Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

clothianidin (ISO); (*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine

EC Number: 433-460-1
CAS Number: 210880-92-5

CLH-O-0000007020-91-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

16 September 2021

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Clothianidin (ISO);

(*E*)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine

EC Number: **433-460-1**

CAS Number: **210880-92-5**

Index Number: **613-307-00-5**

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Version number: 2.0 **Date: July 2020**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON CLOTHIANIDIN (ISO); (*E*)-1-(2-CHLORO-1,3-THIAZOL-5-YLMETHYL)-3-METHYL-2-NITROGUANIDINE

CONTENTS

Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	*Clothianidin (ISO);* *(E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine*
EC number:	*433-460-1*
CAS number:	*210880-92-5*
Annex VI Index number:	*613-307-00-5*
Degree of purity:	*≥ 93 % (w/w)*

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Acute Tox. 4*, H302 Aquatic Acute 1, H400 Aquatic Chronic 1, H410, M = 10
Current proposal for consideration by RAC	Acute Tox. 4, H302 (Oral: ATE = 389 mg/kg bw) Repr. 2, H361fd STOT SE 1, H370 Aquatic Acute 1, H400, M = 10 Aquatic Chronic 1, H410, M = 100
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4, H302 (Oral: ATE = 389 mg/kg bw) Repr. 2, H361fd STOT SE 1, H370 (nervous system) Aquatic Acute 1, H400, M = 10 Aquatic Chronic 1, H410, M = 100

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				Data lacking
2.2.	Flammable gases	-			Conclusive but not sufficient for classification
2.3.	Flammable aerosols	-			
2.4.	Oxidising gases	-			
2.5.	Gases under pressure	-			
2.6.	Flammable liquids	-			
2.7.	Flammable solids	None	-	None	
2.8.	Self-reactive substances and mixtures				Data lacking
2.9.	Pyrophoric liquids	-			Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	None	-	None	
2.11.	Self-heating substances and mixtures	None	-	None	
2.12.	Substances and mixtures which in contact with water emit flammable gases	None	-	None	
2.13.	Oxidising liquids	-			
2.14.	Oxidising solids	None	-	None	
2.15.	Organic peroxides	None	-	None	
2.16.	Substance and mixtures corrosive to metals	None		None	
3.1.	Acute toxicity - oral	Acute Tox. 4, H302	Oral: ATE = 389 mg/kg bw	Acute Tox. 4*, H302	
	Acute toxicity - dermal	None	-	None	This endpoint has not been addressed
	Acute toxicity - inhalation	None	-	None	
3.2.	Skin corrosion / irritation	None	-	None	
3.3.	Serious eye damage / eye irritation	None	-	None	
3.4.	Respiratory sensitisation	None	-	None	Data lacking
3.4.	Skin sensitisation	None	-	None	This endpoint has not been addressed
3.5.	Germ cell mutagenicity	None	-	None	
3.6.	Carcinogenicity	None	-	None	
3.7.	Reproductive toxicity	Repr. 2, H361fd	-	None	
3.8.	Specific target organ toxicity –single exposure	STOT SE 1, H370 (nervous system)	-	None	

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.9.	Specific target organ toxicity – repeated exposure	None	-	None	This endpoint has not been addressed
3.10.	Aspiration hazard	None	-	None	Data lacking
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1, H400 Aquatic Chronic 1, H410	M = 10 M = 100	Aquatic Acute 1, H400 Aquatic Chronic 1, H410 M = 10	
5.1.	Hazardous to the ozone layer	Not applicable			

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: **GHS07, GHS08, GHS09**

Signal word: Danger
Hazard statements: H302 Harmful if swallowed
H361fd Suspected of damaging fertility. Suspected of damaging the unborn child
H370 Causes damage to organs (nervous system)
H410 Very toxic to aquatic life with long lasting effects

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

In the CLP-Regulation (EC) No. 1272/2008 Clothianidin was introduced as Acute Tox. 4*, H302, Aquatic Acute 1, H400 and Aquatic Chronic 1, H410 with M-factor = 10.

2.2 Short summary of the scientific justification for the CLH proposal

For Clothianidin a renewal assessment report was prepared in order to evaluate the application and the supplementary dossier submitted by the applicant to allow a decision on the renewal of the first approval as a pesticide active substance. The current harmonised entry for acute toxicity of Clothianidin (Annex VI) has an asterisk (Acute Tox. 4*, H302) indicating that it is the minimum classification. But based on available data, a more stringent classification is not warranted and Clothianidin meets the criteria to be classified for oral acute toxicity as Acute Tox. 4, H302.

Furthermore, during the re-evaluation procedure of Clothianidin it was noted that the following additional or amended classification (referring to the current entry in Annex VI) is needed:

To include a classification for specific organ toxicity STOT SE 1, H370 based on signs of neurotoxicity (impairment of motor activity) in mice (starting at 50 mg/kg bw) and rats (starting at 100 mg/kg bw) after oral administration. The dose levels did not induce mortalities in tested animals. As the relevant effect dose levels were not below 30 mg/kg bw, it is considered not necessary to derive a SCL for STOT-SE.

To include a classification for developmental toxicity Repr. 2, H361fd based on delayed sexual maturation in males and developmental findings (higher incidence of stillbirths, decreased perinatal viability) in the absence of excessive parental toxicity in the multigeneration study in rats.

For the authorisation and classification of plant protection and biocidal products harmonised M-factors are of high importance in order to ensure the correct classification of these products.

Since the CLP Regulation (EC) No. 1272/2008 came into force in 2009, new classification criteria for the assessment of the long-term aquatic hazards have been introduced with Regulation (EC) No. 286/2011. Hence, a review of the current classification based on available chronic data is necessary. Adequate chronic data is available for classification and an update of the current classification (mainly the derivation of the chronic M factor) is necessary. The acute toxicity (EC_{50} = 0.029 mg/L for *Chironomus riparius*) and the long-term toxicity for insects (EC_{10} = 0.0004 mg/L for *Chironomus riparius*) justifies the classification as Aquatic Acute 1, H400 (acute M-factor = 10) and Aquatic Chronic 1, H410 (chronic M-factor = 100).

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

The current legal classification and labelling regarding environmental hazards is Aquatic Acute 1, H400 “Very toxic to aquatic life” and Aquatic Chronic 1, H410 “Very toxic to aquatic life with long lasting effects” with M-factor = 10. The current harmonised classification and labelling regarding human health hazard is Acute Tox. 4*, H302.

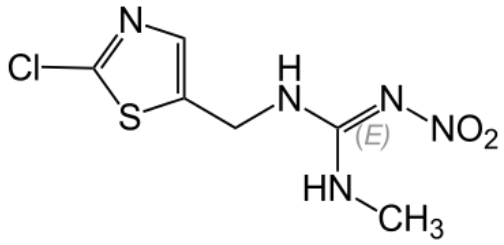
2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

There are 70 notifications within the C&L inventory (date: 14 December 2017) which include the current harmonised classification, without identifying further hazard classes. The group entry of 69 notifiers does not include the harmonised M-factor.

RAC general comment

Clothianidin ((E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine) is an active substance in biocides and plant protection products used as agricultural insecticide. Its chemical structure is shown below:



3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance is an active substance in the meaning of Directive 98/8/EC (repealed by Regulation (EU) No. 528/2012) and in the meaning of Regulation (EC) No. 1107/2009 (replaces Directive 91/414/EEC). Active substances shall normally be subject to harmonised classification and labelling. The existing harmonised classification is updated based on information from the review procedure for active substances.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

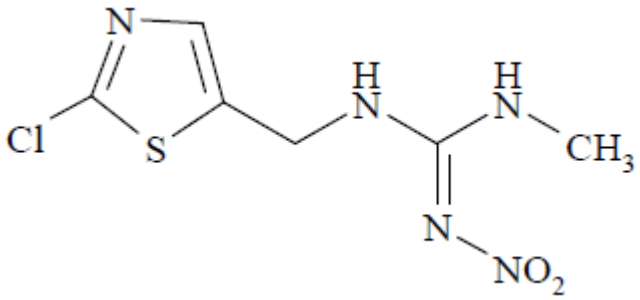
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	433-460-1
EC name:	(*E*)-1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3- methyl-2-nitroguanidine (Clothianidin)
CAS number (EC inventory):	210880-92-5
CAS number:	210880-92-5
CAS name:	Guanidine, N-[(2-chloro-5-thiazolyl)methyl]-N'-methyl-N"-nitro-, [C(E)]-
IUPAC name:	(*E*)-1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
CLP Annex VI Index number:	-
Molecular formula:	C6H8Cl N5O2S
Molecular weight range:	249.7 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
E)-1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3- methyl-2-nitroguanidine (Clothianidin)	97.5 % (w/w)	≥ 95.0 %	

Current Annex VI entry: Index Number 613-307-00-5

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
-			

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
-				

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20 °C and 101,3 kPa	Clear and colourless (Munsell, purity 99.7 %) 5Y 8.3/6 (Munsell, purity 97.6 %) Solid, powder (purity 99.7 % and 97.6 %) Odourless (purity 99.7 % and 97.6 %)	CA report for a.s. Clothianidin	visual assessment
Melting/freezing point	176.8 °C (purity 99.7 %)	CA report for a.s. Clothianidin	Experimental result (OECD 102 (equivalent to EEC A.1), capillary method)
Boiling point	The test substance decomposed before boiling up to 200 °C.	CA report for a.s. Clothianidin	Experimental result (Distilling method, 92/69/EEC)
Relative density	1.61 at 20 °C (purity 99.7 %)	CA report for a.s. Clothianidin	Experimental result (OECD 109 (equivalent to EEC A.3, pycnometer method))
Vapour pressure	1.3×10^{-10} Pa (at 25 °C) (extrapolated) 3.8×10^{-11} Pa (at 20 °C) (extrapolated)	CA report for a.s. Clothianidin	Experimental result (OECD 104 (equivalent to EEC A.4, effusion method))
Surface tension	79.6 mN/m at 20 °C (90 % saturation)	CA report for a.s. Clothianidin	Experimental result (EEC, A.5 and OECD 115, ring method)
Water solubility	pH 4: 0.304 g/l (at 20 °C, buffered solution) pH 10: 0.340 g/l (at 20 °C, buffered solution) pH 10: 0.327 g/l in Milli-Q water (at 20 °C)	CA report for a.s. Clothianidin	Experimental result (OECD 105 (equivalent to EEC A.6, flask method))
Partition coefficient n-octanol/water	pH 4: 0.893 in buffer at 25 °C (shake-flask method) pH 7: 0.905 in buffer at 25 °C (shake-flask method) pH 10: 0.873 in buffer at 25 °C (shake-flask method)	CA report for a.s. Clothianidin	Experimental result (OECD 107 (equivalent to EEC A.8,, flask-shaking method))
Flash point	The study does not need to be conducted because the substance is a solid.	expert judgement	study scientifically not necessary
Flammability	Not highly flammable The substance has no pyrophoric properties and does not liberate flammable gases on contact with water.	Wright, E., (2000) expert judgement	EEC Method A.10
Explosive properties	Non explosive in the sense of the EEC Method A.14 (when heated or not sensitive to	Kramer, H.T., Telleen, K. (2000)	EEC Method A.14 (Koenen steel tube test, drop-weight test and BAM friction

Property	Value	Reference	Comment (e.g. measured or estimated)
	shock and friction) (97,6 % w/w)		test)
Explosives according to the criteria given in section 2.1 of Annex I to Regulation (EC) No 1272/2008	Data lacking		When considering the structure of Clothianidin, there are chemical groups in the molecule associated with explosive properties or self- reactive properties. Recommendation: Thermal stability and exothermic decomposition energy may be estimated using a suitable calorimetric technique such as differential scanning calorimetry (DSC) in a closed crucible. The results from the screening procedure adequately predict when it is not necessary to perform the classification procedures.
Self-reactive substances according to the criteria given in section 2.8 of Annex I to Regulation (EC) No 1272/2008	Data lacking		When considering the structure of Clothianidin, there are chemical groups in the molecule associated with explosive properties or self- reactive properties. Recommendation: Thermal stability and exothermic decomposition energy may be estimated using a suitable calorimetric technique such as differential scanning calorimetry (DSC) in a closed crucible. The results from the screening procedure adequately predict when it is not necessary to perform the classification procedures.
Self-ignition temperature	No self-ignition up to 176.8 °C (97.6 % w/w)	Kramer, H.T., Telleen, K. (2000)	EEC Method A.16
Oxidising properties	No oxidising properties (97,6 % w/w)	Kramer, H.T., Telleen, K. (2000)	EEC Method A.17
Granulometry	-	-	No data available.
Solubility in organic solvents	Heptane: <0.00104 g/l (at 25 °C) Xylene: 0.0128 g/l (at 25 °C) Dichloromethane: 1.32 g/l (at 25 °C) Methanol: 6.26 g/l (at 25 °C) Octanol: 0.938 g/l (at 25 °C) Acetone: 15.2 g/l (at 25 °C) Ethyl acetate: 2.03 g/l (at 25 °C)	CA report for a.s. Clothianidin	Experimental result (OECD 105 (equivalent to EEC A.6, flask method))

Property	Value	Reference	Comment (e.g. measured or estimated)
Dissociation constant	pKa = 11.09 (at 20 °C)		Experimental result (OECD 112 (spectrophoto-metric method))
Viscosity	-	-	Not applicable. The substance is solid.

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

The substance is used as an active substance in the meaning of Directive 98/8/EC (repealed by Regulation (EU) No. 528/2012) and in the meaning of Regulation (EC) No. 1107/2009 (replaces Directive 91/414/EEC). Clothianidin is used as an agricultural insecticide.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

The physical hazards are not considered further in this proposal.

4 HUMAN HEALTH HAZARD ASSESSMENT

This section contains short summaries taken from Vol. 1 (chapter 2.6) of the RAR, which was written for the pesticides procedure. In case more detailed information on the reported effects is needed, it is referred to Volume 3 / chapter B.6 of the RAR. All studies included in this dossier were evaluated and assessed by the dossier submitter.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Results of submitted studies are summarized in Table 10.

Table 9: Summary of absorption, distribution, metabolism and excretion data

Parameter	Conclusion	Reference
Rate and extent of oral absorption	Rapid , over 89-95 % in 24 hours	Anonymous (7), 2000 Anonymous (15), 2000
Distribution	Widely distributed up to 72 hours, slight preference for kidney, liver, urinary bladder	Anonymous (7), 2000 Anonymous (15),2000
Rate and extent of excretion	Rapid, >92 % (low dose) and 58 % (high dose) in urine within 24 hours; < 7 % in faeces; limited enterohepatic circulation	Anonymous (7), 2000 Anonymous (15), 2000
Metabolism	Moderate metabolism (urine, 72 hours, % dose): 56-74 % parent compound, 8-13 % MNG, 7-11 % TZNG, 9 % MTCA, 1-4 % NTG, 1-2 % TMG. + 8 metabolites< 2 %. Oxidative demethylation and C-N cleavage between thiazolyl-nitroimino moiety	Anonymous (7), 2000 Anonymous (8), 2000 Anonymous (15), 2000
Potential for accumulation	No potential for accumulation	Anonymous (7), 2000 Anonymous (8), 2000 Anonymous (15), 2000

Clothianidin was almost completely absorbed via the intestinal tract (90 %) after oral administration of single doses of [nitroimino-^{14}C] or [thiazolyl-2-^{14}C]-labelled compound at 2.5 or 250 mg/kg bw to rats or a single oral dose of 5 mg/kg bw to mice. The rate and extent of absorption was essentially independent of sex, dose or dose rate.

In rats and mice, Clothianidin was widely and homogeneously distributed over various organs (t_{max}=1.5 h), with rapid decrease of residues to levels near or at LOQ at 72 hours. There was no evidence of accumulation, although up to 4 hours post-dosing, higher levels were detected in urinary bladder, kidney and liver reflecting the role of these organs for excretion and metabolism.

At 24 hours after dosing to rats, about 94-96 % of the radioactivity was excreted. The urinary excretion was the dominating route of elimination, accounting for about 89 % (♂) and 95 % (♀) at

termination (72 h). Faecal elimination accounted for about 6 % (♂) and 3 % (♀). The excretion profile after high-dose administration was almost identical to that after low-dose administration, although the plasma concentration exhibited a bi-phasic kinetics, with concentration peaks at 6h and 32 h, suggesting a moderate enterohepatic cycling. The absorption and elimination was similar in mice; 95 % of a single oral dose of 5 mg/kg bw was excreted within 24 hours with excretion in urine accounting for > 88 % of the dose in that time.

Clothianidin was moderately metabolised in the rat, and was excreted unchanged in amounts of 56-74 % at 72 h. The main metabolic pathway was (i) oxidative demethylation, and (ii) cleavage of the nitrogen-carbon bond between the thiazolyl-methyl position and the nitroimino moiety. The main urinary metabolites (the nomenclature of metabolites identified in the rat is given in Table 10.), recovered after low-dose testing were TZNG (7-11 %), MNG (8-13 %) and NTG (1-4 %). In the faeces, MTCA (9 %) and TMG (2 %) were recovered. Other characterized metabolites were present at < 2 % of dose. Unchanged Clothianidin was the major radioactive component in the tissues investigated (> 86 % in all tissues investigated except liver which accounted for 47 %). TZMU, TZNG and MNG were present in all tissues investigated and TMG and MG was present in the liver.

In mice, Clothianidin was metabolised slightly faster than in rats but the route of metabolism was similar. Unchanged parent accounted for approximately 40 % of the dose. The major urinary metabolites were TZNG (~30 %), MNG (9 %), NTG (11 %). The urinary metabolites were also seen in the faeces (0.2-1.3 %), as was TMG (1 %) and 3 unidentified metabolites which accounted for < 1 % of the dose. The proposed metabolic pathway in rats is shown in Figure 1.

Table 10: Nomenclature of identified metabolites of Clothianidin in the rat

Code	Chemical name
TI-435	*(E)*-1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine ; Clothianidin
ACT	5-Aminomethyl-2-chlorothiazole
CTCA	2-Chlorothiazole-5-carboxylic acid
MG	Methylguanidine
MNG	*N*-Methyl-*N'*-nitroguanidine
MTCA	2-Methylthiothiazole-5-carboxylic acid
NTG	Nitroguanidine
THMN	*N*-2-Chlorothiazol-5-ylmethyl-*N*-hydroxy-*N'*-methyl-*N''*-nitroguanidine ; Thiazolhydroxymethylnitroguanidine
TMG	*N*-(2-Chlorothiazol-5-ylmethyl)-*N'*-methylguanidine; Thiazolmethylguanidine
TZG	2-Chlorothiazol-5-ylmethylguanidine; Thiazolguanidine
TZMU	N-(2-Chlorothiazol-5-ylmethyl)-N'-methylurea ; Thiazolylmethylurea
TZNG	N-(2-Chlorothiazol-5-ylmethyl)-N'-nitroguanidine; Thiazolylnitroguanidine
TZU	2-Chlorothiazol-5-ylmethylurea; Thiazolylurea
Urea	Urea

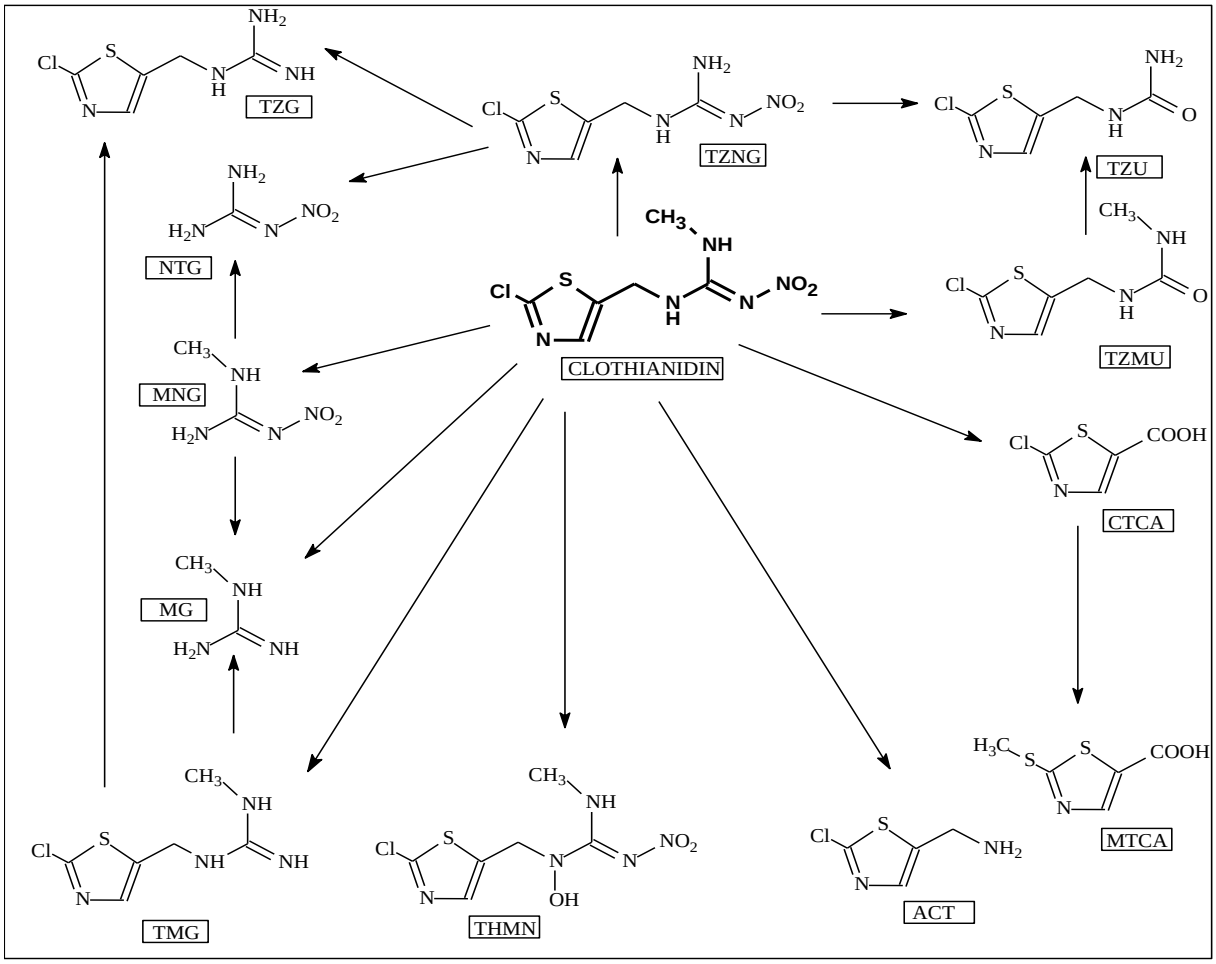


Figure 1: Proposed metabolic pathway in the rat

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

The Acute oral toxicity of Clothianidin was assessed in rats and mice. The results of these studies are summarised in the table below. Further details on materials and methods, guideline (and deviations, if any), doses, number of animals/group and sex, study duration, information on incidences and severities of findings and extent of changes relative to controls are given in the text below or in chapter B.6 of the RAR.

Table 11: Summary table of relevant acute oral toxicity studies

Method	Results	Remarks	Reference
Acute oral toxicity, rat Rat, Crl:CD.BR 5M+5F Dose levels: 1758-2283-2965-3850-5000 mg/kg bw OECD TG 401	LD_{50} >5000 mg/kg bw (m/f)	Purity 96 % w/w	Anonymous (3), 1997a
Acute oral toxicity, rat Rat, F-344/BR, 5M+5F Dose levels: 290-523-1216-2000 mg/kg bw Partly TG OECD 401 Key study	♂: LD_{50} >1216 mg/kg bw ♀: LD_{50} >523 to <1216 mg/kg bw	Purity 96 % w/w	Anonymous (9), 2002 (acute neurotoxicity range-finding)
Acute oral toxicity, mouse Mouse, (Crl:CD-1(ICR)BR 5M+5F Dose levels: 304-380-475-594-742 mg/kg bw OECD 401 Key study	♂: LD_{50} 389 mg/kg bw ♀: LD_{50} 465 mg/kg bw ♂+♀ LD50 425 mg/kg bw	Purity 96 % w/w	Anonymous (4), 1997b

4.2.1.2 Acute toxicity: inhalation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.2.1.3 Acute toxicity: dermal

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.2.1.4 Acute toxicity: other routes

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.2.2 Human information

Not available.

4.2.3 Summary and discussion of acute toxicity

Results on acute oral toxicity are summarised in the table above. Lowest LD_{50} of 389 mg/kg bw was observed in male mice.

4.2.4 Comparison with criteria

The following table presents the critical results for acute oral toxicity used for classification and labelling and further lists the criteria required from CLP regulation

Table 12: Results of acute oral toxicity studies in comparison with CLP criteria

Toxicological result	CLP criteria
Oral LD_{50}, rat: >1216/>523 to <1216 mg/kg (m/f) Oral LD_{50}, mouse: 389/465 mg/kg (m/f)	**Cat 4 (H302): 300 < LD_{50} ≤ 2000 mg/kg (oral)**

4.2.5 Conclusions on classification and labelling

In summary and based on the submitted data, Clothianidin meets the criteria to be classified for acute toxicity (Acute Tox. 4, H302). An ATE of 389 mg/kg bw for the oral route is proposed.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed the classification of clothianidin as Acute Tox. 4, H302 with an ATE = 389 mg/kg bw based on an acute oral toxicity study in mouse performed according to OECD TG 401.

Comments received during consultation

One Member State Competent Authority (MSCA) supported the DS's proposal for classification of clothianidin as Acute Tox. 4, H302.

Assessment and comparison with the classification criteria

The table below summarises all the available studies for assessment of acute toxicity of

clothianidin.

Table: Summary of animal studies on acute oral toxicity with clothianidin.

Study	Dose level	Results	Reference
Crl:CD.BR rats 5 rats/sex OECD TG 401 Gavage	1758-2283-2965-3850-5000 mg/kg bw Purity: 96% w/w Vehicle: 5% w/v aqueous gum Arabic	No mortalities at 1758 and 2283 mg/kg bw 2965 and 3850 mg/kg bw: 1 female mortality, no male mortalities 5000 mg/kg bw: 1 male and 1 female mortalities Clinical signs: palpebral closure, ↓ activity, lethargy, ataxia, hunched posture, vocalisation, tremor, piloerection, hair loss, waisted appearance **LD_{50} > 5000 mg/kg bw**	Anonymous 3, 1997a
F-344/BR rats 5 rats/sex Partly TG OECD 401	290-523-1216-2000 mg/kg bw Purity: 96% w/w	**♂: LD_{50} > 1216 mg/kg bw** **♀: LD_{50} > 523 to < 1216 mg/kg bw**	Anonymous 9, 2002 (acute neurotoxicity range-finding)
(Crl:CD-1(ICR)BR mice 5 mice/sex OECD 401 Gavage	304-380-475-594-742 mg/kg bw Purity: 96% w/w Vehicle: 5% w/v aqueous gum arabic	Male mortalities: 0, 2, 3, 3 and 5 for 304, 380, 475, 594 and 742 mg/kg bw; respectively Female mortalities: 0, 2, 5, 5 and 5 for 304, 380, 475, 594 and 742 mg/kg bw; respectively Clinical signs: ↓ activity, ataxia, tremor, palpebral closure **♂: LD_{50} = 389 mg/kg bw** **♀: LD_{50} = 465 mg/kg bw** **Combined ♂/♀ LD_{50} = 425 mg/kg bw**	Anonymous 4, 1997b

Comparison with the criteria

The lowest LD_{50} (389 mg/kg bw) recorded among three available acute oral toxicity studies was found in the mouse study with males. This LD_{50} falls within the LD_{50} range warranting classification within Cat. 4. The LD_{50} value in female mice is slightly higher but also supports the classification within Cat. 4. Rats seem to be more resistant to clothianidin. One rat species yielded a LD_{50} value higher than 5000 mg/kg bw; while a second rat species showed a LD_{50} for females within the range for classification within Cat. 4. Overall, RAC supports the DS's proposal for **classification of clothianidin as Acute Tox. 4, H302 with an ATE of 390 mg/kg bw.**

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Findings in acute toxicity studies (please refer to chapter 4.2) and acute neurotoxicity studies (please refer to chapter 4.12.1.1) can be used to evaluate the need for a classification for STOT SE. Available studies with single administration were generally conducted in rats and mice according to relevant OECD test guidelines and GLP principles. Non-lethal effects included clinical signs of neurotoxicity. Clothianidin is a known neurotoxic compound; therefore, the observed neuro-toxicological findings are attributed to treatment. Impairments of motor activity were reported in mice (starting at 50 mg/kg bw) and rats (starting at 100 mg/kg bw) upon oral administration. These dose levels did not induce mortality in tested animals. At higher dose levels effects aggravated. No narcotic effects were reported.

