hypoaspis aculeifer* is required. Nevertheless, testing of Dodine 544 SC on both species has been conducted under standard (i.e., *Folsomia candida*, *Hypoaspis aculeifer*) and refined (i.e., *Folsomia candida*) exposure conditions. A summary is provided in the following table :

Table 2.9.4.2-1 Effects of Dodine and the representative formulation on non-target soil meso- and macrofauna (other than earthworms)

Test substance	Test species	Exposure system	Endpoint	Reference	Endpoint used in the risk assessment
Dodine 544 SC	*Hypoaspis aculeifer*	Mixed into substrate 14 d, chronic 5% w/w sphagnum moss content	NOEC = 1846 g f.p./kg dw corresponding to 1000 mg a.s./kg dw [a] EC_{10} > 1846 g f.p./kg dw corresponding to 1000 mg a.s./kg dw [a]	[redacted] (2017a) KCP 10.4.2.1/01	NOEC = 1846 g f.p./kg dw corresponding to 1000 mg a.s./kg dw
Dodine 544 SC	*Folsomia candida*	Mixed into substrate 28 d, chronic artificial soil: 5% w/w sphagnum peat content	NOEC = 5.91 mg f.p./kg dw corresponding to 3.2 mg a.s./kg dw [a] EC_{10} = 12.18 mg f.p./kg dw corresponding to 6.6 mg a.s./kg dw [a]	[redacted] (2017b) KCP 10.4.2.1/02	NOEC = 5.91 mg f.p./kg dw corresponding to 3.2 mg a.s./kg dw
Dodine 544 SC	*Folsomia candida*	Mixed into substrate 28 d, chronic natural soil: LUFA standard soil type 2.2	NOEC = 1000 mg f.p./kg dw corresponding to 541.7 mg a.s./kg dw [a] EC_{10} > 1000 mg f.p./kg dw corresponding to 541.7 mg a.s./kg dw [a]	[redacted] (2021) KCP 10.4.2.1/03	NOEC = 1000 mg f.p./kg dw corresponding to 541.7 mg a.s./kg dw

a.s. active substance
f.p. formulated product
a re-calculation to formulated product equivalents (not given in the study report) is based on the analysed content of 541.7 g Dodine/kg Dodine 544 SC

2.9.5 Summary of effects on soil nitrogen transformation

The effects of Dodine formulated as Dodine 400 SC on soil microbial activity (nitrogen turnover and short-time respiration) are adequately presented and discussed in Volume 3 CP Point B.9.9.

A study on the effects of Dodine formulated as 400 g/L SC on soil microbial activity (nitrogen turnover and short-time respiration) was already submitted for the first EU evaluation for the Annex I inclusion of Dodine ([redacted] 2002 ; KCP 10.5/01). No new studies have been conducted for the purpose of renewal of the approval of Dodine.

Based on the results of [redacted] (2002, KCP 10.5/01), Dodine 400 SC has non unacceptable long-term effects (no effects ≥ 25%) on soil nitrate content and soil nitrate formation rate of soil microflora at the test concentration of 12 mg a.s./kg dry soil. The results of the study are considered supportive only. A data gap has been set to the applicant to submit the soil nitrogen transformation rate expressed in mg nitrate/kg dry weight soil/day between each measurement day for control and all tested concentrations in order to determine the difference in transformation rates as recommended by the OECD 216.

Table 2.9.5-1 Effects of Dodine and the representative formulations on soil microorganisms

Test substance	Endpoint	Exposure system	Results	Reference	Endpoint used in the risk assessment
Dodine 400 SC	N-transformation	28 d, aerobic loamy sand soil	No effects ≥ 25% at 9000 g a.s./ha (corresponding to 12 mg a.s./kg dw) after 28 days	[redacted] (2002) KCP 10.5/01	12 mg a.s./kg dw

a.s. active substance
f.p. formulated product
* RA is considered provisional

2.9.6 Summary of effects on terrestrial non-target higher plants

Studies on the effects of Dodine technical and formulated as 400 g/L SC on the vegetative vigour and seedling emergence of non-target terrestrial plants were already submitted for the first EU evaluation for the Annex I inclusion of Dodine. In addition to the already EU peer reviewed studies, two new studies assessing the effects of the current representative formulation, i.e., Dodine 544 SC, on the vegetative vigour and the seedling emergence of non-target higher plants have been conducted.

The effects of Dodine (technical and formulated) to terrestrial non-target higher plants are adequately presented and discussed in Voume 3 CA B9 Point 9.6 and Volume 3 CP B9 Point B.9.11.

[redacted] (2011b) determined the induced effect of a single application of Dodine 544 SC with respect to the seedling emergence and the seedling growth in a variety of terrestrial plants. The study was performed as a limit test at 0 and 15 kg a.i./ha for six plant species. The most sensitive parameter and plant species was wet weight for ray-grass, lettuce and cabbage: mean weight was reduced by more than 30% as compared to the controls. Significant effects have been observed at the limit concentration, therefore, no endpoint could be derived from the test. Moreover, several deviations from OECD 208 have been noted and the study has not been conducted under GLP. All in all, RMS considers that the study is not valid for risk assessment purposes.

[redacted] (2011a) determined the induced effects of Dodine 544 SC on terrestrial plant growth. The study was performed as a limit test at 0 and 15 kg a.i./ha for nine plant species. The plants were grown from seeds on a natural sandy loam soil to the 2- to 4-true leaf stage. The test item and control treatments were then sprayed on the plant and leaf surfaces at appropriate rates. After the application, the plants were evaluated against water control plants for effects on vigour and growth at various time intervals until 21-28 days after treatment. The plants died within few days for onion, lettuce, radish, tomato, cucumber and cabbage. Ray-grass, oat and soybean survived until the end of the test, but both plant height and plant weight were significantly reduced as compared to the controls. The phytotoxicity consisted of dwarfism and necrosis. Since significant effects above 50% has been observed at the limit concentration, no endpoint could be derived from the test. Moreover, several deviations from OECD 227 has been noted and no GLP statement was provided. All in all, RMS considers that the study is not valid for risk assessment purposes.

[redacted] (2007) determined the effects of Dodine 400 SC on the vegetative vigour, growth and health of three non-target plant species: cabbage (*Brassica oleracea*), cucumber (*Cucumis sativus*) and radish (*Raphanus sativus*)

following one leaf application in comparison to a blank control (deionised water). The experimental design consisted of seven treated groups each one representing a different dodine applied rate at growth stage BBCH 12-14. Based on the results obtained under worst case greenhouse conditions, the ER_{50} was > 4.5 kg a.s./ha for all species tested. However, seedling emergence data were not included in the report, therefore, RMS could not check all validity criteria.

[redacted] (1993a) evaluated the effects of Dodine at its maximum label rate of 2.9 kg a.s/ha on vegetative vigour on ten non-target plant species during critical stages in their development. The validity criteria according to the OECD 227 (the seedling emergence is at least 70 %) cannot be validated, as no data on seedling emergence were reported. However, a parallel study on seedling emergence ([redacted] 1993b) was performed under the same conditions (same study location, test facility, plant species, soil, planting pattern, number of seed per pot and replicates, similar dates and environmental conditions of greenhouse). The lot number of the tested seeds were the same with two exceptions, cucumber and lettuce. Moreover, in [redacted] (1993b), seedling emergence for tomato and lettuce failed in the original study, and it as repeated for this species at a different planting depth. Therefore, taking into account the results on seedling emergence of [redacted] (1993b), RMS is of the opinion that this validity criteria could be considered as met for all expecies excetp to lettuce, tomato and cucumber.

[redacted] (1993b) evaluated the effects of Dodine at its maximum label rate of 2.9 kg as/ha on seedling emergence and other growth characteristics on ten non-target plant species during critical stages in their development. No noticeable phytotoxicity was noted on any day of evaluation. No effects > 25% on phytotoxicity, plant height and dry plant weight were reported. Therefore, it is concluded that the ER_{50} was > 2.9 kg a.s./ha for all species tested except to lettuce, tomato and cucumber.

[redacted] (1993c) studied whether detrimental effects of 25% or greater occurred on one or more plant species after application of dodine technical at the maximum rate of 2.9 kg as/ha. The study was performed in compliance with GLP and following EPA OPP 122-1 guideline (Terrestrial Plant Toxicity Tier I (seedling emergence)). This protocol differs substantially from OECD 208, therefore, the study is considered as supplemenatary information only.

Table 2.9.6-1 Effects of Dodine and the representative formulation on non-target higher plants

Test substance	Test species	Exposure system	Endpoint	Reference	Remarks
Dodine	*Glycine max, Raphanus sativa, Zea mays, Brassica oleraceae, Avena sativa, Lolium perenne, Allium cepa*	21 d Vegetative vigour	ER_{50} phytotoxicity > 2.9 kg a.s./ha ER_{50} plant height > 2.9 kg a.s./ha ER_{50} plant weight > 2.9 kg a.s./ha	[redacted] (1993a) KCA 8.2.2/01	ER_{50} (vegetative vigour) > 2.9 kg a.s./ha ER_{50} (seedling emergence) > 2.9 kg a.s./ha
Dodine	*Glycine max, Lactuca sativa, Raphanus sativa, Lycopersicon esculentum, Zea mays, Cucumis sativus, Brassica oleraceae, Avena sativa, Lolium perenne, Allium cepa*	21 d Seedling emergence	ER_{50} emergence > 2.9 kg a.s./ha ER_{50} plant height > 2.9 kg a.s./ha ER_{50} plant weight > 2.9 kg a.s./ha	[redacted] (1993b) KCA 8.2.2/02	
Dodine	*Glycine max, Lactuca sativa, Raphanus sativa, Lycopersicon esculentum, Zea mays, Cucumis sativus, Brassica oleraceae, Avena sativa, Lolium perenne, Allium cepa*	7 d Seed germination	ER_{50} germination > 2.9 kg a.s./ha	[redacted] (1993c) KCA 8.2.2/03	Supplementary information
Dodine 400 SC	*Brassica oleracea, Cucumis sativus, Raphanus sativus*	21 d Vegetative vigour	ER_{50} phytotoxicity > 4.5 kg a.s./ha ER_{50} plant weight > 4.5 kg a.s./ha	[redacted] (2007) KCP 10.6.2/02	Not accepted

Test substance	Test species	Exposure system	Endpoint	Reference	Remarks
Dodine 544 SC	*Glycine max, Lactuca sativa, Raphanus sativus, Solanum lycopersicum, Cucumis sativus, Brassica oleracea, Avena sativa, Lolium perenne, Allium cepa*	21-28 d Vegetative vigour	LR_{50} < 15 kg a.s./ha (*Allium cepa, Lactuca sativa, Raphanus sativus, Solanum lycopersicum, Cucumis sativus, Brassica oleracea*) ER_{50} plant height > 15 kg a.s./ha (*Glycine max, Avena sativa, Lolium perenne*) ER_{50} plant weight > 15 kg a.s./ha (*Avena sativa*) ER_{50} plant weight < 15 kg a.s./ha (*Glycine max, Lolium perenne*)	[redacted] (2011a) KCP 10.6.2/01	Not accepted
Dodine 544 SC	*Glycine max, Lactuca sativa, Raphanus sativus, Solanum lycopersicum, Cucumis sativus, Brassica oleracea, Avena sativa, Lolium perenne, Allium cepa*	21 d Seedling emergence and seedling growth	ER_{50} emergence > 15 kg a.s./ha ER_{50} plant height > 15 kg a.s./ha ER_{50} plant weight > 15 kg a.s./ha	[redacted] (2011b) KCP 10.6.2/03	Not accepted

a.s. active substance
f.p. formulated product

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

No data available.

2.9.8 Summary of effects on biological methods for sewage treatment

Two studies assessing the effects of Dodine technical to biological methods for sewage treatment by measuring the activated sludge respiration inhibition are available. One study was already submitted for the first EU evaluation for the Annex I inclusion of Dodine while the other study was conducted for the purpose of the renewal of the approval of the active substance. Both studies were conducted according to OECD testing guideline 209 and meet the respective validity criteria. The lowest EC_{50} and EC_{10} endpoints of 22.4 and 3 mg a.s./L for activated sludge respiration inhibition are derived from the newly submitted study.

Table 2.9.8-1 Effects on biological methods for sewage treatment

Test substance	Endpoint	Exposure system	Results	Reference
Dodine	Activated sludge respiration inhibition	30 min, activated sludge from municipal sewage plant	EC_{50} = 52 mg as/L EC_{10} = 9 mg/L	[redacted] (2001) KCP 8.8/02
Dodine	Activated sludge respiration inhibition	3 h, activated sludge of a predominantly domestic sewage	EC_{50} = 22.4 mg/L EC_{10} = 3 mg/L	[redacted] 2020) KCP 8.8/01

a.s. active substance
f.p. formulated product

2.9.9 Summary of product exposure and risk assessment

2.9.9.1 Summary of risk assessment for bird and other terrestrial vertebrates

Birds

The risk assessment for effects on birds is carried out according to the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438). The acute and long-term risks of Dodine formulated as Dodine 544 SC to birds were assessed from toxicity exposure ratios between toxicity endpoints, derived from studies with Dodine technical and estimated exposure based on the maximum residues occurring on food items following applications according to the proposed use pattern.

Acute dietary risk assessment

The geometric mean of the LD_{50} on mallard duck and the LD_{50} on bobwhite quail as derived from the available short-term dietary studies, i.e., 522.8 mg a.s./kg bw, is used in the regulatory acute risk assessment for birds. All TER_A values for Dodine calculated for the relevant exposure scenarios exceed the trigger of 10 at screening step, indicating no potential acute risk for birds following the representative uses of Dodine 544 SC in apples/pear, cherry and peach (Tables 2.9.9.1-1 and 2.9.9.1-2).

Table 2.9.9.1-1 Screening assessment of the acute risk for birds after the use of Dodine 544 SC in apples/pear and cherry (2 × 0.68 kg a.s./ha, 21-day interval)

Intended use		**Apples/pear, cherry**				
Active substance		Dodine				
Application rate [kg a.s./ha]		2 × 0.68				
Acute toxicity						
LD_{50} [mg a.s./kg bw]		522.8				
TER criterion		10				
Crop scenario	**Indicator species**	**SV_{90}**	**MAF_{90}**	**TWA**	**DDD_{90} [mg/kg bw]**	**TER_A**
Orchards	Small insectivorous bird	46.8	1.1	n.a.	35.01	14.9

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio; n.a.: not applicable. TER values shown in bold fall below the relevant trigger.

Table 2.9.9.1-2 Screening assessment of the acute risk for birds after the use of Dodine 544 SC in peach (2 × 0.9 kg a.s./ha, 21-day interval)

Intended use		**Peach**				
Active substance		Dodine				
Application rate [kg a.s./ha]		2 × 0.9				
Acute toxicity						
LD_{50} [mg a.s./kg bw]		522.8				
TER criterion		10				
Crop scenario	**Indicator species**	**SV_{90}**	**MAF_{90}**	**TWA**	**DDD_{90} [mg/kg bw]**	**TER_A**
Orchards	Small insectivorous bird	46.8	1.1	n.a.	46.33	11.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio; n.a.: not applicable. TER values shown in bold fall below the relevant trigger.

Long-term dietary risk assessment

The lowest NOEL of 17 mg a.s./kg bw/d for mallard duck is used in the regulatory long-term/reproductive risk assessment for birds. TER_{LT} values for Dodine calculated for the relevant exposure scenarios are below the trigger of 5 at screening step (Tables 2.9.9.1-3 and 2.9.9.1-4).

Table 2.9.9.1-3 Screening assessment of the long-term/reproductive risk for birds after the use of Dodine 544 SC in apples/pear and cherry (2 × 0.68 kg a.s./ha, 21-day interval)

Intended use	**Apples/pear, cherry**					
Active substance	Dodine					
Application rate [kg a.s./ha]	2 × 0.68					
Reproductive toxicity						
NOEL [mg a.s./kg bw/d]	17					
TER criterion	5					
Crop scenario	**Indicator species**	**SV_m**	**MAF_m**	**TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**
Orchards	Small insectivorous bird	18.2	1.2	0.53	7.87	**2.2**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio; n.a.: not applicable. TER values shown in bold fall below the relevant trigger.

Table 2.9.9.1-4 Screening assessment of the long-term/reproductive risk for birds after the use of Dodine 544 SC in peach (2 × 0.9 kg a.s./ha, 21-day interval)

Intended use	**Peach**					
Active substance	Dodine					
Application rate [kg a.s./ha]	2 × 0.9					
Reproductive toxicity						
NOEL [mg a.s./kg bw/d]	17					
TER criterion	5					
Crop scenario	**Indicator species**	**SV_m**	**MAF_m**	**TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**
Orchards	Small insectivorous bird	18.2	1.2	0.53	10.42	**1.6**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio; n.a.: not applicable. TER values shown in bold fall below the relevant trigger.

Based on Tier 1 assessment, the TER_{LT} values for Dodine calculated for the relevant exposure scenarios exceed the trigger of 5 except for small granivorous birds feeding in treated apples/pear and peach fields and small insectivorous birds feeding in treated apples/pear, cherry and peach fields (Tables 2.9.9.1-5 to 2.9.9.1-7).

Table 2.9.9.1-5 First tier assessment of the long-term/reproductive risk for birds after the use of Dodine 544 SC in apples/pear (2 × 0.68 kg a.s./ha, 21-day interval)

Intended use	**Apples/pear**
Active substance	Dodine
Application rate [kg a.s./ha]	2 × 0.68
Reproductive toxicity	
NOEL [mg a.s./kg bw/d]	17

TER criterion		5				
Growth stage	**Generic focal species**	**SV_m**	**MAF_m**	**TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**
Orchard Crop directed application BBCH 10-19	Small granivorous bird "finch" (100% seeds)	10.1	1.2	0.53	4.37	**3.9**
Orchard Crop directed application BBCH 10-19	Small insectivorous/worm feeding bird "thrush" (100% soil dwelling invertebrates)	2.1	1.2	0.53	0.91	18.7
Orchard Crop directed application BBCH 20-39	Small granivorous bird "finch" (100% seeds)	7.6	1.2	0.53	3.29	5.2
Orchard Crop directed application BBCH 20-39	Small insectivorous/worm feeding bird "thrush" (100% soil dwelling invertebrates)	1.6	1.2	0.53	0.69	24.6
Orchard Crop directed application BBCH ≥ 40	Small granivorous bird "finch" (100% seeds)	3.8	1.2	0.53	1.64	10.3
Orchard Crop directed application BBCH ≥ 40	Small insectivorous/worm feeding bird "thrush" (100% soil dwelling invertebrates)	0.8	1.2	0.53	0.35	49.1
Orchard Spring, Summer	Small insectivorous bird "tit" (100% foliar insects)	18.2	1.2	0.53	7.87	**2.2**

MAF: multiple application factor calculated in line with Appendix H EFSA/2009/1438 for 2 applications with a 21-day interval and assuming a default foliar DT_{50} value of 10 days; TWA: time weighted factor (default); DDD: daily dietary dose. TER values shown in **bold** fall below the relevant trigger.

Table 2.9.9.1-6 First tier assessment of the long-term/reproductive risk for birds after the use of Dodine 544 SC in cherry (2 × 0.68 kg a.s./ha, 21-day interval)

Intended use		Cherry				
Active substance		Dodine				
Application rate [kg a.s./ha]		2 × 0.68				
Reproductive toxicity						
NOEL [mg a.s./kg bw/d]		17				
TER criterion		5				
Growth stage	**Generic focal species**	**SV_m**	**MAF_m**	**TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**
Orchard Crop directed application BBCH ≥ 40	Small granivorous bird "finch" (100% seeds)	3.8	1.2	0.53	1.64	10.3
Orchard Crop directed application BBCH ≥ 40	Small insectivorous/worm feeding bird "thrush" (100% soil dwelling invertebrates)	0.8	1.2	0.53	0.35	49.1
Orchard Spring, Summer	Small insectivorous bird "tit" (100% foliar insects)	18.2	1.2	0.53	7.87	**2.2**

MAF: multiple application factor calculated in line with Appendix H EFSA/2009/1438 for 2 applications with a 21-day interval and assuming a default foliar DT_{50} value of 10 days; TWA: time weighted factor (default); DDD: daily dietary dose. TER values shown in **bold** fall below the relevant trigger.

Table 2.9.9.1-7 First tier assessment of the long-term/reproductive risk for birds after the use of Dodine 544 SC in peach (2 × 0.9 kg a.s./ha, 21-day interval)

Intended use	Peach
Active substance	Dodine

Application rate [kg a.s./ha]	2 × 0.9
Reproductive toxicity	
NOEL [mg a.s./kg bw/d]	17
TER criterion	5

Growth stage	**Generic focal species**	**SV_m**	**MAFm**	**TWA**	**DDD_m [mg/kg bw/d]**	**TER_{LT}**
Orchard Crop directed application BBCH 10-19	Small granivorous bird "finch" (100% seeds)	10.1	1.2	0.53	5.78	**2.9**
Orchard Crop directed application BBCH 10-19	Small insectivorous/worm feeding bird "thrush" (100% soil dwelling invertebrates)	2.1	1.2	0.53	1.20	14.1
Orchard Crop directed application BBCH 20-39	Small granivorous bird "finch" (100% seeds)	7.6	1.2	0.53	4.35	**3.9**
Orchard Crop directed application BBCH 20-39	Small insectivorous/worm feeding bird "thrush" (100% soil dwelling invertebrates)	1.6	1.2	0.53	0.92	18.6
Orchard Crop directed application BBCH ≥ 40	Small granivorous bird "finch" (100% seeds)	3.8	1.2	0.53	2.18	7.8
Orchard Crop directed application BBCH ≥ 40	Small insectivorous/worm feeding bird "thrush" (100% soil dwelling invertebrates)	0.8	1.2	0.53	0.46	37.1
Orchard Spring, Summer	Small insectivorous bird "tit" (100% foliar insects)	18.2	1.2	0.53	10.42	**1.6**

MAF: multiple application factor calculated in line with Appendix H EFSA/2009/1438 for 2 applications with a 21-day interval and assuming a default foliar DT_{50} value of 10 days; TWA: time weighted factor (default); DDD: daily dietary dose. TER values shown in **bold** fall below the relevant trigger.

For these two types of diet guild (granivorous and small insectivorous birds), a higher tier risk assessment is performed by considering focal species, i.e., a real species that actually occur in the crop when the pesticide is being used, and their ecological properties (PT: Proportion of diet obtained in the treated area), by refining the residue decline (DT_{50}) in potential food items of the identified focal species and last by incorporating in the exposure estimation the interception by the crop. However, the refinemenst for the small insectivorous birds of focal specie, PT and DT_{50} are only reliable in the Central Zone. The calculated higher tier TER_{LT} values exceed the trigger of 5 for small insectivorus birds for the intended uses on apple/pear and cherry in the Central Zone and granivorus birds for all the intended uses (Tables 2.9.9.1-8 and 2.9.9.1-9).

Table Table 2.9.9.1-8 Refined long-term TER calculations for small insectivorous birds after the use of Dodine 544 SC in apples/pear, cherry and peach (application on spring-summer) in Central Zone

Intended use	Apples/pear, cherry; 2 × 0.68 kg a.s./ha, 21-day interval	Peach, 2 × 0.9 kg a.s./ha, 21-day interval
Dose [kg a.s./ha]	0.68	0.9
FIR/bw (refined)	0.77	0.77
RUD_m (default)	21	21
Interception factor	1.0	1.0
MAF_m × TWA (refined)	1.06 × 0.34 = 0.36	1.06 × 0.34 = 0.36
PT (refined)	0.673	0.673

PD	1.0	1.0
DDD [mg/kg bw/d]	2.66	3.52
NOEL [mg/kg bw/d]	17	17
TER	6.4	**4.8**

In bold, below the tigger of 5

Table 9.2.1.3-17 Refined long-term TER calculations for granivorous birds after the use of Dodine 544 SC in apples/pear and peach (all EU Zones)

Intended use	Apples/pear, 2 × 0.68 kg a.s./ha, 21-day interval	Peach, 2 × 0.9 kg a.s./ha, 21-day interval	
Growth stage	Crop directed application BBCH 10-19	Crop directed application BBCH 10-19	Crop directed application BBCH 20-39
Dose [kg a.s./ha]	0.68	0.9	0.9
FIR/bw (default)	0.31	0.31	0.31
RUD_m (default)	40.2	40.2	40.2
Deposition factor (refined)	0.4	0.4	0.4
MAF_m × TWA (default)	1.2 × 0.53 = 0.64	1.2 × 0.53 = 0.64	1.2 × 0.53 = 0.64
PT (default)	1.0	1.0	1.0
PD (default)	1.0	1.0	1.0
DDD [mg/kg bw/d]	2.17	2.87	2.87
NOEL [mg/kg bw/d]	17	17	17
TER	7.8	5.9	5.9

Therefore, **no unacceptable long-term risk to birds** following the representative uses of Dodine 544 SC in **apples/pear and cherry in the Central Zone** based on the outcome of the higher tier risk assessment.

However, the following should be considered : all refinements (except interception value) were representative for the Central Zone, their extrapolation to other regulatory zones needs further justification/applicability may need further consideration at Member State level.

Drinking water risk assessment

Based on the ratios of the effective application rate to the relevant toxicity endpoints, an acceptable risk is demonstrated for birds due to exposure to Dodine via contaminated drinking water in puddles (puddle scenario). Further, considering the representative uses of Dodine 544 SC in orchards, no risk to birds is expected via exposure to contaminated drinking water in leaf whorls (leaf scenario).

Secondary poisoning

The log P_{ow} of Dodine does not exceed the trigger value of 3; thus, a risk assessment of secondary poisoning for earthworm- and fish-eating birds is not required.

Mammals

Studies on the acute oral and reproductive/long-term toxicity of Dodine technical to mammals as well as a study investigating the long-term effects of Dodine 400 SC on common voles under field conditions were already submitted for the first EU evaluation for the Annex I inclusion of the active substance. For the renewal of the approval of the active substance a study assessing the acute oral toxicity of the representative product Dodine 544

SC to mammals (i.e., rats) and a semi-field study investigating the long-term effects on common voles in treatment enclosures following Dodine 544 SC applications are newly submitted.

The risk assessment for effects on mammals is carried out according to the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438). The acute and long-term risks of Dodine formulated as Dodine 544 SC to mammals were assessed from toxicity exposure ratios between toxicity endpoints, derived from studies with Dodine technical and estimated exposure based on the maximum residues occurring on food items following applications according to the proposed use pattern.

Acute dietary risk assessment

The geometric mean of the two LD_{50} endpoints on the acute oral toxicity of Dodine technical to rats and, i.e., 1073 mg a.s./kg bw, is used in the regulatory acute risk assessment for wild mammals.

At screening step, acute TERs for mammals were below the trigger values for the representative uses in apples/pear and cherry (2 × 0.68 kg a.s./ha, 21-day interval). Acute TERs for mammals exceed the relevant trigger of 10 for the intended uses in peach (2 × 0.9 kg a.s./ha, 21-day interval). At Tier 1, a high risk is still identified for Small herbivorous mammal "vole" at BBCH < 10 (application crop directed or not crop directed) and BBCH 10-19. Therefore, further refinements are required for the intended use on peach.

Long-term dietary risk assessment

The lowest NOAEL of 26 mg a.s./kg bw/d as derived from the two-generation study in rats is used in the regulatory long-term/reproductive risk assessment for mammals.

At screening step, the long-term TER values for Dodine were below the trigger value of 5 for all the intended uses. At Tier 1, a high risk is still identified for the following scenarios :

Applications on apples/pear (2 × 0.68 kg a.s./ha, 21-day interval) :
- Large herbivorous mammal "lagomorph » at BBCH < 10 (application crop directed or not crop directed)
- Small herbivorous mammal "vole" from BBCH < 10 to ≥ 40
- Frugivorous mammal "dormouse" at BBCH 71-79

Applications on cherry (2 × 0.68 kg a.s./ha, 21-day interval):
- Small herbivorous mammal "vole" at BBCH ≥ 40
- Frugivorous mammal "dormouse" at BBCH 71-79

Applications on peach (2 × 0.9 kg a.s./ha, 21-day interval):

- Large herbivorous mammal "lagomorph » at BBCH < 10 (application crop directed or not crop directed) and BBCH 10 - 19
- Small herbivorous mammal "vole" from BBCH < 10 to ≥ 40

For the above-mentioned exposure scenarios, a higher tier risk assessment was performed by refining the information on their diet composition (PD) and the residue decline (DT_{50}) or the magnitude of residues (i.e., RUD) in potential food items, by incorporating in the exposure estimation the interception by the crop. The results of the available field effect studies could not be considered in the risk assessment as their statistical power were missing. Based on the higher tier information available, no unacceptable long-term risk to mammals is expected following the representative uses of Dodine 544 SC in orchards in Central Zone, except for :

- apple/pear at BBCH<10 (unacceptable for vole identified)
- cherry at postharvest (unacceptable for vole and dormouse identified)
- peach (unacceptable for vole identified).

Refinements were representative for the Central Zone, their extrapolation to other regulatory zones requires further justification and their applicability may need further consideration at Member State level.

Drinking water risk assessment

Based on the ratio of the effective application rate to the relevant toxicity endpoints, an acceptable risk is demonstrated for mammals due to exposure to Dodine via contaminated drinking water in puddles (puddle scenario). Further, considering the representative uses of Dodine 544 SC in orchards, no risk to mammals is expected via exposure to contaminated drinking water in leaf whorls (leaf scenario).

Secondary poisoning

The log P_{ow} of Dodine does not exceed the trigger value of 3; thus, a risk assessment of secondary poisoning for earthworm- and fish-eating mammals is not required

2.9.9.2 Summary of risk assessment for aquatic organisms

The risk assessment were performed following the current EFSA guidance on aquatic organisms (2013). PECsw/sed calculations provided by the applicant were performed for two applications of the representative formulation Dodine 544 SC in orchards at 0.68 and 0.9 kg a.s./ha, in a 21-day interval.

New PECsw/sed calculations of dodine were developed by RMS (see Vol 3CP 8.5). Based on those new PECsw/sed and the adequate endpoints (summarized below), PEC/RAC ratios for dodine have been re-calculated by RMS. For these calculations, only the relevant global maximum FOCUS Steps 3 and 4 PECsw have been considered.

Table 2.9.9.2-1: Endpoints used in the risk assessment for aquatic organisms.

Taxonomic group/exposure regime	Tier I RAC [µg a.s./L]	Higher-tier RAC [µg a.s./L]
Fish/acute	3.12	12.55[a]
Fish/long-term	17	-
Aquatic invertebrates/acute	0.18	ETO-RAC = 0.4 ERO-RAC[b] = 2.5
Aquatic invertebrates/long-term	0.44	
Algae	0.55	
Aquatic invertebrates	8.4	
Sediment dwellers/long-term	88.3	-

[a] Preliminary endpoint, pending on the submission of the statistical robustness of some LC50 by the applicant.
[b] derived from the mesocosm study of [redacted] (2021b) by applying an assessment factor of 3 to the Effect class 3A NOEAEC = 7.5 µg a.s./L

STEP 3

For the intended use of Dodine 544 SC in **apples/pear** (2 x 0.68 kg a.s./ha), both early and late applications, a potential risk for acute and prolonged exposure of aquatic organisms was found, independently if multiple or single applications are considered (PEC/RAC ratios based on both Tier 1 and Higher Tier endpoints were above the relevant trigger value of 1). When mesocosm ERO-RAC is considered, no unacceptable risk for aquatic organisms can be concluded for scenarios D4 pond, D5 pond and R1 pond for late applications. However, further refinement for all scenarios was required.

For **cherry** (2 x 0.68 kg a.s./ha), a potential risk for acute and prolonged exposure of aquatic organisms was found for summer, late and post-harvest applications, independently if multiple or single applications are considered (PEC/RAC ratios based on both Tier 1 and Higher Tier endpoints were above the relevant trigger value of 1). When mesocosm ERO-RAC of 2.5 µg/L is considered, no unacceptable risk for aquatic organisms can be concluded for scenarios D4 pond, D5 pond and R1 pond for summer and late applications. However, further refinement for all scenarios was required.

For **peach** (2 x 0.9 kg a.s./ha), a potential risk for acute and prolonged exposure of aquatic organisms was found for summer, late and post-harvest applications. As occurred for the other uses, when mesocosm ERO-RAC of 2.5 µg/L is considered, no unacceptable risk for aquatic organisms can be concluded for scenarios D4 pond, D5 pond and R1 pond for late applications. However, further refinement for all scenarios was required.

Single applications led the worst-case PECsw values respect to multiple applications, therefore, only PEC/RAC results at FOCUS STEP 3 were presented below (for details of multiple applications, see Vol 3CP 9.4).

Table 2.9.9.2-2: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the active substance Dodine for each organism group based on FOCUS Steps 3 calculations for the use of Dodine 544 SC in apples/pear (2 × 0.68 kg a.s./ha, Pome/stone fruit, early application)

Group	Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophytes	Sed. dwell. prolonged	Higher-tier information	Higher-tier information	Higher-tier information	Higher-tier information

Test species			***Cyprinus carpio***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Raphidocelis subcapitata***	***Lemna gibba***	***Chironomus riparius***	**Fish acute**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC	Geomean LC_{50}	Effect class 2 NOEC	lower Effect class 3A NOEAEC	higher Effect class 3A NOEAEC
[µg/L]			312	200	18	4.4	5.5	63	883	1255	0.8	1.6	7.5
AF			100	10	100	10	10	10	10	100	2.00	3.00	3.00
RAC [µg/L]			3.12	20	0.18	0.44	0.55	6.3	88.3	12.55	0.4	0.53	2.5
Step 3	**PEC $_{sw\ max}$ [µg/L]**	**PEC $_{sed\ max}$ [µg/Kg]**	**PEC/RAC**										
Single applications													
D3/ditch	50.6	132.0	**16.2**	**2.5**	**281.3**	**115.1**	**92.1**	**8.0**	**1.5**	**4.0**	**126.6**	**95.0**	**20.3**
D4/pond	3.0	21.5	**1.0**	0.2	**16.8**	**6.9**	**5.5**	0.5	0.2	0.2	**7.6**	**5.7**	**1.2**
D4/stream	47.9	35.8	**15.3**	**2.4**	**265.9**	**108.8**	**87.0**	**7.6**	0.4	**3.8**	**119.7**	**89.7**	**19.1**
D5/pond	3.0	19.1	**1.0**	0.2	**16.8**	**6.9**	**5.5**	0.5	0.2	0.2	**7.6**	**5.7**	**1.2**
D5/stream	50.2	33.7	**16.1**	**2.5**	**279.1**	**114.2**	**91.3**	**8.0**	0.4	**4.0**	**125.6**	**94.2**	**20.1**
R1/pond	3.0	19.3	**1.0**	0.2	**16.8**	**6.9**	**5.5**	0.5	0.2	0.2	**7.6**	**5.7**	**1.2**
R1/stream	40.9	63.3	**13.1**	**2.0**	**227.2**	**93.0**	**74.4**	**6.5**	0.7	**3.3**	**102.3**	**76.7**	**16.4**
R2/stream	54.3	54.9	**17.4**	**2.7**	**301.4**	**123.3**	**98.7**	**8.6**	0.6	**4.3**	**135.7**	**101.7**	**21.7**
R3/stream	57.9	107.1	**18.6**	**2.9**	**321.9**	**131.7**	**105.3**	**9.2**	**1.2**	**4.6**	**144.9**	**108.6**	**23.2**
R4/stream	40.9	63.5	**13.1**	**2.0**	**227.3**	**93.0**	**74.4**	**6.5**	0.7	**3.3**	**102.3**	**76.7**	**16.4**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 2.9.9.2-3: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the active substance Dodine for each organism group based on FOCUS Steps 3 calculations for the use of Dodine 544 SC in apples/pear (2 × 0.68 kg a.s./ha, Pome/stone fruit, late application)**

Group			**Fish acute**	**Fish prolonged**	**Invertb. acute**	**Invertb. prolonged**	**Algae**	**Aquatic macrophytes**	**Sed. dwell. prolonged**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**
Test species			***Cyprinus carpio***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Raphidocelis subcapitata***	***Lemna gibba***	***Chironomus riparius***	**Fish acute**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC	Geomean LC_{50}	Effect class 2 NOEC	lower Effect class 3A NOEAEC	higher Effect class 3A NOEAEC
[µg/L]			312	200	18	4.4	5.5	63	883	1255	0.8	1.6	7.5
AF			100	10	100	10	10	10	10	100	2.00	3.00	3.00
RAC [µg/L]			3.12	20	0.18	0.44	0.55	6.3	88.3	12.55	0.4	0.53	2.5
Step 3	**PEC$_{sw\ max}$ [µg/L]**	**PEC$_{sed\ max}$ [µg/Kg]**	**PEC/RAC**										
Single applications													
D3/ditch	23.9	60.4	**7.7**	**1.2**	**132.8**	**54.3**	**43.5**	**3.8**	0.7	**1.9**	**59.8**	**44.8**	**9.6**
D4/pond	1.0	5.5	0.3	0.1	**5.8**	**2.4**	**1.9**	0.2	0.1	0.1	**2.6**	**2.0**	0.4
D4/stream	23.4	33.4	**7.5**	**1.2**	**129.9**	**53.2**	**42.5**	**3.7**	0.4	**1.9**	**58.5**	**43.9**	**9.4**
D5/pond	1.0	5.2	0.3	0.1	**5.8**	**2.4**	**1.9**	0.2	0.1	0.1	**2.6**	**2.0**	0.4
D5/stream	25.9	51.2	**8.3**	**1.3**	**143.7**	**58.8**	**47.0**	**4.1**	0.6	**2.1**	**64.7**	**48.5**	**10.3**
R1/pond	1.0	5.4	0.3	0.1	**5.8**	**2.4**	**1.9**	0.2	0.1	0.1	**2.6**	**2.0**	0.4
R1/stream	18.3	32.0	**5.9**	0.9	**101.7**	**41.6**	**33.3**	**2.9**	0.4	**1.5**	**45.8**	**34.3**	**7.3**
R2/stream	24.6	28.6	**7.9**	**1.2**	**136.5**	**55.8**	**44.7**	**3.9**	0.3	**2.0**	**61.4**	**46.1**	**9.8**
R3/stream	25.9	47.8	**8.3**	**1.3**	**143.6**	**58.8**	**47.0**	**4.1**	0.5	**2.1**	**64.6**	**48.5**	**10.3**
R4/stream	18.3	31.0	**5.9**	0.9	**101.7**	**41.6**	**33.3**	**2.9**	0.4	**1.5**	**45.8**	**34.3**	**7.3**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 2.9.9.2-4: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the active substance Dodine for each organism group based on FOCUS Steps 3 calculations for the use of Dodine 544 SC in cherry (2 × 0.68 kg a.s./ha, Pome/stone fruit, summer application)**

Group			**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophytes**	**Sed. dwell. prolonged**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**
Test species			***Cyprinus carpio***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Raphidocelis subcapitata***	***Lemna gibba***	***Chironomus riparius***	**Fish acute**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC	Geomean LC_{50}	Effect class 2 NOEC	lower Effect class 3A NOEAEC	higher Effect class 3A NOEAEC
[µg/L]			312	200	18	4.4	5.5	63	883	1255	0.8	1.6	7.5
AF			100	10	100	10	10	10	10	100	2.00	3.00	3.00
RAC [µg/L]			3.12	20	0.18	0.44	0.55	6.3	88.3	12.55	0.4	0.53	2.5
Step 3	**$PEC_{sw\ max}$ [µg/L]**	**$PEC_{sed\ max}$ [µg/Kg]**	**PEC/RAC**										
Single applications													
D3/ditch	23.9	60.9	**7.7**	**1.2**	**132.6**	**54.3**	**43.4**	**3.8**	0.7	**1.9**	**59.7**	**44.8**	**9.5**
D4/pond	1.0	5.4	0.3	0.1	**5.8**	**2.4**	**1.9**	0.2	0.1	0.1	**2.6**	**2.0**	0.4
D4/stream	23.9	43.2	**7.7**	**1.2**	**132.9**	**54.4**	**43.5**	**3.8**	0.5	**1.9**	**59.8**	**44.9**	**9.6**
D5/pond	1.0	5.9	0.3	0.1	**5.8**	**2.4**	**1.9**	0.2	0.1	0.1	**2.6**	**2.0**	0.4
D5/stream	25.9	51.9	8.3	**1.3**	143.6	58.8	47.0	**4.1**	0.6	**2.1**	**64.6**	**48.5**	**10.3**
R1/pond	1.0	5.5	0.3	0.1	**5.8**	**2.4**	**1.9**	0.2	0.1	0.1	**2.6**	**2.0**	0.4
R1/stream	18.3	32.1	**5.9**	0.9	**101.7**	**41.6**	**33.3**	**2.9**	0.4	**1.5**	**45.8**	**34.3**	**7.3**
R2/stream	24.6	28.6	**7.9**	**1.2**	**136.5**	**55.8**	**44.7**	**3.9**	0.3	**2.0**	**61.4**	**46.1**	**9.8**
R3/stream	25.7	46.5	**8.2**	**1.3**	**142.6**	**58.3**	**46.7**	**4.1**	0.5	**2.0**	**64.2**	**48.1**	**10.3**
R4/stream	17.9	24.2	**5.7**	0.9	**99.4**	**40.7**	**32.5**	**2.8**	0.3	**1.4**	**44.7**	**33.5**	**7.2**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 2.9.9.2-5: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the active substance Dodine for each organism group based on FOCUS Steps 3 calculations for the use of Dodine 544 SC in cherry (2 × 0.68 kg a.s./ha, Pome/stone fruit, late, late application)**

Group			**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophytes**	**Sed. dwell. prolonged**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**
Test species			***Cyprinus carpio***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Raphidocelis subcapitata***	***Lemna gibba***	***Chironomus riparius***	**Fish acute**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC	Geomean LC_{50}	Effect class 2 NOEC	lower Effect class 3A NOEAEC	higher Effect class 3A NOEAEC
[µg/L]			312	200	18	4.4	5.5	63	883	1255	0.8	1.6	7.5
AF			100	10	100	10	10	10	10	100	2.00	3.00	3.00
RAC [µg/L]			3.12	20	0.18	0.44	0.55	6.3	88.3	12.55	0.4	0.53	2.5
Step 3	**$PEC_{sw\ max}$ [µg/L]**	**$PEC_{sed\ max}$ [µg/Kg]**	**PEC/RAC**										
Single applications													
D3/ditch	23.9	59.4	**7.7**	**1.2**	**132.7**	**54.3**	**43.4**	**3.8**	0.7	**1.9**	**59.7**	**44.8**	**9.6**
D4/pond	1.0	5.4	0.3	0.1	**5.8**	**2.4**	**1.9**	0.2	0.1	0.1	**2.6**	**2.0**	0.4
D4/stream	24.0	44.1	**7.7**	**1.2**	**133.1**	**54.5**	**43.6**	**3.8**	0.5	**1.9**	**59.9**	**44.9**	**9.6**
D5/pond	1.0	5.6	0.3	0.1	**5.8**	**2.4**	**1.9**	0.2	0.1	0.1	**2.6**	**2.0**	0.4
D5/stream	25.9	51.8	**8.3**	**1.3**	**143.7**	**58.8**	**47.0**	**4.1**	0.6	**2.1**	**64.7**	**48.5**	**10.3**
R1/pond	1.0	5.3	0.3	0.1	**5.8**	**2.4**	**1.9**	0.2	0.1	0.1	**2.6**	**2.0**	0.4
R1/stream	17.9	24.9	**5.8**	0.9	**99.7**	**40.8**	**32.6**	**2.8**	0.3	**1.4**	**44.9**	**33.6**	**7.2**
R2/stream	24.6	28.7	**7.9**	**1.2**	**136.5**	**55.8**	**44.7**	**3.9**	0.3	**2.0**	**61.4**	**46.1**	**9.8**
R3/stream	25.9	49.8	**8.3**	**1.3**	**143.6**	**58.8**	**47.0**	**4.1**	0.6	**2.1**	**64.6**	**48.5**	**10.3**
R4/stream	18.3	31.7	**5.9**	0.9	**101.7**	**41.6**	**33.3**	**2.9**	0.4	**1.5**	**45.8**	**34.3**	**7.3**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 2.9.9.2-6: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the active substance Dodine for each organism group based on FOCUS Steps 3 calculations for the use of Dodine 544 SC in cherry (2 × 0.68 kg a.s./ha, Pome/stone fruit, early*, post-harvest application)**

Group			Fish acute	Fish prolonged	Inverteb. acute	Invert eb. prolonged	Algae	Aquatic macrophytes	Sed. dwell. prolonged	Higher-tier information	Higher-tier information	Higher-tier information	Higher-tier information
Test species			***Cyprinus carpio***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Raphidocelis subcapitata***	***Lemna gibba***	***Chironomus riparius***	**Fish acute**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC	Geomean LC_{50}	Effect class 2 NOEC	lower Effect class 3A NOEAEC	higher Effect class 3A NOEAEC
[µg/L]			312	200	18	4.4	5.5	63	883	1255	0.8	1.6	7.5
AF			100	10	100	10	10	10	10	100	2.00	3.00	3.00
RAC [µg/L]			3.12	20	0.18	0.44	0.55	6.3	88.3	12.55	0.4	0.53	2.5
Step 3	**$PEC_{sw\ max}$ [µg/L]**	**$PEC_{sed\ max}$ [µg/Kg]**	**PEC/RAC**										
Single applications													
D3/ditch	50.7	136.7	**16.2**	**2.5**	**281.6**	**115.2**	**92.1**	**8.0**	**1.5**	**4.0**	**126.7**	**95.0**	**20.3**
D4/pond	3.0	21.5	**1.0**	0.2	**16.8**	**6.9**	**5.5**	0.5	0.2	0.2	**7.6**	**5.7**	**1.2**
D4/stream	52.6	72.5	**16.8**	**2.6**	**292.0**	**119.5**	**95.6**	**8.3**	0.8	**4.2**	**131.4**	**98.6**	**21.0**
D5/pond	3.0	19.5	**1.0**	0.2	**16.8**	**6.9**	**5.5**	0.5	0.2	0.2	**7.6**	**5.7**	**1.2**
D5/stream	58.2	115.8	**18.7**	**2.9**	**323.6**	**132.4**	**105.9**	**9.2**	**1.3**	**4.6**	**145.6**	**109.2**	**23.3**
R1/pond	3.0	21.5	**1.0**	0.2	**16.8**	**6.9**	**5.5**	0.5	0.2	0.2	**7.6**	**5.7**	**1.2**
R1/stream	41.2	71.1	**13.2**	**2.1**	**229.0**	**93.7**	**74.9**	**6.5**	0.8	**3.3**	**103.1**	**77.3**	**16.5**
R2/stream	54.8	59.1	**17.6**	**2.7**	**304.4**	**124.5**	**99.6**	**8.7**	0.7	**4.4**	**137.0**	**102.7**	**21.9**
R3/stream	58.2	112.2	**18.7**	**2.9**	**323.3**	**132.3**	**105.8**	**9.2**	**1.3**	**4.6**	**145.5**	**109.1**	**23.3**
R4/stream	41.2	70.5	**13.2**	**2.1**	**229.0**	**93.7**	**74.9**	**6.5**	0.8	**3.3**	**103.1**	**77.3**	**16.5**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
PEC/RAC ratios above the relevant trigger of 1 are shown in bold

* In SWASH the user needs to select the crop on which the compound is intended to be used. As spray drift deposition varies considerably for fruit trees and vines, a distinction has been made between their early and late crop growth stage, representing respectively a growth stage with no or few leaves and a growth stage in which the leaves are well developed. The distinction between early and late references is made to the BBCH–codes as mentioned in Table 2.4.2-1 of SW GD. Late applications are only defined up to BBCH 89. However, post-harvest application are made between BBCH 90 and 99, from 50% leaf falling till after leaf falling (autumn). Therefore, in line with groundwater risk assessment, the same drift as an early application should be applied. Consequently, RMS considers that pome/stone fruit early applications FOCUS SW crop should be selected for modelling post-harvest uses.

