

**Committee for Risk Assessment
RAC**

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

1,4-dimethylnaphthalene

EC Number: 209-335-9

CAS Number: 571-58-4

CLH-O-0000006734-69-01/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 public 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
5 December 2019**

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

1,4-dimethylnaphthalene

EC Number: 209-335-9

CAS Number: 571-58-4

Index Number: -

Contact details for dossier submitter:

**Bureau REACH
National Institute for Public Health and the Environment (RIVM)
The Netherlands
bureau-reach@rivm.nl**

Version number: 2.0

Date: December 2018

CONTENTS

1	IDENTITY OF THE SUBSTANCE	1
1.1	NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE.....	1
1.2	COMPOSITION OF THE SUBSTANCE	1
2	PROPOSED HARMONISED CLASSIFICATION AND LABELLING.....	3
2.1	PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA	3
3	HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING	5
4	JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL.....	5
5	IDENTIFIED USES	5
6	DATA SOURCES.....	5
7	PHYSICOCHEMICAL PROPERTIES.....	6
8	EVALUATION OF PHYSICAL HAZARDS	7
8.1	EXPLOSIVES	7
8.1.1	<i>Short summary and overall relevance of the information provided on explosive properties.....</i>	7
8.1.2	<i>Comparison with the CLP criteria.....</i>	7
8.1.3	<i>Conclusion on classification and labelling for explosive properties</i>	7
8.2	FLAMMABLE GASES (INCLUDING CHEMICALLY UNSTABLE GASES).....	7
8.2.1	<i>Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases).....</i>	7
8.2.2	<i>Comparison with the CLP criteria.....</i>	8
8.2.3	<i>Conclusion on classification and labelling for flammable gases</i>	8
8.3	OXIDISING GASES	8
8.3.1	<i>Short summary and overall relevance of the provided information on oxidising gases</i>	8
8.3.2	<i>Comparison with the CLP criteria.....</i>	8
8.3.3	<i>Conclusion on classification and labelling for oxidising gases</i>	8
8.4	GASES UNDER PRESSURE.....	8
8.4.1	<i>Short summary and overall relevance of the provided information on gases under pressure</i>	8
8.4.2	<i>Comparison with the CLP criteria.....</i>	8
8.4.3	<i>Conclusion on classification and labelling for gases under pressure.....</i>	8
8.5	FLAMMABLE LIQUIDS	8
8.5.1	<i>Short summary and overall relevance of the provided information on flammable liquids</i>	9
8.5.2	<i>Comparison with the CLP criteria.....</i>	9
8.5.3	<i>Conclusion on classification and labelling for flammable liquids.....</i>	9
8.6	FLAMMABLE SOLIDS	9
8.6.1	<i>Short summary and overall relevance of the provided information on flammable solids.....</i>	9
8.6.2	<i>Comparison with the CLP criteria.....</i>	9
8.6.3	<i>Conclusion on classification and labelling for flammable solids</i>	9
8.7	SELF-REACTIVE SUBSTANCES	9
8.7.1	<i>Short summary and overall relevance of the provided information on self-reactive substances</i>	9
8.7.2	<i>Comparison with the CLP criteria.....</i>	10
8.7.3	<i>Conclusion on classification and labelling for self-reactive substances.....</i>	10
8.8	PYROPHORIC LIQUIDS.....	10
8.8.1	<i>Short summary and overall relevance of the provided information on pyrophoric liquids</i>	10
8.8.2	<i>Comparison with the CLP criteria.....</i>	10
8.8.3	<i>Conclusion on classification and labelling for pyrophoric liquids</i>	10
8.9	PYROPHORIC SOLIDS	10
8.9.1	<i>Short summary and overall relevance of the provided information on pyrophoric solids</i>	10
8.9.2	<i>Comparison with the CLP criteria.....</i>	10