Reported findings indicate functional disturbance of the nervous system. They are considered as findings of significant or severe toxicity. Findings were already seen at dose levels below 300 mg/kg bw.

It is noted that no signs of neurotoxicity were reported in two acute toxicity studies with dermal or inhalation administration. However, the most sensitive study protocol regarding the observed neurotoxicological findings after oral administration was the acute neurotoxicity study in rats. As no neurotoxicity studies with administration via skin or inhalation are available; it is considered not conclusively proven that no other routes of exposure apart from oral administration may cause the hazard.

4.3.2 Comparison with criteria

Table 13: Comparison of toxicological data with classification criteria for Categories 1 and 2 of specific target organ toxicity-single exposure (C: guidance value)

Toxicological data	CLP criteria	
Acute neurotoxicity screening study, oral, rats (Anonymous (9), 2002): 100 mg/kg bw: FOB: motor activity↓ (males) 200 mg/kg bw: FOB: motor activity ↓ (males and females), body temperature ↓ (males and females) 400 mg/kg bw: FOB: motor activity ↓ (males and females), body temperature ↓ (males and females), Tremors, gait incoordination, pupils pin-point constriction (males and females), ataxia **Acute pharmacological study, oral, rats** (Anonymous (14), 2000): 300 mg/kg bw: body temperature and blood pressure ↓, heart rate ↑ **Acute pharmacological study, oral, mice** Anonymous (14), 2000): 25 mg/kg bw: synergistic effect on convulsions, intestinal transport ↓ 50 mg/kg bw: motor activity ↓, tremor, deep respiration **Acute oral toxicity study in mice (LD50)** (Anonymous (5), 1997c): 304 mg/kg bw: no mortality, clinical signs: activity ↓, ataxia, tremor, palpebral closure ≥380 mg/kg bw: mortality and clinical signs	Category 1 (H370) Oral (rat): C ≤ 300 mg/kg bw Dermal (rat or rabbit): C ≤ 1000 mg/kg bw Inhalative (rat, dust/mist/fume): ≤ 1 mg/L/4 h	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure - reliable and good quality evidence from human cases or epidemiological studies; or - observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations.
Acute oral toxicity study in rats (LD_{50}) (Anonymous (3), 1997a): 1758/2283 mg/kg bw: no mortality, clinical signs (palpebral closure, activity ↓, lethargy, ataxia, hunched posture, vocalisation, tremor, piloerection, hair loss, waisted appearance) **Acute oral toxicity study in rats (LD_{50})** (Anonymous (9), 2002): 250 mg/kg bw: no mortality and no clinical signs 500 mg/kg bw: mortality and clinical signs **Acute inhalative toxicity study in rats (LC_{50})** (Anonymous (13), 1998): 5.538 mg/m³ (approximately 611-742 mg/kg bw): no mortality; clinical signs: ataxia, ptosis, hunched posture, stained body/eyes/nose, lethargy, body weight ↓	Category 2 (H371) Oral (rat): 2000 ≥ C > 300 mg/kg bw Dermal (rat or rabbit): 2000 ≥ C > 1000 mg/kg bw Inhalative (rat, dust/mist/fume): 5 ≥ C > 1 mg/L/4 h	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure - observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

Toxicological data	CLP criteria	
Acute neurotoxicity screening study, oral, male rats (Anonymous (10), 2000): 20/40/60 mg/kg bw: no effects **Acute dermal toxicity study in rats (LD_{50})** (Garder, 1997): 2000 mg/kg bw: no mortality, no clinical signs	No classification	

4.3.3 Conclusions on classification and labelling

In summary and based on the submitted data, Clothianidin meets the criteria to be classified for specific target organ toxicity - single exposure (STOT SE 1, H370 [nervous system]).
As the relevant effect dose levels were not below 30 mg/kg bw, it is considered not necessary to derive a SCL for STOT SE (CLP Guidance Document, Version 5.0, Chapter 3.8.2.6.).

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

DS proposed the classification of clothianidin as STOT SE 1, H370 (nervous system) based on the impairment of motor activity reported in mice starting at 50 mg/kg bw and in rat starting at 100 mg/kg bw. These effects were noted in absence of mortality.

Comments received during consultation

One Member State Competent Authority (MSCA) supported the DS's proposal for classification of clothianidin as STOT SE 1, H370 (nervous system).

One manufacturer company considered the DS's proposal for classification based on neurotoxic clinical signs not to be appropriate because effects representing "significant toxicity" are not seen at dose levels relevant to STOT SE Category 1. Furthermore, the commenter argued that "significant toxicity" is seen at higher dose levels than those relevant for STOT SE classification in Category 2, and that these dose levels are the same as (or are relatively close to) these causing lethality. Furthermore, according to the manufacturer company, the adoption of this classification for clothianidin would be inconsistent with the other neonicotinoid insecticides previously considered by RAC, with special reference to thiamethoxam. The DS replied that clothianidin is a known neurotoxic compound and the observed neurotoxicological findings are attributable to the treatment. They also replied that the proposal for classification is supported because dose levels causing impairment of motor activity (starting at 50 mg/kg bw in mice and at 100 mg/kg bw in rats) did not induce mortalities.

Assessment and comparison with the classification criteria

In addition to the acute oral toxicity studies summarised in the table above, the DS used two different acute oral neurotoxicity studies in rats and one pharmacological study in rats and mice for assessing the STOT SE hazard.

Acute oral neurotoxicity study in rats (Anonymous 11, 2000)

The study was performed observing U.S. EPA FIFRA, Guideline 81-8(SS) and U.S. EPA. Health Effects Test Guidelines OPPTS 870.6200. Fischer 344 rats (12 animals/sex/experimental group) were treated by gavage with 0, 100, 200 and 400 mg/kg bw of clothianidin (purity 95%) in aqueous 0.5% methylcellulose/0.4% Tween 80.

There were no deaths and no effects on body weight or body weight gain. Results of clinical observations and functional observation battery are shown in the table below. At the top-dose and in both males and females, tremors, decreased activity and ataxia were observed. Decreased activity, pin-point pupils, an uncoordinated righting response and decreased body temperature were observed in a number of animals at 400 mg/kg bw and in a few animals at 200 mg/kg bw. Tremors and uncoordinated gait were also observed in high dose animals. A few males showed decreased activity at 100 mg/kg bw but no other signs were noted at this dose level. There were no treatment related effects on remaining endpoints, including forelimb and hindlimb grip strength and landing foot splay. In general, all findings were restricted to the day of treatment. Most endpoints revealed substantial effects at the top dose in both males and females (tremors, hypoactivity, decreased arousal, miosis following light stimulus, decreased aerial righting response, hypothermia), and in addition, gait incoordination and reduced approach response in the males. Effects on arousal were detected at 100 mg/kg bw and above in the males, and at 200 mg/kg bw and above in the females. Biologically significant dose related reductions in motor activity were observed after dosing on day 0 in all male dose groups and in females at 200 and 400 mg/kg bw. There were no treatment related macroscopic or microscopic findings or effects on body weight and brain weight at termination in clothianidin exposed groups.

Table: Functional observation battery and clinical observations in acute neurotoxicity study of clothianidin in rats. The results are shown as incidence/12 animals. * = statistically significant modifications with ANOVA + Dunnetts's t-test for $p < 0.05$. n.d. = dose level tested but not displayed in the CLH report.

Dose (mg/kg bw)		**0**		**100**		**200**		**400**	
Sex		**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
FUNCTIONAL OBSERVATION BATTERY (incidences per 12 animals)									
Home cage observations:									
Tremors	score 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7*	9*
	score 2							1	2
Decreased activity									
score 1		n.d.	n.d.	n.d.	n.d.	1	0	8*	11*
Open field observations:									
Posture:									
standing normally		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7	7
sitting or lying normally								5	5
Tremors	score 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10*	6*
	score 2							1	5
Gait incoordination		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1	0
Arousal:									
sluggish exploratory									

movements	1	0	3	0	4	2	9*	9*
sluggish minimal movement	0	0	1	0	1	1	0	2
Reflex/Physiologic/Manipulative observations:								
Approach response:								
no reaction	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1	0
Touch response:								
no reaction	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0	2
Pupil response on light:								
pin-point constriction	n.d.	n.d.	n.d.	n.d.	1	0	8*	9*
Aerial righting response:								
slightly uncoordinated								
landing on side	0	1	0	1	2	0	2	3
							0	1
Body temperature (°C)	36.5	36.4	36.1	36.3	34.8*	35.1*	32.8*	32.7*
FIGURE-EIGHT MAZE (percentual differences with concurrent controls)								
Motor activity	n.d.	n.d.	↓23%*	-	↓59%*	↓43%*	↓81%*	↓72%*
Locomotor activity	n.d.	n.d.	↓37%*	-	↓62%*	↓45%*	↓88%*	↓83%*

Overall, neurotoxic effects (reduced locomotor activity) were observed in male rats at lowest dose of 100 mg/kg bw and in females at 200 mg/kg bw (reduced locomotor activity, hypothermia).

Acute oral neurotoxicity study in rats (Anonymous 9, 2002)

This study was performed as supplementary to the above-described study (Anonymous 11, 2000). No OECD or EU guidelines were indicated. Animals were sacrificed on day 2 rather than on day 15 of the study; no body weight measurement, and no gross pathology, neither histopathological examination was performed. Fischer 344 rats (12 males/experimental group) were treated by gavage with 0, 20, 40 and 60 mg/kg bw of clothianidin (purity 95.4%) in aqueous 0.5% methylcellulose/0.4% Tween 80.

There were no deaths during the study. No treatment related clinical signs were observed after dosing in any dose group. No treatment related findings were observed in the Functional Observation Battery assessment approximately 4 hours after dosing. No biologically significant dose related reductions in motor activity were observed after dosing on day 0. Overall, there was no evidence of treatment related neurobehavioral changes or signs of toxicity up to the highest dose level of 60 mg/kg bw.

Pharmacological study in rats and mice (Anonymous 14, 2000)

Male CD-1 (ICR) SPF mice were treated with clothianidin (95.5% purity) dispersed in 0.5% arabic gum solution by single gavage at 0, 6.25, 12.5, 25, 50, 75, 100, 200, 225 and 400 mg/kg bw (mice). Treated animals were observed at 1, 2, 4, 6, 8, 12 h and further daily during 1 week to monitor appearance of acute toxic signs and mortalities.

Reductions in spontaneous locomotor activity, tremors and deep respiration were noted at 50 mg/kg bw (table below). Other doses caused a wide array of alterations including, as regard as neurotoxicity, decrease in reactivity, staggering gait, decrease in touch response, decrease in grip strength, inhibition of pinna reflex, inhibition of ipsilateral flexor reflex and inhibition of corneal reflex (table below).

Table: General physical condition and behaviour after clothianidin administration to male mice. Value in table includes number of animals with finding out of 3 animals, degree of response (+ = slight, ++ = moderate, +++ = severe) and duration of clinical sign. Observation conducted before, and 0.5, 1, 3, 6 h, 1 d after dosing.

	Dose (mg/kg bw)			
	50	**100**	**200**	**400**
Decrease in spontaneous locomotor activity	$3^{+;0.5\ h}$	$3^{+,++;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h,1\ d}$
Tremor	$1^{+;0.5\ h}$	$1^{+;0.5\text{-}1\ h}$	$3^{+,++;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h,1\ d}$
Deep respiration	$1^{+;1\ h}$	$3^{+;\ 0.5\text{-}3\ h}$	$2^{+;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Hypothermia	-	$3^{+;\ 1\text{-}3\ h}$	$3^{+;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Decrease in grooming	-	$3^{+;\ 0.5\ h}$	$3^{++;0.5\text{-}1\ h}$	$3^{++;\ 0.5\text{-}6\ h}$
Mydriasis	-	$2^{+;++\ 0.5\text{-}3\ h}$	$2^{+;\ 0.5\text{-}3\ h}$	$2^{+,++,+++;\ 0.5\text{-}6\ h}$
Decrease in reactivity	-	$1^{+;\ 0.5\text{-}3\ h}$	$3^{+;\ 0.5\text{-}3\ h}$	$3^{++;\ 0.5\text{-}6\ h}$
Prone position	-	$1^{+;\ 0.5\text{-}3\ h}$	$3^{+;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Staggering gait	-	$1^{+;\ 0.5\ h}$	$2^{+;\ 0.5\text{-}3\ h}$	$3^{+,++,+++;\ 0.5\text{-}6\ h,1\ d}$
Decrease in body tone	-	$1^{+;\ 0.5\text{-}3\ h}$	$3^{+;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Decrease in abdominal muscle tone	-	$1^{+;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}3\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Decrease in touch response	-	-	$3^{+;0.5\text{-}1\ h}$	$3^{+;\ 0.5\text{-}6\ h}$
Decrease in grip strength	-	-	$2^{+;\ 0.5\ h}$	$3^{+,++;\ 0.5\text{-}6\ h}$
Decreased limb tone	-	-	-	$3^{+,++;\ 1\text{-}6\ h}$
Inhibition of pinna reflex	-	-	-	$3^{+,++;\ 1\text{-}6\ h}$
Inhibition of ipsilateral flexor reflex	-	-	-	$1^{+++;\ 1\ h}$
Inhibition of corneal reflex	-	-	-	$1^{++;\ 1\ h}$
Straub tail	-	-	-	$1^{+;\ 1\ h}$
Skin cyanosis	-	-	-	$1^{++;\ 1\ h}$
Death	-	-	-	$1^{3\ h}3$

Clothianidin also caused synergistic effects on tonic flexor and extensor convulsions caused by subthreshold electric stimulations. This synergistic effect was statistically significant from 25 mg/kg bw and from 75 mg/kg bw it affected to 100% of treated animals (table below). Clothianidin also caused significant increases in sleeping time starting at 225 mg/kg bw and in intestinal transfer rate starting at 75 mg/kg bw (table below).

Table: Specific pharmacological effects after clothianidin administration to male mice. * = Statistically different form control for $p < 0.05$. TF = Tonic flexor. TE = Tonic extensor. n.a. = dose level not tested.

		Dose (mg/kg bw)					
	n	**0**	**6.25**	**12.5**	**25**	**75**	**225**
Synergistic effects on convulsions							
Electroshock TF							
TE	10	1	3	2	8*	10*	10*
	10	1	3	2	8*	10*	10*
Sleeping time	8		n.a.	n.a.	-	↑26%	↑63%*
Intestinal transfer rate	8		n.a.	n.a.	↓7.5%	↓60%*	↓78%*

According to the Guidance on the Application of the CLP Criteria substances that warrant classification as STOT SE are those that, based on evidence from studies in experimental animals, can be presumed to have the potential to impair organ function and produce significant toxicity in humans following single exposure. The table below summarises the effects found in the single dose toxicity studies causing nervous system dysfunction and that, therefore, meet these requirements for warranting STOT SE classification.

Table: Summary of neurotoxic effects found in the single dose toxicity studies. All effects were noted in absence of mortality except entries marked with asterisks.

Species	Effect	Dose (mg/kg bw)	Incidence	Classification (mg/kg bw)
Rat	Motor and locomotor activity	100	↓23-37% males	STOT SE 1 ≤ 300
Rat	Motor and locomotor activity	200	↓59-62% males ↓43-45% females	STOT SE 1 ≤ 300
Mouse	Decrease in spontaneous locomotor activity	50 100 200	3/3 slight 3/3 severe 3/3 severe	STOT SE 1 ≤ 300
Mouse	Tremor	50 100 200	1/3 slight 1/3 slight 3/3 severe	STOT SE 1 ≤ 300
Mouse	Decrease in reactivity	100 200	1/3 slight 3/6 severe	STOT SE 1 ≤ 300
Mouse	Decrease in grip strength	200	2/3 slight	STOT SE 1 ≤ 300
Rat	Tremor (home cage)	400	7/12 males 9/12 females	STOT SE 2 ≤ 2000
Rat	Decreased activity	400	8/12 males 11/12 females	STOT SE 2 ≤ 2000
Rat	Tremors (open field)	400	10/12 males 6/12 females	STOT SE 2 ≤ 2000
Rat	Sluggish exploratory movements	400	9/12 males 9/12 females	STOT SE 2 ≤ 2000
Rat	Motor and locomotor activity	400	↓81-88% males ↓72-83% females	STOT SE 2 ≤ 2000
Mouse*	Decrease in spontaneous locomotor activity	400	3/3 severe	STOT SE 2 ≤ 2000
Mouse*	Tremor	400	3/3 severe	STOT SE 2 ≤ 2000
Mouse*	Decrease in reactivity	400	3/3 moderate	STOT SE 2 ≤ 2000
Mouse*	Inhibition of pinna reflex	400	3/3 severe	STOT SE 2 ≤ 2000
Mouse*	Decrease in grip strength	400	3/3 severe	STOT SE 2 ≤ 2000

Comparison with the criteria

In addition to the affects reported in the table above, it is also described that mice exposed to clothianidin experienced tonic flexor and tonic extensor convulsions after a subthreshold electrical stimulation. These effects were noted at 25, 75 and 225 mg/kg bw. RAC notes that this effect cannot be considered as a toxic effect but is indicative that the nervous system is altered at concentrations warranting classification as STOT SE 1 as a consequence of clothianidin exposure.

RAC also notes that in the table above, the concentrations supporting STOT SE 1 are always above 30 mg/bw and therefore no SCL is needed.

No narcotic effects or respiratory tract irritation were reported in the single dose toxicity studies and therefore RAC notes that classification as STOT SE 3 is not supported. However, in a weight of evidence assessment, RAC supports the DS's proposal for **classification of clothianidin as STOT SE 1, H370 (nervous system).**

4.4 Irritation

4.4.1 Skin irritation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.4.2 Eye irritation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.4.3 Respiratory tract irritation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.5 Corrosivity

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.6 Sensitisation

4.6.1 Skin sensititsation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.6.2 Respiratory sensitisation

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.7 Repeated dose toxicity

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.8 Specific target organ toxicity (CLP regulation) – repeated exposure (STOT RE)

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.9 Germ cell mutagenicity (Mutagenicity)

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.10 Carcinogenicity

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.11 Toxicity for reproduction

The reproductive toxicity of Clothianidin was assessed in a two-generation toxicity study in rats and in two developmental toxicity studies, one in rats and one in rabbits. The results of these studies are summarised in the table below. Further details on materials and methods, guideline (and deviations, if any), doses, number of animals/group and sex, study duration, information on incidences and severities of findings and extent of changes relative to controls are given in the text below or in chapter 4 that contains chapter B.6 of the RAR.

Table 14: Summary table of relevant reproductive toxicity studies

Type of study Doses / (reference)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Adverse effects	
Pilot Reproductive Toxicity Study with TI-435 in the Sprague-Dawley Rat (Anonymous (1), 2000) 0, 50, 100, 500, 1000 ppm	>1000 ppm (59-69 mg/kg bw/d)	-	Parents, offspring, reproduction: No adverse effects	
2-generation reproduction study in Sprague-Dawley rats, 30M, 30F, route: oral/diet (Anonymous (2), 2000), 0, 150, 500, 2500 ppm (approx. ♂): 0, 10; 31; 163 mg/kg bw/d ♀: 0, 10; 31; 161 mg/kg bw/d[1] OECD TG 416 Key study	500 ppm (31 mg/kg bw/d)	2500 ppm (163 mg/kg bw/d)	Parents	Reduced body weight gain; thymus wt, decreased sperm motility (and not significant morphology effects)
	150 ppm (10 mg/kg bw/d)	500 ppm (31 mg/kg bw/d)	Offspring	Delayed preputial separation without decreased body weight At 2500 ppm additionally, decreased bw (gain), thymus & spleen weights, delayed vaginal opening
	500 ppm (31 mg/kg bw/d)	2500 ppm (163 mg/kg bw/d)	Reproduction	No adverse effects on conception rate and fertility indices. Increased number of stillborn pups, decreased perinatal survival, decreased sperm motility (and not signifcant morphology effects) at 2500 ppm.
Note: Takeda batch no. 30037120 (purity 95.2-96.0 % w/w) was used in both studies				
Type of study Doses / (reference)	**NOAEL (mg/kg bw/day)**	**LOAEL (mg/kg bw/day)**	**Adverse effects**	
Developmental toxicity dose range finding study in	Not established	125	Maternal	Mortality at 1000 mg/kg bw/d, clinical signs, reduced body weight and food consumption, reduced spleen weight

Type of study Doses / (reference)	**NOAEL (mg/kg bw/day)**	**LOAEL (mg/kg bw/day)**	**Adverse effects**	
	Not established	125	Developmental	Only limited parameters tested, reduced litter size, reduced foetal weight and foetal anomalies at maternal toxicity
Developmental toxicity in Sprague-Dawley rats, Crl:CD [BR] VAF/Plus SD, 25F/group Oral, pregnancy day 6-190, 10, 40, 125 mg/kg bw/d (Anonymous (19), 1998), OECD 414 Key study	10	40	Maternal	Decreased body weight and food consumption
	125	-	Developmental	No adverse effects
Developmental toxicity dose range finding study in New Zealand White rabbits (Anonymous (17), 1998), 0, 62.5, 125, 250, 500 mg/kg bw/day	Not established	62.5	Maternal	Mortality, abortions, clinical signs of toxicity, decreased body weight and food consumption
	Not established	-	Developmental	No surviving litters at ≥ 125 mg/kg bw/d

Developmental toxicity in New Zealand White rabbits, Hra:[NZW] SPF 23F Oral, pregnancy day 6-28 (Anonymous (19), 1998), 0, 10, 25, 75, 100 mg/kg bw/d OECD 414 Key study	10	25	Maternal	Clinical signs
	25	75	Developmental	Absence of intermediate lung lobe, decreased ossification sternal centres, increased embryolethality, decreased fetal wt
Note: Takeda batch no. 30034708 (purity 96.0 % w/w) was used in both dose range finding studies. Takeda batch 30037120 (purity 95.2% w/w) was used in both main studies				

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Report: Anonymous (2), 2000

Guideline(s): Yes (OECD 416)

Deviations: No

GLP: Yes

Acceptability: Yes

Results and discussions:

Adult animals:

Clinical signs: Alopecia (hair thinning) was detected in F_1♀ at 500 ppm (3/28) and 2500 ppm (1/28).

Body weight, bw gain, and relative feed consumption (see table below): Significant body weight decreases were observed in both ♂ and ♀ of P and F_1 adults at the top dose. Relative feed consumption (g/kg bw/d) was mostly increased (decreases were only noted during the first treatment week, indicating initial poor palatability).

Organ weight (see table below): In both P- and F_1-animals, absolute and relative organ weight changes were in line with the observed body weight loss.

Table 15: Two generation reproduction toxicity in rats: body and organ weight data

Dose (ppm)	**0**		**150**		**500**		**2500**	
ADULTS	P	F_1	P	F_1	P	F_1	P	F_1
BW ♂	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓8-10%**	↓16-20%**

Dose (ppm)	0		150		500		2500	
BW ♀ (d7-70) pm (d0,6,13,20)ge (d0,4,7,14,21)la (d14) la	n.d.	n.d.	n.d.	n.d.	↓6.5%*	n.d.	↓9-15%** ↓12-14%** ↓11-18%**	↓16-19%** ↓13-16%** ↓13-15%**
BW gain ♀ (d0,6,13,20) ge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓17%**	↓8%
Feed consumption ♂	n.d.	n.d.	n.d.	n.d.	↓4%*wk1 pm	-	↓18% **wk1 pm ↑8% *wk3;**wk4,5,8 pm	↑16%**wk1-10 pm
♀	n.d.	n.d.	n.d.	n.d.	↑7.6%*wk10pm	-	↓18% **wk1 pm ↑16% **wk8-10pm ↑11%* d6-13ge ↑13%* d14-21la	↑21%*wk1,5-6 pm **wk2-4,7-10 pm ↑11%* d6-13 ge ↑13%* d7-21 la
Organ weight (a:absolute, r:relative)								
Thymus ♂ a r	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓16%* ↓8%	↓29%* ↓13%*
♀ a r	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓35%* ↓32%*	↓41%* ↓32%*
Ovary a r	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓19%* ↓16%*	↓11% -
Uterus a r	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓25%* ↓20%*	↓21% ↓10%

n.d.: dose level tested but not displayed in CLH report; pm: premating; ge: gestation; la: lactation
Statistically significant modifications *p<0.05, **p<0.01 (parametric data: Anova+Dunnett's, non-parametric: Kruskall-Wallis+Dunnett's or Mann-Whitney U-test (organ weight)

Gross pathology: Occasional gross pathology findings were reported at the top dose (incidence /30 examined) in (i)P-animals: skull malocclusion and ulcer (1♀), kidney adhesion/raised zone (1♀), prolapsed uterus (1), vaginal mass (1), reduced thymus (2♀); (ii)*F_1-animals:* 1 calculus in kidney (1♂), enlarged testicle (1), testicle with abnormal consistency (1), thymus edema (1♀), lung discoloration (1♀), fluid-filled body (1♀), mammary gland cyst (1♀). All findings in other dose groups were observed at low incidence and without dose-response.

Histopathology: Occasional histopathology findings at the top-dose included in the P-animals: adrenal cortical hyperplasia (1♀), necrosis (1♀), vacuolization (2♀), thymus atrophy (2♀), uterus edema (2), haemorrage (1), chronic active inflammation (1), cervix congestion (1), vagina congestion (1), cyst (1), edema (1), abnormal spermatozoa (1), epididymes mineralization (1) and sperm granuloma (1).
In both P and F_1-animals, no difference in number and duration of oestrus cycles between treated and control animals were observed on basis of the smear analyses (see table below).

Table 16: Two generation reproduction toxicity: gross pathology and histopathology

Dose (ppm)	0		150		500		2500	
ADULTS	P	F_1	P	F_1	P	F_1	P	F_1
Gross pathology								
Preputial separation (day pn)	n.a.	41.2	n.a.	41.9	n.a.	42.5**	n.a.	47.9**
Vaginal opening (day pn)	n.a.	32.4	n.a.	32.2	n.a.	32.1	n.a.	34.7**
Histo/Cytopathologie								

Dose (ppm)	**0**		**150**		**500**		**2500**	
Sperm morphology			n.a.	n.a.	n.a.	n.a.		
% normal	83.5	69.3					81.9	67.0
% abnormal	15.9	30.0					16.0	29.0
% detached	0.6	0.7					2.1	4.0
Total sperm count			n.a.	n.a.	n.a.	n.a.		
epididymis	146.1	149.5					141.6	133.7
testis	129.9	105.7					129.5	103.8
Sperm motility			n.a.	n.a.				
% motile	82.9	81.7			82.6	79.0	79.2	73.4**
% progressively motile	64.1	59.9			61.5	53.9	56.2**	46.1**
Estrus cycle			n.d.	n.d.	n.d.	n.d.		
duration (days)	4.2	4.3					4.1	4.4
number	3.3	3.4					3.7	3.4
Estrus stage (sacrifice) (n)								
diestrus	18	19	19	18	25	16	24	25
proestrus	2	0	0	3	1	0	1	1
estrus	9	11	10	9	3	14	2	4
Ovarial follicle count (mean /ovary)								
'non-antral' follicles	12.17	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	12.22
'antral' follicles	3.94	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	4.53
corpora lutea	3.71	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3.24

n.a.: dose level not tested; n.d.: dose level tested but not displayed in CLH report; Statistically significant modifications *p<0.05, **p<0.01 (parametric data: Anova+Dunnett's, non-parametric: Kruskall-Wallis+Dunnett's or Mann-Whitney U-test (organ weight, histopathology), non-parametric dichotomous: χ^2+Fisher Exact Test/Bonferroni adjustment.