Table 2.9.9.2-7: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the active substance Dodine for each organism group based on FOCUS Steps 3 calculations for the use of Dodine 544 SC in <u>peach</u> (2 × 0.9 kg a.s./ha, Pome/stone fruit, <u>early application</u>)**

Group			**Fish acute**	**Fish prolonged**	**Invert eb. acute**	**Invert eb. prolonged**	**Algae**	**Aquatic macrophytes**	**Sed. dwell. prolonged**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**
Test species			***Cyprinus carpio***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Raphidocelis subcapitata***	***Lemna gibba***	***Chironomus riparius***	**Fish acute**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC	Geomean LC_{50}	Effect class 2 NOEC	lower Effect class 3A NOEAEC	higher Effect class 3A NOEAEC
[µg/L]			312	200	18	4.4	5.5	63	883	1255	0.8	1.6	7.5
AF			100	10	100	10	10	10	10	100	2.00	3.00	3.00
RAC [µg/L]			3.12	20	0.18	0.44	0.55	6.3	88.3	12.55	0.4	0.53	2.5
Step 3	**$PEC_{sw\ max}$ [µg/L]**	**$PEC_{sed\ max}$ [µg/Kg]**	**PEC/RAC**										
Single applications													
D3/ditch	67.1	172.9	**21.5**	**3.4**	**372.8**	**152.5**	**122.0**	**10.7**	**2.0**	**5.3**	**167.8**	**125.8**	**26.8**
D4/pond	4.0	28.2	**1.3**	0.2	**22.3**	**9.1**	**7.3**	0.6	0.3	0.3	**10.0**	**7.5**	**1.6**
D4/stream	63.4	47.1	**20.3**	**3.2**	**352.4**	**144.2**	**115.3**	**10.1**	0.5	**5.1**	**158.6**	**119.0**	**25.4**
D5/pond	4.0	25.1	**1.3**	0.2	**22.3**	**9.1**	**7.3**	0.6	0.3	0.3	**10.0**	**7.5**	**1.6**
D5/stream	66.6	44.3	**21.3**	**3.3**	**369.8**	**151.3**	**121.0**	**10.6**	0.5	**5.3**	**166.4**	**124.8**	**26.6**
R1/pond	4.0	25.3	**1.3**	0.2	**22.3**	**9.1**	**7.3**	0.6	0.3	0.3	**10.0**	**7.5**	**1.6**
R1/stream	54.2	82.6	**17.4**	**2.7**	**301.2**	**123.2**	**98.6**	**8.6**	0.9	**4.3**	**135.5**	**101.6**	**21.7**
R2/stream	71.9	71.8	**23.0**	**3.6**	**399.5**	**163.4**	**130.7**	**11.4**	0.8	**5.7**	**179.8**	**134.8**	**28.8**
R3/stream	76.8	140.1	**24.6**	**3.8**	**426.8**	**174.6**	**139.7**	**12.2**	**1.6**	**6.1**	**192.1**	**144.1**	**30.7**
R4/stream	54.2	83.0	**17.4**	**2.7**	**301.3**	**123.3**	**98.6**	**8.6**	0.9	**4.3**	**135.6**	**101.7**	**21.7**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 2.9.9.2-8: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the active substance Dodine for each organism group based on FOCUS Steps 3 calculations for the use of Dodine 544 SC in <u>peach</u> (2 × 0.9 kg a.s./ha, Pome/stone fruit, <u>late application</u>)**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration

Group			**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophytes**	**Sed. dwell. prolonged**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**
Test species			***Cyprinus carpio***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Raphidocelis subcapitata***	***Lemna gibba***	***Chironomus riparius***	**Fish acute**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC	Geomean LC_{50}	Effect class 2 NOEC	lower Effect class 3A NOEAEC	higher Effect class 3A NOEAEC
[µg/L]			312	200	18	4.4	5.5	63	883	1255	0.8	1.6	7.5
AF			100	10	100	10	10	10	10	100	2.00	3.00	3.00
RAC [µg/L]			3.12	20	0.18	0.44	0.55	6.3	88.3	12.55	0.4	0.53	2.5
Step 3	**$PEC_{sw\ max}$ [µg/L]**	**$PEC_{sed\ max}$ [µg/Kg]**	**PEC/RAC**										
Single applications													
D3/ditch	31.6	79.9	**10.1**	**1.6**	**175.8**	**71.9**	**57.5**	**5.0**	0.9	**2.5**	**79.1**	**59.3**	**12.7**
D4/pond	1.4	7.6	0.4	0.1	**7.7**	**3.2**	**2.5**	0.2	0.1	0.1	**3.5**	**2.6**	0.6
D4/stream	30.6	38.7	**9.8**	**1.5**	**170.1**	**69.6**	**55.7**	**4.9**	0.4	**2.4**	**76.6**	**57.4**	**12.2**
D5/pond	1.4	7.7	0.4	0.1	**7.7**	**3.2**	**2.5**	0.2	0.1	0.1	**3.5**	**2.6**	0.6
D5/stream	32.4	36.8	**10.4**	**1.6**	**180.1**	**73.7**	**58.9**	**5.1**	0.4	**2.6**	**81.0**	**60.8**	**13.0**
R1/pond	1.4	7.3	0.4	0.1	**7.7**	**3.2**	**2.5**	0.2	0.1	0.1	**3.5**	**2.6**	0.6
R1/stream	24.2	41.2	**7.8**	**1.2**	**134.6**	**55.0**	**44.0**	**3.8**	0.5	**1.9**	**60.6**	**45.4**	**9.7**
R2/stream	32.6	37.5	**10.4**	**1.6**	**180.9**	**74.0**	**59.2**	**5.2**	0.4	**2.6**	**81.4**	**61.1**	**13.0**
R3/stream	34.0	60.8	**10.9**	**1.7**	**189.0**	**77.3**	**61.9**	**5.4**	0.7	**2.7**	**85.1**	**63.8**	**13.6**
R4/stream	23.7	31.7	**7.6**	**1.2**	**131.7**	**53.9**	**43.1**	**3.8**	0.4	**1.9**	**59.3**	**44.5**	**9.5**

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 2.9.9.2-9: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for the active substance Dodine for each organism group based on FOCUS Steps 3 calculations for the use of Dodine 544 SC in <u>peach</u> (2 × 0.9 kg a.s./ha, Pome/stone fruit, early*, <u>post-harvest application</u>)**

Group			**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophytes**	**Sed. dwell. prolonged**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**	**Higher-tier information**
Test species			***Cyprinus carpio***	***Pimephales promelas***	***Daphnia magna***	***Daphnia magna***	***Raphidocelis subcapitata***	***Lemna gibba***	***Chironomus riparius***	**Fish acute**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**	**Aquatic invertebrates, algae & aquatic plants**
Endpoint			LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	E_rC_{50}	NOEC	Geomean LC_{50}	Effect class 2 NOEC	lower Effect class 3A NOEAEC	higher Effect class 3A NOEAEC
[µg/L]			312	200	18	4.4	5.5	63	883	1255	0.8	1.6	7.5
AF			100	10	100	10	10	10	10	100	2.00	3.00	3.00
RAC [µg/L]			3.12	20	0.18	0.44	0.55	6.3	88.3	12.55	0.4	0.53	2.5
Step 3	**$PEC_{sw\ max}$ [µg/L]**	**$PEC_{sed\ max}$ [µg/Kg]**	**PEC/RAC**										
Single applications													
D3/ditch	67.2	179.2	**21.5**	**3.4**	**373.2**	**152.7**	**122.1**	**10.7**	**2.0**	**5.5**	**167.9**	**125.9**	**26.9**
D4/pond	4.0	28.2	**1.3**	0.2	**22.3**	**9.1**	**7.3**	0.6	0.3	0.3	**10.0**	**7.5**	**1.6**
D4/stream	69.7	94.8	**22.3**	**3.5**	**386.9**	**158.3**	**126.6**	**11.1**	**1.1**	**5.7**	**174.1**	**130.6**	**27.9**
D5/pond	4.0	25.5	**1.3**	0.2	**22.3**	**9.1**	**7.3**	0.6	0.3	0.3	**10.0**	**7.5**	**1.6**
D5/stream	77.2	151.4	**24.7**	**3.9**	**428.8**	**175.4**	**140.3**	**12.3**	**1.7**	**6.3**	**193.0**	**144.7**	**30.9**
R1/pond	4.0	28.2	**1.3**	0.2	**22.3**	**9.1**	**7.3**	0.6	0.3	0.3	**10.0**	**7.5**	**1.6**
R1/stream	54.6	92.9	**17.5**	**2.7**	**303.6**	**124.2**	**99.3**	**8.7**	**1.1**	**4.5**	**136.6**	**102.5**	**21.9**
R2/stream	72.6	77.4	**23.3**	**3.6**	**403.4**	**165.0**	**132.0**	**11.5**	0.9	**5.9**	**181.5**	**136.1**	**29.0**
R3/stream	77.1	146.8	**24.7**	**3.9**	**428.6**	**175.3**	**140.3**	**12.2**	**1.7**	**6.3**	**192.9**	**144.6**	**30.9**
R4/stream	54.6	92.2	**17.5**	**2.7**	**303.5**	**124.2**	**99.3**	**8.7**	**1.0**	**4.5**	**136.6**	**102.4**	**21.9**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration
PEC/RAC ratios above the relevant trigger of 1 are shown in bold

* In SWASH the user needs to select the crop on which the compound is intended to be used. As spray drift deposition varies considerably for fruit trees and vines, a distinction has been made between their early and late crop growth stage, representing respectively a growth stage with no or few leaves and a growth stage in which the leaves are well developed. The distinction between early and late references is made to the BBCH–codes as mentioned in Table 2.4.2-1 of SW GD. Late applications are only defined up to BBCH 89. However, post-harvest application are made between BBCH 90 and 99, from 50% leaf falling till after leaf falling (autumn). Therefore, in line with groundwater risk assessment, the same drift as an early application should be applied. Consequently, RMS considers that pome/stone fruit early applications FOCUS SW crop should be selected for modelling post-harvest uses.

Risk refinements (STEP 4)

Risk refinements based on FOCUS STEP 4 calculations of all scenarios of all intended uses were presented al Vol 3CP B9.4. Single applications led the worst-case PECsw values respect to multiple applications. Then, as conservative approach, only the PEC/RAC results from this application patter was considered for the selection of the mitigation measures at FOCUS STEP 4. In this sense, the following conclusions were reached:

- For the intended used in **Apple/pear** (2 × 0.68 kg a.s./ha):
  - ***Early applications***: no unacceptable risk for aquatic organisms can be concluded for:
    - when ETO-RAC is considered: scenarios D4 pond, D5 pond and R1 pond applying 20 m no-spray buffer zone + 90% drift reduction or 50 m no-spray buffer zone.
    - When ERO-RAC is applied: scenarios D4 pond, D5 pond and R1 pond applying 25 m no-spray buffer zone. All scenarios if 20 m no-spray buffer zone + 90% drift reduction or 50 m no-spray buffer zone are implemented.
  - ***Late applications***: no unacceptable risk for aquatic organisms can be concluded for:
    - when ETO-RAC is considered: scenarios D4 pond, D5 pond and R1 pond applying 25 m no-spray buffer zone. Scenarios R1 stream and R4 stream applying 50 m no-spray buffer zone. All scenarios if 20 m no-spray buffer zone + 90% drift reduction are implemented.
    - When ERO-RAC is applied: all scenarios applying 25 m no-spray buffer zone or 20 m no-spray buffer zone + 90% drift reduction.
- For the intended used in **Cherry** (2 × 0.68 kg a.s./ha):
  - ***Summer applications***: no unacceptable risk for aquatic organisms can be concluded for:
    - when ETO-RAC is considered: scenarios D4 pond, D5 pond and R1 pond applying 25 m no-spray. Scenarios R1 stream and R4 stream applying 50 m no-spray buffer zone. All scenarios if 20 m no-spray buffer zone + 90% drift reduction are implemented.
    - When ERO-RAC is applied: all scenarios if 25 m no-spray buffer zone or 20 m no-spray buffer zone + 90% drift reduction are implemented.
  - ***Late applications***: no unacceptable risk for aquatic organisms can be concluded for:
    - when ETO-RAC is considered: scenarios D4 pond, D5 pond and R1 pond applying 25 m no-spray buffer zone. Scenarios R1 stream and R4 stream applying 50 m no-spray buffer zone. All scenarios if 20 m no-spray buffer zone + 90% drift reduction are implemented.
    - When ERO-RAC is applied: all scenarios applying 25 m no-spray buffer zone or 20 m no-spray buffer zone + 90% drift reduction.
  - ***Post-harvest applications***: no unacceptable risk for aquatic organisms can be concluded for:
    - when ETO-RAC is considered: scenarios D4 pond, D5 pond and R1 pond applying 50 m no-spray buffer zone or 20 m no-spray buffer zone + 90% drift reduction are implemented.

    RMS noted that the recovery option for refining the risk is not an adequate approach for post-harvest applications.
- For the intended used in **Peach** (2 × 0.9 kg a.s./ha):
  - ***Early applications***: no unacceptable risk for aquatic organisms can be concluded for:
    - when ETO-RAC is considered: scenarios D4 pond, D5 pond and R1 pond applying 20 m no-spray buffer zone + 90% drift reduction 50 m no-spray buffer zone.
    - When ERO-RAC is applied: scenarios D4 pond, D5 pond and R1 pond applying 25 m no-spray buffer zone. All scenarios if 20 m no-spray buffer zone + 90% drift reduction are implemented.
  - ***Late applications***: no unacceptable risk for aquatic organisms can be concluded for:
    - when ETO-RAC is considered: scenarios D4 pond, D5 pond and R1 pond applying 25 m no-spray buffer zone. All scenarios if 20 m no-spray buffer zone + 90% drift reduction are implemented.

- When ERO-RAC is applied: all scenarios applying 25 m no-spray buffer zone or 20 m no-spray buffer zone + 90% drift reduction.

- ***Post-harvest applications***: no unacceptable risk for aquatic organisms can be concluded for:
  - when ETO-RAC is considered: scenarios D4 pond, D5 pond and R1 pond applying 20 m no-spray buffer zone + 90% drift reduction 50 m no-spray buffer zone.

RMS noted that the recovery option for refining the risk is not an adequate approach for post-harvest applications.

The risk mitigation measures consisting in « 50 m no-spray buffer zone » or « 20 m no-spray buffer zone + 90% drift reduction » are beyond the 95% limit recommended by the FOCUS landscape and mitigation guidance (FOCUS, 2007; SANCO/10422/2005, version 2.0, September 2007). For these mitigation measures, **supporting evidence of their efficacy reducing drift should be provided. The applicability of each particular mitigation measures should be assessed at the Member State level.**

Evaluation of exposure profiles

However, before a definitive conclusion on the risk acceptability can be drawn, there is a need to consider whether the exposure profiles in the mesocosm study of [redacted] (2021b) are broadly comparable to those predicted in the field. The exposure pattern in the mesocosm study was compared with FOCUS exposure profile predictions to verify whether the mesocosm study is a realistic worst case in terms of exposure of the representative uses (i.e. application to apple/pear, cherry or peach). In this sense, a visual assessment of the overlaying graphs was performed based on envelope curve concept for the exposure scenarios where PECsw(at FOCUS Step 3 or Step 4)/RAC were <1 (safe use). After visual assessment, not all simulated events were covered by the exposure profile of the mesocosms, then higher risk mitigation measures were proposed by the RMS (see overall conclusion table below). For pond scenarios D4p, D5p and R1p for FOCUS step 3 (analysis based on ERO) and step 4 considering 25 m BZ (anlaysis based on ETO) risk mitigation measures were proposed by the RMS in order to obtain maximum PECsw values below Tier 1-RAC. For R4s scenarios, peaks of runoff were not covered by the mesocosm exposure profile when ETO-RAC is considered, consequently, a 20 m of vegetated filter strip zone was applied to these scenarios.

Nevertheless, it was concluded that the mesocosm study showed an exposure profile similar to that shown by initial predicted concentrations from the FOCUS modelling, characterized by an initial peak following spray drift and a rapid decline in the water phase. Therefore, the exposure regime tested in the mesocosms is more or less realistic to worst case relative to the predicted exposure profiles for the different FOCUS scenarios.

Moreover, the exposure period above the ETO-RAC and the time needed for recovery were assessed, as it is also necessary to demonstrate that an acceptable time-course for recovery within 8 weeks can be expected to demostrate an acceptable risk using the ERO-RAC (protection goal according EFSA 2013 Guidance). The duration of the potential total effect period of each scenario, considering the recovery period determined in the mesocosm study of [redacted] (2021b) for the most sensitive population (3 weeks after the second application for *Chroomonas acuta*) were checked. EPAT analyses were run for scenarios where there were exceedances of ETO-RAC, but PECsw (at FOCUS Step 4)/RAC were <1 considering ERO-RAC. The exposure period above the ETO-RAC, for the corresponding exposure scenario assessed, was shorter that the time needed for recovery derived from the mesocosms (see Vol 3CP B9.4.4 for further details). Therefore, the provisional ERO-RAC can be considered as a **definitive ERO-RAC**.

After the risk assessment based on exposure profiles, the risk for aquiatic organisms after a two-fold application of Dodine 544 SC on apples/pear, cherry and peach can be considered acceptable if the mitigation measures reported in the following table are implemented. Unacceptable risk for aquatic organisms was identified for post-harvest applciations on cherry and peach.

Table 2.9.9.2-10. Overview of risk mitigation measures required for each relevant exposure scenario following a two-fold application of Dodine 544 SC on apples/pear, cherry and peach.

Intended use	Exposure scenario									
	D3 ditch	**D4 pond**	**D4 stream**	**D5 pond**	**D5 stream**	**R1 pond**	**R1 stream**	**R2 stream**	**R3 stream**	**R4 stream**
Apples/ pear (early applic.)	50 m NSB* or 20 m NSB + 90% DRN*	25 m NSB*	50 m NSB* or 20 m NSB + 90% DRN*	25 m NSB*	50 m NSB* or 20 m NSB + 90% DRN*	25 m NSB*	50 m NSB* or 20 m NSB + 90% DRN*	50 m NSB* or 20 m NSB + 90% DRN*	50 m NSB* or 20 m NSB + 90% DRN*	50 m NSB* or 20 m NSB + 90% DRN*
Apples/ pear (late applic.)	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 50 m NSB or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 50 m BZ including 20 m VFS or 20 m VFS + 90% DRN
Cherry (summer applic.)	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 50 m NSB or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 50 m BZ including 20 m VFS or 20 m VFS + 90% DRN
Cherry (late applic.)	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 50 m NSB or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 50 m BZ including 20 m VFS or 20 m VFS + 90% DRN
Cherry (post-harvest applic.)	Analysis based on ETO-RAC: No safe use identified Analysis based on ERO-RAC: Application time not covered by the mesocosm.									
Peach (early applic.)	50 m NSB* or 20 m NSB + 90% DRN*	25 m NSB*	50 m NSB* or 20 m NSB + 90% DRN*	25 m NSB*	50 m NSB* or 20 m NSB + 90% DRN*	25 m NSB*	50 m NSB* or 20 m NSB + 90% DRN*	50 m NSB* or 20 m NSB + 90% DRN*	50 m NSB* or 20 m NSB + 90% DRN*	50 m NSB* or 20 m NSB + 90% DRN*
Peach (late applic.)	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN	25 m NSB* or 20 m NSB + 90% DRN
Peach (post-harvest applic.)	Analysis based on ETO-RAC: No safe use identified Analysis based on ERO-RAC: Application time not covered by the mesocosm.									

NSB: no-spray buffer zone, DRN: drift reduction/drift reducing nozzles

*mitigation measures derived from the use of ERO-RAC=2.5 µg/L.

Note: FOCUS Step 4 calculations with spray drift mitigations have been performed for all the representative uses. FOCUS landscape and mitigation guidance advises that the maximum acceptable reduction in spray drift using any combination of mitigation techniques is 95%. For the proposed uses considered in the Step 4 calculations, when possible, two options are presented, one based on mitigations according to FOCUS L&M GD (2007) and other based on mitigations greater than the ceiling of 95%. **Applicant is requested to demonstrate that a drift reduction above 95% is possible under the proposed conditions of use.** Moreover, the choice, combination and appropriateness of available mitigation measures (e.g. drift-reducing nozzles, (vegetated) buffers etc.) should finally be decided at member state level.

2.9.9.3 Summary of risk assessment for bees

The evaluation of the risk for bees was performed in accordance with the recommendations of EFSA (2013)[8], although the guidance document is not yet approved, and certain parts are currently under revision. No risk assessment for bumble bees and solitary bees is performed as recommended in EFSA (2015)[9]. Toxicity endpoints from available studies for honey bees are summarized in the **Table 2.9.3.1-1.**

The acute risk to honeybees by oral and contact exposure following all representative uses of Dodine 544 SC in orchards was calculated to be acceptable at screening level.

The chronic risk to adult honeybees following all representative uses of Dodine 544 SC in orchards was calculated to be unacceptable at Tier 1 for all relevant exposure scenarios except for the treated crop scenario at BBCH ≥ 70.

The chronic risk to honeybee larvae following the representative uses in apples/pear and cherry was calculated to be unacceptable at Tier 1 for all relevant exposure scenarios except for the treated crop scenario at BBCH 10-19, BBCH 20-39 and BCH 40-69 and the weeds scenario at BBCH < 10. For the intended uses in peach, the chronic risk to honeybee larvae is calculated to be unacceptable at Tier 1 for all relevant exposure scenarios except for the treated crop scenario at BBCH 10-19, BBCH 20-39 and BCH 40-69 and the weeds scenario at BBCH < 10 and BBCH 10-19.

An indicative risk assessment was also performed for bumblebees and solitary bees by RMS, by means of an assessment factor of 10 to extrapolate from honeybee endpoints to endpoints for bumblebees and solitary bees.

At Tier 1, acute and chronic risk cannot be excluded for both bumblebees and solitary bees.

Risk assessment for apples/pear and cherry at 2 × 680 g a.s./ha

Table 2.9.9.3-1: Apples/pear, cherry (2 × 680 g a.s./ha) – Screening assessment of the acute contact risk to bees

Test design	Type of bee	LD50 (lab.) [µg a.s./bee]	Single application rate [g a.s./ha]	Application technique	HQcontact	Trigger value
Contact toxicity	Honeybee	> 100	680	Sideward spraying	<6.8	85
	Bumble bee	> 10			**<68**	7
	Solitary bee	> 10			**<68**	8

HQcontact: Hazard quotients for contact exposure. HQ value shown in bold breach the relevant trigger; HQ value < trigger value, indicate an acceptable risk for bees

Table 2.9.9.3-2: Apples/pear, cherry (2 × 680 g a.s./ha) – Screening assessment of the oral toxicity risk to bees

Type of assessment	Type of bee	LD50 (lab.) [µg a.s./bee]	Single application rate [kg a.s./ha]	SV	ETRacute adult oral	Trigger value
Acute oral exposure adult bees	Honeybee	> 55.7	0.68	10.6	<0.13	0.2
	Bumble bee	> 5.57		13.3	**<1.62**	0.036
	Solitary bee	> 5.57		7.3	**<0.89**	0.04
Chronic oral exposure adult bees	Honeybee	1.86	0.68	10.6	**3.875**	0.03
	Bumble bee	0.186		13.3	**48.624**	0.0048
	Solitary bee	0.186		7.3	**26.688**	0.0054
Oral risk to bee larvae	Honeybee	5.7	0.68	6.1	**0.73**	0.2
	Bumble bee	0.57		26	**31.02**	0.2
	Solitary bee	0.57		30.8	**36.74**	0.2

ETRacute oral adult value < trigger value indicates an acceptable risk for bees; SV: Short-cut value for the respective kind of application, application made via sideward spraying

[8] EFSA, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295.

[9] EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924.

Table 2.9.9.3-3: Apples/pear, cherry (2 × 680 g a.s./ha) – First-tier assessment of the acute contact risk to bees

scenario	BBCH	Honeybee		Bumble bee		Solitary bee	
		HQ	trigger	HQ	trigger	HQ	trigger
treated crop	< 10	<6.8	85	<**68.0**	14	<**68.0**	16
treated crop	10 - 19	<6.8	85	<**68.0**	14	<**68.0**	16
treated crop	20 - 39	<6.8	85	<**68.0**	14	<**68.0**	16
treated crop	≥ 40	<6.8	85	<**68.0**	14	<**68.0**	16
weeds	< 10	<6.8	42	<**68.0**	7	<**68.0**	8
weeds	10 - 19	<5.4	42	<**54.4**	7	<**54.4**	8
weeds	20 - 39	<4.1	42	<**40.8**	7	<**40.8**	8
weeds	≥ 40	<2.0	42	<**20.4**	7	<**20.4**	8
field margin	< 10	<2.0	42	<**19.9**	7	<**19.9**	8
field margin	10 - 19	<2.0	42	<**19.9**	7	<**19.9**	8
field margin	20 - 39	<2.0	42	<**19.9**	7	<**19.9**	8
field margin	≥ 40	<2.0	42	<**19.9**	7	<**19.9**	8

Table 2.9.9.3-4: Apples/pear, cherry (2 × 680 g a.s./ha) – First-tier assessment of the acute oral risk to bees

category	scenario	BBCH	Honeybee		Bumble bee		Solitary bee	
			ETR	trigger	ETR	trigger	ETR	trigger
acute	treated crop	< 10	0.01	0.2	**0.11**	0.036	**0.06**	0.04
acute	treated crop	10 - 19	0.13	0.2	**1.62**	0.036	**0.89**	0.04
acute	treated crop	20 - 39	0.13	0.2	**1.62**	0.036	**0.89**	0.04
acute	treated crop	40 - 69	0.13	0.2	**1.62**	0.036	**0.89**	0.04
acute	treated crop	≥ 70	0.00	0.2	0.00	0.036	0.00	0.04
acute	weeds	< 10	0.05	0.2	**0.79**	0.036	**0.28**	0.04
acute	weeds	10 - 19	0.04	0.2	**0.63**	0.036	**0.22**	0.04
acute	weeds	20 - 39	0.03	0.2	**0.48**	0.036	**0.17**	0.04
acute	weeds	40 - 69	0.01	0.2	**0.24**	0.036	**0.08**	0.04
acute	weeds	≥ 70	0.01	0.2	**0.24**	0.036	**0.08**	0.04
acute	field margin	< 10	0.00	0.2	**0.08**	0.036	0.03	0.04
acute	field margin	10 - 19	0.00	0.2	**0.08**	0.036	0.03	0.04
acute	field margin	20 - 39	0.00	0.2	**0.08**	0.036	0.03	0.04
acute	field margin	40 - 69	0.00	0.2	**0.08**	0.036	0.03	0.04
acute	field margin	≥ 70	0.00	0.2	**0.08**	0.036	0.03	0.04
acute	adjacent crop	< 10	0.01	0.2	**0.09**	0.036	**0.05**	0.04
acute	adjacent crop	10 - 19	0.01	0.2	**0.09**	0.036	**0.05**	0.04
acute	adjacent crop	20 - 39	0.01	0.2	**0.09**	0.036	**0.05**	0.04
acute	adjacent crop	40 - 69	0.01	0.2	**0.09**	0.036	**0.05**	0.04
acute	adjacent crop	≥ 70	0.01	0.2	**0.09**	0.036	**0.05**	0.04
acute	next crop	< 10	0.01	0.2	**0.11**	0.036	**0.06**	0.04
acute	next crop	10 - 19	0.01	0.2	**0.11**	0.036	**0.06**	0.04
acute	next crop	20 - 39	0.01	0.2	**0.11**	0.036	**0.06**	0.04
acute	next crop	40 - 69	0.01	0.2	**0.11**	0.036	**0.06**	0.04
acute	next crop	≥ 70	0.01	0.2	**0.11**	0.036	**0.06**	0.04

A Table 2.9.9.3-5: Apples/pear, cherry (2 × 680 g a.s./ha) – First-tier assessment of the chronic oral risk to bees

category	scenario	BBCH	Honeybee		Bumble bee		Solitary bee	
			ETR	trigger	ETR	trigger	ETR	trigger

chronic	treated crop	< 10	**0.14**	0.03	**2.05**	0.0048	**1.29**	0.0054
chronic	treated crop	10 - 19	**2.16**	0.03	**30.01**	0.0048	**19.22**	0.0054
chronic	treated crop	20 - 39	**2.16**	0.03	**30.01**	0.0048	**19.22**	0.0054
chronic	treated crop	40 - 69	**2.16**	0.03	**30.01**	0.0048	**19.22**	0.0054
chronic	treated crop	≥ 70	0.00	0.03	0.00	0.0048	0.00	0.0054
chronic	weeds	< 10	**0.76**	0.03	**15.53**	0.0048	**6.05**	0.0054
chronic	weeds	10 - 19	**0.61**	0.03	**12.42**	0.0048	**4.84**	0.0054
chronic	weeds	20 - 39	**0.46**	0.03	**9.32**	0.0048	**3.63**	0.0054
chronic	weeds	40 - 69	**0.23**	0.03	**4.66**	0.0048	**1.82**	0.0054
chronic	weeds	≥ 70	**0.23**	0.03	**4.66**	0.0048	**1.82**	0.0054
chronic	field margin	< 10	**0.07**	0.03	**1.51**	0.0048	**0.59**	0.0054
chronic	field margin	10 - 19	**0.07**	0.03	**1.51**	0.0048	**0.59**	0.0054
chronic	field margin	20 - 39	**0.07**	0.03	**1.51**	0.0048	**0.59**	0.0054
chronic	field margin	40 - 69	**0.07**	0.03	**1.51**	0.0048	**0.59**	0.0054
chronic	field margin	≥ 70	**0.07**	0.03	**1.51**	0.0048	**0.59**	0.0054
chronic	adjacent crop	< 10	**0.10**	0.03	**1.72**	0.0048	**0.99**	0.0054
chronic	adjacent crop	10 - 19	**0.10**	0.03	**1.72**	0.0048	**0.99**	0.0054
chronic	adjacent crop	20 - 39	**0.10**	0.03	**1.72**	0.0048	**0.99**	0.0054
chronic	adjacent crop	40 - 69	**0.10**	0.03	**1.72**	0.0048	**0.99**	0.0054
chronic	adjacent crop	≥ 70	**0.10**	0.03	**1.72**	0.0048	**0.99**	0.0054
chronic	next crop	< 10	**0.14**	0.03	**2.05**	0.0048	**1.29**	0.0054
chronic	next crop	10 - 19	**0.14**	0.03	**2.05**	0.0048	**1.29**	0.0054
chronic	next crop	20 - 39	**0.14**	0.03	**2.05**	0.0048	**1.29**	0.0054
chronic	next crop	40 - 69	**0.14**	0.03	**2.05**	0.0048	**1.29**	0.0054
chronic	next crop	≥ 70	**0.14**	0.03	**2.05**	0.0048	**1.29**	0.0054

Table 2.9.9.3-6: Apples/pear, cherry (2 × 680 g a.s./ha) – First-tier assessment of the chronic oral risk to honeybee larvae

category	scenario	BBCH	Honeybee		Bumble bee		Solitary bee	
			ETR	trigger	ETR	trigger	ETR	trigger
larva	treated crop	< 10	0.04	0.2	**2.39**	0.2	**1.11**	0.2
larva	treated crop	10 - 19	**0.62**	0.2	**29.82**	0.2	**11.45**	0.2
larva	treated crop	20 - 39	**0.62**	0.2	**29.82**	0.2	**11.45**	0.2
larva	treated crop	40 - 69	**0.62**	0.2	**29.82**	0.2	**11.45**	0.2
larva	treated crop	≥ 70	0.00	0.2	0.00	0.2	0.00	0.2
larva	weeds	< 10	**0.22**	0.2	**31.02**	0.2	**36.74**	0.2
larva	weeds	10 - 19	0.18	0.2	**24.81**	0.2	**29.40**	0.2
larva	weeds	20 - 39	0.13	0.2	**18.61**	0.2	**22.05**	0.2
larva	weeds	40 - 69	0.07	0.2	**9.31**	0.2	**11.02**	0.2
larva	weeds	≥ 70	0.07	0.2	**9.31**	0.2	**11.02**	0.2
larva	field margin	< 10	0.02	0.2	**3.01**	0.2	**3.56**	0.2
larva	field margin	10 - 19	0.02	0.2	**3.01**	0.2	**3.56**	0.2
larva	field margin	20 - 39	0.02	0.2	**3.01**	0.2	**3.56**	0.2
larva	field margin	40 - 69	0.02	0.2	**3.01**	0.2	**3.56**	0.2
larva	field margin	≥ 70	0.02	0.2	**3.01**	0.2	**3.56**	0.2
larva	adjacent crop	< 10	0.03	0.2	**3.54**	0.2	**2.65**	0.2
larva	adjacent crop	10 - 19	0.03	0.2	**3.54**	0.2	**2.65**	0.2
larva	adjacent crop	20 - 39	0.03	0.2	**3.54**	0.2	**2.65**	0.2
larva	adjacent crop	40 - 69	0.03	0.2	**3.54**	0.2	**2.65**	0.2
larva	adjacent crop	≥ 70	0.03	0.2	**3.54**	0.2	**2.65**	0.2
larva	next crop	< 10	0.04	0.2	**2.39**	0.2	**1.11**	0.2
larva	next crop	10 - 19	0.04	0.2	**2.39**	0.2	**1.11**	0.2

larva	next crop	20 - 39	0.04	0.2	**2.39**	0.2	**1.11**	0.2
larva	next crop	40 - 69	0.04	0.2	**2.39**	0.2	**1.11**	0.2
larva	next crop	≥ 70	0.04	0.2	**2.39**	0.2	**1.11**	0.2

Risk assessment for peach at 2 × 900 g a.s./ha

Table 2.9.9.3-7: Peach (2 × 900 g a.s./ha) – Screening assessment of the acute contact risk to honeybees

Test design	Type of bee	LD50 (lab.) [µg a.s./bee]	Single application rate [g/ha]	Application technique	HQcontact	Trigger value
Contact toxicity	Honeybee	> 100	900	Sideward spraying	<9.0	85
	Bumble bee	> 10			<90	7
	Solitary bee	> 10			<90	8

HQcontact: Hazard quotients for contact exposure. HQ value shown in bold breach the relevant trigger; HQ value < trigger value, indicate an acceptable risk for bees

Table 2.9.9.3-8: Peach (2 × 900 g a.s./ha) –Screening assessment of the oral toxicity risk to bees

Type of assessment	Type of bee	LD50 (lab.) [µg a.s./bee]	Single application rate [kg a.s./ha]	SV	ETRacute adult oral	Trigger value
Acute oral exposure adult bees	Honeybee	> 55.7	0.9	10.6	0.17	0.2
	Bumble bee	> 5.57		13.3	**2.15**	0.036
	Solitary bee	> 5.57		7.3	**1.18**	0.04
Chronic oral exposure adult bees	Honeybee	1.86	0.9	10.6	**5.129**	0.03
	Bumble bee	0.186		13.3	**64.355**	0.0048
	Solitary bee	0.186		7.3	**35.323**	0.0054
Oral risk to bee larvae	Honeybee	5.7	0.9	6.1	**0.96**	0.2
	Bumble bee	0.57		26	**41.05**	0.2
	Solitary bee	0.57		30.8	**48.63**	0.2

ETRacute oral adult value < trigger value indicates an acceptable risk for bees; SV: Short-cut value for the respective kind of application, application made via sideward spraying

Table 2.9.9.3-9: Peach (2 × 900 g a.s./ha) – First-tier assessment of the acute oral risk to bees

category	scenario	BBCH	Honeybee		Bumble bee		Solitary bee	
			ETR	trigger	ETR	trigger	ETR	trigger
acute	treated crop	< 10	0.01	0.2	**0.15**	0.036	**0.08**	0.04
acute	treated crop	10 - 19	0.17	0.2	**2.15**	0.036	**1.18**	0.04
acute	treated crop	20 - 39	0.17	0.2	**2.15**	0.036	**1.18**	0.04
acute	treated crop	40 - 69	0.17	0.2	**2.15**	0.036	**1.18**	0.04
acute	treated crop	≥ 70	0.00	0.2	0.00	0.036	0.00	0.04
acute	weeds	< 10	0.06	0.2	**1.05**	0.036	**0.37**	0.04
acute	weeds	10 - 19	0.05	0.2	**0.84**	0.036	**0.30**	0.04
acute	weeds	20 - 39	0.04	0.2	**0.63**	0.036	**0.22**	0.04
acute	weeds	40 - 69	0.02	0.2	**0.32**	0.036	**0.11**	0.04
acute	weeds	≥ 70	0.02	0.2	**0.32**	0.036	**0.11**	0.04
acute	field margin	< 10	0.01	0.2	**0.10**	0.036	0.04	0.04
acute	field margin	10 - 19	0.01	0.2	**0.10**	0.036	0.04	0.04
acute	field margin	20 - 39	0.01	0.2	**0.10**	0.036	0.04	0.04

acute	field margin	40 - 69	0.01	0.2	**0.10**	0.036	0.04	0.04
acute	field margin	≥ 70	0.01	0.2	**0.10**	0.036	0.04	0.04
acute	adjacent crop	< 10	0.01	0.2	**0.12**	0.036	**0.06**	0.04
acute	adjacent crop	10 - 19	0.01	0.2	**0.12**	0.036	**0.06**	0.04
acute	adjacent crop	20 - 39	0.01	0.2	**0.12**	0.036	**0.06**	0.04
acute	adjacent crop	40 - 69	0.01	0.2	**0.12**	0.036	**0.06**	0.04
acute	adjacent crop	≥ 70	0.01	0.2	**0.12**	0.036	**0.06**	0.04
acute	next crop	< 10	0.01	0.2	**0.15**	0.036	**0.08**	0.04
acute	next crop	10 - 19	0.01	0.2	**0.15**	0.036	**0.08**	0.04
acute	next crop	20 - 39	0.01	0.2	**0.15**	0.036	**0.08**	0.04
acute	next crop	40 - 69	0.01	0.2	**0.15**	0.036	**0.08**	0.04
acute	next crop	≥ 70	0.01	0.2	**0.15**	0.036	**0.08**	0.04

Table 2.9.9.3-10: Peach (2 × 900 g a.s./ha) – First-tier assessment of the chronic oral risk to bees

category	scenario	BBCH	Honeybee		Bumble bee		Solitary bee	
			ETR	trigger	ETR	trigger	ETR	trigger
chronic	treated crop	< 10	**0.19**	0.03	**2.72**	0.0048	**1.71**	0.0054
chronic	treated crop	10 - 19	**2.86**	0.03	**39.72**	0.0048	**25.43**	0.0054
chronic	treated crop	20 - 39	**2.86**	0.03	**39.72**	0.0048	**25.43**	0.0054
chronic	treated crop	40 - 69	**2.86**	0.03	**39.72**	0.0048	**25.43**	0.0054
chronic	treated crop	≥ 70	0.00	0.03	0.00	0.0048	0.00	0.0054
chronic	weeds	< 10	**1.01**	0.03	**20.55**	0.0048	**8.01**	0.0054
chronic	weeds	10 - 19	**0.81**	0.03	**16.44**	0.0048	**6.41**	0.0054
chronic	weeds	20 - 39	**0.61**	0.03	**12.33**	0.0048	**4.81**	0.0054
chronic	weeds	40 - 69	**0.30**	0.03	**6.17**	0.0048	**2.40**	0.0054
chronic	weeds	≥ 70	**0.30**	0.03	**6.17**	0.0048	**2.40**	0.0054
chronic	field margin	< 10	**0.10**	0.03	**1.99**	0.0048	**0.78**	0.0054
chronic	field margin	10 - 19	**0.10**	0.03	**1.99**	0.0048	**0.78**	0.0054
chronic	field margin	20 - 39	**0.10**	0.03	**1.99**	0.0048	**0.78**	0.0054
chronic	field margin	40 - 69	**0.10**	0.03	**1.99**	0.0048	**0.78**	0.0054
chronic	field margin	≥ 70	**0.10**	0.03	**1.99**	0.0048	**0.78**	0.0054
chronic	adjacent crop	< 10	**0.13**	0.03	**2.28**	0.0048	**1.31**	0.0054
chronic	adjacent crop	10 - 19	**0.13**	0.03	**2.28**	0.0048	**1.31**	0.0054
chronic	adjacent crop	20 - 39	**0.13**	0.03	**2.28**	0.0048	**1.31**	0.0054
chronic	adjacent crop	40 - 69	**0.13**	0.03	**2.28**	0.0048	**1.31**	0.0054
chronic	adjacent crop	≥ 70	**0.13**	0.03	**2.28**	0.0048	**1.31**	0.0054
chronic	next crop	< 10	**0.19**	0.03	**2.72**	0.0048	**1.71**	0.0054
chronic	next crop	10 - 19	**0.19**	0.03	**2.72**	0.0048	**1.71**	0.0054
chronic	next crop	20 - 39	**0.19**	0.03	**2.72**	0.0048	**1.71**	0.0054
chronic	next crop	40 - 69	**0.19**	0.03	**2.72**	0.0048	**1.71**	0.0054
chronic	next crop	≥ 70	**0.19**	0.03	**2.72**	0.0048	**1.71**	0.0054

Table 2.9.9.3-11: Peach (2 × 900 g a.s./ha) – First-tier assessment of the chronic oral risk to honeybee larvae

category	scenario	BBCH	Honeybee		Bumble bee		Solitary bee	
			ETR	trigger	ETR	trigger	ETR	trigger
larva	treated crop	< 10	0.05	0.2	**3.16**	0.2	**1.47**	0.2
larva	treated crop	10 - 19	**0.82**	0.2	**39.47**	0.2	**15.16**	0.2
larva	treated crop	20 - 39	**0.82**	0.2	**39.47**	0.2	**15.16**	0.2
larva	treated crop	40 - 69	**0.82**	0.2	**39.47**	0.2	**15.16**	0.2
larva	treated crop	≥ 70	0.00	0.2	0.00	0.2	0.00	0.2
larva	weeds	< 10	**0.30**	0.2	**41.05**	0.2	**48.63**	0.2

larva	weeds	10 - 19	**0.24**	0.2	**32.84**	0.2	**38.91**	0.2
larva	weeds	20 - 39	0.18	0.2	**24.63**	0.2	**29.18**	0.2
larva	weeds	40 - 69	0.09	0.2	**12.32**	0.2	**14.59**	0.2
larva	weeds	≥ 70	0.09	0.2	**12.32**	0.2	**14.59**	0.2
larva	field margin	< 10	0.03	0.2	**3.98**	0.2	**4.72**	0.2
larva	field margin	10 - 19	0.03	0.2	**3.98**	0.2	**4.72**	0.2
larva	field margin	20 - 39	0.03	0.2	**3.98**	0.2	**4.72**	0.2
larva	field margin	40 - 69	0.03	0.2	**3.98**	0.2	**4.72**	0.2
larva	field margin	≥ 70	0.03	0.2	**3.98**	0.2	**4.72**	0.2
larva	adjacent crop	< 10	0.04	0.2	**4.69**	0.2	**3.50**	0.2
larva	adjacent crop	10 - 19	0.04	0.2	**4.69**	0.2	**3.50**	0.2
larva	adjacent crop	20 - 39	0.04	0.2	**4.69**	0.2	**3.50**	0.2
larva	adjacent crop	40 - 69	0.04	0.2	**4.69**	0.2	**3.50**	0.2
larva	adjacent crop	≥ 70	0.04	0.2	**4.69**	0.2	**3.50**	0.2
larva	next crop	< 10	0.05	0.2	**3.16**	0.2	**1.47**	0.2
larva	next crop	10 - 19	0.05	0.2	**3.16**	0.2	**1.47**	0.2
larva	next crop	20 - 39	0.05	0.2	**3.16**	0.2	**1.47**	0.2
larva	next crop	40 - 69	0.05	0.2	**3.16**	0.2	**1.47**	0.2
larva	next crop	≥ 70	0.05	0.2	**3.16**	0.2	**1.47**	0.2

According to EFSA Guidance 3295, 2013, risk to honeybees, bumblebees and solitary bees cannot be excluded at Tier 1.