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

8.9.3 Conclusion on classification and labelling for pyrophoric solids 10

8.10 SELF-HEATING SUBSTANCES..... 10

8.10.1 Short summary and overall relevance of the provided information on self-heating substances 11

8.10.2 Comparison with the CLP criteria 11

8.10.3 Conclusion on classification and labelling for self-heating substances..... 11

8.11 SUBSTANCES WHICH IN CONTACT WITH WATER EMIT FLAMMABLE GASES..... 11

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases 11

8.11.2 Comparison with the CLP criteria 11

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases 11

8.12 OXIDISING LIQUIDS..... 11

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids..... 11

8.12.2 Comparison with the CLP criteria 11

8.12.3 Conclusion on classification and labelling for oxidising liquids 12

8.13 OXIDISING SOLIDS 12

8.13.1 Short summary and overall relevance of the provided information on oxidising solids 12

8.13.2 Comparison with the CLP criteria 12

8.13.3 Conclusion on classification and labelling for oxidising solids..... 12

8.14 ORGANIC PEROXIDES..... 12

8.14.1 Short summary and overall relevance of the provided information on organic peroxides 12

8.14.2 Comparison with the CLP criteria 12

8.14.3 Conclusion on classification and labelling for organic peroxides..... 12

8.15 CORROSIVE TO METALS 12

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals 12

8.15.2 Comparison with the CLP criteria 12

8.15.3 Conclusion on classification and labelling for corrosive to metals 12

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 13

9.1 SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION(S) 14

10 EVALUATION OF HEALTH HAZARDS..... 16

10.1 ACUTE TOXICITY - ORAL ROUTE 16

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity 16

10.1.2 Comparison with the CLP criteria 17

10.1.3 Conclusion on classification and labelling for acute oral toxicity..... 17

10.2 ACUTE TOXICITY - DERMAL ROUTE 17

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity..... 17

10.2.2 Comparison with the CLP criteria 17

10.2.3 Conclusion on classification and labelling for acute dermal toxicity 18

10.3 ACUTE TOXICITY - INHALATION ROUTE 18

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity 18

10.3.2 Comparison with the CLP criteria 19

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity 19

10.4 SKIN CORROSION/IRRITATION 21

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation..... 21

10.4.2 Comparison with the CLP criteria 22

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation 22

10.5 SERIOUS EYE DAMAGE/EYE IRRITATION 24

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation 24

10.5.2 Comparison with the CLP criteria 24

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation 25

10.6 RESPIRATORY SENSITISATION..... 26

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation 26

10.6.2 Comparison with the CLP criteria 26

10.6.3 Conclusion on classification and labelling for respiratory sensitisation 26

10.7 SKIN SENSITISATION 26

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

10.7.1	Short summary and overall relevance of the provided information on skin sensitisation	26
10.7.2	Comparison with the CLP criteria	27
10.7.3	Conclusion on classification and labelling for skin sensitisation	27
10.8	GERM CELL MUTAGENICITY	28
10.8.1	Short summary and overall relevance of the provided information on germ cell mutagenicity.....	30
10.8.2	Comparison with the CLP criteria	33
10.8.3	Conclusion on classification and labelling for germ cell mutagenicity	33
10.9	CARCINOGENICITY	35
10.9.1	Short summary and overall relevance of the provided information on carcinogenicity	37
10.9.2	Comparison with the CLP criteria	38
10.9.3	Conclusion on classification and labelling for carcinogenicity	39
10.10	REPRODUCTIVE TOXICITY.....	45
10.10.1	Adverse effects on sexual function and fertility.....	45
10.10.2	Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility.....	45
10.10.3	Comparison with the CLP criteria	47
10.10.4	Adverse effects on development.....	48
10.10.5	Short summary and overall relevance of the provided information on adverse effects on development	49