Litters:

Clinical signs: no relevant findings.

Body weight, bw gain, and relative feed consumption: At the top dose, and in both sexes, a time-dependent decrease of body weight was observed in both generations. Body weight gain was impaired in the parental generation at 500 ppm and above and in both generations at the top dose.

Litter data: The number of early stillborn were increased at 500 ppm (F_2) and at the top dose (F_1, F_2), attaining statistical significance for the pup incidences (F_1: 0, 3, 6, 9*; F_2: 1, 3, 8*, 13* (see table 19 below), corresponding to F_1: 0.0; 10.3, 10.7; 21.4 %; F_2: 0.5, 1.5, 5.0, 5.6 %). All values were within the historical control range, except for the pup incidence at the top dose (HC: F_1: n°= 0-16, 0-3.9 %; F_2: n°=0-13, 0-3.7 %). Additionally, in the absence of a clear dose-effect relationship for the litter incidence (see table 18 below), it was considered that the finding was toxicologically significant only at the top dose.

Organ weight: Decrease of thymus weight was at 500 ppm (♂) and above (♂+♀) and of spleen weight was observed at the top dose. The decreased thymic and splenic weights were considered compound-related.

Gross pathology: Occasional gross pathology findings were reported at the top dose in F_1-animals: hydrocephalus (1) and anophthalmia (1). All findings in other dose groups were also at low incidence and without dose-response.

In the first generation, preputial separation was belated at 500 ppm and above. The notifier considered the change of preputial separation delay at 500 ppm as part of the normal variation of the test lab (±5 %). However, the trend showed a dose-dependency and the post-weaning growth was affected at the top dose, but not at 150 and 500 ppm. Preputial separation was not evaluated in the F2-generation.Vaginal opening was also retarded at the top dose (in parallel with a bw decrease of -13 %).

Table 17: Two generation reproduction toxicity in rats: pup data

Dose (ppm)	**0**		**150**		**500**		**2500**	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Litter data								
N° observed (d21)	24	23	29	25	28	20	29	28
N° pups missing [a]	2	1	2	4	1	4	4	6
N° stillborn pups [a] early [b] late [c] total[a]	 0 1 1	 1 3 4	 3 0 3	 3 1 4	 3 2 4	 5 0 5	 6 1 6	 4 2 5
N° viable pups[a] d0 d4	 14 13	 14 13	 14 14	 14 13	 14 14	 13 13	 13 12	 12 12
Birth index [d]	.898	.918	.905	.934	.945	.874	.940	.878
Live birth index [e]	1.00	.998	.993	.991	.984	.974	.979	.965
Viability index [f]	.992	.980	.994	.981	.987	.988	.924	.970
Lactation index [g]	.995	.995	.996	.985	.978	.974	.960	.960
Pup weight at birth [g]	6.5/6.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BW ♂+♀ d0 d4 d7 d14 d21	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓8%* ↓16%** ↓18%** ↓22%** ↓26%**	↓3% ↓9% ↓13%** ↓16%** ↓21%**
BW gain F1 ♂+♀ day 4-21 cumulative bw gain % of control	100 ♂	100♀	99♂	96♀	94♂	93♀-	71♂	71♀
BW gain F2 ♂+♀ day 4-21 cumulative bw gain % of control	100♂	100♀	100♂	102♀	100♂	101♀	75♂	77♀
Organ weights								
Thymus weight ♂ absolute relative	n.d.	n.d.	n.d.	n.d.	 ↓13%* ↓10%	 - -	 ↓29%** ↓5%	 ↓25%** ↓8%
Thymus weight ♀ absolute relative	n.d.	n.d.	n.d.	n.d.	 ↓10% ↓8%	 - -	 ↓28%** ↓7%	 ↓24%** ↓9%
Spleen weight ♂ absolute relative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	 ↓30%** ↓7%	 ↓31%** ↓16%**

Dose (ppm)	**0**		**150**		**500**		**2500**	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Spleen weight ♀ absolute relative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	 ↓35%** ↓16%**	 ↓30%** ↓17%**

n.a.: dose level not tested; n.d.: dose level tested but not displayed in CLH report; [a] data expressed as litter incidence; Early and late stillborn determined byobservation of [b]non-floating or [c]floating lung;
Indices (data expressed as #pups/litter) defined as:
[d]: pups born/implantation sites, [e]: pups liveborn/total pups, [f]: pups alive (d4)/pups liveborn, [g]: pups alive (d21)/pups post-culling (d4);
Statistically significant modifications *p<0.05, **p<0.01 (parametric data: Anova+Dunnett's, non-parametric: Kruskall-Wallis+Dunnett's or Mann Whitney U-test (organ weight, histopathology), non-parametric dichotomous: χ^2+Fisher Exact Test/Bonferroni adjustment).

Table 18: Litter distribution of perinatal deaths

Parameter	**Number of dead pups in litter**	**0 ppm**		**150 ppm**		**500 ppm**		**2500 ppm**	
		P	F1	P	F1	P	F1	P	F1
Litters evaluated		24	23	29	25	28	21	29	28
Total litter loss at birth		0	0	0	0	0	1	0	0
Litters with any live pups		24	23	29	25	28	20	29	28
Litters with stillborn pups	0 1 2 3 4	24 - - - -	22 1 - - -	26 3 - - -	22 3 - - -	25 2 - - 1	15 4 - - 1	23 3 3 - -	24 - - 3 1
Litters with early postnatal deaths (postnatal day 0-4)	0 1 2 3 12	21 3 - - -	18 3 2 - -	25 - 1 - -	20 3 1 1 -	25 2 1 - -	17 3 - - -	23 4 - - 1	19 8 1 - -

Table 19: F1 male sexual maturation

Parameter		**0 ppm**	**150 ppm**	**500 ppm**	**2500 ppm**
F1 males evaluated		32	32	32	32
Day of preputial separation (mean ± SD)		41.0 ± 1.27	41.8 ± 1.32	42.5**± 1.72	47.8**± 2.74
Cumulative achievement	PND 39 PND 40 PND 41 PND 42 PND 43 PND 44 PND 45 PND 46 PND 47 PND 48 PND 49 PND 50 PND 51 PND 52 PND 58	3 13 21 28 31 32	0 5 14 23 28 31 32	0 4 10 18 21 27 31 32	0 0 0 0 0 3 5 10 16 23 25 29 30 31 32
F1 males with time of achievement > PND 42		4	9	14	32

Histopathology*:* At the top-dose, histopathological findings included a mammary gland cyst and chronic active inflammation (1♂). Total sperm count and sperm morphology were only slightly affected at the top dose compared to controls. A subtle increase of mean incidence of cells with detached spermatozoid heads was remarkable at the top dose in both generations. The increase was due to extreme values (approx. *10x* increase compared to group mean value) in 2/30 (F_1) and 3/30 (F_2) animals at the top dose, and considered biologically relevant. No results of intermediate doses were available. At the top dose, motility was affected (-10 %) in the F_2-♂, and progressive motility in both generations (-12 % to -23 %).
The analysis of ovarian follicle count on serial sections revealed no remarkable differences between top-dose and control animals. Both number and duration of the complete estrus cycles were essentially unaltered in treated animals compared to controls. At termination, diestrus-incidence was slightly higher F_1 at 500 ppm and above and in F_1 and F_2 at the top dose.

Conclusions:

At highest dose level of 2500 ppm (approx. 160 mg/kg bw/d), effects on parental generations (P, F1) included reduced body weight gain during pre-mating, pregnancy and lactation and reduced thymus weights furthermore, decreased sperm (progressive) motility. At 500 ppm, reduced body weight gain was restricted to day 14 of the lactation period, only. However, the substance intake at this dose level was much higher in comparison to pre-mating and gestation period (approx. 64 mg/kg bw/d, lactation week 1 & 2). Therefore, the parental NOAEL is considered to be 500 ppm corresponding to approx. 31 mg/kg bw/d.

Increase in stillbirths observed in F1 offspring at 2500 ppm was reproducible in F2 offspring and is therefore considered to be a test substance-related effect. In this dose group, there was a definite, although small increase in the number of litters in which two or more pups were born dead. This distribution differed from the situation in control and most lower dose group litters. In addition, early postnatal pup viability appeared affected as well. The effects observed at highest dose level are considered the LOAEL for reproductive toxicity.

Sexual maturation of males was delayed at a dose level of ≥500 ppm in the F1 pups; this endpoint has not been evaluated in the F2 generation. At 150 ppm the slight delay is considered not to be biologically relevant. In detail, the delay is just half a day and may depend on the time interval of evaluation. In addition, this delay of preputial separation was statistically not significant. Sexual maturation of females was affected only at highest dose level. As the significant delay in preputial separation was present despite normal post-weaning growth in F1 pups of the 500 ppm group, this dose is considered the LOAEL for offspring.

In conclusion, the $NOAEL_{parental}$ is considered 500 ppm (approx. 31 mg/kg bw/d) based on reduced body weight gain and thymus weight in both sexes at 2500 ppm (approx. 160 mg/kg bw/d).

The $NOAEL_{reproduction}$ is considered 500 ppm (approx. 31 mg/kg bw/d) based on increased stillbirths, affected early postnatal viability and reduced sperm motility and morphology effects at 2500 ppm (approx. 160 mg/kg bw/d).

The NOAEL for offspring is considered 150 ppm (approx. 10 mg/kg bw/d) based on delayed sexual maturation at 500 ppm. At high dose of 2500 ppm the perinatal mortality was increased.

4.11.1.2 Human information

No data available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Report: Anonymous (19), 1998

Guideline(s): Yes (87/302 EEC B.31, OECD draft guideline no 414 (1999), US-EPA OPPTS 870.3700 (1996), Japan MAFF (59 NohSan no. 4200, 1985)

Deviations: No

GLP: Yes

Acceptability: Yes

Results and discussions:

Maternal data (see table below):

Maternal mortality: none.

Clinical signs: no relevant findings.

Body weight, bw change and food consumption: At the top dose, a slight decrease of body weight was observed from d8 on. The body weight change was decreased at the top dose group and essentially attributable to effects during d6-d9. At 40 mg/kg bw/d, the body weight change was altered during period d6-d9.

No modification of gravid uterine weight was observable when compared to the control dams.

Foetal data: Caesarean sectioning observations revealed no remarkable differences between treated and control animals. Foetal alterations occurred at similar incidences in litters of all dosage groups or were comparable to recent historical controls, or showed no consistency with dose (see Table 20).

Table 20: Developmental toxicity study of Clothianidin in rats

Dose (mg/kg bw/d)		0	10	40	125
MATERNAL DATA					
Feed consumption	d6-9 d6-20	20.9 g/d 23.6 g/d	20.5 g/d 23.6 g/d	19 g/d* (↓9%) 22.7g/d (↓3.8%)	11.1 g/d** (↓47%) 19.5 g/d** (↓17%)
Body weight	d20 d20[a]	n.d.	n.d.	n.d.	↓5.3%** ↓6.8%**
Body weight gain	d6-7 d6-8 d6-9 d6-20 d6-20[a]	1.9 7.3 11.8 120.9 46.7	0.7 5.6 10.2 122.6 46.4	-1.1*[b] 0.5**[b] 6.8** (↓42%) 117.0 37.7 (19%)**[b]	-8.4**[b] -11.1**[b] -6.1** (↓152%) 100.8**(↓17%) 25.8** (↓45%)
FOETAL DATA					
Number of pregnant ♀		23	22	24	25
Corpora lutea/ dam		15.9	15.8	15.9	16.0
Implantations/ dam		13.6	14.3	14.2	14.3
Resorptions early/late/ dam		0.6 / 0.0	0.9 / 0.0	0.4 / 0.0	0.6 / 0.1

Dose (mg/kg bw/d)	0	10	40	125
live foetuses / litter	13.0	13.4	13.8	13.6
live ♂ foetuses (%)	49	49	52	48
foetal weight (g) ♂/♀	3.60 / 3.43	3.57 / 3.44	3.63 / 3.46	3.40 / 3.28
foetal variations (%)	34.8	40.9	37.5	40.0
foetal malformations (%)	8.7	4.5	4.2	4.0

[a]: corrected body weight=gestation bw minus gravid uterine weight; n.d.: dose level tested but not displayed in the CLH report
Statistical significance: *p<0.05, **p<0.01, Continuous data: Dunnett's test (homogeneous variances); Kruskall-Wallis/Dunn's test (non-homogeneous variances, ≤ 75% ties) or Fisher's Exact test (>75% ties). Discontinuous data: Kruskall-Wallis procedure.
[b]: t-test, *p<0.05, **p<0.01, additionally performed by RMS for re-evaluation

Table 21: Summary of foetal alterations in the developmental toxicity study of Clothianidin in rats

Location	Parameter	Dose (mg/kg bw/d)			
		0	10	40	125
	N° of litters evaluated	23	22	24	25
External	no remarkable findings	23	22	24	25
Visceral	depressed eye bulge [m]	0 (0.0)	1 (4.5)	1 (4.2)	0 (0.0)
	microphthalmia [m]	0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)
	innominate artery absent [v]	0 (0.0)	0 (0.0)	1 (4.2)	1 (4.0)
	aortic arche dorsal to trachea/oesophagus [v]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
	carotid (l) arises right of subclavian (r) [v]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
Skeletal	small eye socket [m]	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
	fused ribs [m]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
	bifid centrum in thoracic vertebra [v]	1 (4.3)	0 (0.0)	1 (4.2)	2 (8.0)
	cervical rib present at 7th cervical vertebra [v]	0 (0.0)	1 (4.5)	1 (4.2)	2 (8.0)
	incompletely/not ossified sternal centra [v]	5 (21.7)	4 (18.2)	6 (25.0)	5 (20.0)
	incompletely/not ossified pelvis [v]	1 (4.3)	3 (13.6)	3 (12.5)	2 (8.0)

[m]: malformation; [v]: variation; values referring to number of foetuses (litter incidence in %)
(historical control litter incidence: microphthalmia=0.59%, fused ribs=0.46%, small eye socket/depressed eye bulge: not reported)

Conclusions:

Female rats received doses of 0, 10, 40 and 125 mg/kg bw/d on day 6-19 pc. The maternal NOAEL is considered 10 mg/kg bw/d based on significant, however only slight and transient effects on food consumption and body weight gain day 6 to 9 pc. The corrected body weight gain day 6 to 20 was decreased (19%), but without statistical significance.

The developmental NOAEL is considered to be 125 mg/kg bw/d, because no remarkable toxic effects were observed up to the highest dose tested.

Report: Anonymous (18), 1999b

Guideline(s): Yes (Protocol in compliance with test method B.31 of directive 92/69/EEC)

Deviations: No

GLP: Yes

Acceptability: Yes

Results and discussions:

Maternal mortality (see table below): Two animals were found dead at 75 and 100 mg/kg bw/d, and one animal was sacrificed moribund at the high dose.

Clinical signs: Both decreased faecal output and orange/red urine was observed at 25 mg/kg bw/d and above.

Necropsy: no relevant findings

Body weight, bw change and food consumption: Feed consumption was significantly decreased at 75 mg/kg bw/d and above, while uncorrected body weight (i.e. taking into account uterine weight) and body weight gain was only affected at the top dose. The reductions of uterine weight at 75 mg/kg bw/d and above were considered biologically significant.

Table 22: Main developmental toxicity study of Clothianidin in rabbits (maternal data)

Dose (mg/kg bw/d)	0	10	25	75	100
N° rabbits examined	23	23	23	23	23
Mortality found dead sacrificed moribund %	 0 0 0	 0 0 0	 0 0 0	 2[d25,27] 0 8.7	 2[d17,20] 1[d19] 13.0
Clinical signs[a]					
localized alopecia	4(45)	2(18)	3(18)	3(41)	8(61)
scant faeces	1(1)	3(4)	4(10)	10(40) **	16(79) **
no faeces	n.d.	n.d.	n.d.	1 (1)	11(22) **
soft/liquid faeces	n.d.	n.d.	n.d.	n.d.	1 (5)
orange urine	n.d.	n.d.	2(4)	9(38)**	9(64) **
red substance in pan	n.d.	n.d.	1(1)	0	4(8) **
decreased motor activity loss righting reflex	n.d.	n.d.	n.d.	n.d.	1 (1)
Feed consumption (g/d) Day 6-9 Day 9-15 Day 15-21 Day 21-24 Day 24-29 Day 6-29	 141.3 135.4 153.8 143.5 90.0 142.8	 154.2 160.5 167.0 143.1 94.2 145.2	 168.7 149.4 159.3 137.4 85.4 139.5	 142.9 119.0 123.5 101.2* 89.4 118.8*	 90.9* 60.3** 86.4** 104.7* 64.2 89.6*
Body weight (kg) Day 0 Day 29	 4.02 4.36	 4.01 4.40	 4.00 4.37	 3.98 4.22	 4.01 3.98**

Dose (mg/kg bw/d)	0	10	25	75	100
Corrected body weight (kg)[b]	3.84	3.88	3.86	3.83	3.67
Body weight gain (g) Day 0-6 Day 6-9 Day 9-15 Day 15-21 Day 21-29 Day 6-29 Day 0-29	 50 10 90 90 50 320 390	 70 10 120 110 90 320 390	 80 50 90 90 60 300 380	 80 20 30 30 30 170 260	 60 -40** -160 60 -70 -200** 50**
Gravid uterine weight (g)	517.8	524.5	516.5	461.6	420.1

Maternal data are calculated for the gestational period d6-28; Caesarian section data are expressed in mean nbr of foetuses per litter; [a]: litter incidence, and [(-)] =daily incidence, on maximal possible incidence during observation period [i.e. maximally 23 rabbits*29d=667]; [b]: corrected body weight=gestation bw minus gravid uterine weight; n.d.: dose level tested but not displayed in CLH report
Statistical significance: *p<0.05, **p<0.01, Continuous data: Dunnett's test (homogeneous variances); Kruskall-Wallis/Dunn's test (non-homogeneous variances, ≤ 75% ties) or Fisher's Exact test (>75% ties). Discontinuous data: Kruskall-Wallis procedure.

Foetal data (see table below):

A statistical significant decrease in ♀ foetus weight was observed at the top dose. The decrease of the proportion of ♂ life foetuses and the increase of % resorbed foetuses/litter did not attain statistical significance at the top dose, but was probably biologically relevant. There is a dose-dependent increase of animals showing absence of intermediate lung lobe. Despite the relatively high spontaneous incidence of the variation in this strain, the effect is probably also of toxicological significance. Other effects, including small kidney, fused caudal vertebrae, incompletely ossified sternal centra, and absent hindpaw phalanges are minimally but significantly increased on litter incidence base at the top dose. A delay in ossification was observed at 75 mg/kg bw/d and above (sternal centers) and at the top dose (hindlimb phalanges).

Table 23: Main developmental toxicity study of Clothianidin in rabbits (caesarean section)

Dose (mg/kg bw/d)	0	10	25	75	100
Number of surviving pregnant ♀ Aborted[§]/premature[§§]litters (%) Litters with #3 live foetuses Included in analysis	21 3[§](13.0) 0 18	23 0 0 23	22 0 2 20	20 1[§],2[§§] (13.0) 0 17	20 6[§],2[§§](34.8*) 1 11
Corpora lutea	9.4	9.6	9.9	8.7	9.8
Implantations	8.6	8.9	9.2	8.0	8.8
Resorptions early/late	0.1/0.2	0.0/0.1	0.1/0.2	0.0/0.2	0.9/0.4
% resorptions	3.1	1.4	3.6	3.4	12.7
live foetuses	8.3	8.7	8.8	7.8	8.2
live ♂ foetuses (%)	51.8	47.2	50.2	48.8	39.6
foetal weight ♂/♀ (g)	44.2 / 43.0	43.2 / 42.2	40.7 / 40.0	40.7 / 40.2	37.7 / 36.1**
% variations litter incidence foetal incidence	22.2 2.7	30.4 3.5	45.4 9.5**	29.4 4.5	45.4 8.2**
% malformations litter incidence foetal incidence	0.0 0.0	21.7 3.0**	9.1 1.7	11.8 1.5	27.3 5.9**
% litters with ≥ 1 variation	2.6	3.8	13.6	5.0	11.5
% litters with ≥ 1 malformation	0.0	2.9	1.5	1.6	9.0

Table 24: Summary of foetal alterations in rabbits

			Dose (mg/kg bw/d)				
location	parameter	l/f	0	10	25	75	100
external	medially rotated hindlimbs [m]	l f	0	0	1 (4.5) 3 (1.7**)	0	0
visceral	intermediate lung lobe absent [v]	l f	0 0	0 0	0 0	3 (17.6)** 3 (2.2)	5 (45.5)** 8 (9.4)**
	small kidney [m]	l f	0 0	0 0	0 0	0 0	1 (9.1) 3 (3.5)**
skeletal	fused caudal vertebrae [m]	l f	0 0	0 0	1 (4.5) 2 (1.1)	0 0	2 (18.2)** 2 (2.4)**
	incompletely ossified sternal centra [v]	l f	0 0	0 0	0 0	0 0	2 (18.2)** 2 (2.4)
	absent hindpaw phalanges [m]	l f	0 0	0 0	0 0	0 0	2 (18.2)** 2 (2.4)**
Ossification sites: number per foetus/litter							
sternal centers			3.99 / 0.03	3.91 / 0.14	3.94 / 0.13	3.83 / 0.2**	3.76 / 0.25**
hindlimbs phalanx			12.00 / 0.00	12.00 / 0.00	12.00 / 0.00	12.00 / 0.00	11.78 / 0.63**

[m]: malformation; [v]: variation; values referring to number of foetuses; litter (l) or foetal (f) incidence; (…): incidence in %;
Statistical significance: *p<0.05, **p<0.01 (see above).
Historical control incidences:
intermediate lung lobe absent: (i) litter inc.: n=0-5, 0-27.8% (m=10.1%); (ii) foetal inc.: n=0-7, 0-4.4% (m=1.54%)
small kidney: no reported data
fused caudal vertebrae: (i) litter inc. n=0-1, 0-5.9% (m=1.4%); (ii) foetal inc.: n=0-1, 0-1.9% (m=0.21%)
incompletely/not ossified sternebrae: (i) litter inc. n=0-10.5, 0-5.9% (m=1.8%); (ii) foetal inc.: n=0-2, 0-1.2% (m=0.21%)
absent hindpaw phalanx: (i) litter inc. n=0-1, 0-5.9% (m=0.2%); (ii) foetal inc.: n=0-1, 0-0.6% (m=0.02%)
number of sternal center ossification sites: 3.81-3.97 (m=3.91)
number of hindlimb phalanx ossification sites: 11.99-12.00 (m=12)

Conclusions:

In the main developmental toxicity study in rabbits, doses of 0, 10, 25, 75 and 100 mg/kg bw/d were orally given on day 6 to 28 of gestation.

At ≥75 mg/kg bw/d treatment related deaths or sacrifice occurred, reduced food intake and faecal output, but no significant decreased body weight (gain). Additionally, at 100 mg/kg bw/d an increase of abortions or prematurely deliveries were noted. Death and abortion were preceded by reduced food intake, body weight loss, decreased activity and loss of righting reflex, whereas necropsy did not reveal any relevant findings. At 25 mg/kg bw/d single cases of reduced faecal output and orange urine were noted, however not significant. Considering, that these clinical effects increased dose-dependently and reached statistical significance at ≥75 mg/kg bw/d. Based on the clinical effects observed at 25 mg/kg bw/day and above the maternal NOAEL should be set at 10 mg/kg bw/d.

Based on individual data of the study report a reduced number of lung lobes in 3 foetuses out of 3 litters at 75 mg/kg bw/ and 8 foetuses (9.4%) out of 5 litters (45.5%) at 100 mg/kg bw/d were observed. The missing lung lobes appear to indicate a dose-related effect on lung branching morphogenesis at high, maternally toxic doses. Furthermore, ossification was delayed at ≥75 mg/kg bw/d (sternal centers), but within HCD and at the top dose (hindlimb phalanges).

At the top dose of 100 mg/kg bw/d some malformations were slightly, but significantly increased on litter incidence base like small kidney, fused caudal vertebrae, incompletely ossified sternal centra, and absent hindpaw phalanges.

The developmental NOAEL in rabbits is considered to be 25 mg/kg bw/d based on the teratogenic effect, the absence of intermediate lung lobes and retardation, the reduced ossification of sternal centres at 75 mg/kg bw/d, a dose level inducing severe maternal toxicity.

Adverse effects on lactation:

No specific investigations available to DS.

4.11.2.2 Human information

No data available to DS.

4.11.3 Other relevant information

No data available to DS.

4.11.4 Summary and discussion of reproductive toxicity

In the two-generation study in rats, effects on parental generations (P, F1) included reduced body weight gain during pre-mating, pregnancy and lactation as well as reduced thymus weights and a decrease in sperm (progressive) motility (and morphology effects, not significant) at highest dose level of 2500 ppm (approx. 160 mg/kg bw/d). These effects did not influence the fertility of these animals. However, because of differences in sperm parameters between rodents and humans, the effect is considered to be test substance-related and potentially adverse for species with lower sperm production than the rat (e.g. humans). No effects relevant for adverse effects on fertility were reported in repeat-dose studies at dose levels not leading to severe deterioration of animals.

The reproducible increase in stillbirths and decreased perinatal viability observed in both F1 and F2 offspring at 2500 ppm is considered to be test substance related. In the offspring sexual maturation of males was delayed at a dose level of ≥ 500 ppm in the F1 pups, which was not evaluated in the F2 generation. Female sexual maturation was affected at highest dose level, only. The anogenital distance in F2 pups was not affected. Significant delay in sexual maturation (preputial separation) was present despite normal post-weaning growth in F1 pups at 500 ppm.

In the developmental toxicity study in rats, no treatment-related differences in reproductive parameters including the number of corpora lutea, implantation losses, foetal resorptions, and the number of live or dead foetuses were observed. No treatment-related changes in foetuses after external, visceral and skeletal examinations were reported.

In the developmental toxicity study in rabbits, maternal toxicity was observed at doses of ≥ 25 mg/kg bw/d. At 75 and 100 mg/kg bw/d, two and three does were found dead or in moribund condition, respectively. No treatment-related differences in reproductive parameters in females including the mean number of corpora lutea, implantations, losses, and the number of live or dead foetuses were observed. The number of resorptions was increased in top dose and foetal weights were reduced. At 75 and 100 mg/kg bw/d, significantly increased incidences of absent intermediate lung lobes were observed. In top dose group, further malformations were seen during skeletal examination (fused caudal vertebrae, absent hind paw phalanges). The reported findings were considered severe and irreversible.

4.11.5 Comparison with criteria

Adverse effects on sexual function and fertility:

Table 25: Criteria concerning classification for adverse effects on sexual function and fertility

Toxicological result	CLP criteria
	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects
2-generation reproduction study in Sprague-Dawley rats (Anonymous (2), 2000), **Delayed sexual maturation in males despite normal (post-weaning) growth.** **Delayed vaginal opening associated with growth retardation.** Decreased sperm motility (and not significant morphology effects) at the highest dose (2500 ppm). No adverse effects on conception rate and fertility indices.	Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and - where the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

There are no epidemiological data to evaluate effects on fertility; hence Clothianidin cannot be placed in category 1A.

In the submitted multi-generation study delayed sexual maturation in males was observed despite normal (post-weaning) growth, whereas delayed vaginal opening was only associated with growth retardation. Slight effects on sperm motility and morphology were reported, but these effects did not influence the fertility of these animals. However, because of differences in sperm parameters between rodents and humans, these effects are considered to be test substance-related and potentially adverse for species with lower sperm production than the rat (e.g. humans). Therefore, effects on sperms should be considered adverse. However, observations of sperm motility were not reported in other RDT studies. No effects relevant for adverse effects on fertility were reported in repeat-dose studies at dose levels not leading to severe deterioration of animals. In summary, mainly based on the delayed sexual maturation in the absence of affected post-weaning growth observed in males, classification for effects on sexual function/fertility is proposed by the DS.