Dodine posing a low risk to honeybees in laboratory studies, however chronic bee assessment in accordance with EFSA Journal 2013: 11(7):3295 suggest that further investigations are needed (ETR values were above the relevant triggers).

To address the risk on bees, two semi-field studies were submitted to ensure no effects to the whole colony would be overlooked. The semi-filed studies were conducted in the central and in the southern zone according to OECD 75.

The purpose of both studies was to determine the potential effects of Dodine 544 SC on the honeybee (*Apis mellifera*) after two foliar applications at 900 g a.s./ha in a 7-day interval on flowering Phacelia (*Phacelia tanacetifolia*) under semi-field conditions and exposure of bees. Special attention was paid to detailed brood development via photo documentation of initially labelled eggs representing the main endpoint. Further major endpoints were the mortality, foraging activity, bee behavior and the colony and brood development. Additionally, residues of Dodine were determined in flowers, pollen, and nectar.

Overall, no adverse effects on honey bee brood or honey bee colony survival were observed. Effects on mortality were observed for the exposure period in the Germany trial when the application was done during the bee flight, however for the overall exposure there were not any significant effect between test items and control. **Mitigation measures could be proposed to avoid the effects on mortality observed during the exposure period. i.e *to protect bees and other pollinating insects do not use where bees are actively foraging.***

Regarding the risk from exposure to contaminated water, an acceptable risk to honeybees by oral exposure to contaminated surface water and contaminated water in puddles was calculated following all representative uses of Dodine 544 SC in orchards. Although the calculated screening ETR values indicate a potential chronic risk for adult honeybees and honeybee larvae exposed to contaminated guttation water, no accumulation of Dodine in guttation fluids and subsequent exposure of honeybees to guttation water is expected.

The sub-lethal effects were described and reported in each laboratory study as well as in any higher-tier study. In the semi-field studies, the application and subsequent exposure of bees to the test item I, test item II, respectively, did not result in behaviour abnormalities compared to the bees in the control group. No symptoms of apathy, intoxication or any deviations to the normal behaviour of bees occurred. Information about the development of hypopharyngeal glands has not been provided.

Finally, applicant has stated that there are no indications that dodine has accumulative potential. However**, a justification in line with section 8.1.1.3 and pertinent part of Appendix O of EFSA GD (2013) should be provided.**

2.9.9.4 Summary of risk assessment for non-target arthropods

The evaluation of the risk for non-target arthropods has been performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", and in consideration of the recommendations of the guidance document ESCORT 2.

Two laboratory (glass plates) tests on the effects of dodine on non-target arthropods *T. pyri* and *A. rhopalosiphi* were submitted with dodine formulated as 400 g/L SC (Syllit 400 SC). These studies were already evaluated and accepted for the Annex I inclusion. In addition, two new studies have been conducted using the formulation Dodine 544 SC for the purpose of renewal of the approval of Dodine.

Extended laboratory tests were also available with the Annex I inclusion formulation (Syllit 400 SC). In total, five studies on natural substrates investigating the effects of Dodine to the standard specie *Typhlodromus pyri* as well as to additional arthropod species (i.e., *Coccinella septempunctata*, *Orius insidiosus*, *Chrysoperla carnea*).
Since new data with the current formulation are available, the applicant has only considered the results of these new studies at first-tier for the risk assessment. Since all HQ values were below the trigger value, further assessment using higher tier data was not conducted.

However, higher mortality for *Typhlodromus pyri* was observed using the previous formulation. Therefore, the risk assessment has been repeated by RMS considering the endpoint derived from [redacted] (1997b) expressed in grams of dodine/ha. Additionally, since an extended laboratory study was available for *Typhlodromus pyri*, higher tier risk assessment has been carried out as well.

The summary of the risk assessment is included below:

First tier risk assessment (glass plate studies)

Data from the initial laboratory studies on inert substrate with T. pyri and A. rhopalosiphi. were used.
The in-field risk assessment is presented below:

Table 2.9.9.4-1: First-tier assessment of the in-field risk for non-target arthropods due to the use of Dodine 544 SC in apples/pear and cherry (2x 680 g a.s./ha)

Intended use	Apples/pear, cherry		
Active substance/product	Dodine / Dodine 544 SC		
Application rate [g a.s./ha]	2 × 680		
MAF	1.7 (foliar) / 1.9 (soil)		
Test species Tier I	**LR_{50} (lab.) [g a.s./ha]**	**$PER_{in\text{-}field}$ [g/ha]**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 900	1156 (foliar)	1.28
Aphidius rhopalosiphi	> 1530		0.75
Typhlodromus pyri	> 900	1292 (soil)	1.44
Aphidius rhopalosiphi	> 1530		0.84

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

Table 2.9.9.4-2: First-tier assessment of the in-field risk for non-target arthropods due to the use of Dodine 544 SC in peach (2x 900 g a.s./ha)

Intended use	Peach		
Active substance/product	Dodine / Dodine 544 SC		
Application rate [g a.s./ha]	2 × 900		
MAF	1.7 (foliar) / 1.9 (soil)		
Test species Tier I	**LR_{50} (lab.) [g/ha]**	**$PER_{in\text{-}field}$ [g/ha]**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 900	1530 (foliar)	1.7
Aphidius rhopalosiphi	> 1530		1.12
Typhlodromus pyri	> 900	1710 (soil)	1.9
Aphidius rhopalosiphi	> 1530		1.11

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

The in-field HQs at Tier I for the standard laboratory species *Aphidius rhopalosiphi* and *Typhlodromus pyri* are below the trigger value of 2, indicating that risk to non-target arthropods is low in in-field areas following the application of Dodine 544 SC according to the proposed use pattern in apples/pear, cherry and peach.

Risk assessment for off-field exposure
A default correction factor of 10, to account for uncertainty with the extrapolation from Typhlodromus pyri and Aphidius rhopalosiphi as indicator species to all off-field non-target arthropods was used in HQ calculations.
Moreover, a VDF of 5 was used as recommended in the Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA Supporting publication 2019:EN-1673).

Table 2.9.9.4-3: First-tier assessment of the off-field risk for non-target arthropods due to the use of Dodine 544 SC in apples/pear and cherry (2x 680 g a.s./ha)

Intended use	Apples/pear, cherry				
Active substance/product	Dodine / Dodine 544 SC				
Application rate [g a.s./ha]	2 × 680				
MAF	1.7 (foliar) / 1.9 (soil)				
vdf	5				
Test species Tier I	**LR_{50} (lab.) [L/ha]**	**Drift rate**	**$PER_{off\text{-}field}$ [L/ha]**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 900	0.2553	59.02 (foliar)	10	0.66
Aphidius rhopalosiphi	> 1530		59.02 (foliar)		0.39
Typhlodromus pyri	> 900		65.97 (soil)		0.73
Aphidius rhopalosiphi	> 1530		65.97 (soil)		0.43

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

Table 2.9.9.4-4: First-tier assessment of the off-field risk for non-target arthropods due to the use of Dodine 544 SC in peach (2x 900 g a.s./ha).

Intended use	Peach				
Active substance/product	Dodine / Dodine 544 SC				
Application rate [g a.s./ha]	2 × 900				
MAF	1.7 (foliar) / 1.9 (soil)				
vdf	5				
Test species Tier I	**LR_{50} (lab.) [L/ha]**	**Drift rate**	**$PER_{off\text{-}field}$ [L/ha]**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 900	0.2553	78.12 (foliar)	10	0.87
Aphidius rhopalosiphi	> 1530		78.12 (foliar)		0.51
Typhlodromus pyri	> 900		87.31 (soil)		0.97
Aphidius rhopalosiphi	> 1530		87.31 (soil)		0.57

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

The off-field HQs at Tier I for the standard laboratory species *Aphidius rhopalosiphi* and *Typhlodromus pyri* are below the trigger value of 2, indicating that risk to non-target arthropods is low in off-field areas following the application of Dodine 544 SC according to the proposed use pattern in apples/pear, cherry and peach.

Tier 2 risk assessment (extended laboratory studies)
HQ approach is not adequate for Tier 2. At higher tier a trigger value for lethal or sub-lethal effects of 50% after exposure of the test organisms to fresh or aged residues of plant protection products was set in ESCORT 2.

The maximum PERin-field and off-field values resulted from application of Dodine 544 SC to peach**.** So these values are used in the Tier II risk assessment as they are protective of all intended uses.

Table 2.9.9.4-5: In-field risk assessment for non-target arthropods (Tier 2) exposed to Dodine 544 SC.

Intended use	All uses
Active substance	Dodine
Product	Dodine 544 SC
Application rate (g a.s./ha)	2 x 900
MAF	1.9

Crop scenario	Test species Tier II (2D)	LR50 (ext. lab.) [g/ha]	PERin-field [g/ha]	PERin-field below rate with ≤ 50 % effect?
All uses	*T. pyri*	7512	1710	Yes
	Coccinella septempunctata	1800	1710	Yes
	Orius insidiosus	1800	1710	Yes
	Chrysoperla carnea	1800	1710	Yes

Table 2.9.9.4-6: Off-field risk assessment for non-target arthropods (Tier 2) exposed to Dodine 544 SC.

Intended use	All uses
Active substance	Dodine
Product	Dodine 544 SC
Application rate (g a.s/ha)	2 x 900
MAF	1.9
Drift rate (%)	0.2553
VDF	5
CF	5 (2D)

Crop scenario	Test species Tier II (2D)	LR50 (ext. lab.) [g a.s./ha]	ER50 (ext. lab.) [g a.s./ha]	PERoff-field [g/ha][1]	PERoff-field below rate of ≤ 50% effect?
All uses	*T. pyri*	7512	>8000	87.31	Yes
	Coccinella septempunctata	1800	1800	87.31	Yes
	Orius insidiosus	1800	-	87.31	Yes
	Chrysoperla carnea	1800	-	87.31	Yes

[1] Drift rate (higher tier) = application rate * MAF * (drift factor/vegetation distribution factor)* correction factor

The extended laboratory studies showed no unacceptable effect on reproduction and mortality up to the maximum application rate of 2x900 g a.s./ha (mortality and reproductive effects were below 50 %).

The active substance Dodine belongs to the family of guanidine. According to FRAC (Fungicide resistance action committee), it is classified as U12, an unknown mode of action. The proposed target site of Dodine is the disruption of cellular membranes.

Based on the above, the risk for non-target arthropods is acceptable for all proposed used, no further investigation are necessary in semi-field or field scenarios.

2.9.9.5 Summary of risk assessment for non-target soil meso- and macrofauna

2.9.9.5.1 Risk assessment for earthworms

The effects of Dodine (technical or formulated) to earthworms under both acute and long-term exposure conditions are adequately presented and discussed in Volume 3 CA B.9 Point 9.4.1. and Volume 3 CP B.9 Point 9.7.1. Considering the available, complete data set, the worst-case EC_{10} of 62.4 mg a.s./kg dw is proposed for use in the long-term risk assessment for earthworms. This endpoint is provisional pending on the submission of the statistical re-evaluation of [redacted] 2007 (KCP 10.4.1.1/01).

Based on the results of the soil degradation studies, there is no Dodine metabolite found in soil at proportions > 10% AR, 5% AR in two consecutive samples and/or >5% at the end of the study. Thus Dodine is the only compound to be further considered regarding its toxicity to soil organisms.

The evaluation of the risk for earthworms is performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002). The potential long-term risk of Dodine to earthworms is assessed by calculating long-term Toxicity Exposure Ratios ($TER_{LT\ values}$), by comparing the NOEC/EC_{10} values and the maximum PEC_{soil} according to the following equation:

$$TER_{LT} = \frac{NOEC\,(mg/kg)}{PEC_S\,(mg/kg)}$$

The resulting TER_{LT} value is presented in **Table 2.9.9.5.1-1** below.

Table 2.9.9.5.1-1 First-tier assessment of the chronic risk for earthworms due to the use of Dodine 544 SC in peach (early application) [a]

Intended use	Peach (early application), 2 × 0.9 kg a.s./ha, 21-day interval		
Chronic effects on earthworms			
Product/active substance	**EC_{10} [mg a.s./kg dw]**	**PEC_{Soil} [mg a.s./kg dw]**	**TER_{LT} (criterion TER ≥ 5)**
Dodine	62.4*	0.746	83.64

[a] The PEC_{soil} values calculated for the early application in peach represent a worst-case, thus cover also all other intended uses of Dodine 544 SC in orchards

* The EC10 = 62.4 mg a.s./kg dw is considered provisional pending on the submission of the statistiscal re-evaluation of [redacted] 2007 (KCP 10.4.1.1/01).

The long-term TER value for Dodine meet the trigger value of 5, indicating no unacceptable long-term risk to earthworms following the intended uses of Dodine 544 SC in orchards.

2.9.9.5.2 Risk assessment for non-target soil meso- and macrofauna (other than earthworms)

The effects of Dodine formulated as Dodine 544 SC on the survival and reproductive performance of non-target soil meso- and macrofauna (other than earthworms) is adequately presented and discussed in Volume 3 CP B9, Point B.9.7.2. Considering the available data, the NOEC of 1000 mg a.s./kg dw is proposed for use in the risk assessment for *Hypoaspis aculeifer* and the NOEC endpoints of 3.2 and 541.7 mg a.s./kg dw are proposed for use in the Tier 1 and refined tier risk assessment, respectively for *Folsomia candida*. All LogPow values of dodine were < 2, hence correction of the endpoints are not needed.

Based on the results of the soil degradation studies, there is no Dodine metabolite found in soil at proportions > 10% AR and thus Dodine is the only compound to be further considered regarding its toxicity to soil organisms.

The evaluation of the risk for soil meso- and macrofauna (other than earthworms) is performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002). The potential long-term risk of Dodine to for soil meso- and macrofauna (other than earthworms) is assessed by calculating long-term Toxicity Exposure Ratios ($TER_{LT\ values}$), by comparing the NOEC values and the maximum PEC_{soil}.

The resulting TER_{LT} values are presented in **Table 2.9.9.5.2-1** below.

Table 2.9.9.5.2-1 First-tier assessment of the chronic risk for soil meso- and macrofauna (other than earthworms) due to the use of Dodine 544 SC

Chronic effects on *Hypoaspis aculeifer*				
Intended use	**Product/ active substance**	**NOEC [mg/kg dw]**	**PEC_{Soil} [mg/kg dw]**	**TER_{LT} (criterion TER ≥ 5)**
Pome/pear (BBCH 01), 2 × 0.68 kg a.s./ha, 21-day interval	Dodine	1000	0.646	1548
Cherry (BBCH 60), 2 × 0.68 kg a.s./ha, 21-day interval	Dodine	1000	0.517	1934
Peach (BBCH 01), 2 × 0.9 kg a.s./ha, 21-day interval	Dodine	1000	0.746	1340
Chronic effects on *Folsomia candida*				
Intended use	**Product/ active substance**	**NOEC [mg/kg dw]**	**PEC_{Soil} [mg/kg dw]**	**TER_{LT} (criterion TER ≥ 5)**
Pome/pear (BBCH 01), 2 × 0.68 kg a.s./ha, 21-day interval	Dodine	3.2	0.646	**4.9**
Cherry (BBCH 60), 2 × 0.68 kg a.s./ha, 21-day interval	Dodine	3.2	0.517	6.2
Peach (BBCH 01), 2 × 0.9 kg a.s./ha, 21-day interval	Dodine	3.2	0.746	**4.3**

[a] The PEC_{soil} values calculated for the early application in peach represent a worst-case, thus cover also all other intended uses of Dodine 544 SC in orchards

TER values shown in bold fall below the relevant trigger

The tier 1 risk assessment, indicated a high risk for *Folsomia candida* after early applications of dodine on peach. To refine the risk assessment for such uses, one toxicity test with *Folsomia candida* conducted in a natural soil was available.

To date, only two refinement options are available in the current SANCO guidance document (2002): to use of natural soils in laboratory toxicity test or to conduct higher-tier assessmnet with field studies. Moreover, it is stated that the type of the organic matter influences sorption and hence bioavailability. Therefore, a standardised arable soil closer to the scenarios in the exposure assessment would be prefered over the OECD artificial soil. Please, refer to Scientific opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms (EFSA, 2017).

Nevertheless, currently there is no guidance on what natural soil properties would be considered acceptable. Some indications were provided in [redacted] (2011[10]).

Co-RMS refers to the results of two reports, [redacted] (2017[11]) and [redacted] (2017[12]), to support the conclusion reached during 4th central zone harmonisation meeting in 2018 on not using natural soils for the risk assessment as long as no further results from ongoing research projects are available. These report are not available to RMS and without further details about test design, investigated endpoints, exposure conditions, and sensitivity analysis performed, it is difficult to understand the reasoning behind.

We agree with co-RMS that FOCUS SW scenarios are not relevant for PECsoil calculations, not being appropriate to compare soil propertities of LUFA Speyer 2.2 with FOCUS sw scenarios in order to justify its representativeness. However, this soil is commonly used in aerobic degradation test as representative of EU agricultural soils, with soil properties within the recommended ranges of OECD 307 guideline.

Moreover, the LUFA 2.2 soil is on the spectrum of lower organic matter, clay and pH compared to OECD 232 artificial soil. Data on adsorption in four soils suggested that sorption is lower in acid soil compared to basic soils. Therefore, it is expected a higher bioavailability of dodine in LUFA 2.2 soil.

Name	Texture	Organic matter (%)	Clay %	Silt %	Sand %	WHC max	pH
Artificial soil OECD 232	-	5	20	6	74	45	6 (in CaCl2)
Lufa Speyer 2.2	Loamy sand	3.14	6.7	13.2	77.1	49.76	5.3 (in H_2O)

Finally, it is highlighted that the validity criteria of OECD 232 were met and the study is considered aceptable.

Artificial soils do not neccesarily reflect the behaviour of chemicals in the enviroment. In general, the exposure and toxicty of the Plant Protection Products for in-soil organisms in natral agro-ecosystems is influenced by the fate of a substance and their bioavailability in the habitat soil. This bioavailability is influenced by a multitude of factors. Consequently, the use of only one natural soil without a proper sensitivity analysis for a comparison of test performed in other natural soils is highly uncertain and may result in the risk assessment being under-protective. Taking into account that the study is reliable, RMS has proposed to used it as part of a Weight of Evidence approach.

All in all, RMS has concluded that the long-term risk to soil meso- and macrofauna (other than earthworms) can be considered acceptable following de application of Dodine 544 SC in orchards based on the available data:

1.- Dodine exhibits low persistence in soil (normalized geomean DT50 of 5.25 days) with low potential to bioaccumulation (log Pow <2).

2.- An aceptable risk is indicated based on the Tier 1 EC_{10} for *Folsomia* of 6.6 mg as/kg dw. The TER_{LT} value of 10.2 and 8.8 for the uses on pome/pear and peach, respectively.

3.- An aceptable risk is indicated based on the Tier 2 results of the *Folsomia* study performed using an environmentally relevant substrate (i.e. natural soil). Taking into account a NOEC of 541.7 mg a.s./kg dw TER_{LT} for *Folsomia candida* is above the Annex VI trigger of 5, with a high safety margin, indicating an acceptable risk to soil macro-organisms.

4.- Tier 1 risk assessment of standard test species of non-target arthropods, *A. rhopalosiphi* and *T. pyri*, indicated a low risk based on glass plate studies. Moreover, Tier 2 risk assessment based on extended laboratory studies (toxicity of dodine to NTA on natural substrates) on four species of NTA indicated a low risk also.

2.9.9.6. Risk assessment for soil nitrogen transformation

The effects of Dodine formulated as Dodine 400 SC on soil microbial activity (nitrogen turnover and short-time respiration) are adequately presented and discussed in Volume 3 CP Point B.9.9. The maximum tested concentration with lower than 25% effects on nitrogen transformation compared to the control, i.e., 12 mg a.s./kg dw, is used in the risk assessment. The results of the study are considered as supportive information. **A data gap has been set to the applicant to submit the soil nitrogen transformation rate expressed in mg nitrate/kg dry weight soil/day between each measurement day for control and all tested concentrations in order to determine the difference in transformation rates as recommended by the OECD 216.**

Based on the results of the soil degradation studies, there is no Dodine metabolite found in soil at proportions > 10% AR, 5% at two consecutive samplings and/ot >5% at the end of the study. Therefore, Dodine is the only compound to be further considered regarding its toxicity to soil organisms.

The evaluation of the risk for soil microorganisms is performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002). The risk to soil microorganisms was evaluated by comparing the concentration resulting in ≤ 25% effects on nitrogen transformation compared to the control, as derived from the available laboratory test, with the maximum $PEC_{Soil, ini}$ value calculated for Dodine (please, refer to Volume 3 CP B8 Point B.8.2). The results of the comparison expressed as Margin of Safety (MoS) are presented in **Table 2.9.9.6-1** below.

Table 2.9.9.6-1 Assessment of the risk for effects on soil microorganisms due to the use of Dodine 544 SC in peach (early application) [a]

Intended use	Peach (early application), 2 × 0.9 kg a.s./ha, 21-day interval		
N-mineralization			
Product/active substance	**Max. conc. with effects ≤ 25% [mg a.s./kg dw]**	**$PEC_{initial, Soil}$ [mg a.s./kg dw]**	**Margin of safety**
Dodine	12*	0.746	16

a The $PEC_{initial, soil}$ value calculated for the early application in peach represents a worst-case, thus covers also all other intended uses of Dodine 544 SC in orchards

* Results of the study are provisional (see data gap above)

Based on the results of the risk assessment no unacceptable effects on soil microbial activity are expected following application of Dodine 544 SC according to the proposed use pattern.

2.9.9.6. Risk assessment for terrestrial non-target plants

The effects of Dodine (technical and formulated) to terrestrial non-target higher plants are adequately presented and discussed in Voume 3 CA B9 Point 9.6 and Volume 3 CP B9 Point B.9.11. The EU agreed ER_{50} > 2.9 kg a.s./ha for Dodine for both seedling emergence and vegetative vigour is retained in the risk assessment for non-target terrestrial plants.

The formulated product Dodine 544 SC is not an herbicide, following data requirement Commission Regulation (EU) 284/2013 the effects of Dodine 544 SC can be adequately predicted based on active substance data.

The evaluation of the risk for terrestrial non-target higher plants is performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations where spray drift will be the main route of exposure, as non-target plants are non-crop plants located outside the treated area.

The results of the risk assessment for terrestrial non-target plants are shown in **Tables 2.9.9.6-1** and **2.9.9.6-2** below.

Table 2.9.9.6-1 Assessment of the risk for non-target plants due to the use of Dodine 544 SC in apples/pear and cherry

Intended use	Apples/pear, cherry			
Active substance/product	Dodine/Dodine 544 SC			
Application rate [kg/ha]	2 × 0.68			
Test species	**ER_{50} [kg/ha]**	**Drift rate**	**$PER_{off\text{-}field}$ [kg/ha]**	**TER criterion: TER ≥ 5**
Vegetative vigour	> 2.9	0.292	0.199	> 14.6
Seedling emergence	> 2.9	0.292	0.199	> 14.6

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 2.9.9.6-2 Assessment of the risk for non-target plants due to the use of Dodine 544 SC in peach

Intended use	Peach			
Active substance/product	Dodine/Dodine 544 SC			
Application rate [kg/ha]	2 × 0.9			
Test species	**ER_{50} [kg/ha]**	**Drift rate**	**$PER_{off\text{-}field}$ [kg/ha]**	**TER criterion: TER ≥ 5**
Vegetative vigour	> 2.9	0.292	0.263	> 11.0
Seedling emergence	> 2.9	0.292	0.263	> 11.0

PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The calculated TER values are above the trigger of 5, indicating an acceptable risk for non-target terrestrial plants following application of Dodine 544 SC according to the proposed use pattern.

2.10 ENDOCRINE DISRUPTING PROPERTIES

2.10.1 ED assessment for humans

2.10.1.1. Gather all relevant information

The studies included in the volume 3 B.6 of this RAR provided the information used for this assessment, specifically, short-term toxicity studies, long-term toxicity and carcinogenicity studies, reproductive toxicity studies, immunotoxicity studies and studies on endocrine disruption (*in silico*, *in vitro* mechanistic and *in vivo* mechanistic) (Table 2.10.1.1).

Data were populated in the Excel template provided as *Appendix E* to the *EFSA/ECHA guidance for the identification of endocrine disruptors* (2018). According to this template, each study was given a number (Study ID Matrix) for its identification in the data-matrix of the Excel.

Table 2.10.1.1: Outline of dataset considered for mammalian toxicology assessment

Study type (results, source)	**Reference**	**Study ID Matrix**
***In vitro* ToxCast mechanistic studies - OECD framework Level 2**		
Thyroid – T-Bioactivity Model		
Tox21_TRHR_HEK293_Agonist	B.6.8.3.8	1
Tox21_TRHR_HEK293_Antagonist		2
Tox21_TR_LUC_GH3_Agonist		3
Tox21_TR_LUC_GH3_Antagonist		4
Tox21_TR_LUC_GH3_Antagonist_viability		5

Study type (results, source)	Reference	Study ID Matrix
TOX21_TSHR_HTRF_Agonist_ratio		6
TOX21_TSHR_HTRF_Antagonist_ratio		7
TOX21_TSHR_HTRF_wt_ratio		8
Estrogen - ER Bioactivity Model		
TOX21_ERa_BLA_Agonist_ratio	B.6.8.3.9	9
TOX21_ERa_BLA_Antagonist_ratio		10
TOX21_ERa_BLA_Antagonist_viability		11
TOX21_ERa_LUC_VM7_Agonist		12
TOX21_ERa_LUC_VM7_Antagonist_specificity		13
TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2_viability		14
Androgen - AR Bioactivity Model		
TOX21_AR_BLA_Agonist_ratio	B.6.8.3.10	15
TOX21_AR_BLA_Antagonist_ratio		16
TOX21_AR_BLA_Antagonist_viability		17
TOX21_AR_LUC_MDAKB2_Agonist		18
TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881		19
TOX21_AR_LUC_MDAKB2_Antagonist__0.5nM_R1881_viability		20
TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881		21
TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881_viability		22
Steroidogenesis - S-Bioactivity Model		
TOX21_Aromatase_Inhibition	B.6.8.3.11	23
TOX21_Aromatase_inhibition_viability		24
***In vitro* mechanistic studies – OECD framework Level 2**		
Thyroid		
Sodium-Iodide Symporter (NIS) Assay, [redacted] (2021)	B.6.8.3.1	25
Thyroid Peroxidase (TPO) Assay, [redacted] (2021)	B.6.8.3.2	26
Estrogen		
Human Estrogen Receptor-α Transactivation Assay, [redacted] (2021)	B.6.8.3.3	27
Androgen		
Human Androgen Receptor-α Transactivation Assay, [redacted] (2021)	B.6.8.3.4	28
Steroidogenesis		
Aromatase Assay, [redacted] (2021)	B.6.8.3.6	29
Steroidogenesis Assay, [redacted]. (2021)	B.6.8.3.7	30
***In vivo* mammalian toxicity studies – OECD framework Level 3**		
Hershberger Bioassay, [redacted] (2022)	B.6.8.3.5	31
***In vivo* mechanistic study – OECD framework Level 4**		
Short-term mammalian toxicity studies		
28-day repeat dose oral (gavage) toxicity study in rats, [redacted] (1994a)	B.6.3.1.1	32
28-day repeat dose oral (diet) toxicity study in rats, [redacted] (1994b)	B.6.3.1.2	33
28-day repeat dose oral (diet) toxicity study in rats, [redacted] (1997)	B.6.3.1.3	34
7 and 28-day repeat dose oral (diet) toxicity study in rats, [redacted] (1996)	B.6.3.1.4	35
28-day repeat dose dermal toxicity study in rats, [redacted] (1999e)	B.6.3.4.1.1	36
8-week repeat dose oral (diet) toxicity study in mice, [redacted] (1988)	B.6.3.1.5	37
90-day repeat dose oral (diet) toxicity study in rats, [redacted] (1982)	B.6.3.2.1	38
90-day repeat dose oral (diet) toxicity study in mice, [redacted] (1994)	B.6.3.2.4	39
90-day repeat dose oral (capsule) toxicity study in dogs, [redacted] (2005)	B.6.3.2.5	40
6-week repeat dose oral (capsule) toxicity study in dogs, [redacted] (1994)	B.6.3.1.6	41
90-day repeat dose oral (diet) toxicity study in rats, Mitjans, M. and Vinardell, M.P. (1999)	B.6.3.2.2	43
100-day repeat dose oral (diet) toxicity study in rats, Levinskas, G.J. *et al.* (1961)	B.6.3.2.3	44
52-week repeat dose oral (capsule) toxicity study in dogs, [redacted] (1996)	B.6.3.3.1	47
1-year repeat dose oral (diet) toxicity study in dogs, Levinskas, G.J. *et al.* (1961)	B.6.3.3.2	45

Study type (results, source)	Reference	Study ID Matrix
Long-term mammalian toxicity studies		
2-year chronic oral (diet) toxicity study in rats, Levinskas, G.J. *et al.* (1961)	B.6.5.3	46
2-year chronic oral (diet) toxicity study in rats, [redacted] (1998)	B.6.5.1	48
78-week chronic oral (diet) toxicity study in mice, [redacted] (1998)	B.6.5.2	49
Reproduction toxicity studies		
2-generation reproductive toxicity study in rats, [redacted] (1996)	B.6.6.1.1	50
Dose range finding developmental toxicity study in rats, [redacted] (1989a)	B.6.6.2.1	51
Developmental toxicity study in rats, [redacted] 1989b)	B.6.6.2.2	52
Dose-range finding developmental toxicity study in rabbits, [redacted] (1989a)	B.6.6.2.3	53
Developmental toxicity study in rabbits, [redacted] (1989b)	B.6.6.2.4	54
Reproductive toxicity study in rats, Levinskas, G.J. *et al.* (1961)	B.6.6.1.2	55
Immunotoxicity studies		
28-day repeat dose oral (feed) in rats, [redacted] (2013)	B.6.8.2.1	42

2.10.1.2. ED assessment for T-modality

2.10.1.2.1. Dataset sufficiency in T-mediated parameters

	Sufficiently investigated
T-mediated parameters	Yes, based on the availability of the following studies: - Two repeated dose 28-day oral toxicity studies in rodents (OECD TG 407) [a, b] - One repeated dose 90-day oral toxicity studies in rodents (OECD TG 408) [a, b] - A repeated dose 90-day oral toxicity study in non-rodents (OECD TG 409) [a, b] - A 52-week oral toxicity study in non-rodents (OECD TG 452) [b] - A 1-year oral toxicity study in non-rodents (OECD TG 452) [a, b] - A long-term repeated dose toxicity and carcinogenicity study (OECD TG 453) [a, b] - A long-term repeated dose toxicity and carcinogenicity study (OECD TG 453) [b] - Developmental toxicity studies in rats and rabbits (OECD TG 414) - A two-generation reproduction toxicity test (OECD TG 416)

[a] Thyroid weight measured. [b] Thyroid histopathology measured.

The repeated dose 28-day oral toxicity studies (OECD TG 407), the repeated dose 90-day oral toxicity studies in rodents (OECD TG 408), the two-generation reproduction toxicity test (OECD TG 416) and the developmental toxicity studies in rats and rabbits (OECD TG 414) were conducted according to outdated versions of the OECD test guidelines, and consequently, some T-mediated parameters have not been measured.

The long-term repeated dose toxicity and carcinogenicity study in rats (OECD TG 453) was conducted according an outdated version of the guideline, however all the recommended T-mediated parameters in the current guideline were measured.

The repeated dose 90-day oral toxicity study in non-rodents (OECD TG 409) was performed according to the last and updated OECD test guidelines.

The T-mediated parameters that have not been measured in the studies are indicated in table 2.10.1.2.1.

Table 2.10.1.2.1: T-mediated parameters not measured

OECD TG 407 - T-mediated parameters not investigated	
- T3 and/or T4 level	- Thyroid stimulating hormone level (TSH)
OECD TG 408 - T-mediated parameters not investigated	
- T3 and/or T4 level	- Low-density lipoproteins (LDL)
- Thyroid stimulating hormone level (TSH)	- High-density lipoproteins (HDL)
OECD TG 452 - T-mediated parameters not investigated	
- Thyroid weight	

OECD TG 414 - T-mediated parameters not investigated	
- Thyroid weight	- T3 and/or T4 level
- Thyroid histopathology	- Thyroid stimulating hormone level (TSH)
OECD TG 416 - T-mediated parameters not investigated	
- Thyroid weight	

However, it is considered that T-mediated parameters have been sufficiently investigated taking into account the following points:

- The thyroid histopathology has been measured in eleven studies: OECD TG 407 (B.6.3.1.1, ID: 32 and B.6.3.1.2, ID: 33), 6-week repeat dose toxicity study in dogs (B.6.3.1.6, ID: 41, no guideline), OECD TG 408 in rats (B.6.3.2.1, ID: 38), OECD TG 408 in mice (B.6.3.2.4, ID: 39), OECD TG 409 (B.6.3.2.5, ID: 40), OECD TG 452 in dogs (B.6.3.3.1, ID: 47 and B.6.3.3.2, ID: 45), OECD TG 410 (B.6.3.4.1.1, ID: 36), OECD TG 453 in rats and mice (B.6.5.1, ID: 48 and B.6.5.2, ID: 49).

- The thyroid weight has been measured in seven studies: OECD TG 407 (B.6.3.1.1, ID: 32 and B.6.3.1.2, ID: 33), 6-week repeat dose toxicity study in dogs (B.6.3.1.6, ID: 41, no guideline), OECD TG 408 (B.6.3.2.1, ID: 38), OECD TG 409 (B.6.3.2.5, ID: 40), OECD TG 452 in dogs (B.6.3.3.2, ID: 45) and OECD TG 453 (B.6.5.1, ID: 48).

- The thyroid hormones (T3 and T4) levels have been measured only in one study: OECD TG 441 (B.6.8.3.5, ID: 31).

2.10.1.2.2: Lines of evidence for adverse effects and endocrine activity related to T-modality

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
In vitro mechanistic	3	Thyroid receptor	Rat	28 hours	Uptake from the medium (in vitro)		No effect	No effect	A positive result was reported in the NIS assay. However, no cytotoxicity measurements were performed in this study. A positive result was obtained for the thyroid receptor assay in Toxcast, although it was reported at cytotoxic concentration only.	There were no T3, T4 and TSH levels measurements in the overall dataset with the exception of the Hershberger assay in male rats, in which no effects were observed in T3 hormone measurement, and an increase in T4 levels were noted. Overall, more data regarding T-mediated activity parameters are deemed necesary in order to reach a conclusion.	T
	4	Thyroid receptor	Rat	28 hours	Uptake from the medium (in vitro)		Change	Dodine acetate is positive for thyroid receptor at cytotoxic concentration. AC_{50} (Hill model)= 2.894			
	5	Thyroid receptor	Rat	28 hours	Uptake from the medium (in vitro)		No effect	No effect			
	25	Sodium-Iodide-Symporter (NIS)	Rat	1 hour	Uptake from the medium (in vitro)		Decrease	Log IC_{50} values: -5.14; -4.40; -4.06; cytotoxicity measurements not performed			
	26	Thyroperoxidase activity (TPO)	Human	0.5 hours	Uptake from the medium (in vitro)		No effect	No effect; cytotoxicity measurements not performed			
	1	Thyrotropin-releasing hormone receptor	Human	20 hours	Uptake from the medium (in vitro)		No effect	No effect			
	2	Thyrotropin-releasing hormone receptor	Human	20 hours	Uptake from the medium (in vitro)		No effect	No effect			
	6	Thyrotropin-releasing hormone receptor	Human	0.5 hours	Uptake from the medium (in vitro)		No effect	No effect			
	7	Thyrotropin-releasing hormone receptor	Human	0.5 hours	Uptake from the medium (in vitro)		No effect	No effect			
	8	Thyrotropin-releasing hormone receptor	Human	0.5 hours	Uptake from the medium (in vitro)		No effect	No effect			
In vivo mechanistic	31	T3 and T4 level	Rat	10 days	Oral		Increase	Increased T4 levels at high dose (38%) compared with controls. No dose response was noted.	In male rats, increased T4 levels were observed at high dose. This effect was deemed as limited evidence due to this effect was observed in pre-puberal rats		
	51	T3 and T4 level	Rat	10 days	Oral			Not measured			
	52	T3 and T4 level	Rat	10 days	Oral			Not measured			
	53	T3 and T4 level	Rabbit	13 days	Oral			Not measured			
	54	T3 and T4 level	Rabbit	13 days	Oral			Not measured			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	51	Thyroid-stimulating hormone level (TSH)	Rat	10 days	Oral			Not measured	and no additional thyroid hormone measurements were performed in the studies provided within the dossier.		
	52	Thyroid-stimulating hormone level (TSH)	Rat	10 days	Oral			Not measured			
	53	Thyroid-stimulating hormone level (TSH)	Rabbit	13 days	Oral			Not measured			
	54	Thyroid-stimulating hormone level (TSH)	Rabbit	13 days	Oral			Not measured			
T-Mediated	33	Thyroid histopathology	Rat	28 days	Oral		No effect	No effect	Thyroid adenomas and carcinomas were increased in dodine treated rat males in the chronic-carcinogenicity study. Particularly relevant is the increase of thyroid C-cell carcinomas in mid and high dose male groups. Thyroid C-cell adenomas incidence were increased in all dodine treated groups, but without showing a clear dose -relationship. Although these findings were not supported by statistical significance, the incidences exceed the mean and range of the HCD provided for the laboratory. Consequently, these effects were considered relevant for human risk assessment.	Adversity was observed in T-mediated parameters. Thyroid carcinomas and adenomas were increased in dodine-treated groups in male rats, compared with controls, in a dose in which no systemic toxicity was noted. These effects were deemed relevant for human risk assessment. Colloid area, follicular cell height and HDL/LDL ratios were not measured. No adverse effects were related to liver wt.	
	36	Thyroid histopathology	Rat	28 days	Dermal		No effect	No effect			
	38	Thyroid histopathology	Rat	90 days	Oral		No effect	No effect			
	39	Thyroid histopathology	Mouse	90 days	Oral		No effect	No effect			
	40	Thyroid histopathology	Dog	90 days	Oral		No effect	No effect			
	41	Thyroid histopathology	Dog	6 weeks	Oral		No effect	No effect			
	45	Thyroid histopathology	Dog	1 year	Oral	200 ppm	Increase	Shift of follicular epithelium from squamous to cuboidal variety and increased vascularity in 1 dog at 200 ppm and in 2 female dogs at 800 ppm. Evidence of stimulation in thyroid glands on males at 800 ppm, cell type predominantly cuboidal with transition to low columnar and increase in vascularity.			
	47	Thyroid histopathology	Dog	52 weeks	Oral		No effect	No effect			
	48	Thyroid histopathology	Rat	2 years	Oral	200 ppm	Increase	Increased thyroid C-cell adenoma incidences in all male dodine-treated groups, without a dose-response pattern and statistical significance (29%, 38%, 33% and 42% for controls, low, mid and high dose groups). Increased thyroid C-cell carcinoma occurrences in mid and top dose male group, without a dose-response pattern and statistical significance (6%, 2%, 12% and 11% for			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								controls, low, mid and high dose group). Increased combined thyroid C-cell adenoma/carcinomas in all male dodine- treated groups, without statistical significance, but dose-related (35%, 40%, 45% and 53% for controls, low, mid and high dose groups).			
	49	Thyroid histopathology	Mouse	78 weeks	Oral		No effect	No effect			
	51	Thyroid histopathology	Rat	10 days	Oral			Not measured			
	52	Thyroid histopathology	Rat	10 days	Oral			Not measured			
	53	Thyroid histopathology	Rabbit	13 days	Oral			Not measured			
	54	Thyroid histopathology	Rabbit	13 days	Oral			Not measured			
	32	Thyroid weight	Rat	28 days	Oral		No effect	No effect			
	33	Thyroid weight	Rat	28 days	Oral		No effect	No effect			
	38	Thyroid weight	Rat	90 days	Oral		No effect	No effect			
	40	Thyroid weight	Dog	90 days	Oral		No effect	No effect			
	41	Thyroid weight	Dog	6 weeks	Oral		No effect	No effect			
	45	Thyroid weight	Dog	1 year	Oral	50 ppm	Increase	Increase in abs and rel thyroid weight from 50 ppm.			
	48	Thyroid weight	Rat	2 years	Oral		No effect	No effect			
	50	Thyroid weight	Rat	29 weeks	Oral			Not measured			
	51	Thyroid weight	Rat	10 days	Oral			Not measured			
	52	Thyroid weight	Rat	10 days	Oral			Not measured			
	53	Thyroid weight	Rabbit	13 days	Oral			Not measured			
	54	Thyroid weight	Rabbit	13 days	Oral			Not measured			
	31	Liver weight	Rat	10 days	Oral		No effect	No effect	Equivocal effects (decrease/ increase) in liver wt were noted in different toxicity studies and in different species. Overall, no adverse effects in liver weight were found.		
	32	Liver weight	rat	28 days	Oral	100 mg/kg bw	Increase	Increase in relative-to-body liver weight in females at 100 mg/kg bw/day			
	33	Liver weight	rat	28 days	Oral		No effect	No effect			
	34	Liver weight	rat	28 days	Oral	800 ppm	Decrease	Decrease in absolute and relative-to-body liver weight in females at 800 ppm (equiv to 76.7 mg/kg bw)			
	36	Liver weight	rat	28 days	Dermal		No effect	No effect			
	37	Liver weight	mouse	8 weeks	Oral		No effect	No effect			
	38	Liver weight	rat	90 days	Oral		No effect	No effect			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	39	Liver weight	mouse	90 days	Oral	2500 ppm	Increase	Relative-to-body liver weight increased in both sexes at 2500 ppm			
	40	Liver weight	dog	90 days	Oral		No effect	No effect			
	41	Liver weight	dog	6 weeks	Oral		No effect	No effect			
	43	Liver weight	rat	90 days	Oral		No effect	No effect			
	47	Liver weight	dog	52 weeks	Oral	10 mg/kg bw	No effect	Absolute and relative liver weight increased from 10 mg/kg bw/day in females (no histopathological findings associated).			
	48	Liver weight	rat	2 years	Oral		No effect	No effect			
	49	Liver weight	mouse	78 weeks	Oral	1500 ppm	Increase	Relative liver weight was increased in top dose groups (13/14% for males/females)			
	50	Liver weight	rat	29 weeks	Oral	800 ppm	Decrease	Decrease abs liver wt in high dose F1 pups males (18%)			
	50	Liver weight	rat	29 weeks	Oral	800 ppm	Decrease	Decrease abs liver wt in high dose F1 males (16%)			
	50	Liver weight	rat	29 weeks	Oral	800 ppm	Decrease	Decrease abs liver wt in high dose F1 females (12%)			
	50	Liver weight	rat	29 weeks	Oral	800 ppm	Decrease	Decrease abs liver wt in high dose F2 male pups (17%)			
	50	Liver weight	rat	29 weeks	Oral	800 ppm	Decrease	Decrease abs liver wt in high dose F2 female pups (17%)			
Sensitive to, but not diagnostic of, EATS	32	Adrenals weight	rat	28 day	Oral	100 mg/kg bw/day	Increase	At 100 mg/kg bw/day, relative-to-body adrenal weight in males increased 33.3%, relative-to-body adrenal weight in females increased 36.7% and relative-to-brain adrenal weight increased 23.7%.	Changes in adrenal wt and histopathology were recorded but only at MTD.		N
	33	Adrenals weight	rat	28 day	Oral		No effect	No effect			
	36	Adrenals weight	rat	28 day	Dermal		No effect	No effect			
	38	Adrenals weight	rat	90 day	Oral		No effect	No effect			
	39	Adrenals weight	mouse	90 day	Oral		No effect	No effect			
	40	Adrenals weight	dog	90 day	Oral		No effect	No effect			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	41	Adrenals weight	dog	6 week	Oral		No effect	No effect			
	43	Adrenals weight	rat	90 day	Oral		No effect	No effect			
	48	Adrenals weight	rat	2 year	Oral	200 ppm	Decrease	A reduction, without a dose response, were noted in relative adrenal weight in males.			
	49	Adrenals weight	mouse	78 week	Oral		No effect	No effect			
	50	Adrenals weight	rat	29 week	Oral	800 ppm	Increase	Increased left and right adrenal wt (14%) in high dose F0 females.			
	50	Adrenals weight	rat	29 week	Oral	800 ppm	Increase	Increased rel left adrenal (21%) in F1 males at high dose.			
	50	Adrenals weight	rat	29 week	Oral	400 ppm	Increase	Increased rel left (23%) and right (20%) adrenal in F1 females at high dose. Increase rel left adrenal (12%) in mid dose F1 females.			
	32	Adrenals histopathology	rat	28 day	Oral	200 mg/kg bw/day	Increase	Adrenals haemorrhage increased (6/10 vs 0/10 in controls, in both sexes) at 200 mg/kg bw/day (above MTD, pre-terminal). Lower doses not tested.			
	33	Adrenals histopathology	rat	28 day	Oral		No effect	No effect			
	36	Adrenals histopathology	rat	28 day	Dermal		No effect	No effect			
	38	Adrenals histopathology	rat	90 day	Oral		No effect	No effect			
	39	Adrenals histopathology	mouse	90 day	Oral		No effect	No effect			
	40	Adrenals histopathology	dog	90 day	Oral		No effect	No effect			
	41	Adrenals histopathology	dog	6 week	Oral		No effect	No effect			
	43	Adrenals histopathology	rat	90 day	Oral		No effect	No effect			
	47	Adrenals histopathology	dog	52 week	Oral		No effect	No effect			
	48	Adrenals histopathology	rat	2 year	Oral	800 ppm	Increase	Increases of enlarge and white mottling incidences in adrenal gland were found in top dose females groups compared with controls.			
	49	Adrenals histopathology	mouse	78 week	Oral		No effect	No effect			
	50	Adrenals histopathology	rat	29 week	Oral			Not measured			
	50	Adrenals histopathology	rat	29 week	Oral			Not measured			
	36	Brain histopathology examination	rat	28 day	Dermal		No effect	No effect			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	38	Brain histopathology examination	rat	90 day	Oral		No effect	No effect	Increased brain wt were recorded in both sexes in long term and 2-generation toxicity studies. No histopathological alterations were further described.		
	39	Brain histopathology examination	mouse	90 day	Oral		No effect	No effect			
	40	Brain histopathology examination	dog	90 day	Oral		No effect	No effect			
	47	Brain histopathology examination	dog	52 week	Oral		No effect	No effect			
	48	Brain histopathology examination	rat	2 year	Oral		No effect	No effect			
	49	Brain histopathology examination	mouse	78 week	Oral		No effect	No effect			
	50	Brain histopathology examination	rat	29 week	Oral			Not measured			
	50	Brain histopathology examination	rat	29 week	Oral			Not measured			
	32	Brain weight	rat	28 day	Oral		No effect	No effect			
	33	Brain weight	rat	28 day	Oral		No effect	No effect			
	36	Brain weight	rat	28 day	Dermal		No effect	No effect			
	38	Brain weight	rat	90 day	Oral		No effect	No effect			
	39	Brain weight	mouse	90 day	Oral		No effect	No effect			
	40	Brain weight	dog	90 day	Oral		No effect	No effect			
	41	Brain weight	dog	6 week	Oral		No effect	No effect			
	47	Brain weight	dog	52 week	Oral		No effect	No effect			
	48	Brain weight	rat	2 year	Oral	400 ppm	Increase	An increase, without a clear dose response, was noted in relative brain weight in females at mid and high dose (14% and 12%, respectively).			
	49	Brain weight	mouse	78 week	Oral	1500 ppm	Increase	Relative brain weight was increased in top dose groups (8/11% for males/females).			
	50	Brain weight	rat	29 week	Oral	800 ppm	Decrease	Decreased abs brain in F0 males (3%) at high dose.			
	50	Brain weight	rat	29 week	Oral	800 ppm	Increase	Increased rel brain in F0 females (7%) at high dose.			
	50	Brain weight	rat	29 week	Oral	800 ppm	Increase	Increased rel brain wt in F1 pup males at high dose (12%).			
	50	Brain weight	rat	29 week	Oral	800 ppm	Increase	Increased rel brain wt in F1 males at high dose (13%).			
	50	Brain weight	rat	29 week	Oral	800 ppm	Change	Decreased abs brain wt in F1 females at high dose (4%). Increase rel brain wt in F1 females (9%).			
	50	Brain weight	rat	29 week	Oral	800 ppm	Increase	Increase rel brain wt in F2 pup males at high dose (18%).			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	50	Brain weight	rat	29 week	Oral	800 ppm	Increase	Increase rel brain wt in F2 pup females at high dose (15%).			
	50	Fertility (mammals)	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	50	Fertility (mammals)	rat	29 week	Oral		No effect	No effect			
	51	Fertility (mammals)	rat	10 day	Oral		No effect	No effect			
	52	Fertility (mammals)	rat	10 day	Oral		No effect	No effect			
	53	Fertility (mammals)	rabbit	13 day	Oral		No effect	No effect			
	54	Fertility (mammals)	rabbit	13 day	Oral	80 mg/kg bw/day	Decrease	A slight decrease in fertility index was observed in high dose group compared with controls, in which three dams were not pregnant (94%, 94%, 100% and 85% for controls, low, mid and high dose groups, respectively).			
	55	Fertility (mammals)	rat	1 Year	Oral		No effect	No effect			
	52	Foetal development	rat	10 day	Oral		No effect	No effect	No treatment-related effects were observed		
	54	Foetal development	rabbit	13 day	Oral		No effect	No effect	No treatment-related effects were observed		
	50	Gestation length	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	50	Litter size	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	52	Litter size	rat	10 day	Oral		No effect	No effect			
	55	Litter size	rat	1 Year	Oral	800 ppm	Decrease	Smaller sizes were observed in F2 litters of rats treated with dodine than those in controls. This study present important deviations and was deemed no reliable.			
	50	Litter viability	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	52	Litter viability	rat	10 day	Oral		No effect	No effect			
	55	Litter viability	rat	1 Year	Oral		No effect	No effect			
	50	Litter/pup weight	rat	29 week	Oral	400 ppm	Decrease	Bodyweights were significantly decreased in F1 generation for the male and female pups from lactation days 4-21 in the high dose group; and on	Decreased pup wt were recorded in both generations in rat generational study. No relevant decreases were recorded in		