10.10.6	Comparison with the CLP criteria	51
10.10.7	Adverse effects on or via lactation	52
10.10.8	Short summary and overall relevance of the provided information on effects on or via lactation	52
10.10.9	Comparison with the CLP criteria	52
10.10.10	Conclusion on classification and labelling for reproductive toxicity.....	52
10.11	SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE	56
10.11.1	Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure.....	57
10.11.2	Comparison with the CLP criteria	57
10.11.3	Conclusion on classification and labelling for STOT SE.....	58
10.12	SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE	59
10.12.1	Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure	62
10.12.2	Comparison with the CLP criteria	62
10.12.3	Conclusion on classification and labelling for STOT RE	63
10.13	ASPIRATION HAZARD.....	64
10.13.1	Short summary and overall relevance of the provided information on aspiration hazard.....	64
10.13.2	Comparison with the CLP criteria	64
10.13.3	Conclusion on classification and labelling for aspiration hazard	65
11	EVALUATION OF ENVIRONMENTAL HAZARDS.....	65
11.1	RAPID DEGRADABILITY OF ORGANIC SUBSTANCES	65
11.1.1	Ready biodegradability	66
11.1.2	BOD ₅ /COD.....	66
11.1.3	Hydrolysis	67
11.1.4	Other convincing scientific evidence.....	67
11.1.4.1	Field investigations and monitoring data (if relevant for C&L).....	67
11.1.4.2	Inherent and enhanced ready biodegradability tests.....	67
11.1.4.3	Water, water-sediment and soil degradation data (including simulation studies)	67
11.1.4.4	Photochemical degradation.....	68
11.2	ENVIRONMENTAL TRANSFORMATION OF METALS OR INORGANIC METALS COMPOUNDS.....	68
11.2.1	Summary of data/information on environmental transformation.....	68
11.3	ENVIRONMENTAL FATE AND OTHER RELEVANT INFORMATION.....	69
11.4	BIOACCUMULATION	69
11.4.1	Estimated bioaccumulation.....	69
11.4.2	Measured partition coefficient and bioaccumulation test data	69
11.5	ACUTE AQUATIC HAZARD.....	70
11.5.1	Acute (short-term) toxicity to fish.....	71
11.5.2	Acute (short-term) toxicity to aquatic invertebrates	72
11.5.3	Acute (short-term) toxicity to algae or other aquatic plants	73
11.5.4	Acute (short-term) toxicity to other aquatic organisms	73
11.6	LONG-TERM AQUATIC HAZARD	73

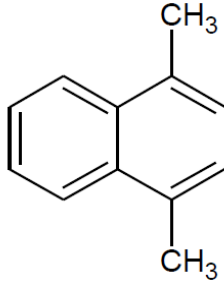
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

11.6.1	<i>Chronic toxicity to fish</i>	74
11.6.2	<i>Chronic toxicity to aquatic invertebrates</i>	75
11.6.3	<i>Chronic toxicity to algae or other aquatic plants</i>	76
11.6.4	<i>Chronic toxicity to other aquatic organisms</i>	77
11.7	COMPARISON WITH THE CLP CRITERIA	77
11.7.1	<i>Acute aquatic hazard</i>	77
11.7.2	<i>Long-term aquatic hazard (including bioaccumulation potential and degradation)</i>	77
11.8	CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS	78
12	EVALUATION OF ADDITIONAL HAZARDS	88
12.1	HAZARDOUS TO THE OZONE LAYER.....	88
12.1.1	<i>Short summary and overall relevance of the provided information on ozone layer hazard</i>	88
12.1.2	<i>Comparison with the CLP criteria</i>	88
12.1.3	<i>Conclusion on classification and labelling for hazardous to the ozone layer</i>	88
13	ADDITIONAL LABELLING	88
14	REFERENCES.....	88
15	ANNEXES.....	88

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	1,4-dimethylnaphthalene
Other names (usual name, trade name, abbreviation)	1,4-DMN
ISO common name (if available and appropriate)	Not available.
EC number (if available and appropriate)	209-335-9
EC name (if available and appropriate)	1,4-dimethylnaphthalene
CAS number (if available)	571-58-4
Other identity code (if available)	CIPAC: 822
Molecular formula	C ₁₂ H ₁₂
Structural formula	
SMILES notation (if available)	Not available.
Molecular weight or molecular weight range	156.23
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	No stereoisomers.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant.
Degree of purity (%) (if relevant for the entry in Annex VI)	Minimum purity 98%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current Annex VI (CLP)	CLH in Table 3.1	Current classification and labelling (CLP)	self- and
1,4-dimethylnaphthalene	98%	None		Asp. Tox. 1, Eye Irrit. 2,	