Adverse effects on development:

Table 26: Toxicological results concerning adverse effects on development

Toxicological result	CLP criteria
	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on development in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects
2-generation reproduction study in Sprague-Dawley rats (Anonymous (2); 2000), Higher incidence of stillbirth, decreased perinatal viability in the absence of excessive parental toxicity.	Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and - the evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study). - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects
Preliminary teratogenicity study, rat (Anonymous (16), 1998a): Limited evaluation of foetuses **Teratogenicity study, rat** (Anonymous (19), 1998): No effects on foetuses reported up to highest dose tested (125 mg/kg bw/d) **Preliminary teratogenicity study, rabbit** (Anonymous (17), 1998): Limited evaluation of foetuses; loss of all litters at doses ≥ 125 mg/kg bw/d **Teratogenicity study, rabbit** (Anonymous (18), 1999b): 75 and 100 mg/kg bw/d: intermediate lung lobe absent ↑	**No classification**

There are no appropriate epidemiological studies available on developmental effects in humans. Hence, classification with Category 1A according CLP regulation is not necessary.

The prenatal developmental toxicity was investigated in rats and rabbits complying with international test guidelines and GLP.

In rats, no findings in offspring relevant for a possible classification for developmental effects were reported. There were no treatment-related differences in reproductive parameters in females including the number of corpora lutea, pre- and post-implantation losses, and the number of foetal resorptions, and the number of live or dead foetuses. There were no treatment-related changes in foetuses after external, visceral and skeletal examinations.

In the teratology study in rabbits no treatment-related differences in reproductive parameters in females, including the mean number of corpora lutea, implantations losses and the numbers of live

or dead foetuses were observed. The number of resorptions was increased in top dose and foetal birth weights were reduced.

At 75 and 100 mg/kg bw/d, significantly increased incidences of absent intermediate lung lobes were observed. According to modern nomenclature, this finding is considered a malformation. In top dose group, further malformations were seen during skeletal examination (fused caudal vertebrae, absent hind paw phalanges). The reported findings are severe and irreversible.

Yet, maternal toxicity was observed in this rabbit study at doses of 25 mg/kg bw/d and above. At 75 and 100 mg/kg bw/d, two and three does were found dead or in moribund condition, respectively. The occurrence of severe findings in rabbit offspring needs to be balanced against the increased incidences of mortalities and moribund condition seen at the same dose levels. Thus, these findings are not considered to justify classification for reproductive toxicity.

In the multi-generation study in rats, higher incidences of still birth and decreased perinatal viability were observed in both F1 and F2 offspring at 2500 ppm. The reported findings are severe and relevant for a possible classification for developmental effects. These findings were seen in the absence of excessive parental toxicity.

Finally, a substance specific mechanism of action for developmental toxicity was not established.

4.11.6 Conclusions on classification and labelling

In summary, classification for developmental toxicity is warranted as Repr. 2, H361fd (H361fd Suspected of damaging fertility. Suspected of damaging the unborn child) based on the findings in the multi-generation study.
It is considered not necessary to derive a SCL for Repr. 2, H361fd, because the relevant effect dose levels were within the medium potency group.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed classification of clothianidin for effects on sexual function/fertility mainly based on the delayed sexual maturation observed in males in the 2-generation reproduction toxicity study in rats. The DS also proposed classification of clothianidin for development based on the high incidences of stillbirth and reduction of perinatal viability observed in both F1 and F2 offspring (in absence of severe maternal toxicity) in this same multi-generation study. Overall, DS proposed classification of clothianidin as Repr. 2, H361fd (Suspected of damaging fertility and unborn child).

Comments received during consultation

During the Stakeholder consultation, reproductive toxicity received a wide range of comments. These comments, together with the answers provided by the DS are summarised in the table below. See the RCOM, Annex 3 and additional information provided by commenters for more detailed information.

In addition to the comments presented in Annex 3, on August 24, 2021 RAC received a new position paper entitled: "*Clothianidin- the delay in preputial separation at 2500 ppm was due to*

general toxicity and does not represent selective reproductive toxicity". As indicated in the title, this position paper defended that the seven-day delay in sexual maturation was a real effect of clothianidin; however, it was in fact as a result of general toxicity and not due to any selective effect on reproduction (fertility). According to this paper delays in preputial separation in male rats can and have been correlated with decreased body weights at weaning (post-natal day 21) because the highest exposure of the F1 generation occurs just prior to, and for the first week, post-weaning. The applicant estimated that the dose in F1 pups at weaning would have been 525 mg clothianidin/kg bw/day rather than the 161 mg clothianidin/kg bw/day estimated for the P animals feed with 2500 ppm. According to the applicant this dose would have caused a notably reduction in food consumption together with the corresponding bodyweight reduction; which would have been responsible of delay in preputial separation. Applicant also described that in other cases where delay in preputial separation have been reported it was caused by increased levels of free glucocorticoids (that have been found in foetuses and pups exposed to food restriction) or caused by an effect on the hormone leptin (involved in the control of food intake and in body-weight homeostasis).

Assessment and comparison with the classification criteria

The reproductive toxicity of clothianidin was assessed in a 2-generation toxicity study in rats and in two developmental toxicity studies, one in rats and one in rabbits.

2-generation reproduction study in rats

The study was performed following OECD TG 416 and observing GLP. Thirty males and thirty female Sprague-Dawley rats were orally exposed via diet to 0, 150, 500 and 2500 ppm of clothianidin (95.7% purity); which corresponded to 0, 10, 31, 163 mg/kg bw/day and 0, 10, 31, 161 mg/kg bw/day for males and females, respectively.

Adult animals

Significant body weight decreases were observed in both males and females of P and F1 adults at the top dose, while relative feed consumption was mostly increased. In both P and F1 animals, absolute and relative organ weight changes were in line with the observed body weight loss (see table below).

Table: Effects of clothianidin on body and organ weight in adult animals in the 2-generation reproduction toxicity study in rats. 30 animals per sex per dose; pm: premating; ge: gestation; la: lactation. * = Statistically different form control for $p < 0.05$. ** = Statistically different form control for $p < 0.01$. n.d. = dose level tested but not displayed in the CLH report.

	0		150		500		2500	
	P	F1	P	F1	P	F1	P	F1
Body weight males	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓8-10%**	↓16-20%**
Body weight females (d7-70) pm (d0,6,13,20) ge (d0,4,7,14,21) la (d14) la	n.d.	n.d.	n.d.	n.d.	↓6.5%*	n.d.	↓9-15%** ↓12-14%** ↓11-18%**	↓16-19%** ↓13-16%** ↓13-15%**
Body weight gain (d0,6,13,20) ge	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓17%**	↓8%
Feed consumption males	n.d.	n.d.	n.d.	n.d.	↓4%* wk1 pm	-	↓18%** wk1pm ↑8%* wk3;**wk4,5,8pm	↑16%** wk1-10pm

Feed consumption females	n.d.	n.d.	n.d.	n.d.	↑7.6%* wk10[pm]	-	↓18% **wk1[pm] ↑16% **wk8-10[pm] ↑11%* d6-13[ge] ↑13%* d14-21[la]	↑21%* wk1,5-6[pm] **wk2-4,7-10[pm] ↑11%* d6-13[ge] ↑13%* d7-21[la]
Organ weight								
Thymus males absolute relative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓16%* ↓8%	↓29%* ↓13%*
Thymus females absolute relative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓35%* ↓32%*	↓41%* ↓32%*
Ovary absolute relative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓19%* ↓16%*	↓11% -
Uterus absolute relative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	↓25%* ↓20%*	↓21% ↓10%

Occasional gross pathology findings were reported at the top dose in (i) female P animals: skull malocclusion and ulcer (1/30), kidney adhesion/raised zone (1/30), prolapsed uterus (1/30), vaginal mass (1/30), reduced thymus (2/30); (ii) F1 animals: 1 calculus in kidney (1/30 males), enlarged testicle (1/30 males), testicle with abnormal consistency (1/30 males), thymus oedema (1/30 females), lung discoloration (1/30 females), fluid-filled body (1/30 females) and mammary gland cyst (1/30 females).

The following occasional histopathology findings were noted in P animals at the top-dose: adrenal cortical hyperplasia (1 female), necrosis (1 female), vacuolization (2 females), thymus atrophy (2 females), uterus oedema (2 females), haemorrhage (1 female), cervix congestion (1 female), vagina congestion (1 female), cyst (1 female), abnormal spermatozoa (1 male), epididymis mineralization (1 male) and sperm granuloma (1 male).

In both P and F1 animals, no difference in number and duration of oestrus cycles between treated and control animals were observed on the basis of the smear analyses. Clothianidin did not affect the sperm morphology and total sperm count. However, sperm motility was statistically reduced in F1 animals exposed to 2500 ppm by 11.5%. The progressive sperm motility was reduced by 28.1 and 12.4% in F1 and P animals exposed to 2500 ppm clothianidin (see table below).

There was a delay of 6.7 days in the day of preputial separation among F1 animals exposed to 2500 ppm clothianidin. The delay was of 1.3 days in the F1 animals exposed to 500 ppm (following two tables). Both delays were statistically significant. Vaginal opening in F1 animals exposed to 2500 ppm was also statistically delayed by 2.3 days as regard F1 control animals (see table below).

Table: Gross pathology and histopathology in adult animals in the 2-generation reproduction toxicity study in rats with clothianidin. 30 animals per sex per dose; * = Statistically significant different from control for p < 0.05. ** = Statistically significant different from control for p < 0.01. n.d. = dose level tested but not displayed in the CLH report. n.a. = dose level not tested.

	0		150		500		2500	
ADULTS	**P**	**F1**	**P**	**F1**	**P**	**F1**	**P**	**F1**
Gross pathology								
Preputial separation (day pn)	n.a.	41.2	n.a.	41.9	n.a.	42.5**	n.a.	47.9**
Vaginal opening (day pn)	n.a.	32.4	n.a.	32.2	n.a.	32.1	n.a.	34.7**
Histo/Cytopathologie								
Sperm morphology			n.a.	n.a.	n.a.	n.a.		
% normal	83.5	69.3					81.9	67.0
% abnormal	15.9	30.0					16.0	29.0
% detached	0.6	0.7					2.1	4.0
Total sperm count			n.a.	n.a.	n.a.	n.a.		
epididymis	146.1	149.5					141.6	133.7
testis	129.9	105.7					129.5	103.8
Sperm motility			n.a.	n.a.				
% motile	82.9	81.7			82.6	79.0	79.2	73.4**
% progressively motile	64.1	59.9			61.5	53.9	56.2**	46.1**
Oestrus cycle			n.d.	n.d.	n.d.	n.d.		
duration (days)	4.2	4.3					4.1	4.4
number	3.3	3.4					3.7	3.4
Oestrus stage (sacrifice)								
dioestrus (n)	18	19	19	18	25	16	24	25
proestrus (n)	2	0	0	3	1	0	1	1
oestrus (n)	9	11	10	9	3	14	2	4
Ovarian follicle count (mean /ovary)								
'non-antral' follicles	12.17	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	12.22
'antral' follicles	3.94	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	4.53
corpora lutea	3.71	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3.24

Table: F1 male sexual maturation in the 2-generation reproduction toxicity study in rats with clothianidin. ** = Statistically significant different from control for p < 0.01.

Parameter		**0 ppm**	**150 ppm**	**500 ppm**	**2500 ppm**
F1 males evaluated		32	32	32	32
Day of preputial separation (mean±SD)		41.0±1.27	41.8±1.32	42.5**±1.72	47.8**±2.74
Cumulative achievement	PND 39	3	0	0	0
	PND 40	13	5	4	0
	PND 41	21	14	10	0
	PND 42	28	23	18	0
	PND 43	31	28	21	0
	PND 44	32	31	27	3
	PND 45		32	31	5
	PND 46			32	10
	PND 47				16
	PND 48				23
	PND 49				25
	PND 50				29
	PND 51				30
	PND 52				31
	PND 58				32
F1 males with time of achievement > PND 42		4	9	14	32

Litters

At the top dose, and in both sexes, a time dependent decrease of body weight was observed in both generations (table below). The number of early stillborn were increased at 500 ppm (F2) and

at the top dose (F1, F2). All values were within the historical control range, except for the pup incidence at the top dose. Additionally, in the absence of a clear dose-effect relationship for the litter incidence, it was considered that the finding was toxicologically significant only at the top dose.

Decrease of thymus weight was observed at 500 ppm (males) and above (males + females), and a reduction of spleen weight was also noted at the top dose (table: 'Gross pathology and histopathology in adult animals…' above). The decreased thymic and splenic weights were considered compound-related. Occasional gross pathology findings were reported at the top dose in F1 animals. It included one case of hydrocephalus and one case of anophthalmia. All findings in other dose groups were also at low incidence and without dose-response.

Table: Pup data in the 2-generation reproduction toxicity study in rats with clothianidin. * = Statistically significant different from control for $p < 0.05$. ** = Statistically significant different from control for $p < 0.01$. n.d. = dose level tested but not displayed in the CLH report.

	0		**150**		**500**		**2500**	
	F1	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**
Litter data								
Observed (day 21)	24	23	29	25	28	20	29	28
Pups missing	2	1	2	4	1	4	4	6
Stillborn pups								
early	0	1	3	3	3	5	6	4
late	1	3	0	1	2	0	1	2
total	1	4	3	4	4	5	6	5
Viable pups								
d0	14	14	14	14	14	13	13	12
d4	13	13	14	13	14	13	12	12
Birth index	.898	.918	.905	.934	.945	.874	.940	.878
Live birth index	1.00	.998	.993	.991	.984	.974	.979	.965
Viability index	.992	.980	.994	.981	.987	.988	.924	.970
Lactation index	.995	.995	.996	.985	.978	.974	.960	.960
Body weight (%)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
d0							↓8*	↓3
d4							↓16**	↓9
d7							↓18**	↓1**
d14							↓22**	↓16**
d21							↓26**	↓21**
Organ weights								
Thymus weight (males)	n.d.	n.d.	n.d.	n.d.				
Absolute (%)					↓13*	-	↓29**	↓25**
Relative (%)					↓10	-	↓5	↓8
Thymus weight (females)	n.d.	n.d.	n.d.	n.d.				
Absolute (%)					↓10	-	↓28**	↓24**
Relative (%)					↓8	-	↓7	↓9
Spleen weight (males)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Absolute (%)							↓30**	↓31**
Relative (%)							↓7	↓16**
Spleen weight (females)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Absolute (%)							↓35**	↓30**
Relative (%)							↓16**	↓17**

Conclusions of this study

At the highest dose level of 2500 ppm (approximately 160 mg/kg bw/day), effects on parental generations (P, F1) included reduced body weight gain during pre-mating, pregnancy and lactation and reduced thymus weights. Decreased sperm (progressive) motility was also noted at 2500 ppm. At 500 ppm, reduced body weight gain was restricted to day 14 of the lactation period, only. However, the substance intake at this dose level was much higher in comparison to pre-mating and gestation period.

Increase in stillbirths observed in F1 offspring at 2500 ppm was reproducible in F2 offspring and is therefore considered a test substance-related effect.

Sexual maturation of males was delayed at a dose level of > 500 ppm in the F1 pups; this endpoint has not been evaluated in the F2 generation. At 150 ppm, the slight delay is considered not to be biologically relevant. In addition, this delay of preputial separation was statistically not significant. Sexual maturation of females was affected only at highest dose level. As the significant delay in preputial separation was present despite normal post-weaning growth in F1 pups of the 500 ppm group it was considered treatment-related.

Developmental toxicity study in rats

The study was performed following OECD TG 414 and observing GLP. Twenty-five naturally mated female Sprague-Dawley rats per group were orally exposed via diet to 0, 10, 40 and 125 mg/kg bw/day of clothianidin (95.2% purity) during gestation days 6-19.

Pregnant females

No moralities and no clinical signs were reported at any dose. At the top dose, a slight decrease of body weight was observed from day 8, the largest body weight gain reduction (152%) was noted between gestation days 6-9, being the body weight reduction between days 6-20 of 17% and the corrected body weight reduction of 45% (table below). Body weight gain in the period between gestation days 6-9 was reduced by 42% at 40 mg/kg bw/day; while at this dose level the corrected body weight gain was also reduced by 19%. The body weight change was decreased at the top dose group and was essentially attributable to effects during days 6-9. At 40 mg/kg bw/day, the body weight change was altered during period days 6-9 (table below). No modification of gravid uterine weight was observable when compared to the control dams. Caesarean sectioning observations revealed no remarkable differences between treated and control animals (table below).

Table: Developmental toxicity study of clothianidin in rats. * = Statistically significant different from control for $p < 0.05$. ** = Statistically significant different from control for $p < 0.01$. [a] corrected body weight = gestation bw minus gravid uterine weight. n.d. = dose level tested but not displayed in the CLH report.

Dose (mg/kg bw/day)	**0**	**10**	**40**	**125**
MATERNAL DATA				
Feed consumption				
day 6-9	20.9 g/day	20.5 g/day	19 g/day* (↓9%)	11.1 g/day** (↓47%)
day 6-20	23.6 g/day	23.6 g/day	22.7g/day (↓3.8%)	19.5 g/day** (↓17%)
Body weight	n.d.	n.d.	n.d.	
day 20				↓5.3%**
day 20[a]				↓6.8%**
Body weight gain				
day 6-7	1.9	0.7	-1.1*	-8.4**
day 6-8	7.3	5.6	0.5**	-11.1**
day 6-9	11.8	10.2	6.8** (↓42%)	-6.1** (↓152%)
day 6-20	120.9	122.6	117.0	100.8**(↓17%)
day 6-20[a]	46.7	46.4	37.7 (19%)**	25.8** (↓45%)
FOETAL DATA				
Nº pregnant females	23	22	24	25
Corpora lutea/ dam	15.9	15.8	15.9	16.0
Implantations/ dam	13.6	14.3	14.2	14.3
Resorptions early/late/ dam	0.6 / 0.0	0.9 / 0.0	0.4 / 0.0	0.6 / 0.1
Live foetuses / litter	13.0	13.4	13.8	13.6
Live males foetuses (%)	49	49	52	48
Foetal weight (g)	3.60/3.43	3.57/3.44	3.63/3.46	3.40/3.28

male/female				
Foetal variations (%)	34.8	40.9	37.5	40.0
Foetal malformations (%)	8.7	4.5	4.2	4.0

Foetal data

Foetal alterations occurred at similar incidences in litters of all dosage groups or were comparable to recent historical controls or showed no consistency with dose (see table below).

Table: Summary of foetal alterations in the developmental toxicity study of clothianidin in rats. [m] malformation; [v] variation. Values referring to number of foetuses (litter incidence in %). Historical control litter incidence: microphthalmia=0.59%, fused ribs=0.46%, small eye socket/depressed eye bulge: not reported).

		Dose (mg/kg bw/day)			
Location	**Parameter**	**0**	**10**	**40**	**125**
	N° of litters evaluated	23	22	24	25
External	No remarkable findings	23	22	24	25
Visceral	Depressed eye bulge[m]	0 (0.0)	1 (4.5)	1 (4.2)	0 (0.0)
	Microphthalmia[m]	0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)
	Innominate artery absent[v]	0 (0.0)	0 (0.0)	1 (4.2)	1 (4.0)
	Aortic arche dorsal to trachea/oesophagus[v]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
	Carotid (l) arises right of subclavian (r)[v]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
Skeletal	Small eye socket[m]	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
	Fused ribs[m]	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.0)
	Bifid centrum in thoracic vertebra[v]	1 (4.3)	0 (0.0)	1 (4.2)	2 (8.0)
	Cervical rib present at 7th cervical vertebra[v]	0 (0.0)	1 (4.5)	1 (4.2)	2 (8.0)
	Incompletely/not ossified sternal centra[v]	5 (21.7)	4 (18.2)	6 (25.0)	5 (20.0)
	Incompletely/not ossified pelvis[v]	1 (4.3)	3 (13.6)	3 (12.5)	2 (8.0)

Conclusions of this study

No remarkable developmental toxicity were observed up to the highest dose (125 mg/kg bw/day) tested. This dose caused negative body weight gain between days 6-9 and a reduction of corrected body weight of 6.8% and of corrected body weight gain of 45% during days 6-20.

Developmental toxicity studies in rabbits

The study was performed following OECD TG 414 and observing GLP. Twenty-three naturally mated female New Zealand White rabbits per group were orally exposed via diet to 0, 10, 25, 75 and 100 mg/kg bw/day of clothianidin (95.2% purity) during gestation days 6-28.

Pregnant females

Two animals were found dead at both 75 and 100 mg/kg bw/day, and one animal was sacrificed moribund at the high dose (table below). Both decreased faecal output and orange/red urine was observed at 25 mg/kg bw/day and above. No relevant findings were noted in the necropsy. Feed consumption was significantly decreased at 75 mg/kg bw/day and above, while body weight and body weight gain was only affected at the top dose. However, no effect on corrected body weight was noted (table below). The reductions of uterine weight at 75 mg/kg bw/day and above were considered biologically significant.

Table: Maternal effects of clothianidin noted in the developmental toxicity study in rabbits. Corrected body weight = gestation bw minus gravid uterine weight; * = Statistically significant different from control for p < 0.05. ** = Statistically significant different from control for p < 0.01. n.d. = dose level tested but not displayed in the CLH report.

Dose (mg/kg bw/day)	0	10	25	75	100
N° rabbits examined	23	23	23	23	23
Mortality					
found dead	0	0	0	$2^{d25,27}$	$2^{d17,20}$
sacrificed moribund	0	0	0	0	1^{d19}
%	0	0	0	8.7	13.0
Clinical signs					
localized alopecia	4	2	3	3	8
scant faeces	1	3	4	10	16
no faeces	n.d.	n.d.	n.d.	1	11
soft/liquid faeces	n.d.	n.d.	n.d.	n.d.	1
orange urine	n.d.	n.d.	2	9	9
red substance in pan	n.d.	n.d.	1	0	4
decreased motor activity, loss righting reflex	n.d.	n.d.	n.d.	n.d.	1
Feed consumption (g/day)					
Day 6-9	141.3	154.2	168.7	142.9	90.9*
Day 9-15	135.4	160.5	149.4	119.0	60.3**
Day 15-21	153.8	167.0	159.3	123.5	86.4**
Day 21-24	143.5	143.1	137.4	101.2*	104.7*
Day 24-29	90.0	94.2	85.4	89.4	64.2
Day 6-29	142.8	145.2	139.5	118.8*	89.6*
Body weight (kg)					
Day 0	4.02	4.01	4.00	3.98	4.01
Day 29	4.36	4.40	4.37	4.22	3.98**
Corrected body weight (kg)	3.84	3.88	3.86	3.83	3.67
Body weight gain (g)					
Day 0-6	50	70	80	80	60
Day 6-9	10	10	50	20	-40**
Day 9-15	90	120	90	30	-160
Day 15-21	90	110	90	30	60
Day 21-29	50	90	60	30	-70
Day 6-29	320	320	300	170	-200**
Day 0-29	390	390	380	260	50**
Gravid uterine weight (g)	517.8	524.5	516.5	461.6	420.1

Foetal data

A statistical significant decrease in female foetus weight was observed at the top dose (table above). The decrease of the proportion of male life foetuses (24%) and the increase (4-times) of % resorbed foetuses/litter at the top dose could be biologically relevant despite did not attain statistical significance at the top dose (table below). There was a dose dependent increase of animals showing absence of intermediate lung lobe. Despite the relatively high spontaneous incidence of the variation in this strain, the effect is probably also of toxicological significance since the incidence was not covered by the historical control data (table: 'Historical control data incidences for alterations…'). Other effects, including small kidney, fused caudal vertebrae, incompletely ossified sternal centra, and absent hind paw phalanges are minimally but significantly increased on litter incidence base at the top dose. Historical control data were also exceeded for these effects (table: 'Historical control data incidences for alterations…'). A delay in ossification was observed at 75 mg/kg bw/day and above (sternal centres) and at the top dose (hind limb phalanges, see table below), although these effects were covered by historical control data (table: 'Historical control data incidences for alterations…').

Table: Main developmental toxicity study of clothianidin in rabbits. [m] malformation; [v] variation; * = Statistically significant different from control for $p < 0.05$. ** = Statistically significant different from control for $p < 0.01$.

Dose (mg/kg bw/d)	0	10	25	75	100
Number of surviving pregnant females	21	23	22	20	20
Aborted/premature litters (%)	3 (13.0)	0	0	1.2 (13.0)	6.2
Litters with 3 live foetuses	0	0	2	17	(34.8*) 1
Included in analysis	18	23	20		11
Corpora lutea	9.4	9.6	9.9	8.7	9.8
Implantations	8.6	8.9	9.2	8.0	8.8
Resorptions early/late	0.1/0.2	0.0/0.1	0.1/0.2	0.0/0.2	0.9/0.4
% Resorptions	3.1	1.4	3.6	3.4	12.7
Live foetuses	8.3	8.7	8.8	7.8	8.2
Live male foetuses (%)	51.8	47.2	50.2	48.8	39.6
Foetal weight (g)					
male	44.2	43.2	40.7	40.7	37.7
female	43.0	42.2	40.0	40.2	36.1**
Variations (%)					
litter incidence	22.2	30.4	45.4	29.4	45.4
foetal incidence	2.7	3.5	9.5**	4.5	8.2**
% malformations					
litter incidence	0.0	21.7	9.1	11.8	27.3
foetal incidence	0.0	3.0**	1.7	1.5	5.9**
Litters with ≥ 1 variation (%)	2.6	3.8	13.6	5.0	11.5
Litters with ≥ 1 malformation (%)	0.0	2.9	1.5	1.6	9.0
External medially rotated hind limbs[m]					
litter					
foetus	0	0	1 (4.5) 3 (1.7**)	0	0
Visceral intermediate lung lobe absent [v]					
litter	0	0	0	3 (17.6)**	5 (45.5)**
foetus	0	0	0	3 (2.2)	8 (9.4)**
Visceral small kidney[m]					
litter	0	0	0	0	1 (9.1)
foetus	0	0	0	0	3 (3.5)**
Skeletal fused caudal vertebrae[m]					
litter	0	0	1 (4.5)	0	2 (18.2)**
foetus	0	0	2 (1.1)	0	2 (2.4)**
Skeletal incompletely ossified sternal centra[v]					
litter	0	0	0	0	2 (18.2)**
foetus	0	0	0	0	2 (2.4)
Skeletal absent hind paw phalanges[m]					
litter	0	0	0	0	2 (18.2)**
foetus	0	0	0	0	2 (2.4)**
Ossification sites: number per foetus/litter					
Sternal centres					
foetus (HCD: 3.81-3.97, mean: 3.91)	3.99	3.91	3.94	3.83	3.76
litter	0.03	0.14	0.13	0.2**	0.25**
Hindlimbs phalanx					
foetus (HCD: 11.99-12.00, mean: 12.0)	12.00	12.00	12.00	12.00	11.78
litter	0.00	0.00	0.00	0.00	0.63**

Table: Historical control data incidences for alterations reported in table: 'Maternal effects of clothianidin noted in the developmental toxicity study in rabbits'.

Effect	Mean (%)		Range (%)	
	Foetal	Litter	Foetal	Litter
Intermediate lung lobe absent	1.54	10.1	0-4.4	0-27.8
Small kidney	no reported data			
Fused caudal vertebrae	0.21	1.4	0-1.9	0-5.9
Incompletely/not ossified sternebrae	0.21	1.8	0-1.2	0-5.9
Absent hind paw phalanx	0.02	0.2	0-0.6	0-5.9

Conclusions of this study

At dose of 75 mg/kg bw/day and higher 2 deaths and at top dose 1 sacrifice occurred, reduced food intake and faecal output, but no significant decreased body weight gain. Additionally, at 100 mg/kg bw/day an increase of abortions or prematurely deliveries were noted. A reduced number of lung lobes in 3 foetuses out of 3 litters at 75 mg/kg bw/day and 8 foetuses (9.4%) out of 5 litters (45.5%) at 100 mg/kg bw/day were observed. The missing lung lobes appear to indicate a dose-related effect on lung branching morphogenesis at maternally toxic doses. At the top dose of 100 mg/kg bw/day some malformations were slightly, but significantly increased on litter incidence base like small kidney, fused caudal vertebrae, incompletely ossified sternal centra, and absent hind paw phalanges.