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								days 4 (precull and postcull, females only), 14 (females only), and 21 (males and females) for the pups in the mid dose group.	developmental toxicity studies.		
	50	Litter/pup weight	rat	29 week	Oral	400 ppm	Decrease	Bodyweights were statistically significantly lower in F2 generation on days 4 (pre-cull and post-cull, males only), 7, 14, and 21 for pups in the 800 ppm dose group and on days 14 (males only) and 21 for pups in the 400 ppm dose group.			
	51	Litter/pup weight	rat	10 day	Oral		No effect	No effect			
	52	Litter/pup weight	rat	10 day	Oral		No effect	No effect			
	53	Litter/pup weight	rabbit	13 day	Oral	70 mg/kg bw/day	Decrease	Decrease not dose related mean foetal wt at high dose (4%) and low dose (8%).			
	54	Litter/pup weight	rabbit	13 day	Oral		No effect	No effect			
	51	Number of implantations, corpora lutea	rat	10 day	Oral		No effect	No effect	Decreased live implants and increased dead implants were recorded in developmental toxicity study in rabbits.		
	52	Number of implantations, corpora lutea	rat	10 day	Oral		No effect	No effect			
	53	Number of implantations, corpora lutea	rabbit	13 day	Oral	100 mg/kg bw/day	Decrease	Decreased mean live implants (18%) at high dose tested.			
	53	Number of implantations, corpora lutea	rabbit	13 day	Oral	70 mg/kg bw/day	Increase	Increased mean dead implants at high dose (19%) and low dose (12%).			
	54	Number of implantations, corpora lutea	rabbit	13 day	Oral	40 mg/kg bw/day	Decrease	Decreased mean live implants at mid (9%) and high dose group (7%), compared with controls.			
	54	Number of implantations, corpora lutea	rabbit	13 day	Oral	10 mg/kg bw/day	Increase	Increased total dead implants (15%, 123% and 53% for low, mid, and high dose groups, respectively).			
	54	Number of implantations, corpora lutea	rabbit	13 day	Oral	10 mg/kg bw/day	Increase	Increased mean dead implants (22%, 111% and 88% for low, mid, and high dose groups, respectively).			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	50	Number of live births	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	52	Number of live births	rat	10 day	Oral		No effect	No effect			
	51	Numbers of embryonic or foetal deaths and viable foetuses	rat	10 day	Oral		No effect	No effect	Increased early and late resorptions were recorded in developmental toxicity study in rabbits.		
	52	Numbers of embryonic or foetal deaths and viable foetuses	rat	10 day	Oral		No effect	No effect			
	53	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	70 mg/kg bw/day	Increase	Increased late resorptions (80%) at high dose and low dose (10%).			
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	10 mg/kg bw/day	Increase	Increased total early resorptions (43%, 114% and 43% for low, mid, and high dose groups, respectively).			
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	10 mg/kg bw/day	Increase	Increased mean early resorptions (40%, 100% and 60% for low, mid, and high dose groups, respectively).			
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	10 mg/kg bw/day	Increase	Increased % early resorptions (40%, 100% and 60% for low, mid, and high dose groups, respectively), compared with controls.			
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	40 mg/kg bw/day	Increase	Increased total late resorptions at mid (350%) and high dose group (150%).			
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	40 mg/kg bw/day	Increase	Increased mean late resorptions at mid (500%) and high dose group (300%).			
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	40 mg/kg bw/day	Increase	Increased % late resorptions at mid (200%) and high dose group (100%), compared with controls.			
	36	Pituitary histopathology	rat	28 day	Dermal		No effect	No effect	No treatment-related effects were observed		
	39	Pituitary histopathology	mouse	90 day	Oral		No effect	No effect			
	40	Pituitary histopathology	dog	90 day	Oral		No effect	No effect			
	47	Pituitary histopathology	dog	52 week	Oral		No effect	No effect			
	48	Pituitary histopathology	rat	2 year	Oral		No effect	No effect			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	49	Pituitary histopathology	mouse	78 week	Oral		No effect	No effect			
	50	Pituitary histopathology	rat	29 week	Oral			not measured			
	50	Pituitary histopathology	rat	29 week	Oral			not measured			
	39	Pituitary weight	mouse	90 day	Oral		No effect	No effect			
	40	Pituitary weight	dog	90 day	Oral		No effect	No effect			
	48	Pituitary weight	rat	2 year	Oral		No effect	No effect			
	52	Post implantation loss	rat	10 day	Oral		No effect	No effect	Increased post implantation loss were recorded in developmental toxicity study in rabbits.		
	54	Post implantation loss	rabbit	13 day	Oral	40 mg/kg bw/day	Increase	Increased post implantation loss in mid and high dodine treated groups (10%, 10%, 19% an 17% for control. low, mid and high dose groups).			
	51	Pre implantation loss	rat	10 day	Oral		No effect	No effect			
	52	Pre implantation loss	rat	10 day	Oral		No effect	No effect			
	53	Pre implantation loss	rabbit	13 day	Oral		Increase				
	54	Pre implantation loss	rabbit	13 day	Oral		No effect	No effect			
	52	Presence of anomalies (external, visceral, skeletal	rat	10 day	Oral		No effect	No effect	No treatment-related effects were observed		
	54	Presence of anomalies (external, visceral, skeletal	rabbit	13 day	Oral		No effect	No effect			
	55	Pup development	rat	1 Year	Oral		No effect	No effect	No treatment-related effects were observed		
	50	Pup survival index	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	52	Pup survival index	rat	10 day	Oral		No effect	No effect			
	50	Sex ratio	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	52	Sex ratio	rat	10 day	Oral		No effect	No effect			
	54	Sex ratio	rabbit	13 day	Oral		No effect	No effect			
Target organ toxicity	33	Kidney histopathology	rat	28 day	Oral	1000 ppm	Increase	In female kidneys, mineralization of the cortico-medullary junction (5/10 vs 2/10 in control) increased at 1000 ppm. In both sexes, very slight increase in fibrosis (1/10 vs 0/10 in control, per sex) at 1000 ppm.Lower doses not tested.	Tubular hyperplasia was found in male mice in chronic toxicity study. No other relevant findings were recorded in the dossier studies. Equivocal histological effects (increase/ decrease) were noted in chronic rat/mice toxicity studies. In 2-	Hepatocellular adenomas were increased in top dose male/female dose groups in the 78-week mice-chronic toxicity study. This effect did not show a clear dose response nor statistical	
	36	Kidney histopathology	rat	28 day	Dermal		No effect	No effect			
	38	Kidney histopathology	rat	90 day	Oral		No effect	No effect			
	39	Kidney histopathology	mouse	90 day	Oral		No effect	No effect			
	40	Kidney histopathology	dog	90 day	Oral		No effect	No effect			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	41	Kidney histopathology	dog	6 week	Oral		No effect	No effect	generation reproductive toxicity study, abs kidney wt, but no rel were reduced in adult and pup rats. Theses findings were not deemed adverse nor biologically relevant.	significance. Signs of systemic toxicity was noted at this dose. No other adverse histopathology findings in the liver were noted in another species in the toxicology studies within the dossier. Overall, these effects were not considered relevant for human risk assessment.	
	47	Kidney histopathology	dog	52 week	Oral		No effect	No effect			
	48	Kidney histopathology	rat	2 year	Oral	200 ppm	Decrease	reduction in pelvic mineralization in kidney (males) incidences were noted in all dodine-treated groups			
	49	Kidney histopathology	mouse	78 week	Oral	200 ppm	Change	Cyst incidences were slightly increased in dodine-treated males, but were decreased in dodine-treated females, compared with controls. Moreover, dilatation pelvis occurrences were reduced in dodine-treated males, whereas hyperplasia of tubular cell incidences were mainly increased in top dose male group compared with controls.			
	50	Kidney histopathology	rat	29 week	Oral			Not measured			
	50	Kidney histopathology	rat	29 week	Oral			Not measured			
	51	Kidney histopathology	rat	10 day	Oral	100 mg/kg bw/day	Increase	In females, low incidences of epithelial pelvic dilatation (10%), pelvic inflammation (10%) and nephritis (10%) at high dose groups. These findings were not further reproduced in the main developmental toxicity study.			
	53	Kidney histopathology	rabbit	13 day	Oral	100 mg/kg bw/day	Increase	20% animal showed kidney inflammation at high dose. These findings were not further reproduced in the main developmental toxicity study.			
	31	Kidney weight	Rat	10 day	Oral		No effect	No effect			
	32	Kidney weight	rat	28 day	Oral		No effect	No effect			
	33	Kidney weight	rat	28 day	Oral	1000 ppm	Decrease	Absolute and relative-to-brain kidney weight			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								reduced in both sexes at 1000 ppm.			
	34	Kidney weight	rat	28 day	Oral		No effect	No effect			
	36	Kidney weight	rat	28 day	Dermal		No effect	No effect			
	37	Kidney weight	mouse	8 week	Oral		No effect	No effect			
	38	Kidney weight	rat	90 day	Oral		No effect	No effect			
	39	Kidney weight	mouse	90 day	Oral		No effect	No effect			
	40	Kidney weight	dog	90 day	Oral		No effect	No effect			
	41	Kidney weight	dog	6 week	Oral		No effect	No effect			
	47	Kidney weight	dog	52 week	Oral		No effect	No effect			
	48	Kidney weight	rat	2 year	Oral	200 ppm	Change	A decreased trend, not statistically significant and not clear dose related, was observed in relative kidney weight in all males dodine treated groups, whereas in females, an increased trend was recorded for relative kidney weight.			
	49	Kidney weight	mouse	78 week	Oral	750 ppm	Increase	Absolute (high dose) and relative (mid and high dose) kidney weights were significantly increased in females.			
	50	Kidney weight	rat	29 week	Oral	800 ppm	Decrease	Decreased abs left kidney in F0 males (5%) at high dose.			
	50	Kidney weight	rat	29 week	Oral	400 ppm	Decrease	Decreased abs left and right kidney at high dose (6%) and mid dose (6%) in F0 females.			
	50	Kidney weight	rat	29 week	Oral	800 ppm	Decrease	Decrease abs left and right kidney wt in high dose F1 male pup (16%).			
	50	Kidney weight	rat	29 week	Oral	400 ppm	Decrease	Decrease abs left (15%) and right (14%) kidney wt in high dose F1 males. Decrease abs kidney wt (7%) in mid dose F1 males.			
	50	Kidney weight	rat	29 week	Oral	400 ppm	Decrease	Decrease abs left (11%) and right (12%) kidney wt in high dose F1 females. Decrease abs left (5%) and			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								right (6%) kidney wt in mid dose F1 female.			
	50	Kidney weight	rat	29 week	Oral	400 ppm	Decrease	Decrease abs left and right kidney wt (16%) in high dose F2 male pups. Decrease abs left (11%) kidney wt in mid dose F2 male pups in mid dose group.			
	50	Kidney weight	rat	29 week	Oral	800 ppm	Decrease	Decrease abs left (14%) and right (17%) kidney wt in high dose F2 female pups.			
	33	Liver histopathology	rat	28 day	Oral		No effect	No effect	Benign tumours (hepatocellular adenomas) were increased after dodine administration. Liver adenomas appeared at a dose in which systemic toxicity was observed and the results were not supported by statistical significance between groups and controls. Besides, although the occurrence of combined adenomas/carcinomas displayed statistically significance in the top dose female group, it is noteworthy that carcinomas incidence was very similar between dodine-treated groups and their respective controls for both sexes.		
	36	Liver histopathology	rat	28 day	Dermal		No effect	No effect			
	37	Liver histopathology	mouse	8 week	Oral	100/1250 ppm	Increase	Mild eosinophilia in liver in both sexes at 100/1250 ppm.			
	38	Liver histopathology	rat	90 day	Oral		No effect	No effect			
	39	Liver histopathology	mouse	90 day	Oral		No effect	No effect			
	40	Liver histopathology	dog	90 day	Oral		No effect	No effect			
	41	Liver histopathology	dog	6 week	Oral		No effect	No effect			
	43	Liver histopathology	rat	90 day	Oral		Change	Not described			
	47	Liver histopathology	dog	52 week	Oral	20 mg/kg bw/day	No effect	Slight increment in liver vacuolization in males at 20 mg/kg bw/day.			
	48	Liver histopathology	rat	2 year	Oral	200 ppm	Decrease	Reduction in bile duct hyperplasia in liver (females) incidences were noted in all dodine-treated groups.			
	49	Liver histopathology	mouse	78 week	Oral	1500 ppm	Increase	An increased incidences of hepatocellular adenomas were observed at high dose groups for both sexes (13%, 12%, 15% and 23% for controls, low, mid and high dose males groups; and 0%, 2%, 2% and 7% for controls, low, mid and high dose females groups, respectively), in which a statistically significant trend was displayed for females.			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								On the other hand, no relevant increases were noted regarding hepatocellular carcinomas in dodine-treated groups (male or females). When the effects were combined, increased incidences were also observed in high dose groups (17%, 12%, 20% and 25% for controls, low, mid and high dose males groups; and 0%, 3%, 2% and 8% for controls, low, mid and high dose females groups, respectively), showing a significant trend test in females, and the only significant group comparison difference with controls was for combined adenomas and carcinomas in females given 1500 ppm dose.			
	50	Liver histopathology	rat	29 week	Oral			not measured			
	50	Liver histopathology	rat	29 week	Oral			not measured			
	53	Liver histopathology	rabbit	13 day	Oral	100 mg/kg bw/day	Increase	20% animal showed liver inflammation			
	40	Pancreas histopathology	dog	90 day	Oral		No effect	No effect	No treatment-related effects were observed		
	47	Pancreas histopathology	dog	52 week	Oral		No effect	No effect			
	48	Pancreas histopathology	rat	2 year	Oral		No effect	No effect			
	49	Pancreas histopathology	mouse	78 week	Oral		No effect	No effect			
	36	Spleen histopathology	rat	28 day	Dermal		No effect	No effect	No adverse treatment-related effects were observed		
	37	Spleen histopathology	mouse	8 week	Oral		No effect	No effect			
	38	Spleen histopathology	rat	90 day	Oral		No effect	No effect			
	39	Spleen histopathology	mouse	90 day	Oral	2500 ppm	No effect	Lymphoid atrophy in spleen in 3/10 females at 2500 ppm vs 0/10 in control (lower doses not analysed).			
	50	Spleen histopathology	rat	29 week	Oral			Not measured			
	50	Spleen histopathology	rat	29 week	Oral			Not measured			
	36	Spleen weight	rat	28 day	Dermal		No effect	No effect			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	37	Spleen weight	mouse	8 week	Oral	100/1250 ppm	Decrease	Absolute spleen weight reduced in females at 100/1250 ppm.			
	38	Spleen weight	rat	90 day	Oral		No effect	No effect			
	39	Spleen weight	mouse	90 day	Oral	1250 ppm	No effect	Absolute spleen weight reduced in both sexes from 1250 ppm. Relative-to-body spleen weight in females decreased from 1250 ppm.			
	50	Spleen weight	rat	29 week	Oral	400 ppm	Decrease	Decrease abs spleen wt (28%) in high dose F2 male pups.			
	50	Spleen weight	rat	29 week	Oral	400 ppm	Decrease	Decrease abs spleen wt (22%) in high dose F2 female pups.			
	53	Stomach histopathology	rabbit	13 day	Oral	100 mg/kg bw/day	Increase	50% of animals showed colour or dark foci areas on stomach, in some cases with epithelium hyperplasia.	No treatment-related effects were observed		
	36	Thymus histopathology	rat	28 day	Dermal		No effect	No effect			
	39	Thymus histopathology	mouse	90 day	Oral	2500 ppm	No effect	Lymphoid necrosis and atrophy in thymus in 4/10 females at 2500 ppm vs 0/10 in control (lower doses not analysed).			
	50	Thymus histopathology	rat	29 week	Oral			Not measured	No adverse treatment-related effects were observed		
	50	Thymus histopathology	rat	29 week	Oral						
	36	Thymus weight	rat	28 day	Dermal		No effect	No effect			
	50	Thymus weight	rat	29 week	Oral	800 ppm	Decrease	Decreased abs thymus in F0 males (17%) at high dose.			
	50	Thymus weight	rat	29 week	Oral	800 ppm	Decrease	Decrease abs thymus wt in F2 female pups (28%) in high dose group.			
	51	Urinary bladder histopathology	rat	10 day	Oral	100 mg/kg bw/day	Increase	Low incidences of epithelial hyperplasia (10%) and chronic inflammation (10%) at high dose group. Ureter inflammation at high dose groups (10%).	No adverse treatment-related effects were observed		
	31	Body weight	Rat	10 day	Oral		No effect	No effect			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
Systemic toxicity	32	Body weight	rat	28 day	Oral	75 mg/kg bw/day	Decrease	Bw reduced in males and bw gain lower in males and females from 100 mg/kg bw/day.	Signs of systemic toxicity occurred mainly at high doses, which included mortality, effects on bodyweight, food consumption, and clinical signs; these signs were related to general toxicity of higher doses. However, a case by case approach may be done, as toxic adverse effects were not observed in all studies.	Overall evidence of systemic toxicity.	
	33	Body weight	rat	28 day	Oral	500 ppm	Decrease	Bw reduced in both sexes at 1000 ppm and bw gain lower in males from 750 ppm and in females from 500 ppm.			
	34	Body weight	rat	28 day	Oral	800 ppm	Decrease	Bw gain reduced in both sexes at 800 ppm.			
	35	Body weight	rat	28 day	Oral	800 ppm	Decrease	Bw gain reduced in both sexes at 800 ppm.			
	36	Body weight	rat	28 day	Dermal	125 mg/kg bw/day	Decrease	Bw gain reduced in males from 125 mg/kg bw/day.			
	37	Body weight	mouse	8 week	Oral	100/1250 ppm	Decrease	Bw reduced in females and bw gain reduced in both sexes at 100/1250 ppm.			
	38	Body weight	rat	90 day	Oral	800 ppm	Decrease	Bw gain reduced in both sexes at 800 ppm.			
	39	Body weight	mouse	90 day	Oral	1250 ppm	Decrease	Bw reduced in males at 2500 ppm and bw gain reduced in males at 1250 ppm and in females at 2500 ppm.			
	40	Body weight	dog	90 day	Oral	20 mg/kg bw/day	Decrease	Bw reduced in females and bw gain reduced in both sexes at 20 mg/kg bw/day.			
	41	Body weight	dog	6 week	Oral	25 mg/kg bw/day	Decrease	Males: bw loss at 50 mg/kg bw/day up to 4 weeks and at 60 mg/kg bw/day for 2 weeks. Bw loss in a male at 25 mg/kg bw/day for 6 weeks. Females: bw loss at 50 mg/kg bw/day up to 5 weeks and at 60 mg/kg/day for 2 weeks.			
	42	Body weight	rat	28 day	Oral	83 mg/kg bw/day	Decrease	Lower body weight gain for high dosed animals.			
	43	Body weight	rat	90 day	Oral		No effect	No effect			
	44	Body weight	rat	100 day	Oral	3200 ppm	Decrease	lower body weight gain for high dosed animals			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	45	Body weight	dog	1 Year	Oral	50 ppm	Decrease	Reduced bw gain			
	46	Body weight	rat	2 year	Oral	800 ppm	Decrease	lower body weight gain for high dosed animals			
	47	Body weight	dog	52 week	Oral	10 mg/kg bw/day	Decrease	Some dogs from 10 mg/kg bw/day exhibited marked bw loss during first weeks that prompted supplemental feeding.			
	48	Body weight	rat	2 year	Oral	800 ppm	Decrease	Slight statistically significant decreases in bodyweight were recorded in top dose male group throughout week 1-37 (5.2-8.2%) and weeks 85-89 (7-8%), whereas in females were noted throughout whole study (4.1-16.6%)			
	49	Body weight	mouse	78 week	Oral	750 ppm	Decrease	Statistically significantly, lower bodyweights were recorded at top male (3-10%) and female (4-14%) dose groups throughout whole study, compared with controls. At mid dose groups, statistically significant reductions were mainly noted from week 30 to study termination for both sexes (2-5% for males and 4-10% for females, respectively), although sporadic reductions were observed the days before week 30. Overall mean bodyweight gain was statistically significant reduced in mid and high dose male groups (5 and 26%) and in dodine-female treated groups (11, 20 and 35% for low, mid and high dose groups, respectively).			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	50	Body weight	rat	29 week	Oral	800 ppm	Decrease	Statistically significantly lower in high dose groups for both sexes throughout study.			
	50	Body weight	rat	29 week	Oral	400 ppm	Decrease	Statistically significantly lower in high (male and females) and mid dose (females) groups throughout study.			
	51	Body weight	rat	10 day	Oral	70 mg/kg bw/day	Decrease	Statistically significant decrease in bodyweight was recorded in gestation day 13 in mid and high dose groups (10% and 8%, respectively), however decreases, not statistically significant and without dose-relationship, were recorder throughout the whole gestation period of mid (8-10%) and top dose dams (2-8%) compared with controls. Statistically significant decrease in bodyweight gain was recorded throughout gestation day 6-13 in mid and high dose groups (26% and 48%, respectively).			
	52	Body weight	rat	10 day	Oral	90 mg/kg bw/day	Decrease	Statistically significant decrease in bodyweights were recorded in gestation day 9 (9%), 13 (8%) and 17 (8%) in high dose group, compared with controls. At high dose group, bodyweight gain was statistically significantly lower from gestation day 6-9 (107%) and 6-17 (20%), compared with the controls. Moreover, corrected bodyweight gain by the			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								uterus weight was statistically significantly lower in top dose group (756%), compared with controls.			
	53	Body weight	rabbit	13 day	Oral	100 mg/kg bw/day	Decrease	Bodyweight loss at high dose group (48%), compared with controls.			
	54	Body weight	rabbit	13 day	Oral		No effect				
	55	Body weight	rat	1 Year	Oral		No effect				
	32	Clinical chemistry and haematology	rat	28 day	Oral	100 mg/kg bw/day	Increase	WBC counts and segmented neutrophils increased in both sexes and RDW increased in males, at 100 mg/kg bw/day.			
	32	Clinical chemistry and haematology	rat	28 day	Oral	100 mg/kg bw/day	Decrease	Lymphocyte count reduced in both sexes at 100 mg/kg bw/day.			
	32	Clinical chemistry and haematology	rat	28 day	Oral	75 mg/kg bw/day	Increase	Alanine aminotransferase increased in both sexes from 75 mg/kg bw/day			
	33	Clinical chemistry and haematology	rat	28 day	Oral	1000 ppm	decrease	Alanine aminotransferase reduced in females at 1000 ppm.			
	38	Clinical chemistry and haematology	rat	90 day	Oral	800 ppm	Increase	Neutrophils increased in males at 800 ppm.			
	38	Clinical chemistry and haematology	rat	90 day	Oral	800 ppm	Decrease	Alanine aminotransferase reduced in females at 800 ppm.			
	39	Clinical chemistry and haematology	mouse	90 day	Oral	2500 ppm	Increase	Neutrophils and RDW increased in males at 2500 ppm.			
	39	Clinical chemistry and haematology	mouse	90 day	Oral	2500 ppm	Increase	BUN in both sexes, phosphorus in males and A/G ratio in females increased at 2500 ppm.			
	40	Clinical chemistry and haematology	dog	90 day	Oral		No effect	No effect			
	41	Clinical chemistry and haematology	dog	6 week	Oral		No effect	No effect			
	42	Clinical chemistry and haematology	rat	28 day	Oral		No effect	No effect			
	43	Clinical chemistry and haematology	rat	90 day	Oral		No effect	No effect			
	44	Clinical chemistry and haematology	rat	100 day	Oral		No effect	No effect			
	46	Clinical chemistry and haematology	rat	2 year	Oral		No effect	No effect			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	47	Clinical chemistry and haematology	dog	52 week	Oral		No effect	No effect			
	48	Clinical chemistry and haematology	rat	2 year	Oral	800 ppm	Decrease	Mean alkaline phosphatase activities were higher at top and mid dose female groups (310% and 150% for top and mid dose groups, respectively) on week 104. No other significant treatment-related variations were noted at any of the scheduled blood sampling periods for any of the parameters assayed.			
	49	Clinical chemistry and haematology	mouse	78 week	Oral		Not measured	Not measured			
	31	Clinical signs	Rat	10 day	Oral		No effect	No effect			
	48	Clinical signs	rat	2 year	Oral	200 ppm	Increase	A statistically significant increase in the absence of grasping was found in top dose male group, compared with controls; whereas a significant trend test was obtained for the absence of grasping, traction and righting reflexes incidences in dodine-male treated groups. On the other hand, a dose-related increase in the hunched posture incidence was revealed in males. Moreover, increased reduced motor activity and piloerection was observed in males dodine-treated groups compared with controls.			
	49	Clinical signs	mouse	78 week	Oral	200 ppm	Increase	Increased incidence of whole body tremors was noted mainly in mid and high dose groups for both sexes (13-14% in males and 11-13% in females compared with controls). Malocclusion occurrences			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								was considerably increased in high male dose group (18.6% vs 5.7% in controls), whereas a slight increase of irregular respiration (4.3% vs 0% in controls) and rough hair coat incidences (11.4% vs 0% in controls) were found in top dose female group. On the other hand, increased dose-related incidences of dilated pupil and excessive salivation were mainly observed in the three male-dodine treated groups and in mid-top male dose groups, respectively, whereas increases,			
	51	Clinical signs	rat	10 day	Oral	100 mg/kg bw/day	Increase	Two females from high dose group exhibited clinical signs: one animal showed wheezing and another female showed piloerection, hunched posture, red/brown staining around face, fore-paws and mild ataxia.			
	52	Clinical signs	rat	10 day	Oral	90 mg/kg bw/day	Increase	Three dams at high dose group showed excessive salivation after dosing for one or 2 days during the treatment period. On the other hand, there was another three animals with red/brown stained fur around the mouth at 90 mg/kg bw/day dose groups.			
	54	Clinical signs	rabbit	13 day	Oral	80 mg/kg bw/day	Increase	15% of rabbits showed liquid faeces, breathing difficulties and emaciation.			
	54	Clinical signs	rabbit	13 day	Oral	80 mg/kg bw/day	Increase	2 abortions (10%) at high dose.			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	32	Food consumption	rat	28 day	Oral	75 mg/kg bw/day	Decrease	Food consumption reduced in both sexes from 75 mg/kg bw/day.			
	33	Food consumption	rat	28 day	Oral	750 ppm	Decrease	Food consumption reduced in both sexes from 750 ppm.			
	34	Food consumption	rat	28 day	Oral	800 ppm	Decrease	Food consumption reduced in males from 800 ppm.			
	35	Food consumption	rat	28 day	Oral		No effect	No effect			
	37	Food consumption	mouse	8 week	Oral		No effect	No effect			
	38	Food consumption	rat	90 day	Oral	800 ppm	Decrease	Food consumption reduced in females at 800 ppm.			
	39	Food consumption	mouse	90 day	Oral	1250 ppm	Decrease	Food consumption reduced in both sexes at 2500 ppm.			
	40	Food consumption	dog	90 day	Oral	20 mg/kg bw/day	Decrease	Food consumption reduced in both sexes at 20 mg/kg bw/day.			
	41	Food consumption	dog	6 week	Oral	25 mg/kg bw/day	Decrease	Decreased food consumption at 50 and 60 mg/kg bw/day and in one male at 25 mg/kg bw/day.			
	42	Food consumption	rat	28 day	Oral	83 mg/kg bw/day	Decrease	statistically significant lower food consumption at high dose animals			
	44	Food consumption	rat	100 day	Oral	3200 ppm	Decrease	Reduced food consumption			
	47	Food consumption	dog	52 week	Oral	10 mg/kg bw/day	Decrease	Some dogs from 10 mg/kg bw/day reduced food consumption. Supplemental feeding required.			
	48	Food consumption	rat	2 year	Oral	800 ppm	Decrease	Food consumption was mostly decreased through sporadic weeks in top dose male (5-12%) and female (4-16%) dose groups without showing a dose relationship and consistency throughout the whole study. On the other hand, isolated decreases or increases were recorded in low and mid dose groups.			
	49	Food consumption	mouse	78 week	Oral	750 ppm	Decrease	Mean food consumption was generally reduced at			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								top dose groups for both sexes throughout whole study (5-16% for males and 5-19 for females, respectively), compared with controls. On the other hand, at mid dose groups, statistically significant reductions were mainly noted at the first half of the study in males (5-8%), and practically through entire study in females (5-16%).			
	50	Food consumption	rat	29 week	Oral	800 ppm	Decrease	Statistically significantly reduced food consumption in high dose groups during premating (male and females), and lactation.			
	50	Food consumption	rat	29 week	Oral	800 ppm	Decrease	Statistically significantly reduced food consumption in high dose groups during premating (male and females), gestation and lactation.			
	51	Food consumption	rat	10 day	Oral	70 mg/kg bw/day	Decrease	Reduction in mean food consumption through days 6-16 in high (24%) and mid dose groups (15%)			
	52	Food consumption	rat	10 day	Oral	45 mg/kg bw/day	Decrease	At high dose group, there was a statistically significantly lower food consumption through gestation day 6-16 (13-37%), whereas at mid dose group, there was a statistically significantly lower food consumption on gestation day 6 (11%) and gestation day 8-10 (12-18%), compared with controls. When time frames were compared, statistical significance was displayed			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								through day 6-10 (30% and 14% for high and mid dose group, respectively), 6-16 (22% and 11% for high and mid dose group, respectively), and 3-19 (14% for high dose group) compared with controls.			
	53	Food consumption	rabbit	13 day	Oral	100 mg/kg bw/day	Decrease	A reduction in absolute food consumption was recorded in top dose group throughout GD 6-18 (31-77%), compared with controls. The mean food consumption was 51% lower than controls for this group			
	54	Food consumption	rabbit	13 day	Oral	80 mg/kg bw/day	Decrease	Statistically significant reduction in food consumption was recorded at high dose group in gestation days 6 (25%), 7 and 8 (30%) compared with controls. Moreover, in this group, sporadic reductions, without statistical significance, were noted during mid to late treatment period.			
	31	Mortality	Rat	10 day	Oral		No effect	No effect			
	32	Mortality	rat	28 day	Oral	75 mg/kg bw/day	Increase	In males, 10/10 at 200 mg/kg bw/day died. In females, 1/10 at 75 mg/kg bw/day, 4/10 at 100 mg/kg bw/day and 10/10 at 200 mg/kg bw/day died.			
	33	Mortality	rat	28 day	Oral		No effect	No effect			
	34	Mortality	rat	28 day	Oral		No effect	No effect			
	35	Mortality	rat	28 day	Oral		No effect	No effect			
	38	Mortality	rat	90 day	Oral		No effect	No effect			
	39	Mortality	mouse	90 day	Oral	2500 ppm	Increase	In females, 4/10 died at 2500 ppm.			
	40	Mortality	dog	90 day	Oral		No effect	No effect			

Grouping	Study ID Matrix	Line(s) of evidence	Species	Exposure	Route of exposure	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	41	Mortality	dog	6 week	Oral		No effect	No effect			
	42	Mortality	rat	28 day	Oral		No effect	No effect			
	44	Mortality	rat	100 day	Oral		No effect	No effect			
	46	Mortality	rat	2 year	Oral		No effect	No effect			
	47	Mortality	dog	52 week	Oral		No effect	No effect			
	48	Mortality	rat	2 year	Oral		No effect	No effect			
	49	Mortality	mouse	78 week	Oral	200 ppm	Decrease	Survival was dose-related increased in male dodine-treated groups, compared to the control.			
	50	Mortality	rat	29 week	Oral		No effect	No effect			
	50	Mortality	rat	29 week	Oral		No effect	No effect			
	51	Mortality	rat	10 day	Oral	100 mg/kg bw/day	Increase	one treatment-related death at high dose group			
	52	Mortality	rat	10 day	Oral		No effect				
	53	Mortality	rabbit	13 day	Oral	100 mg/kg bw/day	Increase	5 treatment related dead animals in high dose group.			
	54	Mortality	rabbit	13 day	Oral	80 mg/kg bw/day	Increase	Three deads were recorded in high dose group			
	51	Necropsy	rat	10 day	Oral	100 mg/kg bw/day	Increase	A slight increase in the kidney incidences (30%; pelvic dilatation and enlarged) and ureters (20%: dilatation) were found in the top dose group, compared with controls			
	52	Necropsy	rat	10 day	Oral		No effect	No effect			
	53	Necropsy	rabbit	13 day	Oral	100 mg/kg bw/day	Increase	At high dose group, half of animals showed liquid contents and gaseous distension in caecum, compared with controls			
	54	Necropsy	rabbit	13 day	Oral	40 mg/kg bw/day	Increase	Increased incidence of dark patches in lung lobes in mid (12.5%) and high dose (20%) groups, in which the half of these animals presented breathing difficulties as clinical signs.			

2.10.1.2.3 Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

Weight of evidence for T-mediated adversity

Table 2.10.1.2.3/1: WoE for T-mediated adversity

- The thyroid weight was measured in two 28-day oral studies in rats, one of the 90-day studies in rats, the 90-day study in dogs, the 6-week study in dogs and a 2-year study in rats, and in none of them an effect on this parameter was observed depending upon dodine administration. It was also measured in one of the two 1-year oral studies in dogs (ID: 45), where increases in the absolute and relative thyroid weights were shown from 50 ppm of dodine. However, this study was considered only as supportive information, due to the number of deviations that presented from the guideline.

- The thyroid histopathology was examined in one of the 28-day oral studies in rats, the 28-day dermal study in rats, one of the 90-day studies in rats, the 90-day study in mice, the 90-day study in dogs, the 6-week study in dogs, the 52-week study in dogs and 78-week study in mice, and in none of them an effect on this parameter was observed depending upon dodine administration.

- Thyroid histopathology was analysed in a 1-year oral study in dogs (ID: 45), where a shift of follicular epithelium from squamous to cuboidal variety and increased vascularity were observed in a dog at 200 ppm and in two female dogs at 800 ppm. Evidence of stimulation were shown in thyroid glands on males at 800 ppm, with cell type predominantly cuboidal with transition to low columnar and increase in vascularity. However, this study was considered only as supportive information, due to the number of deviations that presented from the guideline.

- Thyroid histopathology was analysed in the 2-year study in rats (ID: 48), where increased incidence of thyroid C-cell adenoma was observed in all male dodine-treated groups, without a dose-response pattern and statistical significance, but out of the HCD (29%, 38%, 33% and 42% for controls, low, mid and high dose groups). Furthermore, increased incidence of thyroid C-cell carcinoma was seen in mid and top dose male group, without dose-response pattern and statistical significance, but exceeding the HCD at these doses (6%, 2%, 12% and 11% for controls, low, mid and high dose group). In addition, increased combined thyroid C-cell adenoma/carcinoma was observed in treated males, without statistical significance, but dose-related and out of the HCD (35%, 40%, 45% and 53% for controls, low, mid and high dose groups).

- In one of the 28-day oral study in rats (ID: 32), an increase in the relative-to-body liver weight was seen in females at 100 mg/kg bw/day. In other of the 28-day oral study in rats (ID: 34), a decrease in absolute and relative-to-body liver weight was shown in females at 800 ppm (equivalent to 76.7 mg/kg bw/day). In the two-generation study in rats (ID: 50), the absolute liver weight was reduced in F1 adults, F1 male pups and F2 pups at 800 ppm. In the 90-day oral study in mice (ID: 39), the relative-to-body liver weight was increased in both sexes at 2500 ppm. In the 78-week study in mice (ID: 49), the relative liver weight was increased in males and females at top dose. In the 52-week study in dogs (ID: 47), the absolute and relative liver weights were increased in females from 10 mg/kg bw/day in females (without histopathological findings associated). Equivocal (decreases and increases) effects were noted in liver weight and, therefore, overall, no adverse effects in liver weight were considered.

- Regarding the parameters sensitive to, but not diagnostic of T, several effects were observed. Changes in adrenal weight were recorded after subacute, subchronic and chronic dodine exposure, at doses with excessive toxicity. In the 28-day oral study in rats (ID: 32), the incidence of adrenals with haemorrhage was increased only at 200 mg/kg bw/day, which was above the maximum tolerated dose. In the two-year study in rats, increased incidence of enlarge and white mottled adrenals were found in females only at the highest dose tested. Increased brain weight were recorded in both sexes in long term and 2-generation toxicity studies, but without histopathological alterations associated. No treatment-related effects were

observed in fertility, foetal development, litter size, pituitary weight and histopathology. Decreased pup weight were recorded in F1 and F2 in the two-generation study (ID: 50), but no relevant decreases were recorded in developmental toxicity studies (ID: 51, 52, 53 and 54). Increased post implantation loss was shown in the developmental toxicity study in rabbits (ID: 54). Tubular hyperplasia in kidney was found in male mice in the chronic toxicity study (ID: 49). In the two-generation study, absolute, but not relative, kidney weight, was reduced in adult and pup rats, which was not deemed biologically relevant. An increased incidence of hepatocellular adenomas were observed in mice in the 78-week study, but it was not supported by statistical significance. Although, the occurrence of combined adenomas/carcinomas displayed statistically significance in the top dose female group, carcinomas incidence was not affected by treatment. Overall, no clear evidences were observed, regarding the parameters ´sensitive to, but not diagnostic of T´.

Therefore, taking into account the effects observed, it is considered that dodine causes T-mediated adversity, mainly based on the incidence of thyroid C-cell adenoma and carcinoma found in the two-year study in rats (ID: 48).

Weight of evidence for T-mediated endocrine activity

Table 2.10.1.2.3/2: WoE for T-mediated endocrine activity

- The Danish (Q)SAR Database predictions indicate that dodine lacks the potential to interact with the thyroid receptor. Two inconclusive outcomes are obtained from the Leadscope TPO inhibition and NIS models, although the query compound falls outside the applicability domain of the models. These predictions are considered of low relevance.

- There were eight assays on thyroid activity associated with the EDSP21 tab in the CompTox Chemicals Dashboard (Vol.3, AS, B.6.8.3.8). Based on this battery of *in vitro* assays, dodine was inactive in all of them, but active in the thyroid receptor assay TR_LUC_GH3_Antagonist. However, this positive result is reported at a concentration level that is above the limit of cytotoxicity of dodine.

- TSH levels in serum/plasma have not been measured in the available studies. In the Hershberger bioassay (Vol.3, AS, B.6.8.3.5), T3 and T4 levels were measured in peripubertal, orchidoepididymectomised male Crl:CD (SD) rats. T3 levels in treated males were similar to the vehicle control rats. T4 levels were statistically significantly increased in male rats at 50 mg of dodine/kg bw/day, without a clear dose-response. T3 and T4 were not measured in any other study.

- In the Sodium-Iodide Symporter (NIS) Assay (Vol.3, AS, B.6.8.3.1), dodine gave a positive response. However, measurements of cytotoxicity were not conducted in the study and the positive response obtained could be adequately assessed.

- In the Thyroid Peroxidase (TPO) Assay (Vol.3, AS, B.6.8.3.2), dodine gave a negative response. However, measurements of cytotoxicity were not conducted and the negative response obtained could not be adequately assessed.

Based on the available data, there is not enough evidence of T-mediated endocrine activity, but data is neither enough to reach a conclusion. The observation of the T4 levels increase in peripubertal males was the only information about the effect of dodine on this hormone. It neither makes the RMS to consider it as an evidence itself of T-mediated activity, nor allows concluding that a potential role of dodine in T-mediated activity should be dismissed.

2.10.1.2.4. Initial analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality

Since T-mediated adversity has been found and has been sufficiently investigated and endocrine activity has not been sufficiently investigated according to the ED EFSA/ECHA guidance (2018), it corresponds to the scenario 1b (Table 2.10.1.2.4).

Table 2.10.1.2.4: Selection of relevant scenario for T-modality

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "T-mediated" adversity	
Yes (sufficiently investigated)	Yes/No	1b	**Perform MoA analysis**	**X**
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no T-mediated endocrine activity observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.1.2.5. MoA analysis for T-modality

According to the Scenario 1b, adversity has been found based on T-mediated parameters and T-mediated activity has not been sufficiently investigated. A MoA analysis is required to establish the biological plausibility of the link between the observed endocrine activity and potential adverse effects.

2.10.1.2.6. Conclusion on the assessment of T-modality

Considering the available data, T.mediated adversity has been found based on T-mediated parameters and T-mediated activity has not been sufficiently investigated. It corresponds to Scenario 1b. A MoA analysis is required and a conclusion cannot be reached.

2.10.1.3. ED assessment for EAS-modalities

2.10.1.3.1. Analysis of non-experimental data

In accordance with the OECD Conceptual Framework and the ECHA/EFSA GD on ED, Level 1, EAS-related non-test information was gathered for dodine. Qualitative structural activity relationship (QSAR) data was obtained for dodine from the Danish QSAR database, and results are summarised below.