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
CAS: 571-58-4			Aquatic Acute 1, Aquatic Chronic 1

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not relevant impurities.				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
No additives.					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal		1,4-dimethylnaphthalene	209-335-9	571-58-4	Asp. Tox. 1 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 2	H304 H319 H400 H411	GHS07 GHS08 GHS09 Dgr	H304 H319 H410	-	M = 1	
Resulting Annex VI entry if agreed by RAC and COM		1,4-dimethylnaphthalene	209-335-9	571-58-4	Asp. Tox. 1 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 2	H304 H319 H400 H411	GHS07 GHS08 GHS09 Dgr	H304 H319 H410	-	M = 1	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class needs not to be applied based on chemical structure of the substance.	Yes
Flammable gases (including chemically unstable gases)	Hazard class not applicable	No
Oxidising gases	Hazard class not applicable	No
Gases under pressure	Hazard class not applicable	No
Flammable liquids	Data conclusive but not sufficient for classification	Yes
Flammable solids	Hazard class not applicable	No
Self-reactive substances	Hazard class needs not to be applied based on chemical structure of the substance	Yes
Pyrophoric liquids	Data conclusive but not sufficient for classification	Yes
Pyrophoric solids	Hazard class not applicable	No
Self-heating substances	Hazard class not applicable	No
Substances which in contact with water emit flammable gases	Data conclusive but not sufficient for classification	Yes
Oxidising liquids	Data conclusive but not sufficient for classification	Yes
Oxidising solids	Hazard class not applicable	No
Organic peroxides	Hazard class not applicable	No
Corrosive to metals	Data lacking	No
Acute toxicity via oral route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification	Yes
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification	Yes
Skin corrosion/irritation	Data conclusive but not sufficient for classification	Yes
Serious eye damage/eye irritation	Harmonised classification proposed	Yes
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	Data conclusive but not sufficient for classification	Yes
Carcinogenicity	Data conclusive but not sufficient for classification	Yes
Reproductive toxicity	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Data conclusive but not sufficient for classification	Yes
Aspiration hazard	Harmonised classification proposed	Yes
Hazardous to the aquatic environment	Harmonised classification proposed	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Hazardous to the ozone layer	Data lacking	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

1,4-dimethylnaphthalene has not been previously classified by RAC or TC C&L.

1,4-dimethylnaphthalene is not registered under REACH (October 2017) and has not been notified in the C&L inventory. Eight other dimethylnaphthalene substances have been notified. Dimethylnaphthalene (CAS 28804-88-8) and 1,5-dimethylnaphthalene are both self-classified as eye irrit 2, skin irrit 2 and as STOT SE 3 (H335).

According to the data presented in the DAR (October 2012), the classification of 1,4-dimethylnaphthalene is: Eye irritant Cat 2, Aquatic Acute 1 and Aquatic Chronic 1.

The conclusions on the peer review risk assessment of 1,4-dimethylnaphthalene was published as EFSA conclusion (EFSA Journal 2013;11(10):3229). The classification was unchanged. The DAR can be requested via: <http://dar.efsa.europa.eu/dar-web/provision>. EFSA's peer review is available via the EFSA website (<http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2013.3229/pdf>).

RAC general comment

1,4-dimethylnaphthalene (1,4-DMN) is approved as an active substance in plant protection products. The substance is intended for use as a dormancy enhancer in potatoes during storage and is applied by hot or cold fogging.