Comparison with the criteria

Fertility

The 2-generation reproduction toxicity study with clothianidin reported a reduction in sperm motility (see table: 'Gross pathology and histopathology in adult animals…'). RAC notes that there are notable differences in sperm parameters between rats and humans since the volume of sperm in humans is lower than in rodents. Thus, this reduction in sperm motility caused by clothianidin is an issue of potential concern in humans.

This same 2-generation reproduction toxicity study reported minor (2.3 days) but statistically significant delays in vaginal opening of F1 litters (the effect was not assessed in F2) at the top dose (see table: 'Gross pathology and histopathology in adult animals …'). During the public consultation the applicant submitted a position paper entitled *"Evaluation of the DART Database for Clothianidin"* with a covariate analysis between the day of vaginal opening and pup body weight at post-natal day 21. When this interaction is used as a covariate in the statistical analysis, the delay in vaginal opening at the high dose is no longer statistically significant (table below). Thus, RAC notes that clothianidin did not significantly affect pubertal development in female rats and therefore this effect does not support a classification.

Table: Age at puberty and weight at post-natal day 21 in F1 generation pups reported at the rat reproductive study on clothianidin. ** = Statistically different from control for p ≤ 0.01. [a] Mean ± SE. [b] Mean ± SD.

ppm:	0	150	500	2000
Mean age of preputial separation (days)[a]	41.2 ± 0.27	41.9 ± 0.25	42.5** ± 0.30	47.9** ± 0.53
Mean body weight at preputial separation (g)[b]	190.1 ± 17.6	198.7 ± 12.8	191.9 ± 19.4	189.7 ± 15.4
Mean age of vaginal opening (days)[a]	32.4 ± 0.31	32.2 ± 0.32	32.1 ± 0.46	34.7** ± 0.45
Mean body weight at vaginal opening (g)[b]	104.6 ± 13.0	104.4 ± 10.6	98.8 ± 10.6	90.6 ± 14.3

A more severe and statistically significant delay in male sexual maturation was noted in F1 litters (effect was not assessed in F2) exposed to 500 ppm (1.3 days) and to 2500 ppm (6.7 days) (tables 'Gross pathology and histopathology in adult animals…' , 'F1 male sexual maturation in the 2-generation…' and 'Age at puberty and weight at post-natal day 21…'). On the contrary to the case of vaginal opening delay, the pup weight at post-natal day 21 did not significantly variate with the dose ('Age at puberty and weight at post-natal day 21…'). It suggests that the delay in preputial separation is indeed a clothianidin-related effect. The applicant plotted in the position paper the day of preputial separation versus post-natal day 21 pup weight (see the figure below). As can be seen from the graph, the lines for the control and mid-dose groups have similar slopes and overlap somewhat in the distribution of their data points. In contrast, the slope of the line and distribution of data points for the high dose group differ substantially from that of the control group. The results of this analysis indicate that 2500 ppm clothianidin had a significant effect on preputial separation that was independent of its effect on pup growth in general.

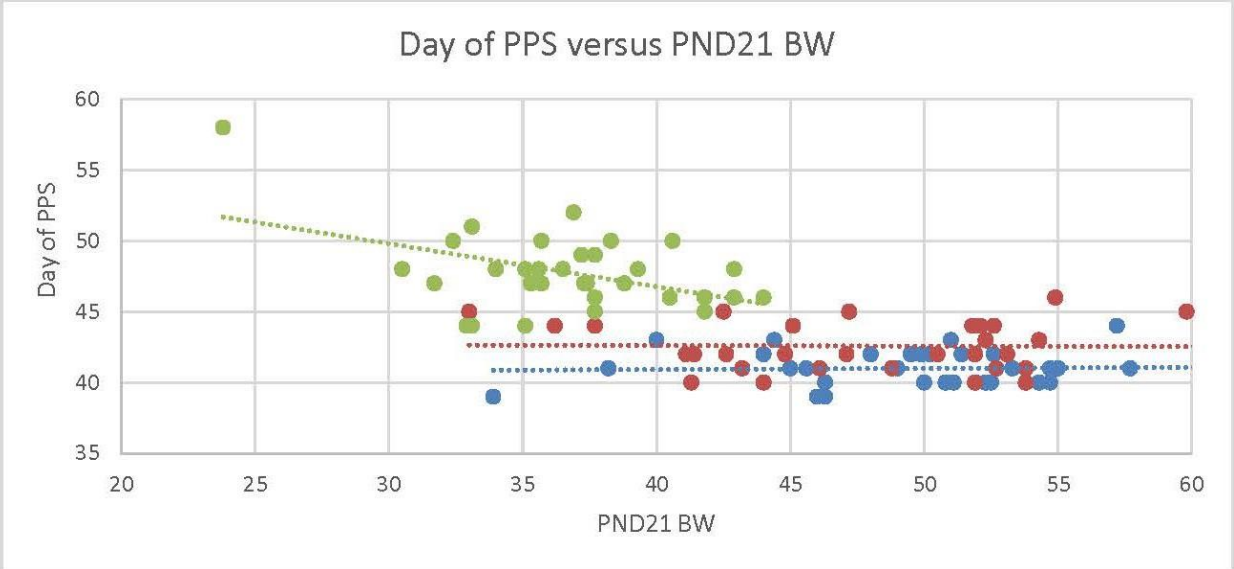


Figure 1. Day of F1 preputial separation (PPS) as a function of the PND 21 body weight (BW) in grams in the rat reproductive study of clothianidin (Freshwater and Astroff, 2000). Blue = control group; red = mid-dose group; green = high dose group.

The applicant considered that the effect on preputial separation at 2500 ppm is secondary to general systemic toxicity derived from undernutrition. However, it is unclear to RAC that general toxicity could justify such a delay since a clear dose-response is observed (1.3 days of delay at 500 ppm and 6.7 days of delay at 2000 ppm). The applicant also argued that no effect on male and female sexual maturation was noted in a developmental neurotoxicity study (see details below in the section "*Supplemental information*") and therefore the effect noted in the 2-generation study would be also incidental. However, RAC notes that in the developmental neurotoxicity study the top dose was slightly lower than in the 2-generation study and therefore the results of this study do not allow ruling out that the effect of clothianidin on male sexual maturation.

In the last position paper submitted by the applicant in August 2021, it was estimated that the actual dose received by F1 pups at weaning time was indeed 3.3 times higher than the P animals fed at the same dose level. Applicant speculated with the possibility that this high dose could have been responsible of a notable bodyweight reduction that could justify the delay in preputial separation. RAC notes that, as the same position paper states, in this multigenerational study, the weekly body weights and food consumption measurements for the F1 generation did not start to be collected until all litters had been weaned. It means that the values presented post-weaning are not accurate compared to body weight and food consumption values collected for the P generation or other general toxicity studies. Therefore, the systemic toxicity is not well documented and it does not allow RAC to diminish the relevance of the 7 days delay in the preputial separation, especially considering the above described dose-response response.

RAC concludes that the delay in preputial separation noted in the 2-generation toxicity study was indeed a treatment-related effect. Therefore, RAC proposes the classification of clothianidin for fertility and sexual function as Repr. 2 (H361f) mainly based in the delayed male sexual maturation and supported by alterations in sperm motility.

Development

The developmental toxicity study in rats showed no remarkable developmental toxicity at the top dose (causing negative body weight gain between days 6-9 and a reduction of corrected body weight gain of 45% during days 6-20). The results of this study do not support a potential classification of clothianidin for developmental toxicity.

The developmental toxicity study in rabbits showed statistically significant increments in the incidences of visceral intermediate lung lobe absent, visceral small kidney, skeletal fused caudal vertebrae, skeletal incompletely ossified sternal centra, skeletal absent hind paw phalanges and in the ossification sites (sternal centers and hind limbs phalanx) in rabbits fed with 2000 ppm clothianidin (table: 'Main developmental toxicity study of clothianidin in rabbits'). The incidences of visceral intermediate lung lobe absent was also statistically increased in rabbits fed with 500 ppm clothianidin (table: 'Main developmental toxicity study of clothianidin in rabbits'). However, RAC notes that in most of the cases, these increased incidences are still within the historical control data and moreover, the top dose of 2000 ppm caused 13% mortality (includes 2 dead and 1 sacrificed animal out of 23 animals); which is above the limit value of 10% of mortality established in the CLP Regulation to be considered as excessive maternal toxicity. Therefore, the data for 2000 ppm dose level shall not be considered by RAC for further evaluation.

The incidence of visceral intermediate lung lobe absent in rabbits fed with 500 ppm clothianidin is statistically significantly higher than the concurrent control and above the historical control data. The applicant argued in the position paper that lobation of the lung in rabbits is similar to that for humans and absence of the intermediate lung lobe occurs due to failure in formation of a lung

fissure and do not involve the absence of lung tissue or alter lung functionality. Moreover, RAC notes that these effects appear at dose causing 9% mortality (borderline with the 10% limit that would avoid considering this effect as supportive of developmental toxicity). Overall, RAC considers that the absence of visceral intermediate lung lobe does not warrant a classification for developmental toxicity.

The CLH report noted an increase in early stillborn pups at 500 ppm (F2 generation) and at 2500 ppm (F1, F2 generations) (table: 'Pup data in the 2-generation reproduction toxicity study…'). However, applicant noted in the released comments that according to the original study report the percent of stillborn pups in both generations did not differ statistically across groups. Although the incidences were increased with treatment (table below), they also were reported to be within the laboratory's historical control range of 0-3.9% (mean 1.9%). Further, there was no significant effect on the live-birth index.

Table: Stillborn pup incidence (% stillborn) and live-birth index reported in the rat reproductive study of clothianidin according to the applicant's comments. [a] No. live pups per litter/total pups per litter x 100

ppm:	0	150	500	2500
		Stillborn pups – # (pup %)		
P generation	0 (0)	3 (0.7)	6 (1.5)	9 (2.3)
F1 generation	1 (0.3)	3 (0.9)	8 (3.1)	13 (3.7)
		Live-birth index[a]		
P generation	100	99	98	98
F1 generation	100	99	97	97

Further support to the lack of effect of clothianidin on stillborn incidence was found in the neurotoxicity and immunotoxicity developmental studies where such effect was not reported (see below supplemental information for details). Overall, RAC notes that the alterations in stillborn incidence reported in the CLH report are not enough for supporting classification as developmental toxicity.

According to the CLH report, the viability index in F1 rats exposed to 2000 ppm clothianidin was reduced to 92.4% (table: 'Pup data in the 2-generation reproduction toxicity study…'). The applicant released comments clarifying that the viability index for the F1 generation at 2500 ppm was miscalculated since included in this calculation a value of 0% viability for a dam (PF3108) that died prior to post-natal day 4. The fact that all of her pups died prior to PND 4 is a function of her death; thus, the value derived from this animal should have been excluded from the calculations. When calculated correctly, the viability index for the F1 generation animals at 2500 ppm is 95.7% (table below); which is a value that RAC does not consider of concern for supporting a classification for developmental toxicity.

Table: Corrected viability index (%) reported in the rat reproductive study of clothianidin.
[a] The value provided in the study reported (92.4%) was miscalculated due to inclusion of dam PF3108 that died prior to PND 4. The value given herein has been calculated based on exclusion of this dam from the calculation.

ppm:	0	150	500	2500
F1 generation	99.2	99.4	98.7	95.7[a]
F2 generation	98.0	98.1	98.8	97.0

Other effects noted in the 2-generation toxicity study was reductions in body, thymus and spleen weights in pups exposed to 2000 ppm clothianidin (table: 'Pup data in the 2-generation reproduction toxicity study…'). However, RAC also notes that the reductions in body weights and in thymus weight was also reported in adult animals and therefore could be interpreted as systemic rather than developmental effects. In the same way, the reductions in spleen weight in pups could also be secondary to reductions in body weight.

In conclusion, RAC notes that none of the developmental effects reported in the CLH report is strong and robust enough for supporting a classification of clothianidin for developmental toxicity.

LACTATION

The classification is intended to indicate when a substance may cause harm due to its effects on or via lactation and is independent of consideration of the reproductive or developmental toxicity of the substance. This can be due to the substance being absorbed by women and adversely affecting milk production or quality, or due to the substance (or its metabolites) being present in breast milk in amounts sufficient to cause concern for the health of a breastfed child. The available reproductive study does not provide evidence of adverse effects in the offspring due to transfer in the milk or adverse effect on the quality of the milk. Toxicokinetic studies do not indicate the likelihood that the substance can be potentially present in breast milk. Thus, there were no effects to warrant classification of clothianidin for effects on or via lactation and RAC proposes no classification for this category hazard.

Overall, RAC considers that **clothianidin should be classified for fertility and sexual function as Repr. 2 (H361f).**

Supplemental information - In depth analyses by RAC

Developmental neurotoxicity study (Hoberman, 2000)

The study was performed according to US-EPA Health Effects Test Guideline OPPTS 870.6300 and observing GLP. Twenty-five naturally mated female Sprague-Dawley rats per group were fed with 0, 150, 500 and 1750 ppm clothianidin (purity 95.9% analysed after dosage completion) from gestation day 0 to 22 post-partum. The dosages corresponded to 0, 12.9, 42.9, 142.0 mg/kg bw/day during gestation and 0, 27.3, 90.0 and 299.0 mg/kg bw/day during lactation.

No treatment-related clinical signs were recorded in dams. Natural delivery observations were unaffected by treatment and all pregnant dams delivered litters. Duration of gestation, the number of implantation sites, the gestation index, the number of dams with stillborn pups and of dams with all pups dying were comparable between all groups.

There was no effect of treatment on either the number of stillborn pups or on the mean number or percent of live born pups observed (table below).

Table: Stillborn pup incidence (% stillborn) and live-birth index reported in the rat developmental neurotoxicity study of clothianidin.

ppm:	0	150	500	1750
Stillborn pups: mean (%)	1 (0.3)	1 (0.3)	6 (1.8)	3 (0.9)
Live born pups: mean (%)	13.5 (99.7)	14.1 (99.7)	14.2 (98.2)	13.8 (99.1)

The day of vaginal opening were 47.1, 46.8, 47.7 and 48.3 for 0, 150, 500 and 1750 ppm; respectively. Therefore, there was no alteration in female sexual maturation in this study. The day of preputial separation were 34.0, 33.7, 35.0 (statistically different from control for $p \leq 0.01$) and 34.1 for 0, 150, 500 and 1750 ppm; respectively. The statistically significant increase reported at 500 ppm does not observe dose-response and therefore this finding is considered incidental and not treatment-related. Therefore, clothianidin did not alter the male sexual maturation in this study.

Developmental immunotoxicity study (Hoberman, 2008)

In the developmental immunotoxicity study clothianidin was administered in the diet at concentrations 0, 150, 500 or 2000 ppm from gestation day 6 through weaning and until sacrifice of the F1 generation pups for a functional assessment of the immune system. The CLH dossier does not contain information about the doses in terms of mg/kg bw/day or whether the study was performed observing a normalized guideline or GLP. The incidences of stillborn and live born pups observed in this study are shown in the table below. Although the mean number of live born pups was statistically ($p \leq 0.05$) reduced at 500 ppm, this was not considered a treatment-related effects as it was not dose-related. Further, no statistically significant effect on the incidence of stillborn pups was observed.

Table: Stillborn pup incidence (% stillborn) and live-birth index reported in the rat developmental immunotoxicity study of clothianidin. * = Statistically different from control for $p \leq 0.05$.

ppm:	0	150	500	2000
Stillborn pups: mean (%)	0 (0)	0 (0)	1 (0.3)	3 (0.9)
Live born pups: mean (%)	13.9 (99.7)	12.2 (100)	11.8* (99.3)	13.2 (99.1)

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Acute neurotoxicity studies, a 90-day neurotoxicity study and a developmental neurotoxicity study were conducted on Clothianidin in the rat. However, in this section only acute neurotoxicity studies were presented, because findings of these studies (and acute toxicity studies) were used to evaluate the need for a classification for STOT SE (please refer to chapter 4.3. Specific target organ toxicity – single exposure, STOT SE). Furthermore, a pharmacological study with Clothianidin was performed with mice and rats *in vivo*.

Acute neurotoxicity studies revealed transient neurotoxic changes consisting of decreased arousal and decreased motor activity but there were no treatment related neuropathological changes. Impairment of motor activity was reported in mice (starting at 50 mg/kg bw) and rats (starting at 100 mg/kg bw) upon oral administration. These dose levels did not induce mortalities in tested animals. At higher dose levels effects aggravated. No narcotic effects were reported. Clothianidin is a known neurotoxic compound; therefore, the observed neurotoxicological findings are attributed to treatment.

Additionally, in the pharmacological study in mice a synergistic effect on convulsions with electroshock at 25 mg/kg bw was noted: Both tonic flexor and tonic extensor convulsions were induced in the presence of subthreshold electroshock application.

Both the 90-day neurotoxicity and the developmental neurotoxicity studies were presented in the RAR.

Further details on materials and methods, guideline (and deviations, if any), doses, number of animals/group and sex, study duration, information on incidences and severities of findings and extent of changes relative to controls are given in the text below or in chapter B.6 of the RAR.

Table 27: Summary of neurotoxicity data

Type of study / (reference)	Dose levels ppm and/or mg/kg bw/day	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Effects at LOAEL
Acute oral neurotoxicity in Fisher 344 rats (Anonymous (10), 2000), Key study	0, 100, 200, 400 mg/kg bw	(male): <100 (female): 100	(male): 100 (female): 200	Reduced locomotor activity in both sexes and hypothermia in females
Supplemental acute oral neurotoxicity in male Fisher 344 rats (Anonymous (12), 2000)	0, 20, 40, 60 mg/kg bw	(male): 60	-	No neurotoxic effects
Acute oral dose range-finding study in Fischer 344 rats (Anonymous (9), 2002)	290, 523, 1216 mg/kg bw	290	523	Decreased activity and tremors, one female death
90-day neurotoxicity in Fisher 344 rats (Anonymous (10), 2000)	0, 150, 1000, 3000 ppm (males): 0, 9.2, 60, 177 mg/kg bw/day (females): 0, 10.6, 71, 200.1 mg/kg bw/day	Systemic tox.: males: 60 females: 71 Neurotox.: males: 177 females: 200	Systemic tox.: males: 177 females: 200 Neurotox.: No effects	Mortality, reduced body weight and food consumption. No neurotoxic effects
Developmental neurotoxicity in Sprague-Dawley rats (Anonymous (6), 2000)	0, 150, 500, 1750 ppm (GD 0-20): 0, 12.9, 42.9, 142.0 mg/kg bw/day (LD 1-22): 0, 27.3, 90.0, 299.0 mg/kg bw/day	Parental tox.: 42.9 systemic tox. offspring: 12.9 offspring Neurotox.: 42.9	Parental tox.: 142 systemic tox. offspring: 42.9 offspring. Neurotox.: 142	Reduced F0 body weight Reduced pup weight F1 motor activity
Acute pharmacological study, oral, rats (Anonymous (14), 2000) Key study	0, 30, 100, 300, 1000, 3000 mg/kg bw	100 mg/kg bw	300 mg/kg bw	Body temperature and blood pressure ↓, heart rate ↑
Acute pharmacological study, oral, mice (Anonymous (14), 2000) Key study	0, 6.25, 12.5, 25, 75, 225 mg/kg bw	12.5 mg/kg bw	25 mg/kg bw	Synergistic effect on convulsions, intestinal transport ↓ 50 mg/kg bw: motor activity ↓, tremor, deep respiration

GD = gestation day; LD = lactation day

Report:	Anonymous (11), 2000; See also: Anonymous (9), 2002
Guideline(s):	U.S. EPA FIFRA, Guideline 81-8(SS) and U.S. EPA. Health Effects Test Guidelines. OPPTS 870.6200
Deviations:	No OECD- and EU-guideline indicated
GLP:	Yes
Acceptability:	Yes

Results and discussions:

The dose levels were 0, 100, 200 and 400 mg/kg bw/d (12 animals/sex/dose). Based on analytical results, the actual doses of Clothianidin were 0-102-213-373 mg/kg bw for males and females.

Mortality: There were no deaths during the study.

Body Weight: There were no treatment related effects on body weight or body weight gain.

Clinical Observations: At the top-dose and in both males and females, tremors, decreased activity and ataxia were observed. In 3/12 females, urine stain was observed and 1/12 females exhibited red nasal and oral stain. Urine stain was also observed in 1/12 females in both control and low-dose group. These signs appeared on the day of treatment, and persisted up to day 1.

Results of clinical observations and functional observation battery are shown in the table below.

FOB: Decreased activity, pin-point pupils, an uncoordinated righting response and decreased body temperature were observed in a number of animals at 400 mg/kg bw and in a few animals at 200 mg/kg bw. Tremors and uncoordinated gait were also observed in high dose animals. A few males showed decreased activity at 100 mg/kg bw but no other signs were noted at this dose level. There were no treatment related effects on remaining endpoints, including forelimb and hindlimb grip strength and landing foot splay.

In general, all findings at either dose or sex were restricted to the day of treatment (d0). Most endpoints revealed substantial effects at the top dose in both males and females (tremors, hypoactivity, decreased arousal, miosis following light stimulus, decreased aerial righting response, hypothermia), with additionally gait incoordination and reduced approach response in the males. Effects on arousal were detected at 100 mg/kg and above in the males, and at 200 mg/kg and above in the females.

Motor and locomotor activity: Biologically significant dose related reductions in motor activity were observed after dosing on day 0 in all male dose groups and in females at 200 and 400 mg/kg bw, as shown in the table below.

Table 28: Group mean motor activity results (% difference from control)[1]

Dose level (mg/kg bw)	Pre-treatment	Day 0	Day 7	Day 14
Males				
100	-8	-23	-5	+1
200	+7	-59	+14	+8
400	-18	-81	-14	-5
Females				
100	+11	+8	+17	-1
200	+3	-43	+12	+3
400	0	-72	+15	-4

[1] = % greater (+) or lower (-) than control, biologically significant differences from control highlighted in bold type (statistically significant differences were observed during intervals 1, 2 or 3 of 9 intervals altogether (see table 30 below); group mean motor activity results over all 9 intervals, as displayed in this table, were not statistically significant)

The biologically significant cut off was determined to be ± 20 % of the control mean based on the degree of intergroup pre-treatment variability. The results in the table above represent the summary of the whole 90-minute assessment that did not result in statistically significant differences. Statistically significant group differences in motor and locomotor activity mirroring the findings in the Table above were noted on day 0 for the first 2-3 test intervals (see table 30 below).

Table 29: FOB and Figure-eight maze observations in acute neurotoxicity study of Clothianidin in rats

Dose (mg/kg bw)		0		100		200		400	
		m	f	m	f	m	f	m	f
FUNCTIONAL OBSERVATION BATTERY[a]									
Home cage observations									
Tremors	score 1 score 2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7* 1	9* 2
Decreased activity	score 1	n.d.	n.d.	n.d.	n.d.	1	0	8*	11*
Open field observations									
Posture	standing normally sitting or lying normally	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	7 5	7 5
Tremors	score 1 score 2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10* 1	6* 5
Gait incoordination		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1	0
Arousal:	sluggish exploratory movemements sluggish minimal movement	1 0	0 0	3 1	0 0	4 1	2 1	9* 0	9* 2
Reflex/Physiologic/manipulative observations									
Approach response:	no reaction	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1	0
Touch response:	no reaction	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0	2
Pupil response on light:	pin-point constriction	n.d.	n.d.	n.d.	n.d.	1	0	8*	9*
Aerial righting response:	slightly uncoordinated landing on side	0	1	0	1	2	0	2 0	3 1

Dose (mg/kg bw)	0		100		200		400	
Body temperature (°C)	36.5	36.4	36.1	36.3	34.8*	35.1*	32.8*	32.7*
FIGURE-EIGHT MAZE [b]								
Motor activity§	n.d.	n.d.	↓23% (1)*	-	↓59% (1,2)*	↓43% (1,2)*	↓81% (1,2)*	↓72% (1,2,3)*
Locomotor activity	n.d.	n.d.	↓37% (1,2)*	-	↓62% (1,2)*	↓45% (1,2)*	↓88% (1,2)*	↓83% (1,2)*

n.d.: dose level tested but not displayed in CLH report; Observations recorded on d0; statistically significant modifications with ANOVA+Dunnetts's t-test *p<0.05;
[a] : Incidences /12 animals (except body temperature); score 1/score2= severity grade of observation; [b]: percentual differences with concurrent control (number of movements during 9*10-minute-intervals); §statistically significant differences were observed during intervals 1, 2 or 3 of the 9 intervals; Comparison of measurements at d0 with the pre-treatment (d-7) values confirmed the historical control variability of ±20%.

Sacrifice and pathology:
There were no treatment related macroscopic or microscopic findings or effects on body weight and brain weight at termination in Clothianidin exposed groups.

Conclusions:

Neurotoxic effects (reduced locomotor activity) were observed in male rats at lowest dose of 100 mg/kg bw and in females at 200 mg/kg bw (reduced locomotor activity, hypothermia).

Report: Anonymous (12), 2000. See also: Anonymous (9) 2002

Guideline(s): Conducted as a supplemental study for US EPA FIFRA Guideline 81-8(SS) and US EPA OPPTS Guideline 870.6200

Deviations: No OECD- and EU-guideline indicated. Animals were sacrificed on d2 rather than on d15 of the study; no body weight measurement, and no gross pathology neither histopathological examination was performed. Since the study was designed to clarify the effects in male animals at d0 (day of treatment) observed during a previous and complete study, and in order to detect the NOAEL for males, the deviations are not considered to invalidate the study results.

GLP: Yes

Acceptability: Yes

Results and discussions:

Mortality: There were no deaths during the study.

Clinical Observations: No treatment related clinical signs were observed after dosing in any dose group.

Body Weight: Body weights were not measured after treatment because a NOEL for body weight changes was established in the previous study.

Neurobehavioural assessment: No treatment related findings were observed in the FOB assessment approximately 4 hours after dosing.

No biologically significant dose related reductions in motor activity were observed after dosing on day 0 as shown in (see table below).

Table 30: Group mean motor activity and locomotor activity results (% difference from control)[1]

	Motor activity		Locomotor activity	
Dose level (mg/kg bw)	Pre-treatment	Day 0	Pre-treatment	Day 0
20	-21	+9	-17	+17
40	-15	+9	-19	+2
60	-11	-5	-13	-9

[1]= % greater (+) or lower (-) than control

The biologically significant cut off was determined to be approximately ± 20 % of the control mean based on the degree of intergroup pre-treatment variability. The results in the table above represent the summary of the whole 90-minute assessment. No statistically significant group differences in motor activity and locomotor activity were observed at any of the test intervals. Habituation was evident by a decrease in activity in all groups, including the control, after the first test interval but was not affected by exposure to Clothianidin.
Sacrifice and pathology: Necropsy not performed.

Conclusion:

There was no evidence of treatment related neurobehavioural changes or signs of toxicity up to the highest dose level of 60 mg/kg bw. Therefore, in combination with the results of the previous study a NOAEL of 60 mg/kg bw for males was established for the acute oral neurotoxicity of Clothianidin.

Report: Anonymous (14), 2000

Guideline(s): No guideline study

Deviations: Not applicable, not in compliance with guidelines;

GLP: Yes

Acceptability: Yes

Materials and methods:

In order to evaluate the pharmacological effects, male CD (SD) SPF rats and male CD-1 (ICR) SPF mice were treated in-vivo (and isolated ileum of Hartley SPF Guinea pigs, but not presented in the CLH Report, but in the RAR). In the in-vivo studies, the substance was dispersed in 0.5% (w:w or v:v) arabic gum solution and administered by single gavage at dose levels specified in the table below (dose volume 10 mL/kg).

Both rats and mice were about 5 weeks of age (body weights rats: 136-174 g, mice: 25-33 g) except for rats in the study of circulatory system: 7 weeks of age (245-319 g).