Table 2.10.1.3.1/1: Results of Danish QSAR database for dodine regarding EAS-modality

	Exp.	Battery	CASE Ultra	Leadscope	SciQSAR
Estrogen Receptor α Binding, Full training set (Human *in vitro*)		INC_OUT	NEG_OUT	NEG_OUT	NEG_OUT
Estrogen Receptor α Binding, Balanced Training Set (Human *in vitro*)		INC_OUT	NEG_OUT	NEG_OUT	NEG_OUT
Estrogen Receptor α Activation (Human *in vitro*)		INC_OUT	NEG_OUT	NEG_OUT	INC_OUT
Estrogen Receptor Activation, CERAPP data (*in vitro*)		N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition (Human *in vitro*)		NEG_IN	NEG_IN	NEG_IN	NEG_IN

Androgen Receptor Binding, CoMPARA data (*in vitro*)	NEG_IN	N/A	N/A	NEG_IN	N/A
Androgen Receptor Inhibition, CoMPARA data (*in vitro*)	NEG_IN	N/A	N/A	NEG_IN	N/A
Androgen Receptor Activation, CoMPARA data (*in vitro*)	NEG	N/A	N/A	NEG_IN	N/A
Key: POS = Positive; NEG = Negative; IN = within the applicability domain of the model; OUT = outside of the applicability domain of the model					

The outcome predictions obtained from the Danish (Q)SAR Database predictions indicate that dodine lacks the potential to interact with estrogen and androgen receptors. A prediction outcome of inconclusive is obtained for estrogen receptor α-binding and activation from the Battery algorithm although these predictions are reported to be outside the applicability domain of the models. For this reason, these predictions are considered of low relevance.

OECD ToolBox
The results of the OECD (Q)SAR Toolbox v.4.2 profilers for dodine in respect to E-related endpoints are displayed below.

Table 2.10.1.3.1/2. Results of the OECD QSAR Toolbox v.4.2 for dodine

Estrogen Receptor Binding, alerts in:	
parent only	Non binder, non cyclic structure
metabolites from *in vivo* Rat metabolism simulator only	Non binder, non cyclic structure
metabolites from Rat liver S9 metabolism simulator only	Non binder, non cyclic structure
rtER Expert System - USEPA, alerts in	
parent only	No alert found
metabolites from *in vivo* Rat metabolism simulator only	No alert found
metabolites from Rat liver S9 metabolism simulator only	No alert found
OECD QSAR Toolbox v.4.2 profilers	
Profiler predictions are supporting information to be used together with the relevant QSAR predictions	

[1] Estrogen receptor binding: Weak binder, OH group

Estrogen receptor (ER) binding is a molecular initiating event similar to protein binding that leads to a series of adverse outcomes, which are typically considered reproductive and development hazards. It is an endpoint where several comprehensive databases exist, which has lead to the development of several approaches for using (Q)SARs to predict ER-binding and possible endocrine disruption.

Since the ER-binding is a receptor mediated event, particular organic functional groups, size and shape are critical to binding potency. Chemicals with a single 5-or 6-member carbon ring structure with an unhindered hydroxyl-group (-OH) (a hydroxyl group in the para- or meta-position on the ring and without ortho substituents to the hydroxyl group) (5) are ER binders. Binding potency is related to the size and shape of non-hydroxylated-ring aspect of the molecule, which can be grossly measured by molecular weight.

The incorporated Toolbox ER binding profiling scheme is based on structural and parametric rules extracted from literature sources and supported by experimental data. The ER-binding profiler classifies chemicals as non-binders or binders depending on molecular weight (MW) and structural characteristics of the chemicals:

1. Very strong binders: Chemicals with MW between 200 and 500 Da and two rings with a hydroxyl group connected to each of them.

2. Strong binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW between 200 and 500 Da.

3. Moderate binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW between 170 and 200 Da.

4. Weak binders: Chemicals with at least one 5-or 6-members carbon ring with an unhindered hydroxyl or amino group and MW less than 170 Da.
If the target chemical does not meet some of the structural and parametric requirements listed above it is classified as Non binder:
- Non binder with impaired hydroxyl or amino group.
- Non binder, MW more than 500 Da.
- Non binders without hydroxyl or amino group.
- Non-binder, non-cyclic.

The OECD Toolbox v.4.2 predicts that dodine lacks the potential to be an ER binder and no alerts were displayed.

[2] rtER Expert System USEPA: Alkoxyphenols

The rtER Expert System ver.1 – USEPA profiler consists of molecular definitions that mimic the structural criteria of chemical classes that are potential estrogen receptor-binders covered by US EPA Estrogen Receptor Expert System (ERES) The ERES profiler is an effects-based automated system used to predict estrogen receptor binding affinity. In the Toolbox, the rtER Expert System ver.1 – USEPA profiler is used for the purpose of categorization based on the structural definitions of the original ERES chemical classes. The rtER Expert System ver.1 – USEPA profiler is intended for categorization purpose and not for predicting relative binding affinity (RBA). rtER Expert System ver.1
USEPA profiler predicts that dodine meets the criteria of chemical classes that are potential ER binders, on the basis that is an alkoxyphenol substance.

The rtER Expert System ver.1 – USEPA profiler consists of molecular definitions mimic the structural criteria of chemical classes potential estrogen receptor-binders covered by US EPA Estrogen Receptor Expert System (ERES) The ERES profiler is an effects-based automated system used to predict estrogen receptor binding affinity. The Estrogen Receptor Expert System (ERES) Profiler is an effects-based automated system used to predict estrogen receptor binding affinity

ToxCast: CERAPP Potency Level (ER-Related activity) and COMPARA (AR-related activity)

The ToxCast Model Dashboard includes predictions of the estrogen receptor activity of dodine acetate, based on the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP[¡Error! Marcador no definido.]). The CERAPP is a large-scale modelling project which has investigated the efficacy of using predictive computational models trained on high-throughput screening data (e.g. from the EDSP21 initiative) to evaluate the ER-related activity of thousands of chemicals, and identify priorities for further testing.

On the other hand, the ToxCast Models Dashboard also includes predictions of the androgen receptor activity of dodine acetate based on the COMPARA. COMPARA is a large scale collaboration between 35 international groups using QSAR models to predict androgen receptor activity using a common training set of 1746 compounds provided by the US EPA. The result is consensus model of AR agonist activity that is run against the DSSTox chemical library that aims to identify priorities for further testing.
The CERAPP and COMPARA predictions for estrogen and androgen activity are summarised in the following table:

Table 2.10.1.3.1/3. Results of the CERAPP and COMPARA predictions for dodine acetate.

Model	Receptor	Agonist	Antagonist	Binding
CERAPP Potency Level (Consensus)	Estrogen	0	0	0
CERAPP Potency Level (From Literature)	Estrogen	Inactive	Inactive	Inactive
COMPARA (Consensus)	Androgen	0	0	0

As noted, dodine acetate displayed inactive results for estrogen and androgen receptor activities.

2.10.1.3.2. US EPA CompTox Chemicals Dashboard

Estrogen receptor bioassays

Dodine acetate was tested in 6 assays included in the ToxCast ER Bioactivity Model component. (Study ID Matrix No: 9-14). Dodine acetate gave negative results in 5 of these assays. A positive result was obtained in the ERa_LUC_VM7_Agonist assay. The AC_{50} value was 3.34 $x10^{-5}$ µM (Hill Model). The limit of cytotoxicity for this assay was reported to be 0.183 µM. The assay presented a flag of 'less than 50% efficacy and AC_{50} less than lowest concentration tested'.

Table 2.10.1.3.2/1: Summary of US EPA ToxCast EDSP21- estrogenic bioactivity assays for dodine acetate.

Assay endpoint	Study ID Matrix	Assay type	Organism	Result	AC_{50} #	Flag
TOX21_ERa_BLA_Agonist_ratio	9	beta lactamase induction	human	Inactive	-	-
TOX21_ERa_BLA_Antagonist_ratio	10	beta lactamase induction	human	Inactive	-	-
TOX21_ERa_BLA_Antagonist_viability	11	ATP content	human	Inactive		
TOX21_ERa_LUC_VM7_Agonist	12	luciferase induction	human	**Active**	3.34 x 10^{-5}	**Less than 50% efficacy. AC50 less than lowest concentration tested**
TOX21_ERa_LUC_VM7_Antagonist_specificity	13	luciferase induction	human	Active	-	-
TOX21_ERa_LUC_VM7_Antagonist_0.5nM_E2_viability	14	ATP content	human	Inactive	-	-

Chemical concentration (in µM) at which 50% of the maximum response is achieved.

Androgen receptor bioassays

Dodine acetate was tested in 8 assays included in the ToxCast AR Bioactivity Model (Study ID Matrix No: 15-22). Dodine acetate is reported to be inactive in 6 assays and is reported active in 2 assays: AR_BLA_Antagonist_viability and AR_LUC_MDAKB2_Antagonist_0.5nM_R1881_viability.

Table 2.10.1.3.2/2: Summary of US EPA ToxCast EDSP21- androgenic bioactivity assays for dodine acetate.

Assay endpoint	Study ID Matrix	Assay type	Organism	Result	AC_{50}#	Flag
TOX21_AR_BLA_Agonist_ratio	15	beta lactamase induction	human	Inactive	-	-
TOX21_AR_BLA_Antagonist_ratio	16	beta lactamase induction	human	Inactive	-	-
TOX21_AR_BLA_Antagonist_viability	17	ATP content	human	**Active**	2.202	**Only highest concentration above baseline, active less than 50% efficacy, borderline active**
TOX21_AR_LUC_MDAKB2_Agonist	18	luciferase induction	human	Inactive	-	-
TOX21_AR_LUC_MDAKB2_Antagonist_0.5nM_R1881	19	luciferase induction	human	**Active**	1.612	**Noisy data**
TOX21_AR_LUC_MDAKB2_Antagonist__0.5nM_R1881_viability	20	ATP content	human	Inactive	-	-

Assay endpoint	Study ID Matrix	Assay type	Organism	Result	AC_{50}[#]	Flag
TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881	21	luciferase induction	human	Inactive	-	-
TOX21_AR_LUC_MDAKB2_Antagonist_10nM_R1881_viability	22	ATP content	human	Inactive	-	-

[#] *Chemical concentration (in µM) at which 50% of the maximum response is achieved*

The reported AC_{50} value for the AR_BLA_Antagonist_viability assay was determined to be 2.20 µM (Hill Model), and flags of 'only highest concentration above baseline, less than 50% efficacy, borderline active' were displayed. On the other hand, the reported AC_{50} value for the AR_LUC_MDAKB2_Antagonist_0.5nM_R1881_viability assay was determined to be 1.612 µM (Hill Model), and a flag of 'noisy data' was displayed.

Both results were above the limit of cytotoxicity for the assays (0.183 µM), so the reliability is low.

Steroidogenesis bioassays

Dodine acetate was tested in 2 assays included in the ToxCast Steroidogenesis Bioactivity Model (Study ID Matrix No.: 23-24), and negative results were obtained for both assays.

Table 2.10.1.3.2/3: Summary of US EPA ToxCast EDSP21- Steroidogenesis bioactivity assays for dodine acetate.

Assay endpoint	Study ID Matrix	Assay type	Organism	Result	AC50 [#]	Flag
TOX21_Aromatase_Inhibition	23	Luciferase induction	human	Inactive	-	-
TOX21_Aromatase_inhibition_viability	24	ATP content	human	Inactive	-	-

2.10.1.3.3. Have EAS-mediated parameters been sufficiently investigated?

The available dataset of *in vivo* mammalian toxicology studies for dodine consists of short-term studies (7 and 28 days) conducted in rodents, sub-chronic studies (42, 56, 90 and 100 days) conducted in rodents and dog, carcinogenicity studies conducted in rodents, one 2-generation toxicity study conducted in rats, and prenatal developmental toxicology studies conducted in rats and rabbits. It was noted that much of the available data pre-dates revisions that were made to the OECD Test Guidelines to include EAS-mediated parameters.

Table 14 of the ECHA/EFSA GD on ED provides a list of relevant EAS-mediated parameters that may be investigated in the OECD CF Level 4 and 5 *in vivo* OECD TG compliant mammalian toxicology studies. Using the currently available set of toxicological data for dodine, the table 2.10.1.3.3/1 summarises the available information on EAS-mediated parameters.

To have the EAS-mediated adversity with regard to humans and mammals sufficiently investigated, all the data requirements of the specific Regulations, must be fulfilled. This should include all the 'EAS-mediated' parameters foreseen to be investigated in an extended one-generation reproductive toxicity study (EOGRTS; OECD TG 443; with cohort 1a/1b including the mating of cohort 1b to produce the F2 generation (OECD, 2012, updated on 2018)) or a two-generation reproductive toxicity study (OECD TG 416; test protocol according to latest version of January 2001 (OECD, 2001)).

Table 2.10.1.3.3/1: Summary of EAS-mediated parameters investigated in mammalian toxicology studies

EAS-mediated parameters	OECD Test guideline	Sufficiently investigated? **Overall conclusion: No (not sufficiently investigated)** Based on the lack of OECD 416 (2001 version) and 443 studies. The following studies are available instead (see parameters covered in table below): -Two generation study in rats (FIFRA 83-4; OECD 416) and repeated dose studies.
Estradiol level	408 (optional)	No data

Follicle stimulating hormone (FSH) level	408 (optional)	No data
Luteinising hormone (LH) level	408 (optional)	No data
Testosterone level	408 (optional)	No data
Accessory sex organs histopathology	408, 421, 451-3	No data
Age at first oestrus	OPPTS 890.1450	No data
Age at balanopreputial separation	426, 416, 443	No data
Age at vaginal opening	426, 416, 443	No data
Anogenital distance (AGD)	414, 421, 426, 416, 443	No data
Cervix histopathology	407, 408, 415, 422, 451-3, 416, 443	Cervix histopathology were performed in the long term/chronic toxicity studies in rodents and in the 2-generation toxicity study in rats.
Coagulating gland histopathology	407, 408, 415, 422, 451-3, 416, 443	Coagulating gland histopathology was only carried out in the 2-generation toxicity study in rats.
Coagulating gland weight	407, 421, 422, 416, 443	Coagulating gland weight was measured together with seminal vesicles in the 2-generation toxicity study in rats.
Cowper's gland weight	421 (optimal), 422 (optional)	No data. (Only in the Hershberger OECD TG 441 assay).
Epididymis histopathology	407, 408, 415 (optional) 421, 422, 451-3, 416, 443	Epididymis histopathology was performed in the 28 and 90 day studies in rodents, in 90 day and 1 year studies in dog, in long term/chronic toxicity studies in rodents and in the 2-generation toxicity study in rats.

Epididymis weight	407, 408, 421, 422, 451-3, 416, 443	Epididymis weight was measured in the 28-day study in rats, in 90-day study in dog, in the long term/chronic toxicity studies in rats and in the 2-generation toxicity study in rats.
Oestrus cyclicity	407 (optional), 408, 421, 422, 416, 443	Oestrus cyclicity was conducted in the 2-generation toxicity study conducted in rat.
Glans penis weight	421 (optimal), 422 (optional)	No data. (Only in the Hershberger OECD TG 441 assay).
Genital abnormalities	414, 415, 421, 422, 416, 443	Genital abnormalities were mainly checked in the 2-generation toxicity study conducted in rats.
LABC weight	421 (optimal), 422 (optional), OPPTS 890.1500	No data. (Only in the Hershberger OECD TG 441 assay).
Mammary gland histopathology (male)	407 (optional), 408, 422, 443, 451-3 (optional)	Mammary gland histopathology (male) was performed in one 28-day toxicity studies in rats, in 90-day study in dogs and in the carcinogenicity studies in rodents.
Mammary gland histopathology (female)	407, 408, 451-3, 443	Mammary gland histopathology (female) was performed in one 28-day toxicity studies in rats, in one 90-day study in mice, in 90-day and 1-year toxicity studies in dogs and in the carcinogenicity studies in rodents.
Nipple development	421, 422, 443	No data
Ovary histopathology	407, 408, 415 (optional) 421, 422, 426, 451-3, 416, 443	Ovary histopathology was performed in one 28, 56 and 90-day toxicity studies in rodents, in 90-day and 1-year study in dogs, in carcinogenicity studies in rodents, and in the 2-generation toxicity study in rats.
Ovary weight	407 (optional), 408, 421 (optional), 422, 451-3, 416, 443	Ovary weight was measured in one 28 and 90-day toxicity studies in rodents, in 42-day and 1-year study in dogs, in carcinogenicity studies in rodents, and in the 2-generation toxicity study in rats.
Oviduct histopathology	408, 415 (optional), 443	Oviduct histopathology was performed in the 2-generation toxicity study in rats and in the 28-day dermal study in rats.
Prostate histopathology (with seminal vesicles and coagulating glands)	407, 408, 415 (optional) 421, 422, 426, 451-3, 416, 443	Prostate histopathology was performed in two 90-day toxicity studies in rodents, in 90-day and 1-year study in dogs, in carcinogenicity studies in rodents, and in the 2-generation toxicity study in rats.

Prostate weight	407, 408, 421, 422, 416, 443	Prostate weight was measured in the 90-day study in dogs, in the carcinogenicity study in rats, and in the 2-generation toxicity study in rats.
Seminal vesicles histopathology	407, 408, 415 (optional), 422, 451-3, 416, 443	Seminal vesicles histopathology was performed in the 28 and 90-day toxicity studies in rodents, in the carcinogenicity studies in rodents, and in the 2-generation toxicity study in rats.
Seminal vesicles weight	407, 408, 421, 422, 416, 443	Seminal vesicles weight was performed in the 2-generation toxicity study in rats.
Sperm morphology	408 (optional), 416, 443	Sperm morphology was performed in the 2-generation toxicity study in rats.
Sperm motility	408 (optional), 416, 443	Sperm motility was performed in the 2-generation toxicity study in rats.
Sperm numbers	408 (optional), 416, 443	Sperm number was performed in the 2-generation toxicity study in rats.
Testis histopathology	407, 408, , 415 (optional) 421, 422, 451-3, 416, 443	Testis histopathology was performed in 28-day toxicity studies in rats, in 90-day studies in rat and mice, in 42 and 90-day and 1-year study in dogs, in carcinogenicity studies in rodents, and in the 2-generation toxicity study in rats.
Testis weight	407, 408, 421, 422, 451-3, 416, 443	Testis weight was measured in 28-day toxicity studies in rats, in 90-day studies in rat and mice, in 42 and 90-day and 1-year study in dogs, in carcinogenicity studies in rodents, and in the 2-generation toxicity study in rats.
Uterus histopathology (with cervix)	407, 408, 415 (optional), 421 (optional), 422, 451-3, 416, 443	Uterus histopathology was performed in one 28-day toxicity study in rats, in 90-day studies in rat and mice, in 90-day and 1-year studies in dogs, in carcinogenicity studies in rodents, and in the 2-generation toxicity study in rats.
Uterus weight (with cervix)	407 (optional), 408, 414 (gravid uterus), 415 (optional), 421 (optional), 422, 451-3, 416, 443	Uterus weight was measured in one 28-day toxicity study in rats, in one 90-day study in dogs, in the carcinogenicity study in rats, in the 2-generation toxicity study in rats, and in the developmental toxicity studies conducted in rat and rabbits.
Vagina histopathology	407, 408, 415 (optional), 422, 451-3, 416, 443	Vaginal histopathology was performed in 90-day and 1-year studies in dogs, in the carcinogenicity study in rats and mice, and in the 2-generation toxicity study in rats.
Vaginal smear	407 (optional), 408, 421, 422, 416, 443	No data

Table 2.10.1.3.3/2: EAS-mediated parameters not measured

OECD TG 407 - EAS-mediated parameters not investigated
- Cervix histopathology - Coagulating gland histopathology - Coagulating gland weight - Prostate histopathology - Prostate weight - Seminal vesicles weight. - Vaginal histopathology
OECD TG 408/409 - EAS-mediated parameters not investigated
- Accessory sex organs histopathology. - Cervix histopathology. - Coagulating gland histopathology - Oestrus cyclicity - Seminal vesicles weight. - Vaginal smear
OECD TG 452/3 - EAS-mediated parameters not investigated
- Accessory sex organs. - Coagulating gland histopathology.
OECD TG 416 - EAS-mediated parameters not investigated
- Anogenital distance measurement -Age at vaginal opening -Age at balanopreputial separation
OECD TG 414 - EAS-mediated parameters not investigated
- Anogenital distance measurement - Genital abnormalities

Regarding to the EAS-mediated endocrine activity:

E-modality: It is considered sufficiently investigated based on the estrogenic activity output data from the US EPA ToxCast Bioactivity Model.

A-modality: It is considered sufficiently investigated based on the output data from “Hershberger bioassay in rats’ (ID::31; OECD TG 441).

S-modality: It is considered sufficiently investigated based on the output data from “H295R Steroidogenesis assay” (ID: 30; OECD TG 456), and the *“In vitro* aromatase inhibition using human recombinant microsomes assay” (ID: 29) in line with OPPTS 890.1200.

Therefore, it is considered that EAS-mediated endocrine activity have been sufficiently investigated.

2.10.1.3.4. Lines of evidence for adverse effects and endocrine activity related to EAS-modality

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
In vitro mechanistic	9	Estrogen receptor	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect	Dodine showed one positive result in one mRNA ESR1 induction assay, however the outcome is of low reliability due to the flags presented by the assay. On the other hand, dodine displayed negative results for ER agonist or antagonist activity in stably transfected hER-HeLa-9903 cell line.	The weight of evidence of the *in vitro* EAS-modalities assays showed that dodine, could be a potential AR-antagonist, without ruling out a possible interaction with components of the steroidogenesis pathway, such as aromatase CYP19.	E
	10	Estrogen receptor	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect			
	11	Estrogen receptor	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect			
	12	Estrogen receptor	Human	22 hour	Uptake from the medium (in vitro)	3.34 x 10-5 µM	Change	Dodine acetate is active for estrogen (ESR1) mRNA induction assays. AC50 (hill model) = 3.34 x 10-5 µM. The assay presents two flags: Less than 50% efficacy and AC50 less than lowest concentration tested.			
	13	Estrogen receptor	Human	22 hour	Uptake from the medium (in vitro)		No effect	No effect			
	14	Estrogen receptor	Human	22 hour	Uptake from the medium (in vitro)		No effect	No effect			
	27	Estrogen receptor agonist	Human	3 hour	Uptake from the medium (in vitro)		No effect	Dodine technical was classified as negative in the ER agonist assay, since the RPCMax did not exceed 10% of the response of the positive control in two			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								independent experiments.			
	27	Estrogen receptor Antagonist	Human	3 hour	Uptake from the medium (in vitro)		No effect	No IC30 values could be derived from any of the replicates for the ER antagonist assay.			
	15	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect	Dodine showed two positive results in agonist and antagonist *in vitro* Toxcast assays. However, the AC50 values derived were higher than the concentration limit for cytotoxicity. On the other hand, antagonist activity was noted in stably transfected AR-EcoScreen cell line in presence of 5α-dihydrotestosterone (DHT). IC30 = 0.05 and 1 µM for each of the two available replicates.		A
	16	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect			
	17	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)	2.2 µM	Change	Dodine acetate is active in HEK293T cell viability assay. AC50 (hill model)= 2.2. The assays presents the flag: Only highest concentration above baseline, active. Less than 50% efficacy. Borderline active. The limit of cytotoxicity was reported to be 0.183 µM.			
	18	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect			
	19	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)	1.612 µM	Change	Dodine acetate is active for androgen receptor (AR) antagonist transcriptional gene expression assay in MDA-kb2 cell line. AC50 (hill			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								model) = 1.612 µM. The assay presents the flag: noisy data. The limit of cytotoxicity was reported to be 0.183 µM.			
	20	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect			
	21	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect			
	22	Androgen receptor	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect			
	28	Androgen receptor Agonist	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect			
	28	Androgen receptor Antagonist	Human	24 hour	Uptake from the medium (in vitro)		Change	Dodine technical was classified as positive in the AR antagonist assay, since an IC30 value could be calculated in two independent runs (0.05 and 1 µM, respectively).			
	23	CYP19	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect	Dodine showed ability to inhibit aromatase activity in an i*n vitro* assays using microsomal proteins. IC50= 56.8 µM. On the other hand, dodine technical was negative for testosterone or estradiol induction.		S
	24	Cellular proliferation	Human	24 hour	Uptake from the medium (in vitro)		No effect	No effect			
	29	Androstenedione (in vitro)	Human	15 Minutes	Uptake from the medium (in vitro)	56.8 µM	Decrease	The outcome of the study indicates that dodine inhibits the activity of aromatase with decreases in aromatase			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								activity at a concentration of 31.6 µM and above. The enzyme activity is reduced to 25% at 316 µM. The calculated mean IC50 value between the three experiments is 56.8 µM. The data fit the 4-parameter regression model and aromatase activity is inhibited more than 50% at the top concentration.			
	30	Estradiol level (in vitro)	Human	48 hour	Uptake from the medium (in vitro)		No effect	No effect			
	30	Testosterone level (in vitro)	Human	48 hour	Uptake from the medium (in vitro)		No effect	No effect			
EAS-mediated	31	Adrenals weight	Rat	10 day	Oral		No effect	No effect	No treatment-related effects were observed	No adverse effects in EAS-mediated parameters were considered. Increases in epididymis and testes wt were not associated with histopathological alternations nor in abnormalities in reproductive parameters that draw attention to	EAS
	31	Bulbourethral gland	Rat	10 day	Oral		No effect	No effect	No treatment-related effects were observed		
	40	Cervix histopathology	dog	90 day	Oral		No effect	No effect	No treatment-related effects were observed		
	49	Cervix histopathology	mouse	78 week	Oral		No effect	No effect			
	50	Cervix histopathology	rat	29 week	Oral		No effect	No effect			
	50	Cervix histopathology	rat	29 week	Oral		No effect	No effect			
	50	Coagulating gland histopathology	rat	29 week	Oral		No effect	No effect	Epididymis rel wt was increased in two year rat study, and in F1 adult males from 2-generation toxicity study.		
	50	Coagulating gland histopathology	rat	29 week	Oral		No effect	No effect			
	36	Epididymis histopathology	rat	28 day	Dermal		No effect	No effect			
	38	Epididymis histopathology	rat	90 day	Oral		No effect	No effect			
	39	Epididymis histopathology	mouse	90 day	Oral		No effect	No effect			
	40	Epididymis histopathology	dog	90 day	Oral		No effect	No effect			
	47	Epididymis histopathology	dog	52 week	Oral		No effect	No effect			
	48	Epididymis histopathology	rat	2 year	Oral		No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	49	Epididymis histopathology	mouse	78 week	Oral		No effect	No effect		a problem in male fertility.	
	50	Epididymis histopathology	rat	29 week	Oral		No effect	No effect			
	50	Epididymis histopathology	rat	29 week	Oral		No effect	No effect			
	36	Epididymis weight	rat	28 day	Dermal		No effect	No effect			
	40	Epididymis weight	dog	90 day	Oral		No effect	No effect			
	48	Epididymis weight	rat	2 year	Oral	400 ppm	Increase	Increased absolute (11% and 6% for mid and high dose groups, respectively) and relative (11% and 12% for mid and high dose groups, respectively) epididymis weight were recorded compared with controls.			
	50	Epididymis weight	rat	29 week	Oral	800 ppm	Increase	Increased rel left and right epididymis wt (13%) at high dose F1 males			
	50	Oestrus cyclicity	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	50	Oestrus cyclicity	rat	29 week	Oral		No effect	No effect			
	50	Genital abnormalities	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	50	Genital abnormalities	rat	29 week	Oral		No effect	No effect			
	31	Glans penis	Rat	10 day	Oral		No effect	No effect	No treatment-related effects were observed		
	31	LABC muscle	Rat	10 day	Oral		No effect	No effect	No treatment-related effects were observed		
	36	Mammary gland histopathology (female)	rat	28 day	Dermal		No effect	No effect	Increased not dose related incidences of malignant adenocarcinomas were revealed in the 2-year toxicity study in rats.		
	39	Mammary gland histopathology (female)	mouse	90 day	Oral		No effect	No effect			
	40	Mammary gland histopathology (female)	dog	90 day	Oral		No effect	No effect			
	47	Mammary gland histopathology (female)	dog	52 week	Oral	2 mg/kg bw/day	Increase	Higher incidence of thickened mammary glands was observed in			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								treated females, although this effect was not dose-response.			
	48	Mammary gland histopathology (female)	rat	2 year	Oral	200 ppm	Increase	An increase, not dose related, of malignant adenocarcinomas incidences were noted in female mid dose group (10, 14.5, 24.6 and 12.9% for control, low, mid and high dose groups).			
	49	Mammary gland histopathology (female)	mouse	78 week	Oral		No effect	No effect			
	39	Mammary gland histopathology (male)	mouse	90 day	Oral		No effect	No effect			
	40	Mammary gland histopathology (male)	dog	90 day	Oral		No effect	No effect			
	48	Mammary gland histopathology (male)	rat	2 year	Oral		No effect	No effect			
	36	Ovary histopathology	rat	28 day	Dermal		No effect	No effect	Equivocal effects (decrease/increase) relative to ovary wt were recorded in the chronic/reproductive toxicity studies. The benign cystadenomas found in the 2-year study in rats were ruled out due to no dose related increase were noted, and no another histopathological findings in ovary were revealed in the other studies.		
	37	Ovary histopathology	mouse	8 week	Oral		No effect	No effect			
	38	Ovary histopathology	rat	90 day	Oral		No effect	No effect			
	39	Ovary histopathology	mouse	90 day	Oral		No effect	No effect			
	40	Ovary histopathology	dog	90 day	Oral		No effect	No effect			
	43	Ovary histopathology	rat	90 day	Oral		No effect	No effect			
	47	Ovary histopathology	dog	52 week	Oral		No effect	No effect			
	48	Ovary histopathology	rat	2 year	Oral	200 ppm	Increase	Benign ovary cystadenomas, not dose related, were noted in 0/37 (0%), 3/32 (9%), 5/36 (14%) and 2/36 (6%) of the females from the 0, 200, 400 and 800 ppm dose groups, respectively.			
	49	Ovary histopathology	mouse	78 week	Oral		No effect	No effect			
	50	Ovary histopathology	rat	29 week	Oral			Not effect			
	50	Ovary histopathology	rat	29 week	Oral			Not effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	36	Ovary weight	rat	28 day	Dermal		No effect	No effect			
	38	Ovary weight	rat	90 day	Oral		No effect	No effect			
	39	Ovary weight	mouse	90 day	Oral		No effect	No effect			
	40	Ovary weight	dog	90 day	Oral		No effect	No effect			
	41	Ovary weight	dog	6 week	Oral		No effect	No effect			
	43	Ovary weight	rat	90 day	Oral		No effect	No effect			
	47	Ovary weight	dog	52 week	Oral		No effect	No effect			
	48	Ovary weight	rat	2 year	Oral	200 ppm	Decrease	A decreased trend, not statistically significant, and not clear dose related, were observed in absolute and relative ovary weight in all dodine treated groups.			
	49	Ovary weight	mouse	78 week	Oral		No effect	No effect			
	50	Ovary weight	rat	29 week	Oral	800 ppm	Increase	Increase rel left ovary/oviduct wt in F1 females (11%) at high dose.			
	38	Prostate histopathology (with seminal vesicles and coagulating glands)	rat	90 day	Oral		No effect	No effect	No treatment-related effects were observed		
	39	Prostate histopathology (with seminal vesicles and coagulating glands)	mouse	90 day	Oral		No effect	No effect			
	40	Prostate histopathology (with seminal vesicles and coagulating glands)	dog	90 day	Oral		No effect	No effect			
	47	Prostate histopathology (with seminal vesicles and coagulating glands)	dog	52 week	Oral		No effect	No effect			
	48	Prostate histopathology (with seminal vesicles and coagulating glands)	rat	2 year	Oral	200 ppm	Increase	An increased, not dose related of atrophy incidences were noted in male treated groups (1.5%, 3.3%, 8.2% y 4.3% for controls, low, mid and high			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								dose groups, respectively).			
	49	Prostate histopathology (with seminal vesicles and coagulating glands)	mouse	78 week	Oral		No effect	No effect			
	50	Prostate histopathology (with seminal vesicles and coagulating glands)	rat	29 week	Oral			No effect			
	50	Prostate histopathology (with seminal vesicles and coagulating glands)	rat	29 week	Oral			No effect			
	40	Prostate weight	dog	90 day	Oral		No effect	No effect			
	48	Prostate weight	rat	2 year	Oral		No effect	No effect			
	50	Prostate weight	rat	29 week	Oral		No effect	No effect			
	32	Seminal vesicles histopathology	rat	28 day	Oral	200 mg/kg bw/day	No effect	Seminal vesicle atrophy (3/10) at 200 mg/kg bw/day (above MTD, pre-terminal). Controls and lower doses not tested.	No treatment-related effects were observed		
	36	Seminal vesicles histopathology	rat	28 day	Dermal		No effect	No effect			
	38	Seminal vesicles histopathology	rat	90 day	Oral		No effect	No effect			
	39	Seminal vesicles histopathology	mouse	90 day	Oral		No effect	No effect			
	48	Seminal vesicles histopathology	rat	2 year	Oral		No effect	No effect			
	49	Seminal vesicles histopathology	mouse	78 week	Oral		No effect	No effect			
	50	Seminal vesicles histopathology	rat	29 week	Oral			No effect			
	50	Seminal vesicles histopathology	rat	29 week	Oral			No effect			
	50	Seminal vesicles weight	rat	29 week	Oral		No effect	No effect			
	50	Seminal vesicles weight	rat	29 week	Oral		No effect	No effect			
	31	Seminal vesicles/coagulating glands	Rat	10 day	Oral		No effect	No effect			
	50	Sperm morphology	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	50	Sperm motility	rat	29 week	Oral		No effect	No effect			
	50	Sperm numbers	rat	29 week	Oral		No effect	No effect			
	33	Testis histopathology	rat	28 day	Oral		No effect	No effect	Increased rel testis wt was increased at high dose in 2-generation toxicity study conducted in rats. No histopathological changes were associated to this finding. In addition, no differences were noted regarding		
	36	Testis histopathology	rat	28 day	Dermal		No effect	No effect			
	38	Testis histopathology	rat	90 day	Oral		No effect	No effect			
	39	Testis histopathology	mouse	90 day	Oral		No effect	No effect			
	40	Testis histopathology	dog	90 day	Oral		No effect	No effect			
	41	Testis histopathology	dog	6 week	Oral		No effect	No effect			
	43	Testis histopathology	rat	90 day	Oral		No effect	No effect			
	47	Testis histopathology	dog	52 week	Oral		No effect	No effect			
	48	Testis histopathology	rat	2 year	Oral		No effect	No effect			
	49	Testis histopathology	mouse	78 week	Oral		No effect	No effect			
	50	Testis histopathology	rat	29 week	Oral		No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	32	Testis weight	rat	28 day	Oral		No effect	No effect	reproductive parameters that draw attention to a problem in male fertility. No alterations regarding testis wt or histopathology abnormalities were recorded in other studies.		
	33	Testis weight	rat	28 day	Oral		No effect	No effect			
	36	Testis weight	rat	28 day	Dermal		No effect	No effect			
	38	Testis weight	rat	90 day	Oral		No effect	No effect			
	39	Testis weight	mouse	90 day	Oral		No effect	No effect			
	40	Testis weight	dog	90 day	Oral		No effect	No effect			
	41	Testis weight	dog	6 week	Oral		No effect	No effect			
	43	Testis weight	rat	90 day	Oral		No effect	No effect			
	47	Testis weight	dog	52 week	Oral		No effect	No effect			
	48	Testis weight	rat	2 year	Oral		No effect	No effect			
	49	Testis weight	mouse	78 week	Oral		No effect	No effect			
	50	Testis weight	rat	29 week	Oral	800 ppm	Increase	Increased rel left (12%) and right (14%) testis wt in high dose F1 adult males.			
	36	Uterus histopathology (with cervix)	rat	28 day	Dermal		No effect	No effect	Equivocal findings (increase/ decrease) regarding uterus wt were noted in different species. The decreased uterus wt observed in the dose-range finding developmental toxicity study in rabbits was not further reproduced in the main study. Histopathological uterus examinations did not revealed adverse effects.		
	38	Uterus histopathology (with cervix)	rat	90 day	Oral		No effect	No effect			
	39	Uterus histopathology (with cervix)	mouse	90 day	Oral		No effect	No effect			
	40	Uterus histopathology (with cervix)	dog	90 day	Oral		No effect	No effect			
	47	Uterus histopathology (with cervix)	dog	52 week	Oral		No effect	No effect			
	48	Uterus histopathology (with cervix)	rat	2 year	Oral	800 ppm	Decrease	There was a reduction in the benign endometrial stromal polyp incidences in the uterus at top dose female group (9% vs 17% in controls).			
	49	Uterus histopathology (with cervix)	mouse	78 week	Oral		No effect	No effect			
	50	Uterus histopathology (with cervix)	rat	29 week	Oral			not measured			
	50	Uterus histopathology (with cervix)	rat	29 week	Oral			not measured			
	36	Uterus weight (with cervix)	rat	28 day	Dermal		No effect	No effect			
	40	Uterus weight (with cervix)	dog	90 day	Oral		No effect	No effect			
	48	Uterus weight (with cervix)	rat	2 year	Oral	800 ppm	Increase	An increase, not statistically significant and not dose related, in the relative uterus weight was observed in			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								top dose female group.			
	50	Uterus weight (with cervix)	rat	29 week	Oral		No effect	No effect			
	50	Uterus weight (with cervix)	rat	29 week	Oral		No effect	No effect			
	51	Uterus weight (with cervix)	rat	10 day	Oral		No effect	No effect			
	52	Uterus weight (with cervix)	rat	10 day	Oral	45 mg/kg bw/day	No effect	A slight increase, not dose related, in the mean uterus weight was found in mid and high dose groups (5% and 7% for mid and high dose groups, respectively)			
	53	Uterus weight (with cervix)	rabbit	13 day	Oral	100 mg/kg bw/day	Decrease	Decreased uterus wt at high dose (35%). This finding was not reproduced in the main developmental toxicity study in rabbits.			
	54	Uterus weight (with cervix)	rabbit	13 day	Oral		No effect	No effect			
	40	Vagina histopathology	dog	90 day	Oral		No effect	No effect	No treatment-related effects were observed		
	47	Vagina histopathology	dog	52 week	Oral		No effect	No effect			
	48	Vagina histopathology	rat	2 year	Oral		No effect	No effect			
	49	Vagina histopathology	mouse	78 week	Oral		No effect	No effect			
	50	Vagina histopathology	rat	29 week	Oral			not measured			
	50	Vagina histopathology	rat	29 week	Oral			not measured			
	31	Ventral prostate	Rat	10 day	Oral		No effect	No effect	No treatment-related effects were observed		
Sensitive to, but not diagnostic of, EATS	32	Adrenals weight	rat	28 day	Oral	100 mg/kg bw/day	Increase	At 100 mg/kg bw/day, relative-to-body adrenal weight in males increased 33.3%, relative-to-body adrenal weight in females increased 36.7% and relative-to-brain adrenal	Increases adrenal wt were recorded in the 28-day study in rats and in the 2-generation toxicity study. A reduction without dose response was observed in the 2-year carcinogenicity study in rats.		N