Currently, there is no entry in Annex VI of CLP regulation for 1,4-DMN. Therefore, the proposal of the dossier submitter (DS) addressed all physical, human health and environmental endpoints.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

1,4-dimethylnaphthalene is an active substance in the meaning of Regulation EC 1107/2009 and therefore no justification is required.

5 IDENTIFIED USES

1,4-dimethylnaphthalene is intended for use as dormancy enhancer in potatoes during storage. The product is applied by hot or cold fogging.

6 DATA SOURCES

This reports has been prepared based on the data on 1,4-dimethylnaphthalene that was submitted for the Annex 1 inclusion and the evaluation in the DAR.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Clear liquid at 21°C	IIA 2.1/02 Doc ID 4373-93-0226-AS	Measured
Melting/freezing point	1°C	IIA 2.3/01, Doc ID 4373-93-0249-AS	Measured
Boiling point	264 °C at 744 mm Hg (=9.91x10 ⁴ Pa)	IIA 2.1/02 Doc ID 4373-93-0226-AS	Measured
Relative density	1.014 at 25 °C	IIA 2.1/02 Doc ID 4373-93-0226-AS	Measured
Vapour pressure	2.50 ± 0.096 Pa at 24.8 ± 0.66 °C (n=7; 1s) 4.85 ± 1.279 Pa at 34.9 ± 0.07 °C (n=6; 1s) 11.7 ± 1.319 Pa at 44.9 ± 0.09 °C (n=6; 1s)	IIA 2.3/01, Doc ID 4373-93-0249-AS	Measured
Surface tension	36.88 dyne cm ⁻¹ at 25 °C (36.88 mN/m)	IIA 2.14/01	Non-GLP public literature study. No information on test method is available. Study not acceptable. However, considering the molecule structure of 1,4-DMN (no hydrophilic groups), surface active properties are not expected.
Water solubility	5.1 ± 0.16 mg L ⁻¹ at 25 ± 1 °C	IIA 2.6/01, Docu ID 4373-93-0250-AS-001	Measured
Partition coefficient n-octanol/water	log P 4.37 ± 0.012 at 22.5 ± 0.5 °C (1s).	IIA 2.8/01	Measured. non-GLP public literature study
Flash point	122 °C at 760 mm Hg (= 1.01x10 ⁵ Pa)	IIA 2.1/02 Doc ID 4373-93-0226-AS	Measured.
Flammability	No data.		
Explosive properties	Not sensitive to impact and Expert statement	IIA 2.1/02 Doc ID 4373-93-0226-AS IIA 2.3/02	Measured. There are no chemical groups present that indicate explosive properties.
Self-ignition temperature	Expert statement	Document N (applicant)	1,4-dimethylnaphthalene is not considered autoflammable.
Oxidising properties	Expert statement	IIA 2.3/02	There are no chemical groups in 1,4-dimethylnaphthalen that indicate oxidising properties
Granulometry	No data.		-
Stability in organic solvents and identity of relevant	No data.		-

Property	Value	Reference	Comment (e.g. measured or estimated)
degradation products			
Dissociation constant	Expert statement	Document N (applicant)	Based on the molecular structure, 1,4-dimethylnaphthalene will not dissociate in water.
Viscosity	Mean viscosity was 6 mPa s at 12 rounds per minute and 6 mPa s at 30 rounds per minute at 25 °C. The kinematic viscosity is 6 mPa.s / 1.014 = 5.9 mm ² /s.	IIA 2.1/02 Doc ID 4373-93-0226-AS	Measured.

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
FIFRA § 63-16 Impact apparatus	Not sensitive to impact at 25°C	-	IIA 2.1/02 Doc ID 4373-93-0226-AS

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

A study (IIA 2.1/02) was conducted to determine the impact explosibility of 1,4-DMN batch H5510 at 25°C using the Bureau of Explosives impact apparatus. The minimum explosive drop height was 31 1/4 inches (maximum drop height). Three replicates were carried out. No explosive behaviour was observed.