The doses were determined in previous range-finding tests were 3 animals/dose were administered 1750, 2280, 2960, 3850 and 5000 mg/kg bw (rats), or 200, 280, 930, 550, and 770 mg/kg bw (mice) and observed during 1, 2, 4, 6, 8, 12h and further daily during 1 week to monitor appearance of acute toxic signs and mortalities.

The tests and doses are summarised in Table 31 below.

Mouse experiments:

Effects on the central nervous system: general physical condition and behaviour. Irwin's multiple observation method included alertness, passivity, strereotypy, grooming, vocalisation, restlessness, irritability, reactivity, locomotor activity, response (touch, pain, startle), reflexes (Straub tail, pinna, corneal, ipsilateral flexor, pupil size), palpebral opening, exophthalmos, urination, salivation, lacrimation, writhing, piloerection, body temperature, skin color, respiration, diarrhea, tremors, twitching, convulsions, body position, gait (staggering, abnormal), muscle tone (limb, grip strength, body, abdominal), mortality. Observation periods after administration: 0.5, 1, 3, 6, 24 h.

Anaesthetic effects: At 1h after administration, hexobarbital was injected i.p. (80 mg/kg) and the animals were laid on a ~37 °C warming plate. Sleeping time, defined as the time between disappearance and recovery of the righting reflex, was recorded.

Synergystic effects on convulsions (subthreshold stimulations):

- electroshock stimulation: At 1h after administration, a current of 8.0 mA was applied via corneal electrodes (pulse duration 5 ms; stimulation interval 10 ms, stimulation period 0.6 ms). Appearance of tonic flexor and extensor convulsions was monitored immediately after the current pulse.

- pentylenetetrazole (PT) stimulation: At 1h after administration, PT was injected s.c. (55 mg/kg). Appearance of clonic and tonic extensor convulsions was monitored for 30' after injection.

Effects on the gastrointestinal system: At 1h after administration (subsequent to a fasting period of about 19h), charcoal (5% suspension in 5% arabic gum) was given by gavage (0.2 mL/animal). Animals were sacrificed by cervical dislocation and gastric tube was isolated 30' after charcoal gavage. Both length from pylorus to the farthest point reached by the carcoal (a) and total small intestine length (b) were measured. Transfer rate was expressed as $^{a}/_{b}$ in %.

Effect on the skeletal muscles: At 1, 3 and 6h after administration, animals were placed with their front paws on a horizontally stretched wire, and observed whether they would grasp the wire with their hind paws within 10 s. Previously, only those animals successful in grasping the wire within 5 s were selected for the experiment.

Rat experiments:

Effect on body temperature: Before and 0.5, 1, 3 and 6h after administration, rectal temperature was registered with a digital thermometer.

Effect on the blood coagulation system: At 1h after administration, blood was sampled from the inferior vena cava under ether anaesthesia. Trisodium citrate (3.2 %) was added (1:9 v:v) and plasma was separated by centrifugation (4 °C, 3000 rpm, 10'), and both prothrombin and activated thromboplastine time was determined using a fully-automatic coagulometer.

Effects on the circulatory system: Before and at 0.5, 1, 3 and 6 h, systolic and mean blood pressure was determined plethysmographically by inserting the tail into the cuff of a non-intrusive automatic blood pressure meter. Heart beat was determined based on the pulse wave.

Results and discussions:

Table 31: Summary of pharmacological studies on Clothianidin

Tests, species	Route	Dose (mg/kg bw)	No. of animals/dose group	LOAEL (mg/kg bw)	NOAEL (mg/kg bw)
Observation of physical condition and behaviour, mice	oral	12.5, 25, 50, 100, 200, 400	3	50	25
Anesthetic effect, mice	oral	25, 75, 225	8	225	75
Synergistic effect on convulsions (electroshock), mice	oral	6.25, 12.5, 25, 75, 225	10	25	12.5
Synergistic effect on convulsions, pentylenetetrazol, mice	oral	25, 75, 225	10	>225	225
Body temperature, rat	oral	30, 100, 300, 1000, 3000	6	300	100
Blood pressure and heart rate, rat	oral	4	4	300	100
Intestinal transport, mice	oral	25, 75, 225	8	25	12.5
Muscle strength, mice	oral	25, 75, 225	8	225	75
Blood coagulation, rat	oral	300, 1000, 3000	6	>3000	3000

General physical conditions and behaviour in mice: Effects were observed at 50 mg/kg and above (see table 33 below). In an additional separate experiment, where Clothianidin was administered at 0, 12.5 and 25 mg/kg bw, no effects were observed.

Table 32: General physical condition and behaviour after administration of Clothianidin to male mice

Endpoint	Dose (mg/kg bw)			
	50	100	200	400
Decrease in spontaneous locomotor activity	$3^{+;0.5h}$	$3^{+,++;\ 0.5\text{-}3h}$	$3^{+,++;\ 0.5\text{-}3h}$	$3^{+,++;\ 0.5\text{-}6h,1d}$
Tremor	$1^{+;0.5h}$	$1^{+;0.5\text{-}1h}$	$3^{+,++;\ 0.5\text{-}3h}$	$3^{+,++;\ 0.5\text{-}6h,1d}$
Deep respiration	$1^{+;1h}$	$3^{+;\ 0.5\text{-}3h}$	$2^{+;\ 0.5\text{-}3h}$	$3^{+,++;\ 0.5\text{-}6h}$
Hypothermia	-	$3^{+;\ 1\text{-}3h}$	$3^{+;\ 0.5\text{-}3h}$	$3^{+,++;\ 0.5\text{-}6h}$
Decrease in grooming	-	$3^{+;\ 0.5h}$	$3^{++;0.5\text{-}1h}$	$3^{++;\ 0.5\text{-}6h}$
Mydriasis	-	$2^{+;++\ 0.5\text{-}3h}$	$2^{+;\ 0.5\text{-}3h}$	$2^{+,++,+++;\ 0.5\text{-}6h}$
Decrease in reactivity	-	$1^{+;\ 0.5\text{-}3h}$	$3^{+;\ 0.5\text{-}3h}$	$3^{++;\ 0.5\text{-}6h}$
Prone position	-	$1^{+;\ 0.5\text{-}3h}$	$3^{+;\ 0.5\text{-}3h}$	$3^{+,++;\ 0.5\text{-}6h}$

Endpoint	Dose (mg/kg bw)			
	50	100	200	400
Staggering gait	-	$1^{+;\ 0.5h}$	$2^{+;\ 0.5\text{-}3h}$	$3^{+,++,+++;\ 0.5\text{-}6h,1d}$
Decrease in body tone	-	$1^{+;\ 0.5\text{-}3h}$	$3^{+;\ 0.5\text{-}3h}$	$3^{+,++;\ 0.5\text{-}6h}$
Decrease in abdominal muscle tone	-	$1^{+;\ 0.5\text{-}3h}$	$3^{+,++;\ 0.5\text{-}3h}$	$3^{+,++;\ 0.5\text{-}6h}$
Decrease in touch response	-	-	$3^{+;0.5\text{-}1h}$	$3^{+;\ 0.5\text{-}6h}$
Decrease in grip strength	-	-	$2^{+;\ 0.5h}$	$3^{+,++;\ 0.5\text{-}6h}$
Decreased limb tone	-	-	-	$3^{+,++;\ 1\text{-}6h}$
Inhibition of pinna reflex	-	-	-	$3^{+,++;\ 1\text{-}6h}$
Inhibition of ipsilateral flexor reflex	-	-	-	$1^{+++;\ 1h}$
Inhibition of corneal reflex	-	-	-	$1^{++;\ 1h}$
Straub tail	-	-	-	$1^{+;\ 1h}$
Skin cyanosis	-	-	-	$1^{++;\ 1h}$
Death	-	-	-	13^{3h}

Observation conducted before, and 0.5, 1, 3, 6h, 1d after dosing; control group (vehicle control): no observable effects. Value in table includes number of animals with finding, degree of response ($^{+}$slight, $^{++}$moderate, $^{+++}$severe) and duration of clinical sign (for all animals observed, i.e. n=3/dose).

Specific pharmacological effects in mice (see table below): The overall detected NOAEL in these experiments was 12.5 mg/kg bw, based upon synergistic effects on convulsions at 25 mg/kg bw.

Table 33: Specific pharmacological effects after administration of Clothianidin to male mice

Endpoint		n	Dose (mg/kg bw)					
			0	6.25	12.5	25	75	225
Synergistic effects on convulsions [a]								
Electroshock [c]	TF TE	10	1 1	3 3	2 2	8* 8*	10* 10*	10* 10*
Pentylenetetrazole	CL TE	10	0 2	n.a. n.a.	n.a. n.a.	2 0	0 0	0 0
Muscle tension suppression	1 h 3 h 6 h	8	0 0 0	n.a. n.a. n.a.	n.a. n.a. n.a.	0 0 0	0 0 0	4 4 2
Sleeping time[b]		8§		n.a.	n.a.	-	↑26%	↑63%*
Intestinal transfer rate[b]		8		n.a.	n.a.	↓7.5%	↓60%*	↓78%*

n: number of mice/dose; convulsion types: TF: tonic flexor, TE: tonic extensor, CL: clonic; statistical significant modifications ([a]: χ^2-test and [b]: Dunnett's test; *p<0.05); §: n=8 mice/dose, except for the top-dose group, where n=6 (2 animals died after hexobarbital injection); [c]: results from two different experiments (trial 1= dose 0, 6.25 and 12.5 mg/kg bw; trial 2= dose 0, 25, 75 and 225 mg/kg bw; incidence equal in both controls); n.a.: dose level not tested.

Specific pharmacological effects in rats (see table 35 below): From the figures, body temperature, heart rate, and blood pressure data were affected at 300 mg/kg bw and above.

Table 34: Specific pharmacological effects after administration of Clothianidin to male rats

Endpoint	n	Dose (mg/kg bw)					
		0	30	100	300	1000	3000
Rectal temperature 0.5 h 1 h 3 h 6 h	6		 - - - -	 - - - -	 ↓1.3% ↓2.1%* ↓2.9%* ↓3.9%*	 ↓2.1%* ↓2.9%* ↓6.3% ↓10.9%	 ↓2.3%* ↓4.2%* ↓10.3% ↓17.0%
Heart rate 0.5 h 1 h 3 h 6 h	4		 n.a. n.a. n.a. n.a.	 - - - -	 ↑12.3%* ↑4.2% ↑4.2% ↑10.5%	 ↑6.5% ↑4.4% ↑6.4% ↑10.0%	 ↑12.3% ↑8.8% ↑7.4% ↑11.0%
Systolic blood pressure 0.5 h 1 h 3 h 6 h	4		 n.a. n.a. n.a. n.a.	 - - - -	 - - - ↓1.8%	 - ↓13%* ↓1.8% ↓7.9%	 ↓3.4% ↓8.9% ↓4.5% ↓11.4%
Mean blood pressure 0.5 h 1 h 3 h 6 h	4		 n.a. n.a. n.a. n.a.	 - - - -	 - - - ↓4.2%	 ↓2.1% ↓15%* ↓5.4% ↓15%*	 ↓4.2% ↓10% ↓7.6% ↓12.5%
PTT, APTT	6		n.a.	n.a.	-	-	-

n: number of rats/dose; statistical significant modifications Dunnett's test trial 2; *p<0.05; PTT: ; APTT:
Results from two different experiments (trial 1= dose 0, 30 and 100; trial 2= dose 0, 300, 1000 and 3000); n.a.: dose level not tested.

Conclusions:

The mouse was the most sensitive species for the effects of Clothianidin as already seen in the toxicity studies: an NOAEL for neurotoxicity of 12.5 mg/kg bw was found in the test on synergistic effect on convulsions with electroshock in male mice. At 25 mg/kg bw and above, both tonic flexor and tonic extensor convulsions were induced in the presence of subthreshold electroshock application.

The NOAEL of observations of physical condition and behaviour was 25 mg/kg bw, slight effects were observed at 50 mg/kg bw (slight tremor in one animal, slight deep respiration in one animal and slightly decreased spontaneous locomotor activity in three animals). More and stronger effects were observed at 100 mg/kg bw and above.

In male rats, body temperature, heart rate and blood pressure were affected at 300 mg/kg bw.

For comparison with classification criteria refer to chapter 4.3.2.

4.12.1.2 Immunotoxicity

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

4.12.1.3 Specific investigations: other studies

This endpoint is not addressed in this CLH report and is outside the scope of the public consultation.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 35: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis 92/69/EEC, C.7	Half-lives at 20 °C: pH 4 and 7 = stable pH 9 = 3.84 years (1401 days)	Hydrolytic stable	Lewis, C.J. (2000a)
Photolysis in water SETAC and US EPA § 161-2	Half-lives: < 1 day in summer > 1 year in winter	photolytically degraded in pure water	Babczinski, P. & Bornatsch, W. (2000)
Photolysis in water ECETOC and UBA, 1992	Half-life: 0.61 h	test with unlabelled substance; arithmetic model	Hellpointer, E. (1999a)
Photolysis in water SETAC and US EPA 161-2	Half-life: 26.6 h	test with labelling on nitroamino and thiazolyl group	Babczinski, P. (2000); report No. MR-391/00 (BCP79)
Photolysis in air Estimation method by AOPWIN (version 1.87, 1.90, 1.91)	Half-life: 2.81 hours (24-hours-mean-day concentration $0.5x10^6$ OH radicals cm^{-3})	photodegradation in air	Hellpointner, E. (1998), Extended by Hellpointer E. in 2005
Ready Biodegradability, 92/69/EEC, C.4-C	1.5% degradation (28d)	Not readily biodegradable	Bealing, D.J. & Watson, S. (1999)
Degradation in water-sediment SETAC and German BBA, Part IV, 5-1	Entire system DT_{50} 48 and 64.8 days, resp. (20 °C), < 4.5% CO_2 (100d)	Two systems tested (pond, gravel pit) 1 degradation product identified: TMG (N-(2-chlorothiazol-5-ylmethyl)-N'-methylguanidine): max. 22.9 %.	Gilges, M. & Brumhard, B. (2000)
Aerobic soil degradation SETAC and US EPA 162-1	DT_{50} 143 – 1328 days (20 °C) Max. 16.9% CO_2 (379 days)	Two laboratory studies with 4 German soils and 6 US soils 4 degradation products identified of which 1 reached levels > 10 %	Gilges, M. (2000), Schad, T. (2000 c);

5.1.1 Stability

Hydrolysis:

Table 36: Hydrolytic degradation

Method /Guideline	pH	Temperature [°C]	Initial TS concentration, C_0 [mg/L]	Reaction rate constant, K_h [1/day]	Half-life, DT_{50}[days]	Coefficient of correlation, r_2	Reference
92/69/EEC, C.7, Preliminary test	4	50	0.3	no degradation	stable		Lewis, C. J. (2000a) A 7.1.1.1.1
	7	50	0.3	no degradation	stable		
	9	50	0.3	0.048	14.4	0.997	
92/69/EEC, C.7, Additional test	9	62	0.3	0.188	3.7	0.997	
	9	74	0.3	1.013	0.68	0.997	
US EPA, Subdivision N, Section 161-1, definitive study	5	25	0.3	no degradation	stable	-	
	7	25	0.3	no degradation	stable	-	
	9	25	0.3	4 %	*	-	

* The first-order reaction model cannot adequately fit the data as degradation is not a first-order rate process.

Clothianidin is stable in sterile buffer solutions at pH 4, 5, and 7, but degrades at pH 9. At relevant temperatures of 20 °C (293.15 K) a half-life of around 1401 days was calculated at pH 9 according to the Arrhenius equation. Relevant amounts of metabolites were formed in pH 9 only at elevated temperatures. The degradation products at pH 9 are CTNU (N-(2-chlorothiazol-5-ylmethyl)-N'-nitrourea), TZMU (N-(2-chlorothiazol-5-ylmethyl)-N'-methylurea), and ACT•HCl (2-chlorothiazol-5-ylmethylamine hydrochloride). The latter seems to be the final transformation product. In conclusion, hydrolysis is not considered to be a significant degradation route for Clothianidin at environmentally relevant temperature and pH.

Photolysis in water:

Table 37: Photolysis in water

Method /Guideline	Initial TS concentration	Total recovery of test substance [% of appl. a.s.]	Photolysis rate constant (k^c_p)	Direct photolysis sunlight rate constant (k_{pE})	Reaction quantum yield (Φ^c_E)	Half-life ($t_{1/2E}$) [hours]	Reference
SETAC and US EPA 161-2	0.3 mg/L	91.0-102.1 (nitroimino label) 94.4-102.0 (thiazolyl label)	no actinometer study	no actinometer study	not given	3.3*	Babczinski, P. & Bornatsch, W. (2000) A 7.1.1.1.2
ECETOC and UBA, 1992	5.1 mg/L	test performed with unlabelled a.s., no balance established	0.0191 min^{-1}*	not given	0.014	0.61*	Hellpointer, E. (1999a) A 7.1.1.1.2/05

Method /Guideline	**Initial TS concentration**	**Total recovery of test substance [% of appl. a.s.]**	**Photolysis rate constant (k^c_p)**	**Direct photolysis sunlight rate constant (k_{pE})**	**Reaction quantum yield (Φ^c_E)**	**Half-life ($t_{1/2E}$) [hours]**	**Reference**
SETAC and US EPA 161-2	269/266 µg/l	96.6-100.4 (nitroimino label) 95.1-100.9 (thiazolyl label)	0.0261 h^{-1}	not given	not given	26.6	Babczinski, P. (2000) A 7.1.1.1.2/03

* mean of two experiments

Under the experimental conditions used, Clothianidin degraded rapidly with an experimental half-life (DT_{50}) of 3.3 hours (mean of two labels; first order rate constant = 0.2088 h-1; r = 0.999). The experimental half-life corresponds to a calculated environmental half-life of 0.6 days under mid-summer solar light conditions at Phoenix / USA. At the same site, the time for disappearance of 90 % (DT_{90}) is calculated to be about 2.0 days. The half-life at sites with less radiation intensity will be longer. Major degradation products (> 10 % of the applied radioactivity) were TZMU (N-(2-chlorothiazol-5-ylmethyl)-N=methylurea), MG (methylguanidine), HMIO (4-hydroxy-2-methylamino-2-imidazolin-5-one), FA (formamide), MU (methylurea), and CO_2. During the continuous irradiation period of 18 days an amount equivalent to 34 % of the applied radioactivity was photo-mineralised to carbon dioxide within the thiazolyl study, whereas in the nitroimino study this value amounted only to 0.8 %. Considering the rapid photolytic breakdown determined at pH and temperature conditions typical for a natural environment, it is concluded from the studies that solar radiation will contribute to the degradation of the test substance in aquatic systems and also can contribute to the elimination of residues by means of mineralisation of the thiazole ring.

However, assessing the so-called environmental half-life by means of an arithmetical model (see Hellpointer, 1999a), besides the laboratory data (experimental quantum yield and molar extinction coefficient) the following additional parameters were taken into account:

- pure water from close to the surface (0-5 cm depth)
- 10th degree of longitude,
- clear sky,
- typical ozone concentration in the atmosphere,
- half-lives integrated over the entire day.

This estimation resulted in half-lives up to 23.4 days for the 50th degree of latitude. Another arithmetic model (see Hellpointer, 1999a), using similar marginal conditions takes cloudiness in Central Europe as an additional parameter into consideration. With this model, especially in autumn and winter high values for the environmental half-life are estimated (maximum > 1a). Although there is no doubt about, that photolysis in water will contribute to the degradation of the Clothianidin in water, assuming a phototransformation in the whole water body would highly overestimate the degradation potential.

Phototransformation in air:

Table 38: Phototransformation in air

Guideline / Test method	Time-dependent OH radical concentration [OH radicals cm^{-3}]	Overall reaction rate constant k [cm^3 $molecule^{-1}$ × s^{-1}]	Half-life [h]	Chemical lifetime [h]	Reference
No Guideline available Estimation method by AOPWIN version 1.87 and 1.90	12-hours-day-time concentration of 1.5 × 10^6	136.97 x 10^{-12}	0.94	1.4	Hellpointer, E. (1998) and extended by Hellpointer, E. in 2005 A.7.3.1
No Guideline available Estimation method by AOPWIN version 1.90 and 1.91	24-hours-mean-day concentration of 0.5 × 10^6	136.97 x 10^{-12}	2.81	4.1	

Based on the half-life and chemical lifetime of Clothianidin, degradation by direct phototransformation processes in the air is expected.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No estimation of biodegradation was conducted.

5.1.2.2 Screening tests

Table 39: Ready biodegradability

Method/ Guideline	Test type	Test parameter	Inoculum			Additional substance	Test substance conc.	Degradation		Reference
			Type	Concentration	Adaptation			Incub. period	Degree [%]	
92/69/EEC, C.4-C	Ready (20.8-22.6 °C pH 7.4-7.5)	CO_2	Activated sludge	15 mg C/L	no	no	51.9-52.0 mg TS/L (equivalent 15 mg C/L)	28 days	1.5	Bealing, D.J. & Watson, S. (1999); report no. 586/162-D2145 R = 1 A 7.1.1.2.1

The ready biodegradability of Clothianidin was determined in a Carbon Dioxide Evolution Test according to Directive 92/69/EEC C.4-C using activated sludge as inoculum. In this test Clothianidin was degraded to a degree of 1.5 % within 28 days. In the toxicity control 77 % of the theoretical CO_2 yield was achieved at the end of incubation. Therefore, Clothianidin is not considered readily biodegradable.

5.1.2.3 Simulation tests

5.1.2.3.1 Surface water

No tests were conducted.

5.1.2.3.2 Water-Sediment

Table 40: Water-sediment degradation studies

Method	Test system	Test subst. conc.	DT_{50} (20°C)	DT_{50} (12°C)	Mine-rali-sation*	Degradation products	Reference
SETAC and German BBA, Part IV, 5-1 (aerobic at 20 °C in the dark, 100 days	System 1: loam, 4.1 % org. C, pH 5.4 System 2: sandy loam, 2.3 % org. C, pH 6.7	50 µg/L water ([nitro-imino-^{14}C] TI-435)	System 1: 30.8 days (water); 48 days (entire system) System 2: 49.8 days (water); 64.8 days (entire system, 20 °C)	System 1: 58.4 days (water); 91 days (entire system) System 2: 94.4 days (water); 122.9 days (entire system,	System I: max. 3.2 % (100 d) System II: max. 4.4 % (100 d)	TMG** (N-(2-chlorothiazol-5-ylmethyl)-N'-methylguanidine). System 1: max. 22.9 % on day 58, System 2: max. 20.6 % on day 100	Gilges, M. & Brumhard, B. (2000); report no. MR-505/99 R = 1 A 7.1.2 and A7.1.2.2.2

* in % of applied radioactivity
** relevant metabolite, determined in the sediment, not detected in the water phase

The dissipation behaviour of Clothianidin applied at a concentration of 50 µg a.s./L water was studied in two German water/sediment systems incubated in the dark at 20 °C over a period of 100 days. Primary degradation (dissipation) of Clothianidin in the water phase and in the entire systems is slow. For systems 1 and 2, first order DT_{50}-values of 30.8 and 49.8 days at 20 °C (58.4 and 91.0 days at 12 °C), respectively, were determined in the water phase and 48.0 and 64.8 days (94.4 and 122.9 days at 12 °C) for the entire systems. In the sediment, the maximum amount of Clothianidin was found on day 7 and was 37.3 % of the applied radioactivity in system 1 and 28.8 % in system 2.

Metabolism of Clothianidin in the water phase of the aerobic water/sediment study is insignificant. Only the parent compound was identified in relevant amounts in both systems. With time Clothianidin steadily decreased in the water phase and amounted to 8.8 % of the applied radioactivity in system 1 and 18.7 % in system 2 on day 100 (end of the study). In the sediments of both systems most of the extractable radioactivity was identified as the parent compound and the metabolite TMG (N-(2-chlorothiazol-5-ylmethyl)-N'-methylguanidine). No further metabolites were observed in significant amounts. Non-extractable residues steadily increased reaching 43.3 % at study end (system 1) and 27.6 % (system 2). Unchanged Clothianidin in the entire systems amounts to 25 – 35 % at study end, demonstrating its limited metabolization.

5.1.2.3.3 Soil

Table 41: Soil degradation studies

Method	Test system	Test subst. conc.	DT_{50} (20°C)	Mineralisation*	Degradation products	Reference
SETAC and US EPA 162-1 (aerobic at 20 °C in the dark, 120 resp. 365 days)	4 EU soils: silt loam, pH 8.1, Corg 0.9 (%) silt, pH 7.8, Corg 2.7 (%) loamy sand, pH 6.0, Corg 2.5 (%) sandy loam, pH 6.7, Corg 1.1 (%)	13.3 µg a.s./100 g soil dw	227 days, 143 days 490 days 1001 days	max. 14.8% in one soil (sandy loam) after 365 days	MNG (N-methyl-N'-nitroguanidine), max. 10.7 % after 120 days. TZNG (N-(2-chloro-5-thiazolylmethyl)-N'-nitroguanidine), TZMU (N-(2-chloro-thiazol-5-ylmethyl)-N'-methylurea), NTG (Nitroguanidine): < 10 %	Gilges, M. (2000); report no. MR-497/99 R = 1 A7.2.1 and A7.2.2.1
SETAC and US EPA 162-1 (aerobic at 20 °C in the dark, 181 days, one soil 379 days)	6 US soils: silt loam, pH 6.7, Corg 1.4 (%) loam, pH 6.7, Corg 1.4 (%) loamy sand, pH 6.7, Corg 0.4(%) loamy sand, pH 6.8, Corg 0.4(%) sand, pH 6.2, Corg 0.7 (%) silt loam, pH 6.7, Corg 3.3 (%)	13.3 µg a.s./100 g soil dw	541 days 1328 days Not calculable 549 days 533 days 808 days	max. 10.7% in one soil (silt loam) after 181 days	TZNG (N-(2-chloro-5-thiazolylmethyl)-N'-nitroguanidine), TZMU (N-(2-chlorothiazol-5-ylmethyl)-N'-methylurea): < 2%	Schad, T. (2000 c); report no. MR-419/99 R = 1 A7.2.1/02 and A7.2.2.1/02

* in % of applied radioactivity

To investigate route and rate of degradation in soil, two aerobic laboratory studies were performed with 4 European and 6 US soils at 20 ºC in the dark. Two different 14C labels were used. In these degradation studies the determined first-order DT_{50} values varied between 143 days and more than one year. Mineralisation reached a maximum rate of 10.7 % after 181 days resp. 16.9 % after 379 days. Four metabolites were detected in the soil extracts: MNG (N-methyl-N'-nitroguanidine) as major as well as TZNG (N-(2-chloro-5-thiazolylmethyl)-N'-nitroguanidine), TZMU (N-(2-chlorothiazol-5-ylmethyl)-N'-methylurea), and NTG (Nitroguanidine) as minor metabolites. Only MNG is predominant with 10.7 % after 120 days in one soil. Bound residues increased during the study to a maximum of 12.8 % in one of the soils after one year.

5.1.3 Summary and discussion of degradation

In a test on ready biodegradability (92/69/EEC, C.4-C) Clothianidin was determined to be not readily biodegradable.

The degradability of Clothianidin was further assessed by considering the results of higher tier biodegradation studies in two water-sediment- and ten soil systems as well as abiotic degradation studies, i.e. hydrolysis.

In both water-sediment systems the DT_{50} (entire system) was >16 days (min. DT_{50} 30.8 days). Taking the simulation studies into account, ultimate degradation of Clothianidin was < 4.5 % in both water-sediment systems and achieved a maximum of 10.7% resp. 16.9% after 181 days resp. 379 days in one soil laboratory study.

Clothianidin is hydrolytically stable under acidic and neutral conditions. In pure water Clothianidin is photolytically degraded with half-lives < 1 day in summer. High environmental half-life (> 1 year) was estimated in autumn and winter. Six major (> 10 % of the applied radioactivity) degradation products were identified.