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								weight increased 23.7%.			
	33	Adrenals weight	rat	28 day	Oral		No effect	No effect			
	36	Adrenals weight	rat	28 day	Dermal		No effect	No effect			
	38	Adrenals weight	rat	90 day	Oral		No effect	No effect			
	39	Adrenals weight	mouse	90 day	Oral		No effect	No effect			
	40	Adrenals weight	dog	90 day	Oral		No effect	No effect			
	41	Adrenals weight	dog	6 week	Oral		No effect	No effect			
	43	Adrenals weight	rat	90 day	Oral		No effect	No effect			
	48	Adrenals weight	rat	2 year	Oral	200 ppm	Decrease	A reduction, without a dose response, were noted in relative adrenal weight in males.			
	49	Adrenals weight	mouse	78 week	Oral		No effect	No effect			
	50	Adrenals weight	rat	29 week	Oral	800 ppm	Increase	Increased left and right adrenal wt (14%) in high dose F0 females.			
	50	Adrenals weight	rat	29 week	Oral	800 ppm	Increase	Increase rel left adrenal (21%) in F1 males at high dose.			
	50	Adrenals weight	rat	29 week	Oral	400 ppm	Increase	Increase rel left (23%) and right (20%) adrenal in F1 females at high dose. Increase rel left adrenal (12%) in mid dose F1 females.			
	32	Adrenals histopathology	rat	28 day	Oral	200 mg/kg bw/day	Increase	Adrenals haemorrhage increased (6/10 vs 0/10 in controls, in both sexes) at 200 mg/kg bw/day (above MTD, pre-terminal). Lower doses not tested.			
	33	Adrenals histopathology	rat	28 day	Oral		No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	36	Adrenals histopathology	rat	28 day	Dermal		No effect	No effect			
	38	Adrenals histopathology	rat	90 day	Oral		No effect	No effect			
	39	Adrenals histopathology	mouse	90 day	Oral		No effect	No effect			
	40	Adrenals histopathology	dog	90 day	Oral		No effect	No effect			
	41	Adrenals histopathology	dog	6 week	Oral		No effect	No effect			
	43	Adrenals histopathology	rat	90 day	Oral		No effect	No effect			
	47	Adrenals histopathology	dog	52 week	Oral		No effect	No effect			
	48	Adrenals histopathology	rat	2 year	Oral	800 ppm	Increase	Increases of enlarge and white mottling incidences in adrenal gland were found in top dose females groups compared with controls.			
	49	Adrenals histopathology	mouse	78 week	Oral		No effect	No effect			
	50	Adrenals histopathology	rat	29 week	Oral			not measured			
	50	Adrenals histopathology	rat	29 week	Oral			not measured			
	36	Brain histopathology examination	rat	28 day	Dermal		No effect	No effect	Increases brain wt were recorded in both sexes in long term and in 2-generation toxicity studies. No histopathological alternations were further described. Clinical signs were recorded only in chronic toxicity studies in rat and mice.		
	38	Brain histopathology examination	rat	90 day	Oral		No effect	No effect			
	39	Brain histopathology examination	mouse	90 day	Oral		No effect	No effect			
	40	Brain histopathology examination	dog	90 day	Oral		No effect	No effect			
	47	Brain histopathology examination	dog	52 week	Oral		No effect	No effect			
	48	Brain histopathology examination	rat	2 year	Oral		No effect	No effect			
	49	Brain histopathology examination	mouse	78 week	Oral		No effect	No effect			
	50	Brain histopathology examination	rat	29 week	Oral		No effect	No effects			
	50	Brain histopathology examination	rat	29 week	Oral		No effect	No effects			
	32	Brain weight	rat	28 day	Oral		No effect	No effect			
	33	Brain weight	rat	28 day	Oral		No effect	No effect			
	36	Brain weight	rat	28 day	Dermal		No effect	No effect			
	38	Brain weight	rat	90 day	Oral		No effect	No effect			
	39	Brain weight	mouse	90 day	Oral		No effect	No effect			
	40	Brain weight	dog	90 day	Oral		No effect	No effect			
	41	Brain weight	dog	6 week	Oral		No effect	No effect			
	47	Brain weight	dog	52 week	Oral		No effect	No effect			
	48	Brain weight	rat	2 year	Oral	400 ppm	Increase	An increase, without a clear			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								dose response, was noted in relative brain weight in females at mid and high dose (14% and 12%, respectively).			
	49	Brain weight	mouse	78 week	Oral	1500 ppm	Increase	Relative brain weight was increased in top dose groups (8/11% for males/females).			
	50	Brain weight	rat	29 week	Oral	800 ppm	Decrease	Decreased abs brain in F0 males (3%) at high dose.			
	50	Brain weight	rat	29 week	Oral	800 ppm	Increase	Increased rel brain in F0 females (7%) at high dose.			
	50	Brain weight	rat	29 week	Oral	800 ppm	Increase	Increase rel brain wt in F1 pup males at high dose (12%).			
	50	Brain weight	rat	29 week	Oral	800 ppm	Increase	Increase rel brain wt in F1 males at high dose (13%).			
	50	Brain weight	rat	29 week	Oral	800 ppm	Change	Decrease abs brain wt in F1 females at high dose (4%). Increase rel brain wt in F1 females (9%).			
	50	Brain weight	rat	29 week	Oral	800 ppm	Increase	Increase rel brain wt in F2 pup males at high dose (18%).			
	50	Brain weight	rat	29 week	Oral	800 ppm	Increase	Increase rel brain wt in F2 pup females at high dose (15%).			
	50	Fertility (mammals)	rat	29 week	Oral		No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	50	Fertility (mammals)	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	51	Fertility (mammals)	rat	10 day	Oral		No effect	No effect			
	52	Fertility (mammals)	rat	10 day	Oral		No effect	No effect			
	53	Fertility (mammals)	rabbit	13 day	Oral		No effect	No effect			
	54	Fertility (mammals)	rabbit	13 day	Oral	80 mg/kg bw/day	Decrease	A slight decrease in fertility index was observed in high dose group compared with controls, in which three dams were not pregnant (94%, 94%, 100% and 85% for controls, low, mid and high dose groups, respectively).			
	55	Fertility (mammals)	rat	1 Year	Oral		No effect	No effect			
	52	Foetal development	rat	10 day	Oral		No effect	No effect	No treatment-related effects were observed		
	54	Foetal development	rabbit	13 day	Oral		No effect	No effect	No treatment-related effects were observed		
	50	Gestation length	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	50	Litter size	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	52	Litter size	rat	10 day	Oral		No effect	No effect			
	55	Litter size	rat	1 Year	Oral	800 ppm	Decrease	Smaller sizes were observed in F2 litters of rats treated with dodine than those in controls. This study present important deviations and was deemed no reliable.			
	50	Litter viability	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	52	Litter viability	rat	10 day	Oral		No effect	No effect			
	55	Litter viability	rat	1 Year	Oral		No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	50	Litter/pup weight	rat	29 week	Oral	400 ppm	Decrease	Bodyweights were significantly decreased in F1 generation for the male and female pups from lactation days 4-21 in the high dose group; and on days 4 (precull and postcull, females only), 14 (females only), and 21 (males and females) for the pups in the mid dose group.	Decreased pup wt were recorded in both generations in rat generational study at doses in which maternal toxicity was described. No relevant decreases were recorded in developmental toxicity studies.		
	50	Litter/pup weight	rat	29 week	Oral	400 ppm	Decrease	Bodyweights were statistically significantly lower in F2 generation on days 4 (pre-cull and post-cull, males only), 7, 14, and 21 for pups in the 800 ppm dose group and on days 14 (males only) and 21 for pups in the 400 ppm dose group.			
	51	Litter/pup weight	rat	10 day	Oral		No effect	No effect			
	52	Litter/pup weight	rat	10 day	Oral		No effect	No effect			
	53	Litter/pup weight	rabbit	13 day	Oral	70 mg/kg bw/day	Decrease	Decrease not dose related mean foetal wt at high dose (4%) and low dose (8%).			
	54	Litter/pup weight	rabbit	13 day	Oral		No effect	No effect			
	51	Number of implantations, corpora lutea	rat	10 day	Oral		No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	52	Number of implantations, corpora lutea	rat	10 day	Oral		No effect	No effect	Decreased live implants and increased dead implants were recorded in developmental toxicity study in rabbits.		
	53	Number of implantations, corpora lutea	rabbit	13 day	Oral	100 mg/kg bw/day	Decrease	Decreased mean live implants (18%) at high dose tested.			
	53	Number of implantations, corpora lutea	rabbit	13 day	Oral	70 mg/kg bw/day	Increase	Increased mean dead implants at high dose (19%) and low dose (12%).			
	54	Number of implantations, corpora lutea	rabbit	13 day	Oral	40 mg/kg bw/day	Decrease	Decreased mean live implants at mid (9%) and high dose group (7%), compared with controls. No statistically significant.			
	54	Number of implantations, corpora lutea	rabbit	13 day	Oral	10 mg/kg bw/day	Increase	Increased total dead implants (15%, 123% and 53% for low, mid, and high dose groups, respectively). No statistically significant and not dose related.			
	54	Number of implantations, corpora lutea	rabbit	13 day	Oral	10 mg/kg bw/day	Increase	Increased mean dead implants (22%, 111% and 88% for low, mid, and high dose groups, respectively). No statistically significant and not dose related.			
	50	Number of live births	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	52	Number of live births	rat	10 day	Oral		No effect	No effect			
	51	Numbers of embryonic or foetal deaths and viable foetuses	rat	10 day	Oral		No effect	No effect	Increased late resorptions (without statistically significance and a		
	52	Numbers of embryonic or foetal deaths and viable foetuses	rat	10 day	Oral		No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	53	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	70 mg/kg bw/day	Increase	Increased late resorptions (80%) at high dose and low dose (10%).	clear dose-relationship) were recorded in developmental toxicity study in rabbits. These findings were seen in presence of maternal toxicity at 80 mg/kg bw/day and without clear maternal toxicity at 40 mg/kg bw/day.		
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	10 mg/kg bw/day	Increase	Increased total early resorptions (43%, 114% and 43% for low, mid, and high dose groups, respectively). No statistically significant and not dose related.			
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	10 mg/kg bw/day	Increase	Increased mean early resorptions (40%, 100% and 60% for low, mid, and high dose groups, respectively). No statistically significant and not dose related.			
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	10 mg/kg bw/day	Increase	Increased % early resorptions (40%, 100% and 60% for low, mid, and high dose groups, respectively), compared with controls. No statistically significant and not dose related.			
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	40 mg/kg bw/day	Increase	Increased total late resorptions at mid (350%) and high dose group (150%). No statistically significant and not dose related.			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	40 mg/kg bw/day	Increase	Increased mean late resorptions at mid (500%) and high dose group (300%). No statistically significant and not dose related.			
	54	Numbers of embryonic or foetal deaths and viable foetuses	rabbit	13 day	Oral	40 mg/kg bw/day	Increase	Increased % late resorptions at mid (200%) and high dose group (100%), compared with controls. No statistically significant and not dose related.			
	36	Pituitary histopathology	rat	28 day	Dermal		No effect	No effect	No treatment-related effects were observed		
	39	Pituitary histopathology	mouse	90 day	Oral		No effect	No effect			
	40	Pituitary histopathology	dog	90 day	Oral		No effect	No effect			
	47	Pituitary histopathology	dog	52 week	Oral		No effect	No effect			
	48	Pituitary histopathology	rat	2 year	Oral		No effect	No effect			
	49	Pituitary histopathology	mouse	78 week	Oral		No effect	No effect			
	50	Pituitary histopathology	rat	29 week	Oral			not measured			
	50	Pituitary histopathology	rat	29 week	Oral			not measured			
	39	Pituitary weight	mouse	90 day	Oral		No effect	No effect			
	40	Pituitary weight	dog	90 day	Oral		No effect	No effect			
	48	Pituitary weight	rat	2 year	Oral		No effect	No effect			
	52	Post implantation loss	rat	10 day	Oral		No effect	No effect	Increased post implantation loss were recorded in developmental toxicity study in rabbits, without showing statistical significance and a clear dose-relationship. These findings were seen in presence of maternal toxicity at 80 mg/kg bw/day and without clear maternal		
	54	Post implantation loss	rabbit	13 day	Oral	40 mg/kg bw/day	Increase	Increased post implantation loss in mid and high dodine treated groups (10%, 10%, 19% an 17% for control. low, mid and high dose groups). No statistically significant and not dose related.			
	51	Pre implantation loss	rat	10 day	Oral		No effect	No effect			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	52	Pre implantation loss	rat	10 day	Oral		No effect	No effect	toxicity at 40 mg/kg bw/day.		
	53	Pre implantation loss	rabbit	13 day	Oral		Increase				
	54	Pre implantation loss	rabbit	13 day	Oral		No effect	No effect			
	52	Presence of anomalies (external, visceral, skeletal	rat	10 day	Oral		No effect	No effect	No treatment-related effects were observed		
	54	Presence of anomalies (external, visceral, skeletal	rabbit	13 day	Oral		No effect	No effect			
	55	Pup development	rat	1 Year	Oral		No effect	No effect	No treatment-related effects were observed		
	50	Pup survival index	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	52	Pup survival index	rat	10 day	Oral		No effect	No effect			
	50	Sex ratio	rat	29 week	Oral		No effect	No effect	No treatment-related effects were observed		
	52	Sex ratio	rat	10 day	Oral		No effect	No effect			
	54	Sex ratio	rabbit	13 day	Oral		No effect	No effect			
Target organ toxicity	33	Kidney histopathology	rat	28 day	Oral	1000 ppm	Increase	In female kidneys, mineralization of the cortico-medullary junction (5/10 vs 2/10 in control) increased at 1000 ppm. In both sexes, very slight increase in fibrosis (1/10 vs 0/10 in control, per sex) at 1000 ppm. Lower doses not tested.	Tubular hyperplasia was found in top dose male mice in chronic toxicity study. On the other hand, low incidences and equivocal histological effects (increase/ decrease) were noted in other toxicity studies. Additionally, in 2-generation reproductive toxicity study, abs kidney wt, but no rel were reduced in adult and pup rats. These findings were not deemed adverse nor biologically relevant.	Hepatocellular adenomas were increased in top dose male/female dose groups in the 78 week mice-chronic toxicity study. This effect did not show a clear dose response nor statistical significance. Signs of systemic toxicity was noted at this dose. No other adverse histopathology findings in the liver were noted in another species in the toxicology studies within the dossier. Overall, these effects were not considered	
	36	Kidney histopathology	rat	28 day	Dermal		No effect				
	38	Kidney histopathology	rat	90 day	Oral		No effect				
	39	Kidney histopathology	mouse	90 day	Oral		No effect				
	40	Kidney histopathology	dog	90 day	Oral		No effect				
	41	Kidney histopathology	dog	6 week	Oral		No effect				
	47	Kidney histopathology	dog	52 week	Oral		No effect				
	48	Kidney histopathology	rat	2 year	Oral	200 ppm	Decrease	reduction in pelvic mineralization in kidney (males) incidences were noted in all dodine-treated groups			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	49	Kidney histopathology	mouse	78 week	Oral	200 ppm	Change	Cyst incidences were slightly increased in dodine-treated males, but were decreased in dodine-treated females, compared with controls. Moreover, dilatation pelvis occurrences were reduced in dodine-treated males, whereas hyperplasia of tubular cell incidences were mainly increased in top dose male group, compared with controls (6.7% vs 0% in controls).		relevant for human risk assessment.	
	50	Kidney histopathology	rat	29 week	Oral			not measured			
	50	Kidney histopathology	rat	29 week	Oral			not measured			
	51	Kidney histopathology	rat	10 day	Oral	100 mg/kg bw/day	Increase	In females, low incidences of epithelial pelvic dilatation (10%), pelvic inflammation (10%) and nephritis (10%) at high dose groups. These findings were not further reproduced in the main developmental toxicity study.			
	53	Kidney histopathology	rabbit	13 day	Oral	100 mg/kg bw/day	Increase	20% animal showed kidney			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								inflammation at high dose. These findings were not further reproduced in the main developmental toxicity study.			
	31	Kidney weight	Rat	10 day	Oral		No effect				
	32	Kidney weight	rat	28 day	Oral		No effect				
	33	Kidney weight	rat	28 day	Oral	1000 ppm	Decrease	Absolute and relative-to-brain kidney weight reduced in both sexes at 1000 ppm.			
	34	Kidney weight	rat	28 day	Oral		No effect				
	36	Kidney weight	rat	28 day	Dermal		No effect				
	37	Kidney weight	mouse	8 week	Oral		No effect				
	38	Kidney weight	rat	90 day	Oral		No effect				
	39	Kidney weight	mouse	90 day	Oral		No effect				
	40	Kidney weight	dog	90 day	Oral		No effect				
	41	Kidney weight	dog	6 week	Oral		No effect				
	47	Kidney weight	dog	52 week	Oral		No effect				
	48	Kidney weight	rat	2 year	Oral	200 ppm	Change	A decreased trend, not statistically significant, and not clear dose related, was observed in relative kidney weight in all males dodine treated groups, whereas in females, an increased trend was recorded for relative kidney weight.			
	49	Kidney weight	mouse	78 week	Oral	750 ppm	Increase	Absolute (high dose) and relative (mid and high dose)			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								kidney weights were significantly increased in females.			
	50	Kidney weight	rat	29 week	Oral	800 ppm	Decrease	Decreased abs left kidney in F0 males (5%) at high dose.			
	50	Kidney weight	rat	29 week	Oral	400 ppm	Decrease	Decreased abs left and right kidney at high dose (6%) and mid dose (6%) in F0 females.			
	50	Kidney weight	rat	29 week	Oral	800 ppm	Decrease	Decrease abs left and right kidney wt in high dose F1 male pup (16%).			
	50	Kidney weight	rat	29 week	Oral	400 ppm	Decrease	Decrease abs left (15%) and right (14%) kidney wt in high dose F1 males. Decrease abs kidney wt (7%) in mid dose F1 males.			
	50	Kidney weight	rat	29 week	Oral	400 ppm	Decrease	Decrease abs left (11%) and right (12%) kidney wt in high dose F1 females. Decrease abs left (5%) and right (6%) kidney wt in mid dose F1 female.			
	50	Kidney weight	rat	29 week	Oral	400 ppm	Decrease	Decrease abs left and right kidney wt (16%) in high dose F2 male pups. Decrease abs left (11%) kidney wt in mid			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								dose F2 male pups in mid dose group.			
	50	Kidney weight	rat	29 week	Oral	800 ppm	Decrease	Decrease abs left (14%) and right (17%) kidney wt in high dose F2 female pups.			
	33	Liver histopathology	rat	28 day	Oral		No effect		Benign tumours (hepatocellular adenomas) were increased after dodine administration. Liver adenomas appeared at a dose in which systemic toxicity was observed and the results were not supported by statistical significance between groups and controls. Besides, although the occurrence of combined adenomas/carcinomas displayed statistically significance in the top dose female group, it is noteworthy that carcinomas incidence was very similar between dodine-treated groups and their respective controls for both sexes.		
	36	Liver histopathology	rat	28 day	Dermal		No effect				
	37	Liver histopathology	mouse	8 week	Oral	100/1250 ppm	Increase	Mild eosinophilia in liver in both sexes at 100/1250 ppm.			
	38	Liver histopathology	rat	90 day	Oral		No effect				
	39	Liver histopathology	mouse	90 day	Oral		No effect				
	40	Liver histopathology	dog	90 day	Oral		No effect				
	41	Liver histopathology	dog	6 week	Oral		No effect				
	43	Liver histopathology	rat	90 day	Oral		Change				
	47	Liver histopathology	dog	52 week	Oral	20 mg/kg bw/day	No effect	Slight increment in liver vacuolization in males at 20 mg/kg bw/day.			
	48	Liver histopathology	rat	2 year	Oral	200 ppm	Decrease	Reduction in bile duct hyperplasia in liver (females) incidences were noted in all dodine-treated groups.			
	49	Liver histopathology	mouse	78 week	Oral	1500 ppm	Increase	An increased incidences of hepatocellular adenomas were observed at high dose groups for both sexes (13%, 12%, 15% and 23% for controls, low, mid and high dose males groups; and 0%,			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								2%, 2% and 7% for controls, low, mid and high dose females groups, respectively), in which a statistically significant trend was displayed for females. On the other hand, no relevant increases were noted regarding hepatocellular carcinomas in dodine-treated groups (male or females). When the effects were combined, increased incidences were also observed in high dose groups (17%, 12%, 20% and 25% for controls, low, mid and high dose males groups; and 0%, 3%, 2% and 8% for controls, low, mid and high dose females groups, respectively), showing a significant trend test in females, and the only significant group comparison difference with			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								controls was for combined adenomas and carcinomas in females given 1500 ppm dose.			
	50	Liver histopathology	rat	29 week	Oral			not measured			
	50	Liver histopathology	rat	29 week	Oral			not measured			
	53	Liver histopathology	rabbit	13 day	Oral	100 mg/kg bw/day	Increase	20% animal showed liver inflammation			
	40	Pancreas histopathology	dog	90 day	Oral		No effect		No treatment-related effects were observed		
	47	Pancreas histopathology	dog	52 week	Oral		No effect				
	48	Pancreas histopathology	rat	2 year	Oral		No effect				
	49	Pancreas histopathology	mouse	78 week	Oral		No effect				
	36	Spleen histopathology	rat	28 day	Dermal		No effect		No adverse treatment-related effects were observed		
	37	Spleen histopathology	mouse	8 week	Oral		No effect				
	38	Spleen histopathology	rat	90 day	Oral		No effect				
	39	Spleen histopathology	mouse	90 day	Oral	2500 ppm	No effect	Lymphoid atrophy in spleen in 3/10 females at 2500 ppm vs 0/10 in control (lower doses not analysed).			
	50	Spleen histopathology	rat	29 week	Oral			not measured			
	50	Spleen histopathology	rat	29 week	Oral			not measured			
	36	Spleen weight	rat	28 day	Dermal		No effect				
	37	Spleen weight	mouse	8 week	Oral	100/1250 ppm	Decrease	Absolute spleen weight reduced in females at 100/1250 ppm.			
	38	Spleen weight	rat	90 day	Oral		No effect				
	39	Spleen weight	mouse	90 day	Oral	1250 ppm	No effect	Absolute spleen weight reduced in both sexes from 1250 ppm. Relative-to-body spleen weight in females decreased from 1250 ppm.			
	50	Spleen weight	rat	29 week	Oral	400 ppm	Decrease	Decrease abs spleen wt (28%)			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								in high dose F2 male pups.			
	50	Spleen weight	rat	29 week	Oral	400 ppm	Decrease	Decrease abs spleen wt (22%) in high dose F2 female pups.			
	53	Stomach histopathology	rabbit	13 day	Oral	100 mg/kg bw/day	Increase	50% of animals showed colour or dark foci areas on stomach, in some cases with epithelium hyperplasia.	No treatment-related effects were observed		
	36	Thymus histopathology	rat	28 day	Dermal		No effect				
	39	Thymus histopathology	mouse	90 day	Oral	2500 ppm	No effect	Lymphoid necrosis and atrophy in thymus in 4/10 females at 2500 ppm vs 0/10 in control (lower doses not analysed).			
	50	Thymus histopathology	rat	29 week	Oral			not measured	No adverse treatment-related effects were observed		
	50	Thymus histopathology	rat	29 week	Oral			not measured			
	36	Thymus weight	rat	28 day	Dermal		No effect				
	50	Thymus weight	rat	29 week	Oral	800 ppm	Decrease	Decreased abs thymus in F0 males (17%) at high dose.			
	50	Thymus weight	rat	29 week	Oral	800 ppm	Decrease	Decrease abs thymus wt in F2 female pups (28%) in high dose group.			
	51	Urinary bladder histopathology	rat	10 day	Oral	100 mg/kg bw/day	Increase	Low incidences of epithelial hyperplasia (10%) and chronic inflammation (10%) at high dose group. Ureter inflammation at	No adverse treatment-related effects were observed		

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								high dose groups (10%).			
Systemic toxicity	31	Body weight	Rat	10 day	Oral		No effect		Signs of systemic toxicity occurred mainly at high doses, which included mortality, effects on bodyweight, food consumption, and clinical signs; these signs were related to general toxicity of higher doses. However, a case by case approach may be done, as toxic adverse effects were not observed in all studies.	Overall evidence of systemic toxicity.	
	32	Body weight	rat	28 day	Oral	75 mg/kg bw/day	Decrease	Bw reduced in males and bw gain lower in males and females from 100 mg/kg bw/day.			
	33	Body weight	rat	28 day	Oral	500 ppm	Decrease	Bw reduced in both sexes at 1000 ppm and bw gain lower in males from 750 ppm and in females from 500 ppm.			
	34	Body weight	rat	28 day	Oral	800 ppm	Decrease	Bw gain reduced in both sexes at 800 ppm.			
	35	Body weight	rat	28 day	Oral	800 ppm	Decrease	Bw gain reduced in both sexes at 800 ppm.			
	36	Body weight	rat	28 day	Dermal	125 mg/kg bw/day	Decrease	Bw gain reduced in males from 125 mg/kg bw/day.			
	37	Body weight	mouse	8 week	Oral	100/1250 ppm	Decrease	Bw reduced in females and bw gain reduced in both sexes at 100/1250 ppm.			
	38	Body weight	rat	90 day	Oral	800 ppm	Decrease	Bw gain reduced in both sexes at 800 ppm.			
	39	Body weight	mouse	90 day	Oral	1250 ppm	Decrease	Bw reduced in males at 2500 ppm and bw gain reduced in males at 1250 ppm and in females at 2500 ppm.			
	40	Body weight	dog	90 day	Oral	20 mg/kg bw/day	Decrease	Bw reduced in females and bw			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								gain reduced in both sexes at 20 mg/kg bw/day.			
	41	Body weight	dog	6 week	Oral	25 mg/kg bw/day	Decrease	Males: bw loss at 50 mg/kg bw/day up to 4 weeks and at 60 mg/kg bw/day for 2 weeks. Bw loss in a male at 25 mg/kg bw/day for 6 weeks. Females: bw loss at 50 mg/kg bw/day up to 5 weeks and at 60 mg/kg/day for 2 weeks.			
	42	Body weight	rat	28 day	Oral	83 mg/kg bw/day	Decrease	Lower body weight gain for high dosed animals.			
	43	Body weight	rat	90 day	Oral		No effect				
	44	Body weight	rat	100 day	Oral	3200 ppm	Decrease	lower body weight gain for high dosed animals			
	45	Body weight	dog	1 Year	Oral	50 ppm	Decrease	Reduced bw gain			
	46	Body weight	rat	2 year	Oral	800 ppm	Decrease	lower body weight gain for high dosed animals			
	47	Body weight	dog	52 week	Oral	10 mg/kg bw/day	Decrease	Some dogs from 10 mg/kg bw/day exhibited marked bw loss during first weeks that prompted supplemental feeding.			
	48	Body weight	rat	2 year	Oral	800 ppm	Decrease	Slight statistically significant			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								decreases in bodyweight were recorded in top dose male group throughout week 1-37 (5.2-8.2%) and weeks 85-89 (7-8%), whereas in females were noted throughout whole study (4.1-16.6%)			
	49	Body weight	mouse	78 week	Oral	750 ppm	Decrease	Statistically significantly lower bodyweights were recorded at top male (3-10%) and female (4-14%) dose groups throughout whole study, compared with controls. At mid dose groups, statistically significant reductions were mainly noted from week 30 to study termination for both sexes (2-5% for males and 4-10% for females, respectively), although sporadic reductions were observed the days before week 30. Overall mean bodyweight gain			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								was statistically significant reduced in mid and high dose male groups (5 and 26%) and in dodine-female treated groups (11, 20 and 35% for low, mid and high dose groups, respectively).			
	50	Body weight	rat	29 week	Oral	800 ppm	Decrease	Statistically significantly lower in high dose groups for both sexes throughout study.			
	50	Body weight	rat	29 week	Oral	400 ppm	Decrease	Statistically significantly lower in high (male and females) and mid dose (females) groups throughout study.			
	51	Body weight	rat	10 day	Oral	70 mg/kg bw/day	Decrease	Statistically significant decrease in bodyweight was recorded in gestation day 13 in mid and high dose groups (10% and 8%, respectively), however decreases, not statistically significant and without dose-relationship,			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								were recorder throughout the whole gestation period of mid (8-10%) and top dose dams (2-8%) compared with controls. Statistically significant decrease in bodyweight gain was recorded throughout gestation day 6-13 in mid and high dose groups (26% and 48%, respectively).			
	52	Body weight	rat	10 day	Oral	90 mg/kg bw/day	Decrease	Statistically significant decrease in bodyweights were recorded in gestation day 9 (9%), 13 (8%) and 17 (8%) in high dose group, compared with controls. At high dose group, bodyweight gain was statistically significantly lower from gestation day 6-9 (107%) and 6-17 (20%), compared with the controls. Moreover, corrected bodyweight gain by the uterus weight was			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								statistically significantly lower in top dose group (756%), compared with controls.			
	53	Body weight	rabbit	13 day	Oral	100 mg/kg bw/day	Decrease	Bodyweight loss at high dose group (48%), compared with controls.			
	54	Body weight	rabbit	13 day	Oral		No effect				
	55	Body weight	rat	1 Year	Oral		No effect				
	32	Clinical chemistry and haematology	rat	28 day	Oral	100 mg/kg bw/day	Increase	WBC counts and segmented neutrophils increased in both sexes and RDW increased in males, at 100 mg/kg bw/day.			
	32	Clinical chemistry and haematology	rat	28 day	Oral	100 mg/kg bw/day	Decrease	Lymphocyte count reduced in both sexes at 100 mg/kg bw/day.			
	32	Clinical chemistry and haematology	rat	28 day	Oral	75 mg/kg bw/day	Increase	Alanine aminotransferase increased in both sexes from 75 mg/kg bw/day			
	33	Clinical chemistry and haematology	rat	28 day	Oral	1000 ppm	decrease	Alanine aminotransferase reduced in females at 1000 ppm.			
	38	Clinical chemistry and haematology	rat	90 day	Oral	800 ppm	Increase	Neutrophils increased in males at 800 ppm.			
	38	Clinical chemistry and haematology	rat	90 day	Oral	800 ppm	Decrease	Alanine aminotransferase reduced in females at 800 ppm.			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	39	Clinical chemistry and haematology	mouse	90 day	Oral	2500 ppm	Increase	Neutrophils and RDW increased in males at 2500 ppm.			
	39	Clinical chemistry and haematology	mouse	90 day	Oral	2500 ppm	Increase	BUN in both sexes, phosphorus in males and A/G ratio in females increased at 2500 ppm.			
	40	Clinical chemistry and haematology	dog	90 day	Oral		No effect				
	41	Clinical chemistry and haematology	dog	6 week	Oral		No effect				
	42	Clinical chemistry and haematology	rat	28 day	Oral		No effect				
	43	Clinical chemistry and haematology	rat	90 day	Oral		No effect				
	44	Clinical chemistry and haematology	rat	100 day	Oral		No effect				
	46	Clinical chemistry and haematology	rat	2 year	Oral		No effect				
	47	Clinical chemistry and haematology	dog	52 week	Oral		No effect				
	48	Clinical chemistry and haematology	rat	2 year	Oral	800 ppm	Decrease	Mean alkaline phosphatase activities were higher at top and mid dose female groups (310% and 150% for top and mid dose groups, respectively) on week 104. No other significant treatment-related variations were noted at any of the scheduled blood sampling periods for any of the parameters assayed.			
	49	Clinical chemistry and haematology	mouse	78 week	Oral						
	31	Clinical signs	Rat	10 day	Oral		No effect				
	48	Clinical signs	rat	2 year	Oral	200 ppm	Increase	A statistically significant increase in the absence of grasping was			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								found in top dose male group, compared with controls; whereas a significant trend test was obtained for the absence of grasping, traction and righting reflexes incidences in dodine-male treated groups. On the other hand, a dose-related increase in the hunched posture incidence was revealed in males. Moreover, increased reduced motor activity and piloerection was observed in males dodine-treated groups compared with controls.			
	49	Clinical signs	mouse	78 week	Oral	200 ppm	Increase	Increased incidence of whole body tremors was noted mainly in mid and high dose groups for both sexes (13-14% in males and 11-13% in females compared with controls).			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								Malocclusion occurrences was considerably increased in high male dose group (18.6% vs 5.7% in controls), whereas a slight increase of irregular respiration (4.3% vs 0% in controls) and rough hair coat incidences (11.4% vs 0% in controls) were found in top dose female group. On the other hand, increased dose-related incidences of dilated pupil and excessive salivation were mainly observed in the three male-dodine treated groups and in mid-top male dose groups, respectively, whereas increases,			
	51	Clinical signs	rat	10 day	Oral	100 mg/kg bw/day	Increase	Two females from high dose group exhibited clinical signs: one animal showed wheezing and another female			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								showed piloerection, hunched posture, red/brown staining around face, fore-paws and mild ataxia.			
	52	Clinical signs	rat	10 day	Oral	90 mg/kg bw/day	Increase	Three dams at high dose group showed excessive salivation after dosing for one or 2 days during the treatment period. On the other hand, there was another three animals with red/brown stained fur around the mouth at 90 mg/kg bw/day dose groups.			
	54	Clinical signs	rabbit	13 day	Oral	80 mg/kg bw/day	Increase	15% of rabbits showed liquid faeces, breathing difficulties and emaciation.			
	54	Clinical signs	rabbit	13 day	Oral	80 mg/kg bw/day	Increase	2 abortions (10%) at high dose.			
	32	Food consumption	rat	28 day	Oral	75 mg/kg bw/day	Decrease	Food consumption reduced in both sexes from 75 mg/kg bw/day.			
	33	Food consumption	rat	28 day	Oral	750 ppm	Decrease	Food consumption reduced in both sexes from 750 ppm.			
	34	Food consumption	rat	28 day	Oral	800 ppm	Decrease	Food consumption			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								reduced in males from 800 ppm.			
	35	Food consumption	rat	28 day	Oral		No effect				
	37	Food consumption	mouse	8 week	Oral		No effect				
	38	Food consumption	rat	90 day	Oral	800 ppm	Decrease	Food consumption reduced in females at 800 ppm.			
	39	Food consumption	mouse	90 day	Oral	1250 ppm	Decrease	Food consumption reduced in both sexes at 2500 ppm.			
	40	Food consumption	dog	90 day	Oral	20 mg/kg bw/day	Decrease	Food consumption reduced in both sexes at 20 mg/kg bw/day.			
	41	Food consumption	dog	6 week	Oral	25 mg/kg bw/day	Decrease	Decreased food consumption at 50 and 60 mg/kg bw/day and in one male at 25 mg/kg bw/day.			
	42	Food consumption	rat	28 day	Oral	83 mg/kg bw/day	Decrease	statistically significant lower food consumption at high dose animals			
	44	Food consumption	rat	100 day	Oral	3200 ppm	Decrease	reduced food consumption			
	47	Food consumption	dog	52 week	Oral	10 mg/kg bw/day	Decrease	Some dogs from 10 mg/kg bw/day reduced food consumption. Supplemental feeding required.			
	48	Food consumption	rat	2 year	Oral	800 ppm	Decrease	Food consumption was mostly decreased through sporadic			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								weeks in top dose male (5-12%) and female (4-16%) dose groups without showing a dose relationship and consistency throughout the whole study. On the other hand, isolated decreases or increases were recorded in low and mid dose groups.			
	49	Food consumption	mouse	78 week	Oral	750 ppm	Decrease	Mean food consumption was generally reduced at top dose groups for both sexes throughout whole study (5-16% for males and 5-19 for females, respectively), compared with controls. On the other hand, at mid dose groups, statistically significant reductions were mainly noted at the first half of the study in males (5-8%), and practically through entire study in females (5-16%).			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	50	Food consumption	rat	29 week	Oral	800 ppm	Decrease	Statistically significantly reduced food consumption in high dose groups during premating (male and females), and lactation.			
	50	Food consumption	rat	29 week	Oral	800 ppm	Decrease	Statistically significantly reduced food consumption in high dose groups during premating (male and females), gestation and lactation.			
	51	Food consumption	rat	10 day	Oral	70 mg/kg bw/day	Decrease	Reduction in mean food consumption through days 6-16 in high (24%) and mid dose groups (15%)			
	52	Food consumption	rat	10 day	Oral	45 mg/kg bw/day	Decrease	At high dose group, there was a statistically significantly lower food consumption through gestation day 6-16 (13-37%), whereas at mid dose group, there was a statistically significantly lower food consumption on gestation day 6 (11%) and gestation day 8-			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								10 (12-18%), compared with controls. When time frames were compared, statistical significance was displayed through day 6-10 (30% and 14% for high and mid dose group, respectively), 6-16 (22% and 11% for high and mid dose group, respectively), and 3-19 (14% for high dose group) compared with controls.			
	53	Food consumption	rabbit	13 day	Oral	100 mg/kg bw/day	Decrease	A reduction in absolute food consumption was recorded in top dose group throughout GD 6-18 (31-77%), compared with controls. The mean food consumption was 51% lower than controls for this group			
	54	Food consumption	rabbit	13 day	Oral	80 mg/kg bw/day	Decrease	Statistically significant reduction in food consumption was recorded at high dose group in gestation days 6 (25%), 7 and 8 (30%) compared			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								with controls. Moreover, in this group, sporadic reductions, without statistical significance, were noted during mid to late treatment period.			
	31	Mortality	Rat	10 day	Oral		No effect				
	32	Mortality	rat	28 day	Oral	75 mg/kg bw/day	Increase	In males, 10/10 at 200 mg/kg bw/day died. In females, 1/10 at 75 mg/kg bw/day, 4/10 at 100 mg/kg bw/day and 10/10 at 200 mg/kg bw/day died.			
	33	Mortality	rat	28 day	Oral		No effect				
	34	Mortality	rat	28 day	Oral		No effect				
	35	Mortality	rat	28 day	Oral		No effect				
	38	Mortality	rat	90 day	Oral		No effect				
	39	Mortality	mouse	90 day	Oral	2500 ppm	Increase	In females, 4/10 died at 2500 ppm.			
	40	Mortality	dog	90 day	Oral		No effect				
	41	Mortality	dog	6 week	Oral		No effect				
	42	Mortality	rat	28 day	Oral		No effect				
	44	Mortality	rat	100 day	Oral		No effect				
	46	Mortality	rat	2 year	Oral		No effect				
	47	Mortality	dog	52 week	Oral		No effect				
	48	Mortality	rat	2 year	Oral		No effect				
	49	Mortality	mouse	78 week	Oral	200 ppm	Decrease	Survival was dose-related increased in male dodine-treated groups, compared to the control.			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
	50	Mortality	rat	29 week	Oral		No effect				
	50	Mortality	rat	29 week	Oral		No effect				
	51	Mortality	rat	10 day	Oral	100 mg/kg bw/day	Increase	one treatment-related death at high dose group			
	52	Mortality	rat	10 day	Oral		No effect				
	53	Mortality	rabbit	13 day	Oral	100 mg/kg bw/day	Increase	5 treatment related dead animals in high dose group.			
	54	Mortality	rabbit	13 day	Oral	80 mg/kg bw/day	Increase	Three dead were recorded in high dose group			
	51	Necropsy	rat	10 day	Oral	100 mg/kg bw/day	Increase	A slight increase in the kidney incidences (30%; pelvic dilatation and enlarged) and ureters (20%: dilatation) were found in the top dose group, compared with controls			
	52	Necropsy	rat	10 day	Oral		No effect				
	53	Necropsy	rabbit	13 day	Oral	100 mg/kg bw/day	Increase	At high dose group, the half of animals showed liquid contents and gaseous distension in caecum (50%), compared with controls			
	54	Necropsy	rabbit	13 day	Oral	40 mg/kg bw/day	Increase	The incidence of dark patches in lung lobes was increased in mid (12.5%) and high dose (20%) groups, in which the half of these animals presented breathing			

Grouping	Study ID Matrix	Effect target	Species	Exposure	Route of administration	Effect dose	Effect direction	Observed effect	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
								difficulties as clinical signs.			

2.10.1.3.5. Assessment of the integrated lines of evidence and weight of evidence for EAS-mediated adversity and endocrine activity.

WoE for EAS-mediated adversity

This section provides the lines of evidence for the *in vivo* mammalian toxicology studies (Level 4 and 5) using test substance dodine in respect of the EAS-modality. The following sections provide an analysis of the integrated lines of evidence and report the weight of evidence in respect of EAS-mediated adversity.

Regarding EAS-mediated parameters

-Most of the EAS-mediated parameters were measured in the long-term/carcinogenicity toxicity studies conducted in rat (ID: 48) and mice (ID: 49), and in the 2-generation toxicity conducted in rats (ID: 50). Furthermore, several short-term/repeated toxicity studies were conducted in rat, mice and dog.

No clear toxicologically significant changes or pattern of effects were reported in the repeated exposure studies in EAS mediated male target organs:

-Increased relative testis weight was increased in high dose F1 males in 2-generation toxicity study conducted in rats (ID: 50). No histopathological changes were associated to this finding. In addition, no differences were noted regarding reproductive parameters that draw attention to a problem in male fertility. No alterations regarding testis weight or histopathology abnormalities were recorded in other toxicity studies contained in the dossier.
- Epididymis relative weight was increased (mid and high doses) in two year rat study (ID: 48), and in F1 adult males (high dose) from 2-generation toxicity study (ID: 50).
-An increased, not dose related of prostate atrophy incidences were noted in male treated groups (1.5%, 3.3%, 8.2% y 4.3% for controls, low, mid and high dose groups, respectively) in 2-year carcinogenicity study conducted in rats.
- Seminal vesicle atrophy (3/10) at 200 mg/kg bw/day (above MTD, pre-terminal) was noted in 28-day study in rats (ID: 32). Controls and lower doses were not tested.
- Regarding sperm evaluation, the available study (ID 50) deviated from current guidelines because sperm morphology was evaluated in 100 sperm/male instead of 200. However, in the 100 sperm examined/male in the study, there were no significant differences in the percent motility, mean sperm concentration, and sperm morphology in P and F1 dodine-treated males compared with controls.

On the other hand, no clear toxicologically significant changes or pattern of effects were reported in the repeated exposure studies in EAS mediated female target organs:

-An increase, not statistically significant and not dose related, in the relative uterus weight was observed in top dose female group in the carcinogenicity study in rats (ID: 48). A slight increase, not dose related, in the mean uterus weight was found in mid and high dose groups (5% and 7% for mid and high dose groups, respectively) in the main developmental toxicity study in rats (ID: 52).
- In the 2-year carcinogenicity study in rats (ID: 48), benign ovary cystadenomas, not dose related, were noted in 0/37 (0%), 3/32 (9%), 5/36 (14%) and 2/36 (6%) of females from the 0, 200, 400 and 800 ppm dose groups, respectively. These findings were ruled out due to no dose related increase were noted, and no another histopathological findings in ovary were revealed in other toxicity studies within the dossier. On the other hand, increase relative left ovary/oviduct wt was described in F1 females (11%) at high dose from 2-generation toxicity study (ID: 50).
- No treatment-related differences were revealed between dodine treated groups and controls regarding oestrus cyclicity in the 2-generation toxicity study (ID: 50).
-Regarding mammary gland histopathology, higher incidence of thickened mammary glands was observed in 1-year treated dogs females (ID: 47), although this effect was not dose-response. On the other hand, in the 2-year carcinogenicity study in rats (ID: 48), an increase, not dose related, of malignant adenocarcinomas incidences were noted in female mid dose group (10, 14.5, 24.6 and 12.9% for control, low, mid and high dose groups).

Regarding sensitive to, but not diagnostic of EATS parameters:

- Increases adrenal wt were recorded in the 28-day study in rats (ID: 32) and in the 2-generation toxicity study (ID: 50). A reduction without dose response was observed in the 2-year carcinogenicity study in rats (ID: 48).
-Increased incidences of relative brain weight were recorded in both sexes in long term (ID: 48-49) and in 2-generation toxicity studies (ID: 50). No histopathological alternations were further described. Potential neurotoxic clinical signs were mainly recorded in chronic and developmental toxicity studies.

-In the two-generation toxicity study conducted in rats (ID: 50):

.No effects were noted on fertility in males or females.

.No effects were observed in reproductive/pregnancy parameters in females.

.No genital abnormalities were noted.

.Decreased pup weight were recorded in both generations in rat generational study at doses in which maternal toxicity was described. No relevant decreases were further recorded in developmental toxicity studies.

-In the main developmental toxicity studies conducted in rats (ID: 52) and rabbits (ID 54):

.No effects were observed in reproductive/pregnancy parameters in the main developmental toxicity study in rats (ID 52).

.Increased of post implantation losses and late resorption were recorded in the developmental toxicity study in rabbits (ID: 54) at mid (40 mg/kg bw/day) and high doses (80 mg/kg bw/day), without showing statistical significance and a clear dose-relationship. These findings were seen in presence of maternal toxicity at 80 mg/kg bw/day and without clear maternal toxicity at 40 mg/kg bw/day.

.No effects on foetal development, no external, skeletal or visceral abnormalities have been reported in the developmental studies (rats and rabbits).

Target organ toxicity

-In the chronic/carcinogenicity study in mice (ID: 49), increased incidences of hepatocellular adenomas were observed at high dose groups for both sexes (13%, 12%, 15% and 23% for controls, low, mid and high dose males groups; and 0%, 2%, 2% and 7% for controls, low, mid and high dose females groups, respectively), in which a statistically significant trend was displayed for females. On the other hand, no relevant increases were noted regarding hepatocellular carcinomas in dodine-treated groups (male or females). When the effects were combined, increased incidences were also observed in high dose groups (17%, 12%, 20% and 25% for controls, low, mid and high dose males groups; and 0%, 3%, 2% and 8% for controls, low, mid and high dose females groups, respectively), showing a significant trend test in females, and the only significant group comparison difference with controls was for combined adenomas and carcinomas in females given 1500 ppm dose. Signs of systemic toxicity were described at mid and high dose dose. Additionally, no other adverse histopathology findings in the liver were noted in another species in the toxicology studies within the dossier. Overall, these effects were not considered relevant for human risk assessment.

-No other relevant findings were observed regarding target organ toxicity in the subacute, subchronic or chronic toxicity studies provided in the dossier.

WoE for EAS-mediated activity

The available dataset of *in vitro* mechanistic assays showed positive/active results for ER and AR bioactivity models. Toxcast ER bioactivity model showed one active/positive assay (ID: 12). The reported AC_{50} value for this assay is less than the lowest concentration tested in the experiment. The data and curve fitting for this study also appear to be scattered and do not show a clear response, so the relevance of this result is low. Furthermore, dodine technical is also reported inactive in the CERAPP models.

On the other hand, Toxcast AR bioactivity model displayed two active/positive result (ID: 17 and 19). Both positive results are reported at a concentration level that is above the limit of cytotoxicity of dodine and presented flags that compromise the reliability of the assays. Additionally, predictions from COMPARA model indicate that dodine is inactive for AR agonist, antagonist, and AR binding.

To end, negative results were displayed in the only two available Toxcast Steroidogenesis bioactivity models.

E-modality: An *in vitro* estrogen receptor transactivation (ER-STTA) assay, according to OECD TG 455 was conducted (ID 27). Dodine displayed negative results for agonist and for antagonist effects on the estrogen receptor.

A-modality: An *in vitro* androgen receptor transactivation (AR-STTA) assay, according to OECD 458 was conducted (ID 28). The outcome of the AR agonist assay showed that the RPCmax value was below 10% of the positive control in two replicates. On the other hand, the outcome of the AR antagonist assay showed that dodine was able to reduce AR-transactivation of luciferase gene expression in presence of dihydrotestosterone (DHT). In this assay, IC_{50} values could not be derived, but IC_{30} values could be derived from replicate 1 (0.05µM) and replicate

3 (1 µM). Based on these results, dodine technical may be deemed positive in the AR antagonist assay and negative for AR agonist assay.

A Hershberger assay (ID 31) was conducted in order to follow up the positive results *in vitro* in the AR-STTA antagonist assay. Both agonist and antagonist groups (three doses of 5, 15, 50 mg/kg bw each) and corresponding controls were included in the study. No changes were observed in the five androgen-dependent organs (Cowper's gland, seminal vesicles, LABC muscle, glans penis and ventral prostate) upon exposure to dodine, both in the agonist and antagonist part of the assay.

Considering the mixed positive/negative results observed *in vitro* for the androgen modality, and the clearly negative results observed in the *in vivo* Hershberger assay, the effects of dodine seen *in vitro* are not relevant in the *in vivo* model. Therefore, overall the database shows no endocrine activity with regard to the androgen modality.

S-modality: An aromatase assay according to OPPTS 890.1200 was conducted (ID: 29). This is a cell-free assay that directly measures the activity of the aromatase enzyme. Dodine caused inhibition of the aromatase enzyme with an IC_{50} of 56.8 µM. Furthermore, a steroidogenesis assay according to OECD 456 was conducted (ID 30). Dodine did not induce or inhibit the production of testosterone and 17β-estradiol in two independent experiments up to the cytotoxicity limit (0.5 µM). Consequently, unlike previous aromatase activity test cell-free assay, neither aromatase (CYP19) enzymatic activity, nor the activity of other enzymes from steroidogenesis pathway was affected in the H295R steroidogenesis assays.

On the other hand, the Hershberger assay (ID 31) conducted with dodine also provides relevant information on possible effects on the steroid pathways. This study serves as a mechanistic *in vivo* screening assay for androgen agonists or antagonists and 5α-reductase inhibitors. So, the lack of effects in androgen dependent tissues (agonist or antagonist) shows that no effects occurred in the levels of steroid hormones upon exposure to dodine.

Overall, although the enzyme-based assay showed inhibition of the aromatase enzyme, no effect on steroidogenesis/aromatase enzyme gene transactivation was seen in cell-based assays. In addition, in the *in vivo* mechanistic study (Hershberger assay), no effects were observed that could be related to changes in the steroid hormones pathways. Therefore, the dataset did not shows endocrine activity with regard to the steroidogenesis modality.

2.10.1.3.6. Selection of relevant scenario for the ED assessment of EAS-modality

No OECD TG 443 or OECD TG 416 (according to latest version of January 2001) studies have been conducted with dodine. However, based on a weight of evidence approach, no EAS-mediated adversity have been identified in the available dataset. On the other hand, the positive anti-androgenic activity displayed *in vitro* was not further confirmed in the *in vivo* Hershberger assay. Moreover, the positive *in vitro* inhibition of aromatase activity in a cell-free system was not further confirmed in the *in vitro* cell based steroidogenesis assay nor in the *in vivo* Hershberger assay. Overall, it can be concluded that no EAS-mediated adversity nor activity were found, therefore it corresponds to the scenario 2a (ii).

The relevant scenario for the EAS-modality is identified as 2a (ii).

Table 2.10.1.3.6: Identification of relevant scenario for EAS-modality

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not "EAS-mediated" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no EAS-mediated endocrine activity observed	x
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EAS-mediated" parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

2.10.1.3.7. MoA analysis for EAS-modalities

According to the ED EFSA/ECHA guidance (2018), in cases of Scenario 2a (ii), a MoA analysis for EAS-modalities is not required.

2.10.1.3.8. Conclusion on the assessment of EAS-modalities

Considering all the data available, there is no indication of endocrine activity and no adversity for the EAS modalities. Therefore, ED criteria are not met because no EAS-mediated effects have been observed and scenario 2a (ii) is selected.

2.10.1.4. Overall conclusion on the ED assessment for humans

T-modality:

Considering the available data, T-mediated adversity has been found based on T-mediated parameters and T-mediated activity has not been sufficiently investigated. It corresponds to Scenario 1b. A MoA analysis is required and a conclusion cannot be reach.

EAS-modality:

Based on the available data, EAS-mediated adversity has been considered not sufficiently investigated, whereas EAS-mediated endocrine activity has been deemed sufficiently investigated.

E-modality: It is considered sufficiently investigated based on the estrogenic activity output data from the US EPA ToxCast Bioactivity Model.

A-modality: It is considered sufficiently investigated based on the output data from "Hershberger bioassay in rats' (ID: 31; OECD TG 441).

S-modality: It is considered sufficiently investigated based on the output data from "H295R Steroidogenesis assay" (ID: 30; OECD TG 456), and the *"In vitro* aromatase inhibition using human recombinant microsomes assay" (ID: 29) in line with OPPTS 890.1200.