8.1.2 Comparison with the CLP criteria

No explosive behaviour was observed in an impact explosibility study. In addition, there are no chemical group in 1,4-dimethylnaphthalene that indicate explosive properties as given in section 2.1.4.2 of the CLP guidance. The impurities are largely comparable to the active substance and also do not include groups that may induce explosive behaviour in the sense of EC A14. Therefore, no classification is proposed.

8.1.3 Conclusion on classification and labelling for explosive properties

No classification is proposed.

8.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable. 1,4-dimethylnaphthalene is not a gas.

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

Not relevant.

8.2.2 Comparison with the CLP criteria

Hazard class not applicable.

8.2.3 Conclusion on classification and labelling for flammable gases

Hazard class not applicable (1,4-dimethylnaphthalene is not a gas).

8.3 Oxidising gases

Hazard class not applicable. 1,4-dimethylnaphthalene is not a gas.

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

Not relevant.

8.3.2 Comparison with the CLP criteria

Hazard class not applicable.

8.3.3 Conclusion on classification and labelling for oxidising gases

Hazard class not applicable. 1,4-dimethylnaphthalene is not a gas.

8.4 Gases under pressure

Hazard class not applicable (1,4-dimethylnaphthalene is not a gas).

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

Not relevant.

8.4.2 Comparison with the CLP criteria

Hazard class not applicable.

8.4.3 Conclusion on classification and labelling for gases under pressure

Hazard class not applicable (1,4-dimethylnaphthalene is not a gas).

8.5 Flammable liquids

Table 9: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
FIFRA § 63-15 (ASTM D 93-90, CIPAC MT12.3) Pensky-Martens apparatus equivalent to EEC A9	Flashpoint: 122 °C at 760 mm Hg (= 1.01x10 ⁵ Pa)	-	IIA 2.1/02 Doc ID 4373-93-0226-AS
FIFRA § 63-6 (ASTM D 1120-89, ebulliometric method) equivalent to OECD TG103 and EEC A2.	Boiling point: 264 °C at 744 mm Hg (=9.91x10 ⁴ Pa)	-	IIA 2.1/02 Doc ID 4373-93-0226-AS

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

A study was conducted to determine the flammability of 1,4-DMN (batch H5510) using a test guideline equivalent to EEC A9 (IIA 2.1/02). The test was run in duplicate. The mean flash point corrected to a barometric pressure of 760 mm Hg was 122°C.

An experiment was conducted to determine the boiling point of 1,4-DMN (batch H5510) (IIA 2.1/02). The test was run in duplicate. The test was repeated with a standard material (ethylene glycol) of known boiling point. The atmospheric pressure was recorded in mm Hg at the conclusion of each test. The mean boiling point was 264°C at 744 mm Hg.

8.5.2 Comparison with the CLP criteria

According to the CLP Guidance a substance should be classified as a flammable liquid when:

Category 1: Flash point < 23 °C and initial boiling point ≤ 35 °C

Category 2: Flash point < 23 °C and initial boiling point > 35 °C

Category 3: Flash point ≥ 23 °C and initial boiling point ≤ 60 °C

Considering the flashpoint of 122°C and the boiling point of 264°C 1,4-dimethylnaphthalene does not need to be classified as a flammable liquid.

8.5.3 Conclusion on classification and labelling for flammable liquids

No classification is proposed.

8.6 Flammable solids

Hazard class not applicable (1,4-dimethylnaphthalene is not a solid).

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

Not relevant.

8.6.2 Comparison with the CLP criteria

Hazard class not applicable.

8.6.3 Conclusion on classification and labelling for flammable solids

Hazard class not applicable (1,4-dimethylnaphthalene is not a solid).

8.7 Self-reactive substances

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

No specific study available.

8.7.2 Comparison with the CLP criteria

No specific study has been submitted. However, 1,4-dimethylnaphthalene does not contain any chemical groups associated with explosive or self-reactive properties in accordance with Table 2.8.4.2 of the CLP guidance.

8.7.3 Conclusion on classification and labelling for self-reactive substances

No classification is proposed.

8.8 Pyrophoric liquids

No specific study was carried out.