Based on the information available, Clothianidin is considered not rapidly degradable for the purposes of classification.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Table 42: Adsorption/desorption Clothianidin

Method /Guideline Tested soils	Tested Soils (classification acc. To USDA)	Adsor-bed a.s. [%]	K_a[1]	K_{aOC}[2]	K_d [3]	K_{dOC} [4]	K_a / K_d[5]	Reference
OECD 106 and US EPA 163-1								Lewis, C. J. (2000b) A 7.1.3
Quincy	loamy sand	24 – 42	0.52	129	0.62	154	0.84	
Elder	sandy loam	74 – 90	4.14	345	4.58	382	0.90	
Crosby	clay loam	47 – 70	1.48	123	1.67	139	0.89	
Laacher Hof	sandy loam	53 – 76	1.77	84	1.99	95	0.89	
BBA 2.1	sand	28 – 43	0.59	119	0.85	170	0.69	

[1] **K_a = Adsorption coefficient [mg g^{-1}]**
[2] **K_{aOC} = Adsorption coefficient based on organic carbon content [mg g^{-1}]**
[3] **K_d = Desorption coefficient [mg g^{-1}]**
[4] **K_{dOC} = Desorption coefficient based on organic carbon content [mg g^{-1}]**
[5] K_a / K_d = Adsorption / Desorption distribution coefficient [-]

Based on the adsorption/desorption study and in accordance with the classification principle by McCall et al. (1980), Clothianidin is considered to be medium to highly mobile in soil (). The arithmetic mean value of Ka_{OC} amounts to 160 mL g^{-1} and the arithmetic mean value of Kd_{OC} amounts to 188 mL g^{-1}.

5.2.2 Volatilisation

The vapour pressure of Clothianidin (3 .8 x 10^{-11} Pa at 20 °C) and the Henry's law constant (2.9 x 10^{-11} Pa m^3 mol^{-1} at 20 °C) are low. Thus, no substantial volatilisation of Clothianidin is expected. The half-life and chemical lifetime of Clothianidin in the troposphere was estimated to be 2.81 hours and 4.1 hours, respectively considering a global 24-hours mean concentration (OH-concentration 5 x 10^5 radicals/cm^3). According to these results, an accumulation of Clothianidin in the air is not to be expected.

5.2.3 Distribution modelling

No distribution studies were conducted in addition to the screening methods according to US EPA § 163-1. Two lysimeter studies found that all metabolites remained below 0.1 µg/L and no Clothianidin parent compound occurred in the leachates (A 7.2.3.2/01, A 7.2.3.2/02).

5.3 Aquatic Bioaccumulation

Table 43: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
QSAR (according to Guidance on the Biocidal Products Regulation, Volume IV)	BCF = 0.78	Log Kow: 0.7	-

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

An approximate estimation of the bioconcentration factor BCF_{fish} was performed using the standard equation given in the Guidance on the Biocidal Products Regulation – Volume IV Environment (2015):

$\log BCF = 0.85 \cdot \log Kow - 0.7 = 0.595 - 0.7 = -0.105$

$BCF_{fish} = 0.78$

This value indicates a low potential for bioaccumulation.

Although according to the EU Guidance (2015) the linear relationship for estimating the bioconcentration factor is not applicable to Clothianidin with its log K_{ow} below the QSAR validity range ($2 < \log K_{ow} < 6$), the calculated BCF is acceptable, as it describes the order of magnitude in which the BCF is expected. Furthermore, no other indicators like surface active property, structural features or adsorption potential point to an intrinsic potential for bioconcentration; the surface tension, for instance, is 79.6 mN/m and thus lies above the trigger value of ≤ 50 mN/m.

5.3.1.2 Measured bioaccumulation data

A study on the bioaccumulation behaviour of Clothianidin is not available.

5.3.2 Summary and discussion of aquatic bioaccumulation

Measured data on bioaccumulation are not available for Clothianidin. Therefore, the assessment of bioaccumulation has to be based on the estimation of bioaccumulation behaviour. With a log K_{ow} = 0.7 Clothianidin has a low potential for bioaccumulation.

5.4 Aquatic toxicity

Table 44: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
OECD 203 *Oncorhynchus mykiss*	96h-LC_{50} >100 mg/L	-	Anonymous 21 (1998a)
US EPA OPPTS Draft Guideline No. 850.1400 *Pimephales promelas*	33d-NOEC ≥ 20 mg/L	-	Anonymous 20 (2000)
US EPA Guideline OPPTS No. 850.1035 and ASTM Standard E729-88a *Americamysis bahia* (salt water mysids)	96h-EC_{50} = 0.053 (m.m.)	-	K.R. Drottar, J.A. MacGregor, H.O. Krueger (2000a)
US EPA Guideline OPPTS No 850.1350 and ASTM Standard E1191-90 *Americamysis bahia* (saltwater mysid)	39d-NOEC = 0.0097 (m.m.)	-	K.R. Drottar, J.A. MacGregor, H.O. Krueger (2000b)
FIFRA Guideline 123-2, OPPTS Guideline 850.5400, OECD Guideline 201 *Skeletonema costatum* (saltwater diatom)	72h-ErC_{50} = 33.2 (nom.)	-	Banman et al. (2012b)
FIFRA Subdivision J Series 123-2 *Selenastrum capricornutum* (green alga)	NOErC = 15 (72, 96h) 30 (120 h)	-	Sutherland, C.A. & MacGregor, J.A. & Krueger, H.O. (2000)
no recommended guideline *Chironomus riparius*	48h-EC_{50} = **0.029**	-	Mattock, S.D. (2001)
proposal for a BBA guideline (1995) *Chironomus riparius*	28d-EC_{10} = **0.0004**	-	Heimbach, F. (1999)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Table 45: Acute toxicity of Clothianidin to fish

Method/ Guideline	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]			Remarks	Reference
			design	duration	LC_0	LC_{50}	LC_{100}		
OECD 203	*Oncorhynchus mykiss* (rainbow trout)	mortality/ limit test	static	96 h	≥ 100	>100	>100	nominal test concentrations confirmed by analytical monitoring	Anonymous 22 (1998a); project no. 970714TA/FAR54472/CFF54472A7_4_1_1
OECD 203	*Lepomis macrochirus* (bluegill sunfish)	mortality	static	96 h	≥ 120	>120	>120	nominal test concentrations	Anonymous 23 (2000a); report no. 110003/149 A-123
US EPA OPPTS Draft Guideline 850.1075	*Cyprinodon variegatus* (Sheepshead minnow)	mortality	semi-static, limittest	96 h l	>100 (nom.)	>100 (nom.)	>100 (nom.)	Test substance TI-435 technical nominal test concentrations confirmed by analytical monitoring	Anonymous 24 (1999a) THW-0028/ M-027244-01-1

The acute toxicity of Clothianidin to rainbow trout was investigated in a static limit test by Anonymous (1998). The test was conducted according to OECD No. 203 (1992) in compliance with the Directive 92/69/EEC C.1. Seven animals were held per 15 L - vessel filled with 10 L tab water. The nominal test concentration was 100 mg a.s./L. Concentrations of the test substance in test and control samples were measured at the beginning and at the end of the test and were within ±20 % of the nominal concentration. Mortality was determined after 24, 48, 72, and 96 h. None of the test animals died during exposure time. Therefore, the LC_{50} is >100 mg a.s./L. The validity criteria are fulfilled and the test is acceptable.

In a further limit test by Anonymous (1999) the acute toxicity to sheepshead minnow (*Cyprinodon variegatus*) was determined. A semi-static limit test with daily renewal of the test medium was conducted with the single concentration level of 102.5 mg/L corresponding to 100 mg/L a.s. selected on the basis of a preliminary semi-static test. The study was conducted over a duration of 96 hours. Ten test organisms were exposed to the test concentration and control. No vehicles were used to dissolve the test substance. Sedimentation of the test substance was observed in the test medium throughout the test period.

Analytical results showed that the active ingredient concentrations were between 80 and 120 % of the nominal concentration. The dose-limit concentration of 102.5 mg/L caused no mortality or other symptoms of toxicity throughout the 96-hour exposure period. The LC_{50} value (96 hours) value was > 102.5 mg/L.

The results from both studies was confirmed by Anonymous (2000 a), who investigated the acute toxicity of Clothianidin to the bluegill sunfish *Lepomis macrochirus* according to OECD 203. They determined the 96h-LC_{50} to be above the highest test concentration of 120 mg/L. The effect value is based on nominal concentrations, as no analytical monitoring was performed.

5.4.1.2 Long-term toxicity to fish

Table 46: Long-term toxicity of Clothianidin to fish

Method/ Guideline	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]		Remarks	Reference
			design	duration	NOEC	LOEC		
US EPA OPPTS Draft Guideline No. 850.1400 (1996)	*Pimephales promelas* (fathead minnow)	Hatching, mortality and growth / ELS test	Flow-through	33 d	≥ 20	> 20	nominal test concentrations confirmed by analytical monitoring	Anonymous (2000); report no.110163/149 A-124B A7_4_3_2

The toxicity of Clothianidin to early life-stages of *Pimephales promelas* (fathead minnow) was determined by Anonymous (2000). The test was performed according to US EPA OPPTS draft guideline No. 850.1400 (1996), US EPA-FIFRA Subdivision E, Series 72-4 (1982) and ASTM Standard E1241-88 (1988) in compliance with OECD 210. The duration was 33 days including a 5-day embryo hatching period as well as a 28-day post hatch period. Four replicates with twenty fish embryos per vessels were prepared. As Clothianidin was diluted with DMF, a solvent control (DMF 0.1 ml/L) and a water control were applied. The embryos were exposed to nominal concentrations of 0, 1.3, 2.5, 5.0, 10, and 20 mg a.s./L. The mean measured concentrations of Clothianidin during the test ranged from 92 to 110% of nominal concentrations. Hatching, mortality and growth of the fish were observed. Exposure to Clothianidin at the concentrations tested showed no statistically significant effects on hatching success, larval survival, total length and growth as compared to the controls. Therefore, the NOEC is 20 mg a.s./L. The validity criteria are fulfilled and the test is acceptable.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Table 47: Acute toxicity of Clothianidin to invertebrates

Method/ Guideline	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]			Remarks	Reference
			design	duration	EC_0	EC_{50}	EC_{100}		
OECD 202	*Daphnia magna* (water flea)	immobility/ acute toxicity	static	48 h	≥ 120	> 120	> 120	nominal test concentrations confirmed by analytical monitoring	Palmer, S.J., & MacGregor, J.A. & Krueger, H.O (2000b); report no. 110004/149A-122 A7_4_1_2_01
OECD 202	*Daphnia magna* (water flea)	immobility/ acute toxicity	static	48 h	3.2	26*	270	nominal test concentrations confirmed by analytical monitoring	Noack, M. and Geffke, T. (1997); project no. 970714TA A7_4_1_2_02
US EPA Guideline OPPTS No. 850.1035 and ASTM Standard E729-88a	*Americamysis bahia* (salt water mysids)	mortality	flow through	96 h		0.053 (m.m.)		Mean measured concentrations	K.R. Drottar, J.A. MacGregor, H.O. Krueger 2000a THW-0057/ M-019551-01-1 PPP

* = in the evaluation of the same test according to PPPD an EC_{50} of 40 mg/L was derived. However, 70 % effect was reported at 32 mg/L test concentration. Therefore, a recalculation of the EC_{50} value was performed resulting in an EC_{50} of 26 mg/L.

The acute toxicity of Clothianidin to *Daphnia magna* was determined in two static tests which were performed according to OECD No. 202 (1984) in compliance with the Directive 92/69/EEC C.2. In the first test (Palmer et al., 2000b), ten Neonates < 24 h were held in a 250 mL- vessel filled with 200 mL of dilution water. Two test vessels per concentration were prepared. The following nominal test concentrations were used: 0, 7.5, 15, 30, 60, and 120 mg a.s./L. Concentrations of test substance were measured in test and control samples at 0 h and 48 h and were within ±20 % of the nominal concentration. Immobility was investigated daily. None of the daphnids in the test and control solutions showed any adverse effects. Therefore, the EC_{50} is >120 mg a.s./L. The validity criteria are fulfilled and the test is acceptable.

In the second test (Noack and Geffke, 1997), five Neonates (<24 h) were held in a 50 mL- vessel filled with 20 mL of dilution water. Four test vessels per concentration were prepared. The following nominal test concentrations were used: 0, 1.0, 3.2, 10, 32, 100, and 270 mg a.s./L. Concentrations of test substance were confirmed by analytical monitoring. Immobility was recorded

daily. A reference substance was tested once per month. After 48h, immobile daphnids were observed at test concentrations ≥ 10 mg a.s./L. An EC_{50} (48 h) of 26 mg/L can be calculated. The validity criteria are fulfilled and the test is acceptable.

Furthermore, Drottar et al. (2000a) exposed juvenile saltwater mysids, *Mysidopsis bahia*, less than 24 hours old, to a geometric series of six test concentrations of Clothianidin technical and a saltwater control for 96 hours in a flow-through system. Nominal test concentrations were 0.035, 0.060, 0.10, 0.17, 0.29 and 0.50 mg/L. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, after 48 hours and at test termination to 0.040, 0.067, 0.11, 0.19, 0.34 and 0.56 mg/L. Observations were made to determine the number of mortalities. The number of individuals exhibiting clinical signs of toxicity or abnormal behaviour also was evaluated. Observations were made approximately 6, 24, 48, 72, and 96 hours after test initiation.

Mysids in the negative control appeared healthy and normal throughout the test. After 96-hours of exposure, mortality in the 0.040, 0.067, 0.11, 0.19, 0.34 and 0.56 mg/L treatment groups was 20, 85, 85, 100, 100 and 100 %, respectively. The 48 and 96-hour LC_{50} values were 0.082 and 0.053 mg/L, respectively, based on mean measured concentrations. Based on the mortality and observation data, the 48-hour no-mortality concentration and NOEC were 0.040 mg/L (mean measured). At 96 hours they were both <0.040 mg/L, the lowest concentration tested.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Table 48: Long-term toxicity of Clothianidin to invertebrates

Method/ Guideline	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]		Remarks	Reference
			design	duration	NOEC	LOEC		
OECD No. 211	*Daphnia magna*	mortality, reproduction	semi-static	21 d	0.12	0.37	nominal test concentrations confirmed by analytical monitoring	Noack, M. and Geffke, T. (1998); project no. 970714TA/DRE54471/CDR54471 A7_4_3_4
US EPA Guideline OPPTS No 850.1350 and ASTM Standard E1191-90	*Americamysis bahia* (saltwater mysid)	Reproduction, mortality	flow-through	39 d	0.0097 (m.m.)		Mean measured concentrations	K.R. Drottar, J.A. MacGregor, H.O. Krueger 2000b THW-0058/M-026384-01-1 PPP

The long-term toxicity of Clothianidin to *Daphnia magna* was examined under semi-static conditions according to OECD-Guideline 211 (revised draft, April 1997) by Noack and Geffke (1998). Ten daphnids (individually held) per treatment were exposed to nominal concentrations of 0.041, 0.12, 0.37, 1.1, 3.3, 9.9, and 29.7 mg/L and a control for 21 days. The nominal concentrations of the test substance were confirmed by analytical monitoring. Potassium dichromate was used as reference substance. Mortality of parent daphnids, first appearance of juveniles and

number of young daphnids were checked daily. Significant mortality of parent test organisms occurred at test concentrations ≥ 1.1 mg/L. At test concentrations ≥ 0.37 mg/L the number of juveniles per parent was significantly reduced compared to the control. First appearance of juveniles was significantly delayed at the concentrations 9.9 and 29.7 mg/L. Therefore, the NOEC for reproduction is 0.12 mg/L (nominal) and the NOEC for mortality of parent animals is 0.37 mg/L (nominal). The validity criteria are fulfilled and the test is acceptable.

Drottar et al. (2000b) exposed *Mysidopsis bahia (Americamysis bahia*) neonates, less than 24 hours old, to a geometric series of six test concentrations of Clothianidin technical and a saltwater control for 39 days in a flow-through system. Nominal test concentrations were 0.63, 1.3, 2.5, 5.0, 10 and 20 µg/L. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, at weekly intervals during the test and at test termination and were 0.62, 1.2, 2.5, 5.1, 9.7 and 19 µg/L, representing > 90% of the nominal concentrations, respectively. Mean measured concentrations were used to express the results. On Day 17 of the test, female and male adults were paired, and the reproduction of the paired mysids was monitored through Day 39. Observations of mortality, clinical signs of toxicity, and reproduction were made daily. At test termination, the lengths and dry weights of all surviving first-generation mysids were measured.

There were no statistically significant effects on survival or growth of the mysid shrimp (*Mysidopsis bahia*) exposed to Clothianidin technical at concentrations up to 19 µg/L for 39 days. Reproduction was the most sensitive biological endpoint measured in this study. Mysid shrimp exposed to 19 µg/L had a significantly reduced reproduction rate in comparison to the negative control. Consequently, the LOEC for this study, based on reproduction, was 19 µg/L and the NOEC was 9.7 µg/L.

5.4.3 Algae and aquatic plants

Table 49: Toxicity of Clothianidin to algae and aquatic plants

Method/ Guideline	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]			Remarks	Reference
			design	duration	NOE_rC	EbC_{50}[1]	E_rC_{50}[2]		
FIFRA Subdivision J Series 123-2	*Selenastrum capricornutum* (green alga)	Cell density, biomass, growth rate/ Inhibition test	static	120 h	15 (72, 96h) 30 (120 h)	70 (72h) 55 (96h) 56 (120 h)	> 120 (72, 96, 120 h)	nominal test concentrations confirmed by analytical monitoring	Sutherland, C.A. & MacGregor, J.A. & Krueger, H.O. (2000); report no. 197A-102 A7_4_1_3
OECD 201	*Scenedesmus subspicatus* (green alga)	Cell density, biomass, growth rate/ Inhibition test	static	120 h	180	228	> 270		Wilhelmy, H. & Geffke, T. (1998b); project no. 970714TA/ SSO54471/ CSO54471

FIFRA Guideline 123-2, OPPTS Guideline 850.5400, OECD Guideline 201	*Skeletonema costatum* (saltwater diatom)	Growth rate	static	72 h	-	-	33.2	Test substance Clothianidin technical nominal test concentrations confirmed by analytical monitoring	Banman et al. 2012b THW-0390/M-421990-01-1 PPP
FIFRA Guideline 123-2, OPPTS Guideline 850.5400, OECD Guideline 201	*Navicula pelliculosa* (freshwater diatom)	Growth rate	static	72 h	16	-	67.2	Test substance Clothianidin technical nominal test concentrations confirmed by analytical monitoring	Banman et al. 2012c THW-0391/M-421991-01-1 PPP

[1] calculated from the area under the growth curve; [2] calculated from growth rate

The toxicity of Clothianidin to the green alga *Selenastrum capricornutum* was investigated under static conditions by Sutherland et al. (2000). The test was conducted according to FIFRA Subdivision J Series 123 2 in compliance with EC Directive 92/69/EEC, C.3. Deviating from the guideline EC Directive 92/69/EEC, C.3, the test duration was 120 h instead of 72 h and the composition of test medium differed from the recommendations. The initial cell concentration was approximately 3.0 x 103 cells/mL. The following nominal test concentrations were used: 0, 3.8, 7.5, 15, 30, 60, and 120 mg a.s./L. Algal cells were transferred in 250 mL-Erlenmeyer flasks with the correspondent test solution. Three flasks per concentration were prepared and maintained in an environmental chamber under continuous shaking and lightning. Samples for analytical monitoring were taken from additional vessels at the beginning and at the end of exposure time. Due to the lag phase during the first 24 h, the effect concentrations after 72 h cannot be used. The EC_{50}-values for cell density and biomass after 96 h were calculated to be 52 mg a.s./L and 55 mg a.s./L respectively (57 mg a.s./L and 56 mg a.s./L respectively after 120 h). The corresponding NOEC values for cell density and biomass were 7.3 mg a.s./L (72 h) and 15 mg a.s./L (120 h). For growth rate an E_rC_{50} of > 120 mg a.s./L and a NOEC of 15 mg a.s./l (96 h) resp. 30 mg a.s./L (120 h) was determined. However, the growth rate related effect values cannot be used due to the high variability between control replicates (7-24 %) and control growth rate (45 %).

In a study according to OECD 201, Wilhelmy and Geffke (1998b) examined the toxicity of Clothianidin to the green algae *Scenedesmus subspicatus*. In a static test system without analytical monitoring, a 120h-ErC_{50} > 270 mg/L was obtained.

A static 96-hour algal growth test was conducted by Banman et al. (2012b) to determine the growth effects of Clothianidin technical to the saltwater diatom, *Skeletonema costatum*. The saltwater diatoms were exposed under static (shaken cultures) conditions for 96-hours. Nominal (mean measured) concentrations were control, 2.56 (2.67), 6.4 (6.4), 16 (16), 40 (41), and 100 (100) mg a.s./L. Mean measured recoveries were within the range of 99 to 104 % of the nominal concentrations. All measured recoveries were within the range of ± 20 % of the nominal concentrations therefore toxicity values were calculated based on the nominal concentrations.

After 72 hours, a significant difference ($p \leq 0.5$) in growth rate from the control was seen at test concentrations of 16, 40 and 100 mg a.s./L, with the % inhibition exceeding 50% at the two highest

concentrations. After 96 hours, a significant difference ($p \leq 0.5$) in growth rate from the control was again seen at the test concentrations of 16, 40 and 100 mg a.s./L. No physical abnormalities were observed in the control or in the treatment groups during the study. The 72 and 96-hour growth rates were calculated based on the nominal concentrations. The 72hour EC_{50} value for growth rate (E_rC_{50}) is 33.2 (95 % confidence interval: 30.8 to 35.6) mg a.s./L with LOEC and NOEC values of 16 and 6.4 mg a.s./L, respectively. The 96-hour EC_{50} value for growth rate is 37.8 (95 % confidence interval: 34.3 to 41.4) mg a.s./L with LOEC and NOEC values of 16 and 6.4 mg a.s./L, respectively.

A static 96-hour algal growth test was conducted by Banman et al. (2012c) to determine the growth effects of Clothianidin technical to the freshwater diatom, *Navicula pelliculosa*. The freshwater diatoms were exposed under static (shaken cultures) conditions for 96-hours. Nominal (mean measured) concentrations were control, 2.56 (2.35), 6.4 (6.0), 16 (15), 40 (37), and 100 (95) mg a.s./L. Mean measured recoveries were within the range of 92 to 95 % of the nominal concentrations. All measured recoveries were within the range of ± 20 % of the nominal concentrations therefore toxicity values were calculated based on the nominal concentrations.

After 72 hours, a significant difference ($p \leq 0.5$) in growth rate from the control was seen at test concentrations of 40 and 100 mg a.s./L, with the % inhibition exceeding 50% at the highest test concentration. After 96 hours, a significant difference ($p \leq 0.5$) in growth rate from the control was again seen at the test concentration of 100 mg a.s./L only and this did not exceed 50 %. No physical abnormalities were observed in the control or in the treatment groups during the study. The 72 and 96-hour growth rates were calculated based on the nominal concentrations. The 72 hour EC_{50} value for growth rate (ErC_{50}) is 67.2 (95 % confidence interval: 59.8 to 75.5) mg a.s./L with LOEC and NOEC values of 40 and 16 mg a.s./L, respectively. The 96-hour EC_{50} value for growth rate is >100 mg a.s./L with LOEC and NOEC values of 100 and 40 mg a.s./L, respectively.

5.4.4 Other aquatic organisms (including sediment)

Acute toxicity

Table 50: Acute toxicity of Clothianidin to sediment dwelling organisms

Method/ Guideline	Species	Endpoint / Type of test	Exposure		Results [mg/L]			Remarks	Reference
			design	duration	EC_0	EC_{50}	EC_{100}		
no recommended guideline	*Chironomus riparius*	mortality, immobility/ acute	static	48 hours	< 0.007	**0.029**	0.050	Initial measured concentration	Mattock, S.D. (2001); report no. 586/218-D2145 A 7.4.3.5.1/03

The acute toxicity of Clothianidin to larvae of the midge *Chironomus riparius* was determined in a 48h static toxicity test without sediment (Mattock, 2001). First instar larvae (approximately 2 days old) were exposed to the test substance in ASTM medium at nominal concentrations of 0.007, 0.013, 0.025, 0.050 and 0.10 mg/L. An aqueous 10 mg/L stock solution with the test substance was prepared and further diluted with ASTM medium to obtain the single test treatments. The stock solution was sonicated to aid dissolution.

The pH, temperature and concentration of dissolved oxygen were determined in the control and test treatments at the start and end of the exposure period. Total hardness and alkalinity of the control medium was determined at the start and end of the exposure period. All parameters were in an acceptable range.

Analysis of test substance concentrations using HPLC-UV at 254 nm was performed at the start of the tests. The measured concentrations of the test substance were 0.0068, 0.0115, 0.0211, 0.0412 and 0.0816 mg/L, which is very close to the nominal concentrations.

150 mL glass crystallizing dishes were used as test vessels, each filled with 100 mL of test medium. Five test organisms per vessel and four replicates per test concentration and the control were used. The midges were not fed during the test.

Test parameter was immobility. The larvae were considered immobile if they did not move during a 15 second period of observation.

After 24 and 48 hours, the numbers of immobilized midges were recorded. A 24 h-EC_{50} of 0.072 mg/L and a 48h-EC_{50} of 0.029 mg/l was determined indicating, as expected, a high toxicity of the test substance to insects. The highest concentration causing no immobility was < 0.007 mg/L and the lowest concentration causing 100 % immobility was 0.05 mg/L.

Chronic toxicity

Table 51: Chronic toxicity of Clothianidin to sediment dwelling organisms

Method/ Guideline	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]			Remarks	Reference
			design	duration	EC_{10}	EC_{15}	EC_{50}		
proposal for a BBA guideline (1995)	*Chironomus riparius*	emergence, development	static	28 d	0.00065 **0.0004** (recalc. to mean conc.)	0.00072	0.00106		Heimbach, F. (1999); report no. HBF/Ch 28 A7_4_3_5

The long-term toxicity of Clothianidin to *Chironomus riparius* in a water-sediment system was determined in a test under static conditions according to the proposed BBA guideline (1995) by Heimbach (1999). Observed endpoints were emergence and development of midges after 28 days.

3 L glass beakers (average diameter: 13.5 cm) were used as test vessels. The bottom of the test vessel was covered with a 2 cm-layer of test sediment and filled with 2.65 L of test water.

Test and breeding water (M7 medium) were prepared using deionised water and adding mineral salts and vitamins. The water was aerated and temperated to 20 °C in an in-house preparation tank. The artificial sediment consisted of 69 % fine quartz sand, 10 % dried finely ground peat, 20 % kaolin and about 1 % calcium carbonate to adjust the pH to 6 ± 0.5. The organic carbon content of the sediment was 4.5 %, the water holding capacity 93 g H_2O/100 g dw sediment and the particle size distribution 78.1% sand, 9.3% silt, 12.6% clay. The cation exchange capacity was 10.0 meq/100 g sediment.

First instar (L1) larvae (< 2-3 days old; 25 larvae per test vessel) were exposed to initial nominal concentrations of 0.10, 0.32, 0.56, 1.0, 1.8, 3.2, and 10 µg Clothianidin/L for 28 days. Stock solutions of the test substance were prepared in test water and were stirred on a magnetic stirrer for

15 minutes. In order to reach the desired concentrations, appropriate volumes of the stock solutions were applied into the overlying water column. Gently mixing of the water ensured homogeneous distribution without disturbing the sediment. 4 replicates for the control and 1 or 2 replicates per test concentration were used.

The midges were fed during the test with an aqueous suspension of a commercial ornamental fish food extract (about 1 mg /larvae/day) in 1- to3-day intervals.

The test animals were observed at least 3 times per week for behaviour. The time of emergence, the number and the sex of emerged midges were recorded daily during the period of emergence.

Analytical monitoring of the test concentrations was performed by HPLC for the 0.56, 1.8 and 10 µg/L vessels. The analysis was carried out on day 0, 7 and 28 in the overlying water and in the pore water (sediment filtered by vacuum (mesh size 1 µm)). Limit of quantification was 0.01 mg/L Temperature, pH and dissolved oxygen were measured in the replicates of each treatment and control at days -1, 6, 13, 20 and 28 after larvae transfer.