No signs of adversity were found in the available dataset consistent in subacute, subchronic, chronic and reproductive toxicity studies conducted with dodine in different species. On the other hand, the positive anti-androgenic activity and the capacity of dodine to inhibit aromatase activity were not further confirmed in the follow up *in vitro* and *in vivo* studies, so the relevance of a potential dodine activity for the EAS modalities was ruled out. Therefore, the dodine does not meet the ED criteria for the EAS modalities and the scenario 2a (ii)) applies.

2.10.2 ED assessment for non-target organisms

Where the available evidence indicates that the ED criteria are not met for mammals as non-target organisms, the assessment for non-target organisms should proceed by considering fish and amphibians, because these are the taxa where standardised test methods and knowledge on how to interpret the results are available. Information on other organism (e.g. birds) should be considered if available.

For wild mammals, the main source of information are the regulatory toxicological studies performed with mammals in the laboratory for human safety purposes, which are summarized in Volume 3 (AS) Section B.6. The assessment for wild mammals should be based on the same data package. However, the determination of adverse effects will be different, as for wild mammals the focus is on effects that are relevant at the population level. It is noted that a separate assessment for wild mammals have not been submitted by applicant and can be requested once the conclusion on ED assessment for human heaalth is reached. Regarding T- modality for mammals, T-mediated adversity was found based on T-mediated parameters and T-mediated activity was not sufficiently investigated. Then, a MoA analysis is required and a conclusion cannot be reached. Nonetheless, potential ED properties of Dodine on non-target organisms other than mammals was assessed for this modality.

In the case of the EAS modalities, the dataset for mammals showed that Dodine does not meet the criteria (see Point 2.3). Therefore, according to the strategy recommended in the ECHA/EFSA Guidance (2018), further consideration on the potential ED properties on non-target organisms other than mammals for these modalities is required.

The available studies in the RAR for Dodine were considered. A summary of all studies is provided below.

Data were populated in the Excel template provided as Appendix E to the EFSA/ECHA guidance for the identification of endocrine disruptors (2018). According to this template each study was given a unique identification number (Study ID Matrix) that is important for its identification in the data-matrix and Lines of Evidence (LoE) spreadsheets of the Excel.

Table 2.10.3-1 Outline of dataset considered for ED assessment of non-target organisms

Type of toxicity	Study type	Study ID Matrix
***In vitro* mechanistic**	Refer to Table 2.10.1-1	-
Avian reproduction	Avian reproduction test in bobwhite quail (OECD 206)	56
	Avian reproduction test in bobwhite quail (OECD 206)	57
	Avian reproduction test in mallard duck (OECD 206)	58
Long-term and chronic toxicity to fish	Fish early life stage toxicity in fathead minnow (OECD 210)	59
Endocrine disruption	Fish short-term reproduction assay (OECD 229)	60
	Amphibian metamorphosis assay (OECD 231)	61

Avian data

The avian reproduction toxicity test (level 4 of the OECD CF), provide only apical endpoints that might be endocrine-sensitive but which cannot be considered specific for the identification of an endocrine MoA.

Three studies are available in the dossier investigating the effects of dodine on avian reproduction, two with bobwhite quail (one of them accepted as supporting information) and other with mallard duck.

KCA 8.1.1.3./03; [redacted] 1999. Bobwhite quail (OECD 206): The reproductive toxicity of Dodine to bobwhite quail (*Colinus virginianus*), fed ad libitum in the diet for a period of 21 weeks, was investigated. Effects of nominal test concentrations of 200, 600 and 1000 mg Dodine/kg diet, respectively, over 1 generation were compared to a control group. No treatment related effects were observed on body weight or food consumption of adults. Statistically significant differences in survival rate for offspring were noted on day 14 in the high dose group, therefore, a NOEC of 600 mg a.s./kg of feed was set. However, high mortality in the control (16%, 25% in females) identified a concern regarding the reliability of this study. Consequently this study was accepted as supporting information.

KCA 8.1.1.3./03; [redacted] 1999. Bobwhite quail (OECD 206): The reproductive toxicity of Dodine to bobwhite quail (*Colinus virginianus*), fed ad libitum in the diet for a period of 21 weeks, was investigated. Effects of nominal test concentrations of 75, 150 and 300 mg Dodine/kg diet, respectively, over 1 generation were compared to a control group. No signs of toxicity were observed at any of the concentrations tested. There w0ere no apparent treatment-related effects in adults body weight, feed consumpt ion and reproductive performance. Therefore, NOEC was 300 mg a.s./kg feed, the highest concentration tested (equivalent to 27 mg a.s./kg bw/d, based parental toxicity and reproduction).

KCA 8.1.1.3./05; [redacted] 1994b. Mallard duck (OECD 206): The reproductive toxicity of Dodine to mallard duck (*Anas platyrhynchos*), fed ad libitum in the diet for a period of 20 weeks, was investigated. Nominal test

concentration were : 0 (control), 200, 600 and 1000 mg a.s./kg diet. Statistically significant differences on body weigh at 1000 mg a.s./kg feed and on food consumption at 600 and 1000 mg a.s./kg feed were observed in adults. Furthermore, significant differences on several reproductive effects and on the F1 generation in the mallard duck were reported (eggs laid per hen per day, %viable embryos, % live 21-day embryos of viable embryos, %14-day old survivos, dosy weight of 1-day old survivors, mean thinckness). Therefore, a NOEC of 200 mg a.s./kg feed was set (equivalent to 17.0 mg a.s./kg body weight/day, based parental toxicity and reproduction).

Fish data

A 34 d fish early life stage test (OECD TG 210, CF level 4) with fathead minnow (*Pimephales promelas*) and a 21-day Fish Short Term Reproduction Assay (OECD TG 229, CF level 3) with the same species were available.

KCA 8.2.2.1/01, [redacted] 1995. Fish early life stage toxicity test with fathead minnow (*Pimephales promelas*) (OECD 210): A 35-day early life-stage limit test under flow-through conditions was conducted on fathead minnow (*Pimephales promelas*). Embryos were exposed to 22, 44, 87, 170 and 350 µg Dodine/L, a solvent control containing methanol (0.01mL/L) and a dilution water control. At the end of hatching period (day 5), no effects on hatching of embryos were observed. Following 30-days post-hatch exposure (day 35), larval survival was reduced at the highest concentration (40% respect to a 91% reported in controls). Total length and weight of larval at 170 µg a.s./L was significantly reduced. No effects were observed at the remaining treatments. The lowest NOEC for chronic toxicity of Dodine technical to fathead minnow was 170 µg/L based on effects on larval survival, length and wet weight.

KCA 8.2.3/01; [redacted] 2021. Fish Short Term Reproduction Assay with fathead minnow (*Pimephales promelas*) (OECD 229): The study was performed under flow-through conditions to evaluate potential endocrine activity of dodine. Sexually mature and reproducing fish were exposed to nominal concentrations of 4.0, 20 and 100 µg/L (mean measured concentrations: 3.4, 18 and 85 µg/L). No significant differences on body weight, body length and fecundity were found. Secondary sexual characteristics in male fish did not indicate endocrine activity (no differences in number of tubercules per male fish were observed). A significant increase of plasma VTG levels compared to the pooled controls was found in males exposed to the highest concentration (85 µg/L), while in females, a decreased was reported. No correlation of the effects on VTG levels to other endpoints has been found. Histopathological results could not be assessed by RMS (images not available, **DATA GAP**). **Therefore, a conclusion on the ED properties of the test item could not be reached.** In addition, RMS has **concerns** about the results relevance, as the selected doses did not cover the MTC (as recommended in the ED guidance and OECD 229) and could be too low to elicit any possible ED mediated effect. The results of the study should be used in the risk assessment with caution, as it only showed the ED effects up to the highest concentration tested.

Amphibian data

An amphibian metamorphosis assay (AMA) according to OECD TG 231 is available.

KCA 8.2.3/02;[redacted] 2022. Amphibian metamorphosis assay (AMA) (OECD 231): Lethal and sublethal effects as well as effects on the normal function of the hypothalamic-pituitary-thyroid (HPT) axis on tadpoles of *Xenopus laevis*, caused by the test item Dodine technical, were investigated. The tadpoles were exposed in a flow-through test during a period of 21 days to the nominal concentrations of 2.0, 10.0 and 50.0 µg/L (mean measured: 2.4, 5.8 and 29 µg/L), and to a control group consisting of aqueous test media and a solvent control group, containing the solvent dimethyl sulfoxide (DMSO). The study fulfilled the performance criteria reported in the OECD 231 guideline, except with the related to the variability of measured test concentrations over time, as CV was slightly above the limit. Considering the observed behavior of the test item in test medium, the outcome was considered acceptable. No significant differences on the development stage were observed. A reduction of wet weight (22%) and of the SVL (11%) were observed at the middle test concentration (5.8 µg/L) at day 7, and a slight increase of both parameters at the lowest concentration at the end of the assay were found (2.4 µg/L). These effects on wet weight and SVL were considered no dose-related. The normalized Hind Limb Length (by SVL) of larvae was significantly reduced at day 7 at the lowest concentration, and in all treatments at the end of the study for larvae at NF $\leq$ 60 (reduction 10-14%), but not for tadpoles above stage NF 60. However, as no acceleration of HLL development was found, the effects observed on this paramenter were not considered thyroid-related. Normal morphological development of tadpoles was reported, then, no asynchronous development was identified. Histopathological results could not be assessed by RMS (images not available, **DATA GAP**). **Therefore, a conclusion on ED properties according the decision logic scheme reported in OECD 231 could not be reached.** In addition, RMS has **concerns** about the results relevance, as the selected doses did not cover the MTC (as recommended in the ED guidance and OECD 231) and could be too low to elicit any possible ED mediated effect. The results of the study should be used in the risk assessment with caution, as it only showed the ED effects up to the highest concentration tested.

2.10.2.1 ED assessment for T-modality

A summary of all studies considered for non-target organisms other than mammals, including the Study ID Matrix is outlined in Table 2.10.3.1-1.

Table 2.10.3.1-1: Have T-mediated parameters been sufficiently investigated?

	Sufficiently investigated
T-mediated adversity	No, based on lack of the following study: LAGDA, OECD TG 241 The following studies are available which include endpoints sensitive to, but not diagnostic of, EATS modalities: - Avian reproduction test, OECD TG 206, study IDs: 56 (**KCA 8.1.1.3./02; [redacted] 1994a)** - Avian reproduction test, OECD TG 206, study IDs: 57 (**KCA 8.1.1.3./03; [redacted] 1999)** - Avian reproduction test, OECD TG 206, study IDs: 58 (**KCA 8.1.1.3./05; [redacted] 1994b)** - Fish early life stage assay, OECD TG 210, study ID: 59 (**KCA 8.2.2.1/01, [redacted] 1995**) - Fish short term reproduction assay, OECD TG 229, study ID: 60 (**KCA 8.2.3/01; [redacted] 2021**) - Amphibian Metamorphosis Assay, OECD TG 231, study ID: 61 (**KCA 8.2.3/02; [redacted] 2022**)
T-mediated endocrine activity	The following study is available which includes endpoints related to T-mediated endocrine activity: - Amphibian Metamorphosis Assay, OECD TG 231, study ID: 61 (**KCA 8.2.3/02; [redacted] 2022**)

2.10.2.1.1 Lines of evidence for adverse effects and endocrine activity related to T-modality

The tables below presented the lines of evidence based on applicant's proposal updated by RMS to reflect RMS's conclusion for each study.

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
61	EATS-mediated	Developmental stage	*Xenopus laevis*	21	days	Uptake from water	> 29	µg ai/L	No effect	No statistically significant differences were found in median sage (NF stage) at day 7 and day 21 up to the highest concentration tested.	No effect	The delayed development cannot be conclusively identified as generalized toxicity or antagonistic thyroid activity, since there were no significant effects on other indicators of developmental delays or relevant histopathological changes in the thyroid gland. **RMS**: a reliable conclusion could not be reached, as histopahtology could not be assessed by RMS (images not available, **DATA GAP**). Additionally, RMS has **concerns** about representativeness of doses tested.	T
61		Hind limb length	*Xenopus laevis*	21	days	Uptake from water	2.4	µg ai/L	Decrease	A significant decrease of normalised HLL (by SVL) was observed at day 7 in larvae exposed to 2.4 µg ai/L and at day 21 in all treatments in larvae NF<60.	Delayed development		
61		Thyroid histopathology (amphibian)	*Xenopus laevis*	21	days	Uptake from water	> 29	µg ai/L	No effect	No significant differences observed up to the highest concentration tested. Mild hypertrophy of follicular cells (grade 1) found in all groups, including controls. **RMS**: results were not assessed by RMS, images not available (**DATA GAP**).	Effect could not be discarted by RMS (images not available)		
56	Sensitive to, but not diagnostic of, EATS	Body weight (bird)	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral		ppm	No effect	No significant differences on body weight of adults were found up to 1000 mg a.s./kg feed. **RMS**: Results should be considered with caution due to the high mortality observed in controls.	A decrease of body weight of birds was reported in one of three studies performed. This decrease was observed in mallard duck, in adults at 1000 mg a.s./kg feed and in 1-day-old survivors from 600 mg a.s./kg. No effects in bobwhite quail were found.	**RMS:** Evidence of adverse effects on parameters sensitive to, but not diagnostic of, EATS-mediated parameters could not be discarted (effects on body weight of birds and fish, and length of fish reported)	N

56		Body weight (bird)	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral		ppm	No effect	No significant differences on body weight 1+14 d old survivorsup to 1000 mg a.s./kg feed . **RMS**: Results should be taken into account with coution due to the high mortality observed in controls.			
57		Body weight (bird)	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral		ppm	No effect	No significant differences on body weight of adults were found up to 300 mg a.s./kg feed .			
57		Body weight (bird)	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral		ppm	No effect	No significant differences on body weight 1+14 d old survivors up to 300 mg a.s./kg feed			
58		Body weight (bird)	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	1000	ppm	Decrease	A significant decrease of body weight of adults was found up to 1000 mg a.s./kg feed			
58		Body weight (bird)	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	600	ppm	Decrease	A significant decrease of body weight of 1-day-old survivors was found at 600 and 1000 mg a.s./kg feed			
59		Body weight (fish)	Fathead minnow *(Pimephales promelas)*	35 (=30 day post hatch)	Days	Uptake from water	170	ug ai/L	Decrease	A significant decrease of wet weight was observed in larvae exposed to 170 µg ai/L.	A decrease of body weight was observed in one of two studies performed with fish (early life-stage exposure). RMS has **concerns** about representativeness of doses tested in FSTRA.		
60		Body weight (fish)	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	Up to 85	µg ai/L	No effect	No significant differences on body weight up to the highest dose tested (85 µg ai/L) were found. **RMS**: RMS has concerns about representativeness of doses tested.			
61		Body weight (amphibian)	*Xenopus laevis*	21	days	Uptake from water	2.4	µg ai/L	Increase	A significant decrease of wet weight was observed at day 7 in larvae exposed to 5.8 µg ai/L at day 7. While at day 21, a significant increase in larvae exposed to 2.4 µg ai/L was found.	Not dose-related effect		
59		Length (fish)	Fathead minnow *(Pimephales promelas)*	35 (=30 day post hatch)	Days	Uptake from water	200	µg ai/L	Decrease	A significant decrease of body length of larvae was found at 170 µg ai/L.	A decrease of body length was observed in one of two studies performed with fish (early life-stage exposure). RMS has **concerns** about		

60		Length (fish)	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	Up to 85	µg ai/L	No effect	No significant differences on body length of fish up to the highest dose tested was found (85 µg ai/L). **RMS**: RMS has concerns about representativeness of doses tested.	representativeness of doses tested in FSTRA.		
61		Snout-vent length/growth	*Xenopus laevis*	21	days	Uptake from water	2.4	µg ai/L	Increase	A significant decrease of SVLwas observed at day 7 in larvae exposed to 5.8 µg ai/L at day 7. While at day 21, a significant increase was found in larvae NF<60 at 2.4 µg ai/L. **RMS**: RMS has concerns about representativeness of doses tested.	Increase of Snout-vent length/growth not dose-related		
60		Morphological abnormalities	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	Up to 85	µg ai/L	No effect	No significant differences were found up to the highest dose tested	No malformations observed in fish up to the highest dose tested		
61		Malformations	*Xenopus laevis*	21	Days	Uptake from water		mg/L water	No effect	No significant differences were found up to the highest dose tested	No malformations observed in amphibians up to the highest dose tested		
56		Behaviour	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested	No effect		
56	Systemic toxicity	Mortality	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral		ppm	No effect	**RMS**: Results should be considered with caution due to the high mortality observed in controls.	No treatment-related mortality in fish, birds and amphibians	No evidence of systemic toxicity. Considered not sufficient to show absence of adversity.	-
57		Mortality	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral		ppm	No effect	No significant differences were found up to the highest dose tested			
58		Mortality	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral		ppm	No effect	No significant differences were found up to the highest dose tested			
60		Survival (fish)	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	Up to 85	µg ai/L	No effect	No significant differences were found up to the highest dose tested			

61		Mortality (amphibian)	*Xenopus laevis*	21	days	Uptake from water	Up to 29	µg ai/L	No effect	No significant differences were found up to the highest dose tested			
56		Feed consumption (offspring)	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested	Effects on feed consumption of birds was observed in one of three studies performed. This reduction was found only in adults of mallard duck		
56		Feed consumption (adult)	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
57		Feed consumption (offspring)	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
57		Feed consumption (adult)	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
58		Feed consumption (offspring)	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
58		Feed consumption (adult)	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	600	ppm	Decrease	Decreased food consumption			

2.10.2.1.2 Assessment of the integrated lines of evidence and weight of evidence

For non-target organisms other than mammals, no T-mediated activity and adversity were observed in the Amphibian Metamorphosis Assay up to the highest dose tested (OECD TG 231; study ID 61). Delayed development of larvae (decrease of HLL) was observed but it was not correlated to significants effects on developmental stage, asynchronous develpment or histophatological changes. The alterations in the thyroid glands found in tadpoles after 21-days of exposure were considered not test item-related. However, a reliable conclusion could not be reached, as histopahtology could not be assessed by RMS (images not available, **DATA GAP**). Additionally, RMS has **concerns** about representativeness of doses tested. In the ecotoxicological studies with birds (OECD TG 206; study IDs: 56, 57 and 58) and with fish (OECD TG 210 and 229; study ID: 59 and 60), adverse effects on body weight and length were observed. Therefore, adverse effects on parameters rated as "sensitive to but not diagnostic of EATS" were found, although not assignable to a specific modality. No evidence of systemic toxicity in fish, birds and amphibians were found.

The studies which include information on the T-mediated endocrine activity and adversity are discussed in Table 2.10.3.1.2-1. For T-mediated adversity, only studies which included endpoints sensitive to, but not diagnostic of, EATS modalities are available.

Table 2.10.3.1.2-1: Weight of evidence of T-mediated endocrine activity and adversity for non-mammalian vertebrates

WoE for T-mediated activity: - Amphibians: in the Amphibian Metamorphosis Assay (OECD TG 231; study ID 61), no indications for advanced or asynchronous development or relevant histopathological changes induced by Dodine in the thyroid glands. The effects indicating delayed development (i.e. reduction of normalized HLL only in the NF < 60 tadpoles) cannot be conclusively identified as generalized toxicity or antagonistic thyroid activity. It should be noted that HLL alone is ranked 3 for potential antagonist thyroid effects according to Borgert et al. (2014) [13], due to systemic toxicity and non-hormonal activity. Dodine could be considered apparent thyroid inactive, although results should be considered with caution, histopathological images were not checked by RMS and there were concerns regarding the doses tested (it was performed below the MTC of the test item).
WoE for T-related parameters "sensitive to but not diagnostic of EATS": - Birds: in the Avian reproduction test (OECD TG 206; study IDs: 56, 57 and 58), only parameters not assignable to a specific modality were evaluated. Relevant effects on body weight of adults and 1-day old survivors were reported in mallard duck, but not in bobwhite quail. These effects on body weight were dose-related, therefore can be considered biologically relevant and adverse on a (sub)population level, but they cannot assignable to a specific modality. - Fish: in the Fish early life stage assay (OECD TG 210; study ID: 59) adverse effects on body weight and length (decrease) were observed at 170 µg/L, but they cannot assignable to a specific modality. Instead, in the Fish short-term reproduction assay (OECD 229; IDs: 60), no adverse effects on relevant parameters were found at the the highest doses tested. However, it is noted that the last study was performed probably below the MTC of the test item. - Amphibians: in the Amphibian Metamorphosis Assay (OECD TG 231; study ID 61), no adverse effects or not dose-related on sensitive to, but not diagnostic of, EATS-mediated parameters were observed (body weight, Snout-vent length/growth). However, there were concerns regarding the doses tested (it was performed below the MTC of the test item) that could influence on reliability of resulst.
WoE for systemic toxicity: - Birds: in the Avian reproduction tests (OECD TG 206; study IDs: 56, 57 and 58), no evidences of systemic toxicity were reported. Effects on feed consumption of birds was observed in only in adults of mallard duck.

[13] Borgert, C. J., Stuchal, L. D., Mihaich, E. M., Becker, R. A., Bentley, K. S., Brausch, J. M., Coady, K., Geter, D. R., Gordon, E., Guiney, P. D., Hess, F., Holmes, C. M., LeBaron, M. J., Levine, S., Marty, S., Mukhi, S., Neal, B. H., Ortego, L. S., Saltmiras, D. A., Snajdr, S., Staveley, J., Tobia, A. (2014): Relevance weighing of tier 1 endocrine screening endpoints by rank order. Birth Defects Research Part B. Developmental and Reproductive Toxicology.; 101(1): 90 - 113

- Fish: in the Fish Early Life Stage Assay (OECD TG 210; study ID: 59) and in the Fish short-term reproduction assay (OECD 229; IDs: 60), no evidences of systemic toxicity were reported.
- Amphibians: in the Amphibian Metamorphosis Assay (OECD TG 231; study ID 61), no evidences of systemic toxicity were reported.

2.10.2.1.3 Initial analysis of the evidence and identification of the relevant scenario

No T-mediated adversity was observed. In order to consider T-mediated adversity with regard to non-target organisms sufficiently investigated, in principle a LAGDA (OECD TG 256) would be needed. This study was not performed; therefore, adversity is considered not sufficiently investigated. Moreover, adverse effects on sensitive to, but not diagnostic of, EATS-mediated parameters were reported in birds and fish, although not assignable to a specific modality. Regarding T-mediated activity, an amphibian metamorphosis assay was performed, in which the T-mediated parameters were apparently negative (development delay observed but not correlated with other apical indicators). According to the ECHA/EFSA guidance, a negative result in T-mediated parameters in the AMA is sufficient to support that T-mediated adversity is unlikely because no T-related endocrine activity has been observed. However, (i) histopathological results were not checked by RMS (DATA GAP), then a reliable conclusion could not be reached; and (ii) RMS highlighted concerns regarding if the highest tested concentration in AMA was enough to to elicit any possible ED mediated effect, as they were not close to the MTC (no mortality observed). Consequently, there are uncertainities to reach a conclusion on the ED properties of the substance with the AMA performed, T-mediated activity was not considered sufficiently investigated.

Overall, this leads to the selection of scenario 2a(iii) “generate missing level 2 and 3 information. Alternatively, generate missing “EATS-mediated” parameters. Depending on the outcome move to corresponding scenario” (see Table 2.10.3.1.3-1).

In order to avoid unnecessary animal testing, outcome of ED assessment in humans should be considered before generate more information.

Table 2.10.3.1.3-1 Selection of relevant scenario

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is not “T-mediated” adversity	-
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	-
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	-
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no T-mediated endocrine activity observed	-
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing “EATS-mediated” parameters. Depending on the outcome move to corresponding scenario	X
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	-

2.10.2.1.4 MoA analysis for T-modality

Not relevant.

2.10.2.1.5 Conclusion on the ED assessment for T-modality

The outcome of the assessment reported above for humans also applies to wild mammals as non-target organisms. A MoA analysis is required, as T-mediated adversity was found based on T-mediated parameters and T-mediated activity was not sufficiently investigated.

For non-target organisms other than mammals, the endocrine disruption potential of dodine through the T-modality could not be drawn since the endocrine activity/endocrine adversity was not sufficiently investigated. There are uncertainities to reach a conclusion on the ED properties of the substance. Further information should be generated, scenario 2a(iii). In order to avoid unnecessary animal testing, outcome of ED assessment in humans should be considered before generate more information.

2.10.2.2 ED assessment for EAS-modality

A summary of all studies considered for non-target organisms other than mammals, including the Study ID Matrix is outlined in Table 2.10.3.2-1.

Table 2.10.3.2-1 Have EAS-mediated parameters been sufficiently investigated?

	Sufficiently investigated?
EAS-mediated adversity	No, based on lack of the following studies: MEOGRT (OECD TG 240) or FLCTT (OPPTS 850.1500). The following studies are considered in ED assessment which include endpoints sensitive to, but not diagnostic of, EATS modalities: - Avian reproduction test, OECD TG 206, study IDs: 56 (**KCA 8.1.1.3./02; [redacted] 1994a)** - Avian reproduction test, OECD TG 206, study IDs: 57 (**KCA 8.1.1.3./03; [redacted] 1999)** - Avian reproduction test, OECD TG 206, study IDs: 58 (**KCA 8.1.1.3./05; [redacted] 1994b)** - Fish early life stage assay, OECD TG 210, study ID: 59 (**KCA 8.2.2.1/01, [redacted] 1995**) - Fish short term reproduction assay, OECD TG 229, study ID: 60 (**KCA 8.2.3/01; [redacted] 2021**) - Amphibian Metamorphosis Assay, OECD TG 231, study ID: 61 (**KCA 8.2.3/02; [redacted] 2022**)
EAS-mediated endocrine activity	The following study is available which includes endpoints related to EAS-mediated endocrine activity: - Fish short term reproduction assay, OECD TG 229, study ID: 61 (**KCA 8.2.3/01; [redacted] 2021**)

2.10.2.2.1 Lines of evidence for adverse effects and endocrine activity related to AES-modality

The tables below presented the lines of evidence based on applicant's proposal updated by RMS to reflect RMS's conclusion for each study.

Table 2.10.3.2-1 Lines of evidence for adverse effects and endocrine activity related to EAS-modalities

Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
60	In vivo mechanistic	Vitellogenin (VTG) in females	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	85	µg ai/L	Decrease	A significant decrease in VTG in females was found at the highest dose tested (85 µg ai/L)	Effects considred unrelated to endocrine disruption	No indication of EAS related activity, since no correlation of effects on VTG to other endpoints found. **RMS: a reliable conclusion could not be reached**, as histopahtology could not be assessed (images not available, DATA GAP). Additionally, RMS has **concerns** about representativeness of doses tested.	EAS
60		Vitellogenin (VTG) in males	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	85	µg ai/L	Increase	A significant increase in VTG in males was found at the highest dose tested (85 µg ai/L)			
60	EATS-mediated	Male 2nd sex characteristics in females	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	> 85	µg ai/L	No effect	No significant differences up to the highest dose tested were found	No effect	**RMS**: Evidences of EAS-mediated adversity could not be discarted. A reliable conclusion could not be reached, as histopahtology could not be assessed by RMS (images not available, DATA GAP). Additionally, RMS has concerns about representativeness of doses tested.	A
60		Male 2nd sex characteristics in males	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	> 85	µg ai/L	No effect	No significant differences up to the highest dose tested were found	No effect		EAS
60		Specific gonad histopathology	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	> 85	µg ai/L	No effect	No significant differences up to the highest dose tested were found. **RMS**: **results were not assessed by RMS,** images not available (**DATA GAP**).	Effect could not be discarted by RMS (images not available)		

60		Specific gonad histopathology	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	> 85	µg ai/L	No effect	No significant differences up to the highest dose tested were found. **RMS**: r**esults were not assessed by RMS**, images not available (**DATA GAP**).	Effect could not be discarted by RMS (images not available)		
56	Sensitive to, but not diagnostic of, EATS	Body weight (bird)	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences on body weight of adults were found up to 1000 mg a.s./kg feed. **RMS**: Results should be considered with caution due to the high mortality observed in controls.	A decrease of body weight of birds was reported in one of three studies performed. This decrease was observed in mallard duck, in adults at 1000 mg a.s./kg feed and in 1-day-old survivors from 600 mg a.s./kg. No effects in bobwhite quail were found.	**RMS**: Adverse effects observed on parameters sensitive to, but not diagnostic of EATS-mediated parameters	N
56		Body weight (bird)	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences on body weight 1+14 d old survivorsup to 1000 mg a.s./kg feed . **RMS**: Results should be considered with coution due to the high mortality observed in controls.			
57		Body weight (bird)	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences on body weight of adults were found up to 300 mg a.s./kg feed .			
57		Body weight (bird)	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences on body weight 1+14 d old survivorsup to 300 mg a.s./kg feed			
58		Body weight (bird)	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	1000	ppm	Decrease	A significant decrease of body weight of adults was found up to 1000 mg a.s./kg feed			
58		Body weight (bird)	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	600	ppm	Decrease	A significant decrease of body weight of 1-day-old survivors was found at 600 and 1000 mg a.s./kg feed			
59		Body weight (fish)	Fathead minnow *(Pimephales promelas)*	35 (=30 day post hatch)	Days	Uptake from water	170	ug ai/L	Decrease	A significant decrease of wet weight was observed in larvae exposed to 170 µg ai/L.	A decrease of body weight was observed in one of two studies performed with fish (early life-stage exposure). RMS has **concerns** about representativeness of doses tested in FSTRA.		
60		Body weight (fish)	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	Up to 85	µg ai/L	No effect	No significant differences on body weight up to the highest dose tested (85 µg ai/L) were found. **RMS**: RMS has concerns about representativeness of doses tested.			

61		Body weight (amphibian)	*Xenopus laevis*	21	days	Uptake from water	2.4	µg ai/L	Increase	A significant decrease of wet weight was observed at day 7 in larvae exposed to 5.8 µg ai/L at day 7. While at day 21, a significant increase in larvae exposed to 2.4 µg ai/L was found. **RMS**: RMS has concerns about representativeness of doses tested.	Not dose-related effect		
56		Cracked eggs	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested	No effect on cracked eggs of birds was observed		
57		Cracked eggs	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
58		Cracked eggs	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
56		Egg production	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested	A decrease of egg production was found in one of three studies performed. This reduction was observed in mallard duck, but not in bobwhite quail.		
57		Egg production	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
58		Egg production	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	600	ppm	Decrease	A significant decrease in egg production was observed at 600 and 1000 mg a.s./kg feed			
56		Egg viability (% viable embryo of egg set)	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested	A decrease of egg viability was found in one of three studies performed. This reduction was observed in mallard duck, but not in bobwhite quail.		
57		Egg viability (% viable embryo of egg set)	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
58		Egg viability (% viable embryo of egg set)	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	600	ppm	Decrease	A significant decrease in viable embryos was observed at 600 and 1000 mg a.s./kg feed			

56		Eggshell thickness	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested	A decrease of eggshell thickness was found in one of three studies performed. This reduction was observed in mallard duck, but not in bobwhite quail.		
57		Eggshell thickness	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
58		Eggshell thickness	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	1000	ppm	Decrease	A significant decrease in eggshell thickness was found at 1000 mg a.s./kg feed			
56		Gross pathology (bird)	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested	No effect on gross pathology of birds was observed		
57		Gross pathology (bird)	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
58		Gross pathology (bird)	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
56		Hatchability	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested	Effects on hatchability observed in one of three studies performed was not dose-related.		
57		Hatchability	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
58		Hatchability	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	600	ppm	Decrease	A significant decrease in hatchability at 600 mg a.s./kg feed was found. Not dose-related			
56		No of 14 day-old survivors	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	1000	ppm	Decrease	A significant decrease in 14-d survivors was observed at 1000 mg a.s./kg feed. **RMS**: Results should be taken into account with caution due to the high mortality observed in controls.	Effects on survival of 14 day-old birds. This effect was observed in two of three studies performed.		
57		No of 14 day-old survivors	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			

58		No of 14 day-old survivors	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	600	ppm	Decrease	A significant decrease in 14-d survivors was observed at 600 and 1000 mg a.s./kg feed			
59		Survival of embryos	Fathead minnow *(Pimephales promelas)*	35 (=30 day post hatch)	Days	Uptake from water		ug ai/L	No effect	No significant differences were found up to the highest dose tested	No effect on survival of fish embryos was observed		
56		Viable embryos	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested. **RMS**: Results should be taken into account with caution due to the high mortality observed in controls.	Decrease of viable embryos observed in one of three studies performed. This reduction was observed in mallard duck, but not in bobwhite quail.		
57		Viable embryos	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
58		Viable embryos	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	600	ppm	Decrease	A significant decrease in viable embryos was observed at 600 and 1000 mg a.s./kg feed			
60		Reproduction (fecundity, fertility)	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	> 85	µg ai/L	No effect	No significant differences were found up to the highest dose tested. **RMS**: RMS has concerns about representativeness of doses tested	No effect on fish reproduction observed		
59		Larval survival and length	Fathead minnow *(Pimephales promelas)*	35 (=30 day post hatch)	Days	Uptake from water	400	ug ai/L	Decrease	A decrease in larval survival was found at 170 µg ai/L.	Effects on larval survival		
59		Length (fish)	Fathead minnow *(Pimephales promelas)*	35 (=30 day post hatch)	Days	Uptake from water	200	ug ai/L	Decrease	A decrease of body length of larvae was found at 170 µg ai/L.	A decrease of body length of fish was observed in one of two studies performed (early life-stage exposure). RMS has **concerns** about representativeness of doses tested in FSTRA.		

60		Length (fish)	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	Up to 85	µg ai/L	No effect	No significant differences on body length of fish up to the highest dose tested was found (85 µg ai/L). **RMS**: RMS has concerns about representativeness of doses tested.			
61		Snout-vent length/growth	*Xenopus laevis*	21	days	Uptake from water	2.4	µg ai/L	Increase	A significant decrease of SVLwas observed at day 7 in larvae exposed to 5.8 µg ai/L at day 7. While at day 21, a significant increase was found in larvae NF<60 at 2.4 µg ai/L. **RMS**: RMS has concerns about representativeness of doses tested.	Increase of Snout-vent length/growth not dose-related		
60		Morphological abnormalities	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	Up to 85	µg ai/L	No effect	No effect	No malformations observed in fish up to the highest dose tested		
61		Malformations	*Xenopus laevis*	21	Days	Uptake from water		mg/L water	No effect	No effect	No malformations observed in amphibians up to the highest dose tested		
56	Systemic toxicity	Mortality	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral		ppm	No effect	**RMS**: Results should be taken into account with coution due to the high mortality observed in controls.	No treatment-related mortality in fish, birds and amphibians	No evidence of systemic toxicity in fish, birds and amphibians	-
57		Mortality	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral		ppm	No effect	No significant differences were found up to the highest dose tested			
58		Mortality	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral		ppm	No effect	No significant differences were found up to the highest dose tested			
60		Survival (fish)	Fathead minnow *(Pimephales promelas)*	21	Days	Uptake from water	Up to 85	µg ai/L	No effect	No significant differences were found up to the highest dose tested			
61		Mortality (amphibian)	*Xenopus laevis*	21	days	Uptake from water	Up to 29	µg ai/L	No effect	No significant differences were found up to the highest dose tested			
56	[Not in list]	Behaviour	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested	No effect		

56		Feed consumption (offspring)	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested	Effects on feed consumption of birds was observed in one of three studies performed. This reduction was found only in adults of mallard duck		
56		Feed consumption (adult)	Bobwhite Quail *(Colinus virginianus)*	24	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
57		Feed consumption (offspring)	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
57		Feed consumption (adult)	Bobwhite Quail *(Colinus virginianus)*	21	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
58		Feed consumption (offspring)	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	-	ppm	No effect	No significant differences were found up to the highest dose tested			
58		Feed consumption (adult)	Mallard Duck (*Anas platyrhynchos*)	20	Weeks	Oral	600	ppm	Decrease	A decrease of food consumption was observed at 600 and 1000 mg a.s./kg feed			

2.10.2.2.2 Assessment of the integrated lines of evidence and weight of evidence

Based on the statistically significant effects of Dodine on the plasma vitellogenin levels in female and male fish at the highest mean measured test concentration of 85 µg/L observed in the fish short-term reproduction assay (OECD TG 229; study ID 60), it cannot be excluded that Dodine technical affects the normal function of the HPG axis in fathead minnow. Taking into account that neither tubercle number and tubercle score, nor fecundity and histopathology indicate any endocrine effect, the effect on the plasma VTG levels is most likely a false positive result. This is supported by the reported high variability of the VTG concentration in males and females, rendering VTG levels alone without any relation to effects on other endpoints indicative of endocrine disruption an unreliable indicator. However, a reliable conclusion could not be reached, as histopahtology could not be assessed by RMS (images not available, **DATA GAP**), and RMS has **concerns** about representativeness of doses tested. In the ecotoxicological studies with birds (OECD TG 206; study IDs: 56, 57 and 58) and with fish (OECD TG 210 and 229; study ID: 59 and 60), several adverse effects on parameters rated as "sensitive to but not diagnostic of EATS" were found, although not assignable to a specific modality. No evidence of systemic toxicity in fish, birds and amphibians were found.

The studies which include information on the EAS-mediated endocrine activity and adversity are discussed in Table 2.10.3.2.2-1.

Table 2.10.3.2.2-1: Weight of evidence of EAS-mediated endocrine activity and adversity for non-mammalian vertebrates

WoE for EAS-mediated activity: - Fish: significant effects on vitellogenin content (VTG) in males and females were observed in the Fish Short-Term Reproduction Assay (OECD TG 229; study ID 60). However, there was no indication of EAS related activity, since no correlation of effects on VTG to other endpoints found (secondary sexual characteristics, histophatolocical assessment of gonads, fecundity), although results should be considered with caution, histopathological images were not checked by RMS and there were concerns regarding the doses tested (it was performed below the MTC of the test item).
WoE for EAS-mediated adversity: - Fish: in the Fish Short-Term Reproduction Assay (OECD TG 229; study ID 60), no relevant effects were observed in the gonadal histopathology, nor were any of secondary sexual characteristics affected. Results should be considered with caution, histopathological images were not checked by RMS and there were concerns regarding the doses tested (it was performed below the MTC of the test item).
WoE for EAS-related parameters "sensitive to but not diagnostic of EATS": - Birds: in the Avian reproduction test (OECD TG 206; study IDs: 56, 57 and 58), only parameters not assignable to a specific modality were evaluated. A decrease of body weight of adults and 1-day old survivors, egg production, egg viability, embryos viability and eggshell thickness were reported in mallard duck, but not in bobwhite quail. Effects on survival of 14 day-old birds was also foun in both species. All these adverse effects were dose-related, therefore can be considered biologically relevant and adverse on a (sub)population level, but they cannot assignable to a specific modality. - Fish: in the Fish Early Life Stage Assay (OECD TG 210; study ID: 59), adverse effects (decrease) on body weight, survival of embryos, larval survival and length were observed at 170 µg/L. All these effects were dose-related, and they can be considered biologically relevant and adverse on a (sub)population level, but they cannot assignable to a specific modality. Instead, in the Fish short-term reproduction assay (OECD 229; IDs: 60), no adverse effects on relevant parameters were found up to 85 µg/L (e.g. body weight, length, fecundity, fertility, length, malformations). However, it is noted that the FSTRA was performed below the MTC of the test item (RMS has **concerns** about representativeness of doses tested). - Amphibians: in the Amphibian Metamorphosis Assay (OECD TG 231; study ID 61), no adverse effects or not dose-related on sensitive to, but not diagnostic of, EATS-mediated parameters were observed (body weight, Snout-vent length/growth). However, there were **concerns** regarding the doses tested (it was performed below the MTC of the test item) that could influence on reliability of results.
WoE for systemic toxicity: - Birds: in the Avian reproduction tests (OECD TG 206; study IDs: 56, 57 and 58), no evidences of systemic toxicity were reported. Effects on feed consumption of birds was observed in only in adults of mallard duck.

- Fish: in the Fish Early Life Stage Assay (OECD TG 210; study ID: 59) and in the Fish short-term reproduction assay (OECD 229; IDs: 60), no evidences of systemic toxicity were reported.
- Amphibians: in the Amphibian Metamorphosis Assay (OECD TG 231; study ID 61), no evidences of systemic toxicity were reported.

2.10.2.2.3 Initial analysis of the evidence and identification of the relevant scenario

In order to consider EAS-mediated adversity with regard to non-target organisms sufficiently investigated, a Medaka extended one-generation test (MEOGRT; OECD TG 240) or Fish life cycle toxicity test (FLCTT; OPPTS 850.1500) would be needed. These studies were not performed, therefore, adversity is considered not sufficiently investigated. The endocrine activity for the EAS-modalities could be considered sufficiently investigated since Fish Short-Term Reproduction Assay (OECD TG 229) was performed, and in which not test substance-related effects on EAS-mediated activity were observed (effects on VTG content but not correlated with other apical endpoints). However, (i) histopathological results were not checked by RMS (DATA GAP), and (ii) RMS highlighted concerns regarding if the highest tested concentration in FSTRA was enough to elicit any possible ED mediated effect, as they were not close to the MTC (no mortality observed). Consequently, there are uncertainities to reach a conclusion on the ED properties of the substance with this test, and EAS-mediated activity was not considered sufficiently investigated. Moreover, several adverse effects on "sensitive to, but not diagnostic of, EATS-mediated parameters" were reported in birds and fish that can be considered biologically relevant and adverse on a (sub)population level, although not assignable to a specific modality.

Overall, this leads to the selection of scenario 2a(iii) "generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario (see Table 2.10.3.1.3-1). In order to avoid unnecessary animal testing, outcome of ED assessment in humans should be considered before generate more information.

Table 2.10.3.1.3-1 Selection of the relevant scenario

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is no "EAS-mediated" effect	-
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	-
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	-
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no EAS mediated effect observed	-
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	X
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	-

2.10.2.2.4 MoA analysis for EAS-modalities

Not relevant.

2.10.2.2.5 Conclusion on the ED assessment for EAS-modality

The outcome of the assessment reported above for humans also applies to wild mammals as non-target organisms.

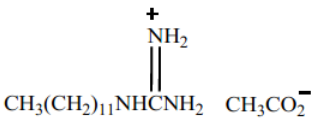
For non-target organisms other than mammals, the endocrine disruption potential of dodine through the EAS-modalities could not be drawn since the endocrine activity/endocrine adverse was not sufficiently investigated. There are uncertainities to reach a conclusion on the ED properties of the substance. Furhter information should be generated scenario 2a(iii).

2.11 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA *[SECTIONS 1-6 OF THE CLH REPORT]*

2.11.1 Identity of the substance [section 1 of the CLH report]

2.11.1.1 Name and other identifiers of the substance

Table 72: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1-dodecylguanidinium acetate
Other names (usual name, trade name, abbreviation)	dodecylguanidine monoacetate
ISO common name (if available and appropriate)	dodine
EC number (if available and appropriate)	219-459-5
EC name (if available and appropriate)	
CAS number (if available)	2439-10-3
Other identity code (if available)	
Molecular formula	$C_{15}H_{33}N_3O_2$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	287.4 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The active substance is not a mixture of isomers. Therefore, consideration of isomeric composition is not relevant.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not a UVCB substance CONFIDENTIAL information - data provided separately (Volume 4)
Degree of purity (%) (if relevant for the entry in Annex VI)	≥ 980 g/kg

2.11.1.2 Composition of the substance

Table 73: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Dodine; 1-dodecylguanidinium acetate CAS No.: 2439-10-3	Minimum 980 g/kg	Acute Tox. 4 * Skin Irrit. 2 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	Acute Tox. 4 * Skin Irrit. 2 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1
The remaining components of dodine are confidential			

Table 74: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
Confidential information – available on volume 4				

Table 75: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
Dodine does not contain additives					

Table 76: Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**

2.11.2 Proposed harmonized classification and labelling

2.11.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 77: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	**Hazard statement Code(s)**	**Pictogram, Signal Word Code(s)**	**Hazard statement Code(s)**	**Suppl. Hazard statement Code(s)**		
Current Annex VI entry	607-076-00-X	dodine (ISO); dodecylguanidinium acetate	219-459-5	2439-10-3	Acute Tox. 4* Eye Irrit. 2 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H319 H315 H400 H410	GHS07 GHS09 Wng	H302 H319 H315 H410			
Dossier submitters proposal	607-076-00-X	dodine (ISO); dodecylguanidinium acetate	219-459-5	2439-10-3	**Retain** Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1 **Add** Acute Tox. 2 STOT RE 2 Carc. 2 **Modify** Acute Tox. 4 Eye Dam. 1	**Retain** H315 H400 H410 **Add** H330 H373 H351 **Modify** H302 H318	**Retain** GHS09 **Add** GHS05 GHS06 GHS08 **Modify** Dng **Remove** GHS07	**Retain** H315 H410 **Add** H330 H373 H351 **Modify** H302 H318		**Add –** oral: ATE = 817 mg/kg bw inhalation: ATE = 0.44 mg/L (dust/mist) M=100 M=1	
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	607-076-00-X	dodine (ISO); dodecylguanidinium acetate	219-459-5	2439-10-3	Acute Tox. 2 Acute Tox. 4 Eye Dam. 1 Skin Irrit. 2 STOT RE 2 Carc. 2 Aquatic Acute 1 Aquatic Chronic 1	H330 H302 H318 H315 H373 H351 H400 H410	GHS05 GHS06 GHS08 GHS09 Dng	H330 H302 H318 H315 H373 H351 H410		oral: ATE = 817 mg/kg bw inhalation: ATE = 0.44 mg/L (dust/mist) M=100 M=1	

2.11.2.2 Additional hazard statements / labelling

No additional hazards statements/labelling.