Emergence was not reduced at the nominal test concentrations of 0.10, 0.32, and 0.56 µg a.s./L. At 1.0 µg a.s./L, the emergence of midges was significantly lower compared to the control. No adult midges emerged in the higher concentrations of 1.8 to 10 µg/L. The relevant EC_{10} based on nominal concentrations was determined to 0.65 µg a.s./Lby probit analysis. For the endpoint development rate, there was no statistical difference between the test concentrations with emergence and the control.

Analytical monitoring of the test substance in overlying and pore water for 3 test concentrations showed a significant decrease in test substance concentration over the exposure period. While the measured concentrations in the overlying water on day 0 varied between 84 and 146 % of nominal, they decreased after 7 days to between 57 and 69 % and after 28 days to between 0 and 53 % of nominal concentrations . The concentration of the test substance in the pore water was almost negligible. As no measurement of Clothianidin concentration in the sediment was performed, it is assumed for reasons of precaution that the decrease in test substance concentration in the water phase was mainly due to degradation than due to adsorption onto the sediment. This means that the use of nominal concentrations underestimates the toxicity of Clothianidin to *Chironomus*. To consider this decline in test substance concentration, the geometric mean of the measured concentrations for the time 0 (0.472 µg/L), day 7 (0.322 µg/L) and day 28 (< 0.3 µg/L) for the nominal concentration 0.56 µg/L (concentration near to EC_{10}) is calculated. This results in a mean measured concentration of 0.357 µg/L, corresponding to a recovery of 63 %. Applying this recovery to the nominal EC_{10} of 0.65 µg/L results in a concentration of 0.4 µg/L.

The validity criteria of the guideline are fulfilled, since 90% of the inserted larvae matured to adults in the controls. and the test is acceptable.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

A ready biodegradation test resulted in 1.5 % degradation (based on theoretical carbon dioxide production) at day 28. On this basis, it is concluded that Clothianidin is not readily biodegradable. Mineralisation was only a minor element of dissipation of Clothianidin in aquatic water/sediment systems. On this basis Clothianidin is not considered to undergo rapid ultimate degradation and is considered **not rapidly degradable** for the purposes of classification.

Measured data on bioaccumulation are not available for Clothianidin. Therefore, the assessment of bioaccumulation has to be based on the estimation of bioaccumulation behaviour and with a log K_{ow} = 0.7 Clothianidin has a **low potential for bioaccumulation**.

Short-term (acute) aquatic hazard

For Clothianidin acute studies are available for fish, invertebrates/insects and algae. Insects are the most sensitive group of organisms and the lowest effect value is a **96h-EC_{50} = 0.029 mg/L** for *Chironomus riparius* for the endpoint mortality/immobilisation.

The criterion for classification as **H400 "Very toxic to aquatic life**" is a $LC_{50} \leq 1$ mg/L. Hence, Clothianidin fulfils this criterion and has to be classified as H400. Due to an acute toxicity in the range $0.01 < EC_{50} \leq 0.1$ mg/L an **M-factor = 10** has to be applied.

Long-term (chronic) aquatic hazard

For Clothianidin adequate chronic toxicity data is available for all three trophic levels and therefore the long-term (chronic) aquatic hazard classification has to be based on chronic toxicity data. The most sensitive trophic level are invertebrates/insects with *Chironomus riparius* being the most sensitive species with a **28d-EC_{10} = 0.0004 mg/L** for the endpoint emergence.

For not rapidly degradable substances the criterion for classification as H410 "**Very toxic to aquatic life with long lasting effects"** is EC_{10}/NOEC ≤ 0.1 mg/L. Clothianidin fulfils this criterion and has to be classified accordingly. Due to a chronic toxicity in the range 0.0001 < NOEC ≤ 0.001 mg/L an **M-factor = 100** has to be applied.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Classification of Clotianidin:

Aquatic Acute 1; H400, M = 10

Aquatic Chronic 1; H410, M = 100

Labelling:

Signal word: Warning

Pictogram: GHS 09

Hazard statement: H410: "Very toxic to aquatic life with long lasting effects"

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Clothianidin has an existing entry for environmental hazard classification as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) with a generic M-factor of 10 in Annex VI of CLP.

During the re-evaluation procedure of clothianidin under the PPP regulation, a new chronic toxicity study became available for classification and a potential update of the current classification (mainly the derivation of new M-factors) was proposed. Based on the new data, retention of the existing hazard categories and new M-factors for Aquatic Acute and Aquatic Chronic have been proposed by the DS.

Overall, the DS concluded that clothianidin is 'not rapidly degradable', has a low potential for bioaccumulation and proposed classification based on aquatic acute and chronic toxicity in aquatic insects:

Aquatic Acute 1 with an M-factor of 10, based on the lowest measured 48-hour EC_{50} value of 0.029 mg/L for *Chironomus riparius*; and

Aquatic Chronic 1 with an M-factor of 100, based on the lowest measured 28-d EC_{10} of 0.0004 mg/L for *C. riparius*.

Degradation

A ready biodegradability test (Carbon Dioxide Evolution Test - Directive 92/69/EEC C.4-C) showed 1.5% biodegradation of clothianidin after 28 days (Bealing and Watson, 1999). Therefore, clothianidin was considered as "not readily biodegradable" by the DS.

The results of a hydrolysis study (92/69/EEC, C.7; US EPA, Subdivision N, Section 161-1) showed that clothianidin is stable in sterile buffer solutions at pH 4, 5, and 7, but degrades at pH 9 (Lewis, 2000a). Nevertheless, relevant amounts of metabolites (CTNU (N-(2-chlorothiazol-5-ylmethyl)-N'-nitrourea), TZMU (N-(2-chlorothiazol-5-ylmethyl)-N'-methylurea), and ACT•HCl (2-chlorothiazol-5-ylmethylamine hydrochloride)) were formed in pH 9 only at elevated temperatures (50°C). At an environmentally relevant temperature (20°C) the calculated half-live was 1401 days. Therefore, the DS considered that hydrolysis is not a significant degradation route for clothianidin at environmentally relevant temperature and pH.

Regarding photolysis in pure water under the experimental conditions used, clothianidin degraded rapidly with an experimental half-life (DT_{50}) of 3.3 hours (SETAC and US EPA 161-2). The experimental half-life corresponds to a calculated environmental half-life of < 0.6 day under midsummer solar light conditions in Phoenix, USA (Babczinski and Bornatsch, 2000). However, assessing environmental half-lives by means of an arithmetical model (Hellpointer, 1999a), with additional parameters, besides the laboratory data, resulted in half-lives up to 23.4 days for the 50th degree of latitude. Also, with another arithmetic model (Hellpointer, 1999a), which takes cloudiness in Central Europe as an additional parameter into consideration, there were estimated high values for the environmental half-lives in autumn and winter (over 1 year). Therefore, the DS considered that although there is no doubt that photolysis in water will contribute to the degradation of clothianidin in water. However, assuming photo transformation in the whole water body would highly overestimate the degradation potential.

Photolysis in air (estimation method by AOPWIN) showed a half-life of 2.81 hours indicating photodegradation in air (Hellpointer, 1998; Extended by Hellpointer, 2005).

Regarding the water/sediment system, one study following BBA part IV (Gilges and Brumhard, 2000) was provided. The dissipation behaviour of clothianidin applied at a concentration of 50 µg a.s./L water was studied in two German water/sediment systems incubated in the dark at 20°C over a period of 100 days. Primary degradation (dissipation) of clothianidin in the water phase and in the entire systems was slow. For systems I and II, first order DT_{50} values of 30.8 and 49.8 days at 20°C (58.4 and 91.0 days at 12°C), respectively, were determined in the water phase and 48.0 and 64.8 days (94.4 and 122.9 days at 12°C) for the entire systems. Max mineralisation of 3.2% (100 d) was at system I and 4.4% at system II. Therefore, metabolism of clothianidin in the water phase of the aerobic water/sediment study is insignificant. Only the parent compound was identified in

relevant amounts in both systems.

Overall, due to the results summarised above, the DS concluded that the available degradation information does not provide sufficient evidence to show that clothianidin is ultimately degraded to above 70% within 28 days (equivalent to a half-life of less than 16 days) or being transformed to non-classifiable products. Therefore, clothianidin was considered to be not rapidly degradable by the DS, according to the CLP criteria.

Aquatic Bioaccumulation

No measured BCF data are available for clothianidin, although a determined Log K_{OW} of 0.7 is available. However, an approximate estimation of the bioconcentration factor BCF_{fish} of 0.78 was performed using the standard equation given in the Guidance on the BPR. Although according to the EU Guidance (2015) the linear relationship for estimating the bioconcentration factor based on Log K_{OW} is not applicable to clothianidin as its Log K_{OW} is below the QSAR validity range (2 < Log K_{OW} < 6), the calculated BCF was accepted by the DS as it describes the order of magnitude in which the BCF is expected. Therefore, the DS concluded that clothianidin has a low potential for bioaccumulation.

Aquatic Toxicity

The aquatic toxicity test results from available acute and chronic studies for all trophic levels of clothianidin are summarised in the following table and sections. Only the valid acute and chronic studies on clothianidin which are relevant for hazard classification purposes are included in the following table and relevant endpoints from these studies are discussed in further detail below. The most sensitive trophic group for acute and chronic toxicity are insects (Midge larvae: *C. riparius)*.

Table: Aquatic Acute toxicity mm: mean measured concentration, nom: nominal concentration

Test organism	Guideline, test method	Short-term result (endpoint)	Reference
Fish			
Rainbow trout *(Oncorhynchus mykiss)*	OECD TG 203	96h LC_{50} > 100 mg/L (nom. confirmed by analytical monitoring)	Anonymous 22, 1998a
Bluegill sunfish *(Lepomis macrochirus)*	OECD TG 203	96h LC_{50} > 120 mg/L (nom)	Anonymous 23, 2000a
Sheepshead minnow *(Cyprinodon variegates)*	US EPA OPPTS Draft Guideline 850.1075	96h LC_{50} > 100 mg/L (nom. confirmed by analytical monitoring)	Anonymous 24, 1999a
Aquatic invertebrates			
Water flea *(Daphnia magna)*	OECD TG 202	48h EC_{50} > 120 mg/L (nom. confirmed by analytical monitoring)	Palmer *et al*., 2000b
Water flea *(Daphnia magna)*	OECD TG 202	48h EC_{50} = 26 mg/L (nom. confirmed by analytical monitoring)	Noack and Geffke, 1997
Salt-water mysids *(Americamysis bahia)*	US EPA Guideline OPPTS No. 850.1035 and ASTM Standard E729-88a	48h EC_{50} = 0.082 mg/L (mm) 96h EC_{50} = 0.053 mg/L (mm)	Drottar *et al*., 2000a

Algae / other aquatic plants			
Green algae *(Selenastrum capricornutum)*	FIFRA Subdivision J Series 123-2	72h E_bC_{50} = 70 mg/L 72h E_rC_{50} > 120 mg/L (nom. confirmed by analytical monitoring)	Sutherland *et al*., 2000
Green algae *(Scenedesmus subspicatus)*	OECD TG 201	120h E_bC_{50} = 228 mg/L 120h E_rC_{50} > 270 mg/L (mm)	Wilhelmy and Geffke, 1998b
Saltwater diatom *(Skeletonema costatum)*	OECD TG 201 FIFRA Guideline 123-2, OPPTS Guideline 850.5400	72h E_rC_{50} = 33.2 mg/L (nom. confirmed by analytical monitoring)	Banman *et al*., 2012b
Freshwater diatom *(Navicula pelliculosa)*	OECD TG 201 FIFRA Guideline 123-2, OPPTS Guideline 850.5400	72h E_rC_{50} = 67.2 mg/L (nom. confirmed by analytical monitoring)	Banman *et al*., 2012c
Sediment dwelling organisms			
Midge larvae *(Chironomus riparius)*	No recommended guideline	**48h EC_{50} = 0.029 mg/L** (nominal concentration)	Mattock, 2001

Three studies have been submitted on the acute toxicity of clothianidin to fish. The reported 96-hour LC_{50} values of clothianidin in all studies with fish were above 100 mg/L based on nominal test concentration confirmed by analytical monitoring.

To address acute toxicity of clothianidin to invertebrates two studies with *Daphnia magna* and one study with *Americamysis bahia* were submitted. The reported 48-hour EC_{50} values of clothianidin in studies with *D. magna* were 26 mg/L and > 120 mg/L, respectively, based on nominal test concentrations confirmed by analytical monitoring. The reported 48-hour and 96-hour EC_{50} values of clothianidin in the study with *A. bahia* were 0.082 and 0.053 mg/L, respectively, based on mean measured concentrations.

Two studies on the green algae, one study on saltwater diatom and one study on freshwater diatom were submitted to address acute toxicity of clothianidin to algae. The reported 72-hour and 120-hours E_rC_{50} values in green alga study were above 100 mg/L. The reported E_rC_{50} values with salt and fresh-water diatoms were in the range between > 10 – < 100 mg/L based on nominal test concentrations confirmed by analytical monitoring.

One 48-hour static toxicity test without sediment with larvae of the midge *C. riparius* has been submitted. The results indicate a high toxicity of the clothianidin to insects with the test parameter being immobility. The larvae were considered immobile if they did not move during a 15 second period of observation. After 24-hours and 48-hours, the numbers of immobilized midges were recorded. Based on the measured immobilisation, the 24-hour EC_{50} of 0.072 mg/L and 48-hour EC_{50} of 0.029 mg/l were calculated based on nominal concentrations.

Overall, the DS proposed to classify clothianidin as Aquatic Acute 1 based on the 48-hour EC_{50} for *C. riparius* of 0.029 mg/L based on nominal concentrations for the endpoint mortality/immobilisation. As this acute toxicity value falls within the $0.01 < L(E)C_{50} \leq 0.1$ mg/L range, the acute M-factor proposed by the DS was 10.

Table: Aquatic Chronic toxicity mm: mean measured concentration, nom: nominal concentration

Test organism	Guideline, test method	Long-term result (endpoint)	Reference / Test item
Fish			
Fathead minnow *(Pimephales promelas)*	US EPA OPPTS Draft Guideline No. 850.1400	33d NOEC ≥ 20 mg/L (nom. confirmed by analytical monitoring)	Anonymous, 2000
Aquatic invertebrates			
Water flea *(Daphnia magna)*	OECD TG 211	21d NOEC = 0.12 mg/L (nom. confirmed by analytical monitoring)	Noack and Geffke, 1998
Salt-water mysids *(Americamysis bahia)*	US EPA Guideline OPPTS No 850.1350 and ASTM Standard E1191-90	39d NOEC = 0.0097 mg/L (mm)	Drottar *et al*., 2000b
Algae /other aquatic plants			
Green algae *(Selenastrum capricornutum)*	FIFRA Subdivision J Series 123-2	72 / 96h NOE_rC = 15 mg/L 120h NOE_rC = 30 mg/L (nom. confirmed by analytical monitoring)	Sutherland *et al*., 2000
Green algae *(Scenedesmus subspicatus)*	OECD TG 201	120h NOE_rC = 180 mg/L (mm)	Wilhelmy and Geffke, 1998b
Freshwater diatom *(Navicula pelliculosa)*	OECD TG 201 FIFRA Guideline 123-2, OPPTS Guideline 850.5400	72h NOE_rC = 16 mg/L (nom. confirmed by analytical monitoring)	Banman *et al*., 2012c
Sediment dwelling organisms			
Midge larvae *(Chironomus riparius)*	BBA Guideline proposal (1995)	28d EC_{10} = 0.0004 (recalculated to mm)	Heimbach, 1999

The toxicity of clothianidin to early life-stages of Fathead minnow (*Pimephales promelas*) was performed according to US EPA OPPTS draft guideline No. 850.1400 (1996), US EPA-FIFRA Subdivision E, Series 72-4 (1982) and ASTM Standard E1241-88 (1988) in compliance with OECD TG 210. Exposure to clothianidin at the concentrations tested showed no statistically significant effects on hatching success, larval survival, total length, or growth when compared to the controls. The reported 33-days NOEC of clothianidin was ≥ 20 mg/L based on nominal test concentrations confirmed by analytical monitoring.

Two studies have been submitted on the chronic toxicity of clothianidin to invertebrates. The reported chronic toxicity values were a 21-day NOEC of 0.12 mg/L for *D. magna* based on nominal test concentrations confirmed by analytical monitoring and a 39-day NOEC of 0.0097 mg/L for *A. bahia*, based on mean measured concentration.

Two studies on the green algae and one study with a freshwater diatom were submitted to address chronic toxicity of clothianidin to algae. The reported 72-hours, 96-hours and/or 120-hours chronic toxicity values were in range of NOE_rC > 10 – < 100 mg/L for *Selenastrum capricornutum* and *Navicula pelliculosa* based on nominal test concentrations confirmed by analytical monitoring. The reported 120-hours chronic toxicity value for *Scenedesmus subspicatus* was above 100 mg/L based on mean measured concentration.

One 28-day static toxicity test in a water-sediment system was determined with larvae of

the midge *C. riparius* with the observed endpoints being emergence and development of midges after 28 days. However, no measurement of clothianidin concentration in the sediment was performed and it was assumed that the decrease in test substance concentration in the water phase is mainly due to degradation than due to adsorption onto the sediment. Therefore, use of nominal concentrations underestimates the toxicity of clothianidin to *Chironomus*. To consider this decline in test substance concentration, the geometric mean of the measured concentrations for the time 0, day 7 and day 28 for the nominal concentration was calculated. This resulted in a recalculated mean measured concentration of EC_{10} = 0.0004 mg/L for the emergence endpoint.

Overall, as clothianidin is considered not rapidly degradable the DS proposed to classify clothianidin as Aquatic Chronic 1 based on the 28-day EC_{10} for *C. riparius* of 0.0004 mg/L, based on recalculated mean measured concentration for the emergence endpoint. As chronic toxicity falls within the 0.0001 < NOEC ≤ 0.001 mg/L range, the chronic M-factor proposed by the DS was 100.

Comments received during consultation

One MSCA and one National Authority (NA) provided comments on the proposal. The MSCA agreed with proposed classification and made only one editorial remark.

The NA in principle agreed with the proposed classification but raised questions in order to confirm the proposed classification. The first question raised by the NA was whether the DS considered research of literature data on the aquatic toxicity of clothianidin to mayfly, as the harmonised classification of other neonicotinoids are based on data for mayfly, which is very sensitive to these substances.

In addition, the NA pointed out that the acute key endpoint for *C. riparius* is based on initial measured concentrations. Test concentrations were not measured at the end of the 48-hour exposure period to verify that these remained within 80-120% of the nominal. In parallel, the acute mysid (*A. bahia*) study used similar test concentrations and mean measured concentrations are provided in the CLH report. Therefore, the NA asked for information on the actual concentrations for this mysid study in order to support that the test substance was stable from 0 – 48 or 96 hours and support the *C. riparius* endpoints.

The last question raised by the NA was related to the proposed Aquatic Chronic classification as is it based on a *C. riparius* study which included sediment in the test system. While aquatic phase monitoring was conducted indicating loss of the active substance, sediment analysis was not included. Therefore, the NA asked for further information to help understand test substance concentrations in the sediment phase during the course of the experiment in order to help consider if the aquatic phase endpoint is reliable for hazard classification.

In answer, the DS confirmed that there are no indications that mayflies are more sensitive to clothianidin than *Chironomus*. The DS provided the study of Macaulay *et al*. (2019) which investigated the chronic toxicity of several neonicotinoids (among them clothianidin and imidacloprid) to nymphs of the mayfly *Deleatidium spp*., which indicated:

- The 28-day LC_{50} for clothianidin was 0.00136 mg/L for mayfly. This is in the same range as the 28-day acute toxicity value of EC_{50} value of 0.00106 mg/L for *Chironomus*.
- The 28-day LC_{50} for imidacloprid was 0.00028 mg/L, indicating a higher toxicity of

imidacloprid compared to clothianidin. The study authors also concluded from the available literature, that mayflies and midges showed similar sensitivity to clothianidin.

With regards to providing further information on acute mysid test actual concentrations and *C. riparius* chronic test substance concentrations in the sediment phase during the course of the experiment, the DS did not reflect.

Assessment and comparison with the classification criteria

Degradation

A ready biodegradation study with clothianidin indicated 1.5% degradation after 28 days, indicating that clothianidin is not readily biodegradable.

Clothianidin is stable in sterile buffer solutions at pH 4, 5 and 7, but degrades at pH 9. However, at relevant temperatures of 20°C the degradation is very slow with calculated half-life of 1401 days.

Under photolysis in pure water under the experimental conditions used, clothianidin degraded rapidly with an experimental half-life of 3.3 hours. The experimental half-life corresponds to a calculated environmental half-life of < 0.6 day under midsummer solar light conditions at Phoenix / USA. However, assessing environmental half-life by means of an arithmetical model (with additional parameters), besides the laboratory data, half-lives resulted in up to 23.4 days for the 50th degree of latitude. As well with another arithmetic model, which takes cloudiness in central Europe as an additional parameter into consideration, estimated half-life was over 1 year (especially in autumn and winter).

In water/sediment systems, primary degradation of clothianidin in the water phase and in the entire systems was slow. For systems I and II, first order DT_{50} values of 30.8 and 49.8 days at 20°C (58.4 and 91.0 days at 12°C), respectively, were determined in the water phase and 48.0 and 64.8 days (94.4 and 122.9 days at 12°C) for the entire systems. Max mineralisation of 3.2% (100 d) was at system I and 4.4% at system II.

Overall, due to the results summarised above, RAC agrees with the assessment of the DS that clothianidin is not ultimately degraded to > 70% within 28 days (equivalent to a half-life < 16 days), or rapidly degraded via primary degradation routes to non-classifiable products. Consequently, RAC agrees that clothianidin should be considered as not rapidly degradable under the CLP regulation.

Aquatic Bioaccumulation

No measured data on bioaccumulation (BCF_{fish}) are available for clothianidin. However the derived Log K_{OW} value of 0.7 is well below the CLP trigger value for indication of bioaccumulation (Log K_{OW} < 4). In addition, the calculated BCF_{fish} of 0.78 was performed using standard equations given in the Guidance of BPR. Although according to the EU Guidance (2015) the linear relationship for estimating the bioconcentration factor is not applicable to clothianidin as derived Log K_{OW} of 0.7 is below the QSAR validity range (2 < Log K_{OW} < 6), still calculated BCF_{fish} of 0.78 could be used as supportive information as it describes the order of magnitude in which the BCF is expected.

Therefore, based on the derived Log K_{OW} that is well below 4 and a calculated BCF_{fish} well below 500 used as supportive information, RAC agrees with the DS that clothianidin has a low potential for bioaccumulation according to the CLP criteria.

Aquatic Toxicity

RAC notes that there are reliable acute and chronic aquatic toxicity data for all trophic levels. RAC agrees that based on provided data in the CLH dossier the most acutely and chronically sensitive trophic group is aquatic insects. RAC recognises that based on comments and answers in the RCOM there are no indications that mayflies will be more sensitive to clothianidin than *Chironomus* according to the results from study of Macaulay *et al*. (2019). However, RAC notes that this particular study was not part of the CLH dossier and has not been evaluated by RAC. Furthermore, RAC also notes that although the salt-water mysid (*A. bahia*) study (Drottar *et al*., 2000a) summary has been included in the CLH report, the robust study summary was not available to the RAC.

Regarding the 48-hour static toxicity test without sediment using larvae of the midge *C. riparius*, RAC acknowledges that nominal test concentrations of clothianidin were 0.007, 0.013, 0.025, 0.050 and 0.10 mg/L. The concentration ranges selected for the definite tests were based on range-finding tests. The range-finding tests were not reported, although the results of the range-finding tests were consistent with those of the definite tests. After 24 and 48 hours, the numbers of immobilised *C. riparius* were recorded. Individual animals were considered immobile if they did not move during a 15 second period of observation. Analysis of test substance concentrations was performed at the start of each of the definite tests. The pH, temperature and concentration of dissolved oxygen were determined in the control and test treatments at the start and end of the exposure period. Total hardness and alkalinity of the control medium was determined at the start and end of the exposure period. The 24-hour and 48-hour EC_{50} values (with 95% confidence limits) were determined by Probit analysis of the data. The highest test concentration causing no immobility and the lowest test concentration causing 100% immobility was based on observation rather than on calculation. The measured concentrations of the test substances were 0.0068, 0.0115, 0.0211, 0.0412 and 0.0816 mg/L which was close to the nominal concentrations. Analytical results indicate that measured concentrations were within 20% of nominal so the toxicity of clothianidin is based on the nominal exposure concentrations throughout the test. Based on immobilisation, a 24-hour EC_{50} of 0.072 mg/L (95% confidence limit) and a 48-hour EC_{50} of 0.029 mg/L (95% confidence limit) were calculated, based on nominal concentration. Based on the measured immobilisation, the highest concentrations causing no immobility of < 0.007 mg/L and lowest concentrations causing 100% immobility of 0.050 mg/L were determined based on nominal concentration.

Regarding the 28-day static toxicity test in a water-sediment system RAC acknowledges that the adsorption coefficient based on organic carbon content (K_{OC}) varies between 84 - 345 mL/g (depending on soil type and concentration) with an arithmetic mean value of 160 mL/g. Therefore, the decrease in test substance concentration in the water phase is assumed to be mainly due to degradation rather than adsorption onto the sediment due to the low Log K_{OW} and adsorption coefficient. Hence, clothianidin will not partition to sediment to a great extent indicating that the test is valid for classification. RAC finds this approach acceptable and a that it provides a suitable worst-case value for chronic toxicity in larvae of the midge *C. riparius*.

RAC also acknowledges that no measurement of clothianidin concentration in the sediment was performed. However adopting a conservative approach, it was assumed that the decrease in the test substance concentration in the water phase was mainly due to degradation rather than due to adsorption onto the sediment. Nevertheless, that means that the use of nominal concentrations underestimates the toxicity of clothianidin to *Chironomus*.

To consider this decline in test substance concentration, the geometric mean of the measured concentrations for the time 0 (0.000472 mg/L), day 7 (0.000322 mg/L) and day 28 (<0.0003 mg/L) for the nominal concentration of 0.00056 mg/L (concentration near to EC_{10}) was calculated. This results in a mean measured concentration of 0.000357 mg/L, corresponding to a recovery of 63%. Applying this recovery to the nominal EC_{10} of 0.00065 mg/L results in a concentration of 0.0004 mg/L.

Consequently, RAC agrees that 48-hour static toxicity test without sediment and 28-day static toxicity test in a water-sediment system with larvae of the midge *C. riparius* are reliable and acceptable for classification. RAC agrees that the lowest acute endpoint for aquatic acute classification is the 48-hour EC_{50} for *C. riparius* of 0.029 mg/L based on nominal concentration for the endpoint mortality/immobilisation. The lowest chronic endpoint for aquatic chronic classification is the 28-day EC_{10} for *C. riparius* of 0.0004 mg/L, based on recalculated mean measured concentrations for the emergence endpoint.

Conclusion on classification

Clothianidin is considered as not rapidly degradable and does not fulfil the criteria for bioaccumulation. Based on the available and reliable information, RAC agrees with the DS that **clothianidin warrants classification as**:

Aquatic Acute 1 based on EC_{50} = 0.029 mg/L for *C. riparius.* As this acute toxicity value falls within the 0.01 < $L(E)C_{50}$ ≤ 0.1 mg/L range, the **acute M-factor is 10**.

Aquatic Chronic 1 based on EC_{10} = 0.0004 mg/L for *C. riparius*. As this chronic toxicity value falls within the 0.0001 < NOEC ≤ 0.001 mg/L range, the **chronic M-factor is 100.**

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON CLOTHIANIDIN (ISO); (*E*)-1-(2-CHLORO-1,3-THIAZOL-5-YLMETHYL)-3-METHYL-2-NITROGUANIDINE

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7 ANNEXES

Annex I (non confidential): Robust Study Summaries of the Assessment-Report for Clothianidin