Table 78: Reason for not proposing harmonised classification and status under CLH public consultation

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Explosives	Data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Hazard class not applicable	No
Flammable solids	Data conclusive but not sufficient for classification	Yes
Self-reactive substances	Data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	Hazard class not applicable	No
Pyrophoric solids	Data conclusive but not sufficient for classification	Yes
Self-heating substances	Data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Hazard class not applicable	No
Oxidising solids	Data conclusive but not sufficient for classification	Yes
Organic peroxides	Data conclusive but not sufficient for classification	Yes
Corrosive to metals	Data conclusive but not sufficient for classification	Yes
Acute toxicity via oral route	**Harmonised classification proposed: Acute Tox. 4 (H302)**	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	**Harmonised classification proposed: Acute Tox. 2 (H330)**	Yes
Skin corrosion/irritation	**Harmonised classification proposed: Skin Irrit. 2 (H315)**	Yes
Serious eye damage/eye irritation	**Harmonised classification proposed: Eye Dam. 1 (H318)**	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	**Harmonised classification proposed: Carc. 2 (H351)**	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	**Harmonised classification proposed: STOT RE 2 (H373)**	Yes

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Aspiration hazard	Data conclusive but not sufficient for classification.	Yes
Hazardous to the aquatic environment	**Aquatic Acute 1** **H400, M = 100** **Aquatic Chronic 1** **H410, M = 1**	Yes
Hazardous to the ozone layer	Data conclusive but not sufficient for classification	Yes

2.11.3 History of the previous classification and labelling

Dodine is a fungicide used as an active substance in plant protection products (PPP). It was included in Annex I to Directive 91/414/EEC (Commission Directive 2011/9/EC) and it has been deemed to be approved under Commission Implementing Regulation (EU) No 540/2011 in accordance with Regulation (EC) No 1107/2009. With Commission Implementing Regulation (EU) No 2020/2007, the expiry date of the approval of Dodine was set to 31.08.2024.

Regarding the renewal of dodine as an active substance in the context of PPP Regulation, a Renewal Assessment Report (RAR) in accordance with Commission Regulation (EC) No. 2020/1740 is being developed by the Spanish CA.

At the time of submission of this CLH report, dodine is currently listed in Annex VI of Regulation (EC) 1272/2008 and it is classified as Aquatic Acute 1, Aquatic Chronic 1 without setting M factors. The actual classification derives from Directive 67/548/EEC.In this CLH report, harmonized classification is maintained and M factors are proposed as M = 100 and M = 1 for acute and chronic hazard, respectively.

2.11.4 Identified uses

Dodine is an active substance used for plant protection products. It is used as a as a fungicide on pome fruits and stone fruits.

The representative uses evaluated comprise foliar spraying against scab in apples and pears, and against leaf curl and leaf spot diseases in peaches and cherries, respectively.

2.11.5 Data sources

This CLH Report is based on the available information provided within the dossier submitted for the renewal process (AIR IV) of dodine as active susbtance as plant protection product under regulation (EC) 1107/2009. Data are exposed and evaluated in the respective volume 3 of the dRAR (2023) performed by Spain.

2.12 RELEVANCE OF METABOLITES IN GROUNDWATER

No major metabolites were identified for the active substance. Hence, information under this point is not required. Consequently, an assessment of the relevance of the metabolites according to the principles set out in the relevant guidance document, SANCO/221/2000, is not required.

2.12.1 STEP 1: Exclusion of degradation products of no concern

No major metabolites were identified for the active substance. Hence, information under this point is not required. Consequently, an assessment of the relevance of the metabolites according to the principles set out in the relevant guidance document, SANCO/221/2000, is not required.

2.12.2 STEP 2: Quantification of potential groundwater contamination

No major metabolites were identified for the active substance. Hence, information under this point is not required. Consequently, an assessment of the relevance of the metabolites according to the principles set out in the relevant guidance document, SANCO/221/2000, is not required.

2.12.3 STEP 3: Hazard assessment – identification of relevant metabolites

2.12.3.1 STEP 3, Stage 1: screening for biological activity

2.12.3.2 STEP 3, Stage 2: screening for genotoxicity

2.12.3.3 STEP 3, Stage 3: screening for toxicity

No major metabolites were identified for the active substance. Hence, information under this point is not required. Consequently, an assessment of the relevance of the metabolites according to the principles set out in the relevant guidance document, SANCO/221/2000, is not required.

2.12.4 STEP 4: Exposure assessment – threshold of concern approach

No major metabolites were identified for the active substance. Hence, information under this point is not required. Consequently, an assessment of the relevance of the metabolites according to the principles set out in the relevant guidance document, SANCO/221/2000, is not required.

2.12.5 STEP 5: Refined risk assessment

No major metabolites were identified for the active substance. Hence, information under this point is not required. Consequently, an assessment of the relevance of the metabolites according to the principles set out in the relevant guidance document, SANCO/221/2000, is not required.

2.12.6 Overall conclusion

No major metabolites were identified for the active substance. Hence, information under this point is not required. Consequently, an assessment of the relevance of the metabolites according to the principles set out in the relevant guidance document, SANCO/221/2000, is not required.

2.13 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

2.13.1 Identity and physical chemical properties

Since the active substance is not an isomeric compound, the isomeric composition has not to be taken into consideration.

2.13.2 Methods of analysis

Since the active substance is not an isomeric compound, the isomeric composition has not to be taken into consideration.

2.13.3 Mammalian toxicity

Since the active substance is not an isomeric compound, the isomeric composition has not to be taken into consideration.

2.13.4 Operator, Worker, Bystander and Resident exposure

Since the active substance is not an isomeric compound, the isomeric composition has not to be taken into consideration.

2.13.5 Residues and Consumer risk assessment

Since the active substance is not an isomeric compound, the isomeric composition has not to be taken into consideration.

2.13.6 Environmental fate

Since the active substance is not an isomeric compound, the isomeric composition has not to be taken into consideration.

2.13.7 Ecotoxicology

Since the active substance is not an isomeric compound, the isomeric composition has not to be taken into consideration.

2.14 RESIDUE DEFINITIONS

2.14.1 Definition of residues for exposure/risk assessment

Food of plant origin: (fruits) RD-RA 1: Dodine
RD-RA 2: Guanidine (tentative)

Food of animal origin: Dodine. Definition set to parent compound by default.

Soil: Dodine

Groundwater: Dodine

Surface water: Dodine

Sediment: Dodine

Air: Dodine

2.14.2 Definition of residues for monitoring

Food of plant origin: Dodine

Food of animal origin: Dodine. Definition set to parent compound by default.

Soil: Dodine

Groundwater: Dodine

Surface water: Dodine

Sediment: Dodine

Air: Dodine

Level 3

DODINE

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4

		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		Dodine is a foliar fungicide with protective and some curative action. According to its mode of action, dodine is a multisite inhibitor acting mainly on the fungus membranes with not systemic but translaminar action. It penetrates partially in the leaves and stops the disease. The representative formulation Dodine 544 SC is intended to be used up to 2 times per season to apples/pear, cherry and peach with minimum application intervals of 21 days. The maximum application rates (per application) range between 0.68 kg a.s./ha (apples/pear and cherry) to 0.9 kg a.s./ha (peach), equivalent to 1.25 L product/ha and 1.65 L product/ha, respectively. *Fate and behaviour in the environment:* • **Groundwater:** - *80th percentile annual average concentration in leachate at 1 m depth (PECgw) for dodine are below the trigger value of 0.1 µg/L in all relevant scenarios. Therefore, it is concluded that an unacceptable risk to groundwater after application of Dodine 544 according to the GAP is not expected.* *Ecotoxicology:* • **Birds and mammals:** - *Exposure of birds to dodine according to the GAP shows no unacceptable risks in **apples/pear and cherry in the Central Zone** based on the outcome of the higher tier risk assessment. However, all refinements (except interception value) were representative for the Central Zone, their extrapolation to other regulatory zones needs further justification/applicability may need further consideration at Member State level.*

				- *Exposure of mammals to dodine according to the GAP shows no unacceptable risks, with the exception of:*
				  o *apple/pear at BBCH<10 (unacceptable risk for vole identified)*
				  o *cherry at postharvest (unacceptable risk for vole and dormouse identified)*
				  o *peach (unacceptable risk for vole identified).*
				Refinements were representative for the Central Zone, their extrapolation to other regulatory zones requires further justification and their applicability may need further consideration at Member State level.
				
				• **Aquatic organisms:**
				- *Exposure of aquatic organisms to dodine according to the GAP shows no unacceptable risks for the intended uses if the following mitigation measures are implemented.*
				  o *When ETO-RAC is considered: 20 m no-spray buffer zone + 90% drift reducing nozzles for late applications on apple/pear, cherry and peach, and summer applications on cherry. Unnaceptable risk was identified for early applications on apple/pear and peach.*
				  o *When ERO-RAC is considered:*
				  ▪ *50 m no-spray buffer zone for early applications on apple/pear and peach.*
				  ▪ *25 m no-spray buffer zone for late applications on apple/pear, cherry and peach and summer applications on cherry.*
				Supporting evidence of efficacy of the mitigation measures requiring a drift reduction above 95% should be provided. The suitability of the use of ERO-RAC in the risk assessment (extrapolation to other regulatory zones) and the applicability of each particular mitigation measure should be assessed at the Member State level.
				
				For the posthaverst use on cherry and peach, unacceptable risk for aquatic organisms was identified.

				• **Bees**: - *Exposure of bees to dodine according to the GAP shows no unacceptable risks on honey bee brood or honey bee colony survival for all the intended uses based on the results of two semi-field studies carried out in the central and in the southern zone. Since some effects on mortality were observed in one trial for the exposure period, the following mitigation measure is proposed:* ▪ *To protect bees and other pollinating insects do not use where bees are actively foraging.* • **Non-target arthropods**: - *Exposure of Non-target arthropods to dodine according to the GAP shows no unacceptable risks for the all the intended uses* • **Non-target soil meso- and macrofauna:** - *Exposure of earthworms and other non-target soil meso- and macrofauna to dodine according to the GAP shows no unacceptable risks for the all the intended uses.* • **Soil nitrogen transformation (microbial processes):** - *Exposure of soil microorganisms to dodine according to the GAP shows no unacceptable risks for the all the intended uses. The results of the risk assessment are considerd provisional.* • **Non-target terrestrial plants:** - *Exposure of non-target terrestrial plants to dodine according to the GAP shows no unacceptable risks for the all the intended uses based on the risk assessment performed with active substance toxicity endpoints.*

3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted	X		
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because:			

	(a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.	X		
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.	X		Three plant metabolism studies investigating the metabolic pathway of dodine on fruits crops (apples, strawberries, pecans) are available and considered valid in compliance to OECD guideline 501. However, qualitative differences are observed between apples/strawberry and pecans. In order to cover the representative uses with long PHIs, the RMS deems advisable to submit a new study in another fruit crop. For the evaluation of the metabolism in livestock, a goat metabolism study is available and considered acceptable. It is expected that the levels of the metabolites identified in edible tissues would be very low at the dietary intake calculated. A complete residue data package is available to support the representative uses of Dodine in pome fruits and peach, in southern and northern zone. However not enough trials are available for cherries according to the intended GAP in both zones (data gap). One hydrolysis study was submitted and considered acceptable and three apple processing studies were performed. The number of trials is considered sufficient to derive robust processing factors for apple juice, wet pomace and canned apples. Processing factor for apple juice may be extrapolated to other pome (pear) or stone fruits (peach).

				One study for determination of residues of dodine in phacelia honey under semi-field conditions was submitted and considered acceptable. Representative uses (apple, cherry, peach) are perennial crops, therefore, data on the metabolism or magnitude of residues in rotational crops are not required
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.			
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		Dodine is intended to be used as a fungicide against scab (*Venturia inaequalis/Venturia pyrina*) on pome fruits (Apple/Pear), leaf spot (*Blumeriella jaapii*) on cherry and leaf curl (*Taphrina deformans*) on peach, nectarine. Dodine is fungitoxic in action preventing disease infection and establishment. Dodine has a translaminar action and penetrates partially in the leaves and stops the disease. It is a multisite inhibitor acting mainly on the fungus membranes. Dodine is currently used as a fungicide on pome fruits (apple/pear/quince/medlar/loquat), on cherry, peach and nectarine, olives, walnut, chestnut and pistachios, almonds, and poplar. Representative uses for this application are pome fruit, cherry, and peach. FRAC evaluates the general risk for development of resistance against Dodine as low to medium. The crop safety of the representative uses has already been evaluated under Uniform Principles for national registration and found acceptable. Therefore, no specific data on phytotoxicity is required. The representative uses have already been evaluated under Uniform Principles for national registration and found acceptable. Therefore, no information regarding observations on other undesirable or unintended side effects is required.
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		Based on the results of soil degradation studies, no metabolites weere found in soil at proportions > 10% AR, 5% AR in two consecutive samples and/or >5% at the end of the study. Thus dodine is the only compound to be further considered in groundwater risk assessment.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of	X		*The minimum purity of the active substance Dodine proposed by the applicant is 980 g/kg* *Purity for the first approval (Commission Directive 2011/9/EU): 950 g/Kg*

	impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.			*It does not contain impurities of toxicological, ecotoxicological and/or environmental concern.*
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	X		*FAO specification (101/TC/S (1988) (AGP:CP/236)): Dodine: min. 950 g/kg*
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted			*Not applicable*
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.		X	*For active substance: HPLC-UV (specificity not demonstrated). Please see Level 2, point 2.5.1.1* *There are not relevant impurities* *For significant impurities: LC-MS/MS and GC-MS/MS. Please see Vol 4 of DRAR (confidential)*
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.		X	***Crops:*** *Hight Water content plants (GC-MSD with LOQ of 0.05 mg/kg), Acidic Crop Matrices (LC/MS/MS with LOQ of 0.01 mg/kg), High Oil content plants (LC/MS/MS with LOQ of 0.01 mg/kg) and Dry Crop Matrices (LC/MS/MS with LOQ of 0.01 mg/kg).* ***Animal origin:*** *LC/MS/MS with LOQ of 0.01 mg/kg.* ***ILV required for honey*** ***Soil:*** *GC/MS with LOQ of 0.01 mg/kg and LC/MS/MS with LOQ of 0.01 mg/kg* ***Surface Water:*** *LC/MS/MS with LOQ of 0.008 µg/L and LC/MS/MS with LOQ of 0.05 µg/L* ***Drinking Water:*** *LC/MS/MS with LOQ of 0.008 µg/L and LC/MS/MS with LOQ of 0.05 µg/L.* ***ILV required for drinking water*** ***Air:*** *LC/MS/MS with LOQ 0.00850 mg/absorber (0.1xC-level)* ***Validation required at LOQ ≤ 0.00173 mg dodine/absorber*** ***Body fluids and tissues:*** *in tissues (liver) dodine is determined by LC/MS/MS with LOQ of 0.01 mg/kg and the metabolite hydroxy-dodecylguanidine with LOQ of 5.0 µg/kg* ***(a confirmatory method is required for the metabolite)*** *; in body fluids (human blood and urine) dodine and the metabolite hydroxy-dodecylguanidine are determined by LC/MS/MS with LOQ of 2 µg/L* ***(a confirmatory method is required for dodine and the metabolite)****.*
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				

Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		Toxicological reference values can be established. After the assessment, the following values are proposed: ADI = 0.02 mg/kg bw/day AOEL = 0.008 mg/kg bw/day ARfD = 0.1 mg/kg bw
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	Dodine has been tested for potential genotoxic properties (gene mutation, clastogenicity and aneugenicity) in a group of in vitro and in vivo assays. In any of them dodine showed evidence of genotoxic potential. Therefore, the evaluation of available information leads to the conclusion that dodine should not be classified as mutagenic.
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**		X	Two long-term chronic and carcinogenicity studies were presented with dodine, one in rats and one in mice. In the chronic toxicity/carcinogenicity study in rats, an increase in the incidence of combined thyroid adenomas and carcinomas was observed. In the chronic toxicity/carcinogenicity study in mice, increased incidences of hepatocellular adenomas were observed, but they were not considered as treatment-related adverse effects. The RMS proposes the classification of dodine as Carc. 2 (H351).
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for		X	Neither adverse effects on sexual function and fertility, nor developmental effects in the absence of maternal toxicity, nor effects on or via lactation, have

	the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**			been identified. Therefore, no classification of dodine is expected as toxic for reproduction.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE identified as having endocrine disrupting properties** in accordance with the provisions of point 3.6.5 in Annex II of Regulation (EC) No 1107/2009			Considering the available data, T-mediated adversity has been found. It corresponds to Scenario 1b: a MoA analysis is required. Based on the available data, EAS-mediated adversity has been considered not sufficiently investigated, whereas EAS-mediated endocrine activity has been deemed sufficiently investigated. Therefore, the scenario 2a(ii) applies: the dodine does not meet the ED criteria for the EAS modalities.
ii)	Linked to above identification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	**1.- Persistence criterion** **Soil system:** The aerobic degradation of Dodine was determined in 5 different soils at 20/25ºC. Dodine degraded rapidly in soil with persistence DT50 values

				at 20ºC ranging from 2.9 to 17 days (not corrected for moisture). DT90 values ranged from 9.7 to 35.7 days. The persistence criterion was not fulfilled in any soil. A data gap has been identified for a new study investigating anaerobic degradation in soil.. Moreover, photolysis does not play a significant role for Dodine degradation in soil. Please, refer to Level 2 pont 2.8.1.2 for further details **Based on all available data, it is concluded that the P-criteria in soil is not fulfilled for dodine.** **Aquatic system:** Dodine is hydrolytically stable in water under without significative pH dependence. It was found to be not readily biodegradable under the conditions of the modified Sturm test. DT50 values for [14C]guanidine-labelled Dodine in natural surface water were calculated to be 2.3 days. Dodine dissipated rapidly (DT50water < 1 day) from the water phase by mineralisation (up to 89 % AR after 84 days), and partitioning to the sediment (up to 27.5 % AR after 1 hour). In the sediment dodine dissipates rapidly, decreasing to < 5 % AR after 5 days. Unextracted sediment residues increased to 58 % AR and 35 % AR after 1 day, and decreased to 33 % AR and 14 % AR in the total system at study end. DT50 in the whole water sediment systems < 1d **Based on all available data, it is concluded that the P-criteria in water is not fulfilled for dodine.** **<u>2.- Bioaccumulation criterion</u>** Although Dodine is stable in water (less than 90% loss of the original substance over 24 h via hydrolysis), it has a log Pow < 3 and therefore no test investigating its bioconcentration potential in fish was required. **Dodine does not meet the criterion of bioaccumulation.** **<u>3.- Toxicity criterion</u>** **Dodine is very toxic to aquatic organisms.** The effects on invertebrates and algae drive the aquatic risk assessment.

				4.- Atmospheric Long-range transport The DT50 in air is estimated using the Atkinson calculation. The total OH rate constant was determined at 1.0876 x 10^{-10} cm^3 molec. sec. Half-life in the troposphere was calculated to be 1.180 hours for overall OH rate constant. Therefore, long-range transport is not relevant (trigger is DT50 >2 days).
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	Please refer to paragraph above
Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	Please refer to paragraph above
Ecotoxicology				
		Yes	No	
i	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		*Please, refer to Level* 2.9 for further details • **Birds and mammals:** • *Exposure of birds to dodine according to the GAP shows no unacceptable risks in* ***apples/pear and cherry in the Central Zone*** *based on the outcome of the higher tier risk assessment. However, all refinements (except interception value) were representative for the Central Zone, their extrapolation to other regulatory zones needs further justification/applicability may need further consideration at Member State level.* • *Exposure of mammals to dodine according to the GAP shows no unacceptable risks, with the exception of:* o *apple/pear at BBCH<10 (unacceptable risk for vole identified)* o *cherry at postharvest (unacceptable risk for vole and dormouse identified)* o *peach (unacceptable risk for vole identified).*

				Refinements were representative for the Central Zone, their extrapolation to other regulatory zones requires further justification and their applicability may need further consideration at Member State level. • **Aquatic organisms:** • *Exposure of aquatic organisms to dodine according to the GAP shows no unacceptable risks for the intended uses if the following mitigation measures are implemented.* o *When ETO-RAC is considered: 20 m no-spray buffer zone + 90% drift reducing nozzles for late applications on apple/pear, cherry and peach, and summer applications on cherry. Unnaceptable risk was identified for early applications on apple/pear and peach.* o *When ERO-RAC is considered:* ▪ *50 m no-spray buffer zone for early applications on apple/pear and peach.* ▪ *25 m no-spray buffer zone for late applications on apple/pear, cherry and peach and summer applications on cherry.* *Supporting evidence of efficacy of the mitigation measures requiring a drift reduction above 95% should be provided. The ERO-RAC is representative for the Central Zone, the suitability of its use in the risk assessment (extrapolation to other regulatory zones) and the applicability of each particular mitigation measure should be assessed at the Member State level.* *For the posthaverst use on cherry and peach, unacceptable risk for aquatic organisms was identified.* • **Bees**: - *Exposure of bees to dodine according to the GAP shows no unacceptable risks on honey bee brood or honey bee colony survival for all the intended uses based on the results of two semi-field studies carried out in the central and in the southern zone. Since some effects on mortality were observed in one trial for the exposure period, the following mitigation measure is proposed:*

				▪ *To protect bees and other pollinating insects do not use where bees are actively foraging.* • **Non-target arthropods***:* - *Exposure of Non-target arthropods to dodine according to the GAP shows no unacceptable risks for the all the intended uses* • ***Non-target soil meso- and macrofauna:*** - *Exposure of earthworms and other non-target soil meso- and macrofauna to dodine according to the GAP shows no unacceptable risks for the all the intended uses.* • ***Soil nitrogen transformation (microbial processes):*** - *Exposure of soil microorganisms to dodine according to the GAP shows no unacceptable risks for the all the intended uses. Results of the RA are considered provisional.* • ***Non-target terrestrial plants:*** - *Exposure of non-target terrestrial plants to dodine according to the GAP shows no unacceptable risks for the all the intended uses based on the risk assessment performed with active substance toxicity endpoints.*
ii	It is considered that, the substance **SHOULD BE identified as having endocrine disrupting properties** that may cause adverse effects on non-target organisms in accordance with the provisions of point 3.8.2 in Annex II of Regulation (EC) No 1107/2009.			**The ED assessment for non target organisms was not finished**. Regarding T- modality for mammals, T-mediated adversity has been found based on T-mediated parameters and T-mediated activity has not been sufficiently investigated. Then, a MoA analysis is required and a conclusion cannot be reached. For non-target organisms other than mammals, further information should be generated for T- and EAS-modalities (scenario 2a(iii)), although outcome of ED assessment in humans should be considered before generate more information in order to avoid unnecessary animal testing.
iii	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.		X	
iv	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant			

	protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.			
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		**Residue definition for exposure/risk assessment** • Food of plant origin: RD-RA 1: Dodine RD-RA 2: Guanidine (tentative) • Food of animal origin: Dodine. Definition set to parent compound by default. • Soil: Dodine • Groundwater: Dodine • Surface water: Dodine • Sediment: Dodine • Air: Dodine **Residue definition for monitoring** • Food of plant origin: Dodine • Food of animal origin: Dodine. Definition set to parent compound by default. • Soil: Dodine • Groundwater: Dodine • Surface water: Dodine • Sediment: Dodine • Air: Dodine
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration			

	of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.			

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution			*[If yes identify the criteria considered met by the substance* *i.e.* *its ADI, ARfD or AOEL is significantly lower than those of the majority of the approved active substances within groups of substances/use categories,* *— it meets two of the criteria to be considered as a PBT substance* *— there are reasons for concern linked to the nature of the critical effects (such as developmental neurotoxic or immunotoxic effects) which, in combination with the use/exposure patterns, amount to situations of use that could still cause concern, for example, high potential of risk to groundwater; even with very restrictive risk management measures (such as extensive personal protective equipment or very large buffer zones),* *— it contains a significant proportion of non-active isomers,* *— it is or is to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as carcinogen category 1A or 1B, if the substance has not been excluded in accordance with the criteria laid down in point 3.6.3,* *— it is or is to be classified, in accordance with the provisions of Regulation (EC) No 1272/2008, as toxic for reproduction category 1A or 1B if the substance has not been excluded in accordance with the criteria laid down in point 3.6.4,* *— if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, reviewed by the Authority, it is considered to have endocrine disrupting properties that may cause adverse effects in humans if the substance has not been excluded in accordance with the criteria laid down in point 3.6.5.]*

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** If the active substance is not a micro-organism, in particular it is considered that: (a) the substance **should NOT be classified or proposed for classification** in accordance to Regulation (EC) No 1272/2008 as any of the following: — carcinogenic category 1A, 1B or 2, — mutagenic category 1A, 1B or 2, — toxic to reproduction category 1A, 1B or 2, — skin sensitiser category 1, — serious damage to eye category 1, — respiratory sensitiser category 1, — acute toxicity category 1, 2 or 3, — specific Target Organ Toxicant, category 1 or 2, — toxic to aquatic life of acute and chronic category 1 on the basis of appropriate standard tests, — explosive, — skin corrosive, category 1A, 1B or 1C; (b) it has **not been identified as priority substance under Directive 2000/60/EC**; (c) it is **not deemed to be an endocrine disruptor** in accordance to Annex II of Regulation (EC) No 1107/2009; (d) it **has no neurotoxic or immunotoxic effects**; (e) it **is not persistent** (half-life in soil is more than 60 days) or its **bio-concentration factor is lower than 100**. (f) it is a **semiochemical** and verifies points (a) to (d).		X	Dodine is proposed to be classified as: - Acute Toxicity, category 2, H330 “Fatal if inhaled”. - Serious damage to eye, category 1 (H318) “Causes serious eye damage”. - Specific Target Organ Toxicant (STOT RE), category 2, (H373) “May cause damage to organs (undetermined) through prolonged or repeated oral exposure”. - Carcinogenic, category 2 (H351) “Suspected of causing cancer”.

	Paragraph (e) doesn't apply to naturally occurring active substances. If the active substance is a micro-organism, in particular it is considered that at strain level the micro-organism has not demonstrated multiple resistance to anti-microbials used in human or veterinary medicine. If the active substance is a baculovirus, in particular it has not demonstrated adverse effects on non-target insects.			

3.1.4 List of studies to be generated, still ongoing or available but not peer reviewed

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
A signed certificate of technical specifications should be provided				
More information on the method of manufacture and the origin of impurities. MSDS of starting materials				
Clarification on the composition of some batches used in tox/ecotox studies (confidential Vol 4)				
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
Physical state and colour of the technical material		X		
Surface tension with purified active substance		X		
3.1.4.3 Data on uses and efficacy				
None				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
None				

3.1.4.5 Methods of analysis				
For the active substance in technical material : Specificity of the HPLC-UV method not demostrated. A confirmatory method required.		X		
For risk assessment : Complete validation for dodine in feeding solutions for honey bee (method in [redacted] 2016)			X	
For monitoring : ILV required for drinking water			X	
For monitoring : A confirmatory method for dodine and the metabolite hydroxy-dodecylguanidine at the LOQ of 0.002 mg/kg in urine and blood A confirmatory method for the metabolite hydroxy-dodecylguanidine at the LOQ of 0.005 mg/kg in liver		X		
For monitoring : An ILV for dodine in honey			X	
For monitoring : A method for dodine in air validated to cover the trigger value C = 0.00173 mg dodine / absorber		X		
3.1.4.6 Toxicology and metabolism				

3.1.4.7 Residue data				
Additional residue trials on cherry (6 NEU / 1 SEU) are required to support the representative use on cherry	Additional residue trials on cherry (6 NEU / 1 SEU) are required to support the representative use on cherry	Additional residue trials on cherry (6 NEU / 1 SEU) are required to support the representative use on cherry		
Additional residue trials could be required to exclude the formation of guanidine in fruits after treatments, in order to conclude on the RD for RA in plants (pending the submission and assessment of further tox data for guanidine).	Additional residue trials could be required to exclude the formation of guanidine in fruits after treatments, in order to conclude on the RD for RA in plants (pending the submission and assessment of further tox data for guanidine).	Additional residue trials could be required to exclude the formation of guanidine in fruits after treatments, in order to conclude on the RD for RA in plants (pending the submission and assessment of further tox data for guanidine).		
3.1.4.8 Environmental fate and behaviour				
Exposure of the active substance to anaerobic conditions cannot be excluded for the intended uses on cherry and peach (autumn applications). A new anaerobic degradation study should be submitted.	Cherry and peach (applications after harvest)			
3.1.4.9 Ecotoxicology				
A statistical re-evaluation of field effect studies on mammals, KCP 10.1.2.2/01 and KCP 10.1.2.2/02, including the statistical power of the the study or MDDs should be provided.	All intended uses			
The study and summary of Rinke (1991) with its corresponding KCA number should be provided.	All intended uses			

New residue decline studies in arthropods and ground vegetation covering Southern European conditions should be submitted.	All intended uses			
New risk assessment for birds following the recommendations of the Northern Zone B&M GD version 2.1, December 2021.	All intended uses			
New calculations of the LC_{50} considering geomean of measured concentrations and the statistical reliability of the endpoint (95% CI and normalised CI) should be provided in the Study B.9.2.1/01 [redacted] (1990).	All intended uses			
The statilstical robustness of the calculated LC50 (95% CI and normalised CI) should be provided to assess the reliability of the endpoint obtained in Study B.9.2.1/03 [redacted] (1992a), Study B.9.2.1/04 [redacted] (2005)	All intended uses			
New calculations of EC_{50} based on TWA-mean measured concentrations and the statistical reliability of the endpoint (95% CI and normalised CI) should be provided in the Study B9.2.4.1/01, [redacted] (1989)	All intended uses			
Supporting evidence of efficacy of the mitigation measures requiring a drift reduction above 95% should be provided.	All intended uses			
Eisenia fetida EC10 = 62.4 mg a.s./kg dw is considered provisional pending on the submission of the statistiscal re-evaluation of [redacted] 2007 (KCP 10.4.1.1/01).	All intended uses			
Histological images of Amphibian Metamorphosis Assay (KCA 8.2.3/02; [redacted] 2022) and Fish	All intended uses			

Short Term Reproduction Assay (KCA 8.2.3/01; [redacted] 2021) should be provided.				

3.1.5 Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	Relevance in relation to representative use(s)
1. ED assessment on T-modality	All uses
2. ED assessment for non-target organisms: Regarding T- modality for mammals, T-mediated adversity was found based on T-mediated parameters and T-mediated activity was not sufficiently investigated, then, a MoA analysis is required. Considering the available information of non-target organisms other than mammals, RMS has concluded that neither adversity nor EAST-mediated endocrine activity has been sufficiently investigated. Further information should be generated (scenario 2a(iii)) to reach a conclusion on the ED properties of the substance.	All uses

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable

influence on the environment.

Critical area of concern identified	Relevance in relation to representative use(s)
	[specify if concern relates to all or specific representative use/use scenario/product or to all uses/products]

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)
All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		Apple/Pear	Cherry	Peach
Operator risk	Risk identified			
	Assessment not finalised	X[1]	X[1]	X[1]
Worker risk	Risk identified			
	Assessment not finalised	X[1]	X[1]	X[1]
Bystander risk	Risk identified			
	Assessment not finalised	X[1]	X[1]	X[1]
Consumer risk	Risk identified			
	Assessment not finalised	X[1]	X[1]	X[1]
Risk to wild non target terrestrial vertebrates	Risk identified	X	X	X
	Assessment not finalised			
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	X	X	X
	Assessment not finalised			
Risk to aquatic organisms	Risk identified	X	X	X
	Assessment not finalised			
Groundwater exposure active substance	Legal parametric value breached			
	Assessment not finalised			
Groundwater exposure metabolites	Legal parametric value breached			
	Parametric value of 10µg/L[(a)] breached			
	Assessment not finalised			
Comments/Remarks				

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	Justification
Refined risk assessment of non-target soil macro- and mesofauna	The Tier 1 risk assessment indicated a high risk for *Folsomia candida* after early applications of dodine on peach. To refine the risk assessment, one toxicity test with *Folsomia candida* conducted in a natural soil was available. Currently there is no guidance on what natural soil properties would be

	acceptable. RMS considers that this is a general issue that should be discussed with MMSS and EFSA.

3.1.9 Critical issues on which the Co RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**

3.2 PROPOSED DECISION

It is proposed that:

DODINE can be approved or renewed under Regulation (EC) No 1107/2009

It is considered that the following be specified in Part B of the Commission Implementing Regulation as areas requiring particular attention from Member States when evaluating applications for product authorisation(s):

- *the potential long-term risk to birds and mammals;*
- *the risk to aquatic organisms and ensure that conditions of use impose adequate*
- *risk mitigation measures;*

It is considered that it should be specified that conditions of use shall include risk mitigation measures, where appropriate.

It is proposed that the Member States concerned shall request the submission of confirmatory information:
(a) where new data requirements are established during the evaluation process, or
(b) as a result of new scientific and technical knowledge, or
(c) to increase confidence in the decision.

3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH THE APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

3.3.1 Particular conditions proposed to be taken into account to manage the risks identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
	[specify if measure relates to a specific representative use/use scenario/product or to all uses/products]

3.4 APPENDICES

GUIDANCE DOCUMENTS USED IN THIS ASSESSMENT

General

Section identity, physical chemical and analytical methods

Section physico chemical properties

Section analytical methods

Section Data on application and efficacy

Section Toxicology

- ECHA Guidance on the application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 5.0 July 2017.
- Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, EFSA/ECHA (2018), Adopted on 5 June 2018.
- Guidance on dermal absorption (2017). Buist, H., Craig, P., Dewhurst, I., Hougaard Bennekou, S., Kneuer, C., Machera, K., Pieper, C., Court Marques, D., Guillot, G., Ruffo, F. and Chiusolo, A. EFSA Journal 2017; 15(6):4873. doi: 10.2903/j.efsa.2017.4873.
- Guidance of EFSA. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/20091. EFSA Journal 2011; 9(2): 2092.
- EFSA 2016. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology. EFSA supporting publication 2016:EN-1074. 24 pp.
- EFSA 2020. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in mammalian toxicology. EFSA supporting publication 2020:EN-1837. 26 pp. doi:10.2903/sp.efsa.2020.EN-1837
- Scientific opinion: clarification of some aspects related to genotoxicity assessment. November 2017. EFSA Journal. doi: 10.2903/j.efsa.2017.5113.
- Retrospective analysis of the immunotoxic effects of plant protection products as reported in the Draft Assessment Reports for their peer review at EU level (Dewhurst, I., Koshy, L, Samuel, S. and Shillaker, D., 2015, EFSA supporting publication 2015:EN-782).
- Guidance for immunotoxicity risk assessment for chemicals. IPCS harmonization project document; no. 10. World Health Organization and International Programme on Chemical Safety. (2012).
- Scientific opinion. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579.
- OECD Test Guideline 402: Acute Dermal Toxicity: Fixed Dose Procedure (2017).
- OECD Test Guideline 403: Acute Inhalation Toxicity (2009).
- OECD Test Guideline 404: Acute Dermal Irritation/Corrosion (2015).
- OECD Test Guideline 405: In Vivo Eye Irritation/Serious Eye Damage (2021).
- OECD Test Guideline 406: Skin Sensitisation Guinea Pig Maximisation Test and Buehler Test (2021).
- OECD Test Guideline 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents (2008).

- OECD Test Guideline 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents (2018).
- OECD Test Guideline 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents (1998).
- OECD Test Guideline 410: Repeated Dose Dermal Toxicity: 21/28-day Study (1981).
- OECD Test Guideline 414: Prenatal developmental toxicity study (2018).
- OECD Test Guideline 416: Two-Generation Reproduction Toxicity Study (2001).
- OECD Test Guideline 417: Toxicokinetics (2010).
- OECD Test Guideline 420: Acute Oral Toxicity – Fixed Dose Procedure (2001).
- OECD Test Guideline 423: Acute Oral Toxicity – Acute Toxic Class Method (2001).
- OECD Test Guideline 425: Acute Oral Toxicity – – Up-and-Down-Procedure (UDP) (2008).
- OECD Test Guideline 428: Skin absorption: *in vitro* method (2004).
- OECD Test Guideline 441: Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties (2009).
- OECD Test Guideline 452: Chronic Toxicity Studies (2018).
- OECD Test Guideline 453: Combined chronic toxicity\carcinogenicity studies (2018).
- OECD Test Guideline 455: Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists (2021).
- OECD Test Guideline 456: H295R Steroidogenesis Assay (2022).
- OECD Test Guideline 458: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals (2020).
- OECD Test Guideline 471: Bacterial Reverse Mutation Test (2020).
- OECD Test Guideline 473: *In Vitro* Mammalian Chromosomal Aberration Test (2016).
- OECD Test Guideline 474: Mammalian Erythrocyte Micronucleus Test (2016).
- OECD Test Guideline 476: *In Vitro* Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes (2016).
- OECD Test Guideline 490: *In Vitro* Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene (2016).
- US EPA TG OPPTS 870.7800: Immunotoxicity (1998).
- US EPA TG OPPTS 890.1200: Aromatase Assay (Human Recombinant) (2009)

Section Residue and consumer risk assessment

- EC (European Commission), Appendix A. Metabolism and distribution in plants. 7028/IV/95-rev.3, 1997.
- EC (European Commission), Appendix B. General recommendations for the design, preparation and realization of residue trials. Annex 2. Classification of (minor) crops not listed in the Appendix of Council Directive 90/642/EEC. 7029/VI/95-rev.6, 1997.
- EC (European Commission), Appendix E. Processing studies. 7035/VI/95-rev. 5, 22 July 1997.
- EC (European Commission), Appendix H. Storage stability of residue samples. 7032/VI/95-rev. 5, 22 July 1997.
- EC (European Commission), Appendix I. Calculation of maximum residue level and safety intervals. 7039/VI/95, 1997. As amended by the document: classes to be used for the setting of EU pesticide maximum residue levels (MRLs). SANCO 10634/2010.

- EC (European Commission). Technical guidelines on data requirements for setting maximum residue levels, comparability of residue trials and extrapolation of residue data on products from plant and animal origin. SANTE/2019/12752.
- EFSA (European Food Safety Authority). Guidance on the establishment of the residue definition for dietary risk assessment EFSA Panel on Plant Protection Products and their Residues. EFSA Journal 2016;14(12):4549
- EFSA (European Food Safety Authority). Use of EFSA Pesticide Residue Intake Model (EFSA PRIMo revision 3). EFSA Journal 2018;16(1):5147
- OECD (Organisation for Economic Co-operation and Development), OECD MRL Calculator: User Guide. In: Series on Pesticides No 56. ENV/JM/MONO(2011)2, 01 March 2011.
- OECD (Organisation for Economic Co-operation and Development), Metabolism in Crops. OECD guideline for the testing of chemicals No 501, 8 January 2007.
- OECD (Organisation for Economic Co-operation and Development), Stability of Pesticide Residues in Stored Commodities. OECD guideline for the testing of chemicals No 506, 16 October 2007.
- OECD (Organisation for Economic Co-operation and Development), Nature of the Pesticide Residues in Processed Commodities – High Temperature Hydrolysis. OECD guideline for the testing of chemicals No 507, 16 October 2007.
- OECD (Organisation for Economic Co-operation and Development), Magnitude of the pesticide residues in processed commodities. OECD guideline for the testing of chemicals No 508, 3 October 2008.
- OECD (Organisation for Economic Co-operation and Development), Crop Field Trial. OECD guideline for the testing of chemicals No 509, 7 September 2009.
- Uwe Meier (editor), Growth Stages of mono-and dicotyledonous plants. BBCH Monograph, 2. Edition. Federal Biological Research Centre for Agriculture and Forestry, 2001

Section fate and behavior in environment

- European Commission (2014) - Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU" Report of the FOCUS Ground Water Work Group, EC Document Reference Sanco/13144/2010 version 3, 613 pp.
- EFSA - 2014. EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662, 37 pp., doi:10.2903/j.efsa.2014.3662
- FOCUS (1997) - Soil persistence models and EU Registration - The Final Report of the Soil Modelling Workgroup of FOCUS (Forum for the Co-ordination of Pesticide Fate Models and their Use) – 29 February 1997.
- FOCUS (2001) - FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.1. 221 pp.
- FOCUS (2006) - Guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration. Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005, version 2.0, 434 pp.
- FOCUS (2008) - Pesticides in Air: Considerations for Exposure Assessment. Report of the FOCUS Working Group on Pesticides in Air, EC Document Reference SANCO/10553/2006 Rev 2 June 2008. 327 pp.
- FOCUS (2014a) - Generic guidance for Tier 1 FOCUS groundwater assessments. Version 2.2, May 2014.
- FOCUS (2014b) - Generic Guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration, version 1.1
- FOCUS (2015) - Generic guidance for FOCUS surface water Scenarios, Version: 1.4, Date: May 2015

- SANCO (2003) - Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under Council directive 91/414/EEC. Sanco/221/2000-rev.10-final, 25 February 2003.
- DG SANCO Working Document on "Evidence Needed to Identify POP, PBT and vPvB Properties for Pesticides", 25.09.2012 – rev. 3.
- EFSA (2019) - Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers. EFSA Journal 2019;17(8):5804.

Section ecotoxicology

- EFSA (European Food Safety Authority), 2009. Guidance on the Risk Assessment for Birds and Mammals: EFSA Journal 2009; 7(12):1438
- EFSA (European Food Safety Authority), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters: EFSA Journal 2013;11(7):3290
- Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods ESCORT II (2000)
- EFSA (European Food Safety Authority) Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees) Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC: SANCO/10329/2002
- EFSA (European Food Safety Authority), 2011. Guidance Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009: EFSA Journal 2011;9(2):2092
- ECHA and EFSA (European Chemicals Agency and European Food Safety Authority), 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (EFSA/ECHA, 2018). EFSA Journal, Vol 16, Issue 6, June 2018, e05311 https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5311.
- OECD (2019), Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, OECD Series on Testing and Assessment, OECD Publishing, Paris, https://doi.org/10.1787/0ed2f88e-en.

DOSSIER PC COMMENTS

No comments were received during the public consultation of the dossier.

The PC on the MRL dossier for honey was held between 16/02/2024 and 19/03/2024 and no comments were received

3.5 REFERENCE LIST

Section identity, physical chemical and analytical methods

Section data on application and efficacy

- Portugal. Report prepared in the context of the application for first inclusion of dodine in Annex I of the Council Directive 91/414/EEC (2006).
- Portugal. Additional Report to the DAR - public version - August 2009.
- Final addendum to the Draft Assessment Report (DAR) and Additional Report - public version - April 2010.

Section toxicology

- Portugal. Report prepared in the context of the application for first inclusion of dodine in Annex I of the Council Directive 91/414/EEC (2006).
- Portugal. Additional Report to the DAR - public version - August 2009.
- Final addendum to the Draft Assessment Report (DAR) and Additional Report - public version - April 2010.

Section residue and consumer risk assessment

- Portugal. Report prepared in the context of the application for first inclusion of dodine in Annex I of the Council Directive 91/414/EEC (2006).
- Portugal. Additional Report to the DAR - public version - August 2009.
- Final addendum to the Draft Assessment Report (DAR) and Additional Report - public version - April 2010.
- EFSA (European Food Safety Authority), 2010; Conclusion on the peer review of the pesticide risk assessment of the active substance dodine. EFSA Journal 2010; 8(6):1631. [54 pp.]. doi:10.2903/j.efsa.2010.1631.
- EFSA (European Food Safety Authority), 2013b. Reasoned opinion on the modification of the existing MRLs for dodine in pome fruit, apricots and olives. The EFSA Journal 2013;11(2):3112, 25 pp. doi:10.2903/j.efsa.2013.3112.
- EFSA (European Food Safety Authority), 2015. Reasoned opinion on the review of the existing maximum residue levels (MRLs) for dodine according to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2015;13(1):3946, 48 pp.
- Portugal, 2009. Additional report to the draft assessment report on the active substance dodine prepared by the rapporteur Member State Portugal in the framework of Council Regulation (EC) No 33/2008, August 2009.
- SCFCAH, 2010. Review report for the active substance dodine finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 23 November 2010 in view of the inclusion of dodine in Annex I of Directive 91/414/EEC; SANCO/12248/2010 final, November 2010.

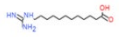
Section fate and behavior in environment

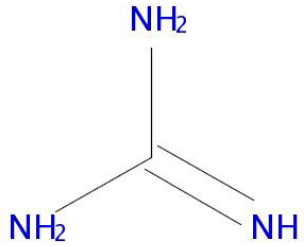
- Portugal. Report prepared in the context of the application for first inclusion of dodine in Annex I of the Council Directive 91/414/EEC (2006).
- Portugal. Additional Report to the DAR - public version - August 2009.
- Final addendum to the Draft Assessment Report (DAR) and Additional Report - public version - April 2010.
- EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer

Section ecotoxicology

- Portugal. Report prepared in the context of the application for first inclusion of dodine in Annex I of the Council Directive 91/414/EEC (2006).
- Portugal. Additional Report to the DAR - public version - August 2009.
- Final addendum to the Draft Assessment Report (DAR) and Additional Report - public version - April 2010.
- EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.
- EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673

3.6 TABLE OF METABOLITES

Name	IUPAC name, SMILES, InChi	Structure	Remarks
Hydroxydodecylguanidine	Dodecylanolguanidine No SMILES notation provided No inchi notation provided	**Figure 1.1.** 	
Dodecylguanidine carboxylic acid	12-carbamimidamidododecanoic acid NC(=N)NCCCCCCCCCCCC(O)=O No inchi notation provided	**Figure 1.2.** 	
Octylguanidine carboxylic acid	8-carbamimidamidooctanoic acid NC(=N)NCCCCCCCC(O)=O No inchi notation provided	**Figure 1.3.**	

			
guanidine	guanidine NC(=N)N InChI=1/CH5N3/c2-1(3)4/h(H5,2,3,4)	**Figure 1.4.** 	
Hexylguanidine carboxylic acid	6-carbamimidamidohexanoic acid NC(=N)NCCCCCC(O)=O No inchi notation provided	**Figure 1.5.**