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

No specific data derived in accordance with the recommended test method in CLP has been provided. However, 1,4-dimethylnaphthalene has been handled extensively in air within all studies available in the dossier and there are no reports of self-ignition.

8.8.2 Comparison with the CLP criteria

According to the additional classification considerations in CLP Annex I, 2.9.4, the classification procedure for pyrophoric liquids need not be applied when experience in manufacture or handling shows that the liquid does not ignite spontaneously on coming into contact with air at normal temperatures. 1,4-dimethylnaphthalene has been extensively handled in all studies (physicochemical studies, toxicity studies etc.) without reports of self-ignition. Therefore, no classification is proposed.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

No classification is proposed.

8.9 Pyrophoric solids

Hazard class not applicable (1,4-dimethylnaphthalene is not a solid).

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

Not relevant.

8.9.2 Comparison with the CLP criteria

Hazard class not applicable.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Hazard class not applicable (1,4-dimethylnaphthalene is not a solid).

8.10 Self-heating substances

Hazard class not applicable (1,4-dimethylnaphthalene is a liquid).

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

Not relevant

8.10.2 Comparison with the CLP criteria

Hazard class not applicable.

8.10.3 Conclusion on classification and labelling for self-heating substances

Hazard class not applicable (1,4-dimethylnaphthalene is a liquid).

8.11 Substances which in contact with water emit flammable gases

No data.

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

No specific data derived in accordance with the recommended test method in CLP has been provided. However, 1,4-dimethylnaphthalene has been handled in water within many of the studies available in the dossier and there are no reports of violent reaction and emission of gas

8.11.2 Comparison with the CLP criteria

Based on experience in handling of 1,4-dimethylnaphthalene, it is not a substance which in contact with water emit flammable gases.

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

No classification is proposed..

8.12 Oxidising liquids

No data.

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

No specific study was submitted.

8.12.2 Comparison with the CLP criteria

The CLP Guidance states that for organic substances or mixtures the classification procedure for this hazard class need not to be applied if:

- a. the substance or mixture does not contain oxygen, fluorine or chlorine; or
- b. the substance or mixture contains oxygen, fluorine or chlorine and these elements are chemically bonded only to carbon or hydrogen.

As 1,4-dimethylnaphthalene does not contain these chemical groups no classification for oxidizing properties is required.

8.12.3 Conclusion on classification and labelling for oxidising liquids

No classification is proposed.

8.13 Oxidising solids

Hazard class not applicable. 1,4-dimethylnaphthalene is not a solid.

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

Not relevant.

8.13.2 Comparison with the CLP criteria

Hazard class not applicable.

8.13.3 Conclusion on classification and labelling for oxidising solids

Hazard class not applicable (1,4-dimethylnaphthalene is not a solid).

8.14 Organic peroxides

Hazard class not applicable (1,4-dimethylnaphthalene is not an organic peroxide).

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

Not relevant.

8.14.2 Comparison with the CLP criteria

Hazard class not applicable.

8.14.3 Conclusion on classification and labelling for organic peroxides

Hazard class not applicable (1,4-dimethylnaphthalene is not an organic peroxide).

8.15 Corrosive to metals

No data has been provided that addresses this hazard class.

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

No data has been provided that addresses this hazard class.

8.15.2 Comparison with the CLP criteria

No data has been provided that addresses this property.

8.15.3 Conclusion on classification and labelling for corrosive to metals

No classification is proposed due to lack of data.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

No classification is proposed by the DS for explosive properties as no structural alerts for explosive properties were identified and no explosive behaviour was observed in an impact explodability study at 25 °C (EPA OPP 63-16, GLP).

No classification as flammable liquid is proposed as the substance does not fulfilled the CLP criteria (flash point < 23°C or ≥ 23°C and ≤ 60°). Indeed, a flash point of 122 °C was observed in a study equivalent to test method EEC A9.

The DS proposed no classification for self-reactivity as the substance does not contain any structural alerts associated with self-reactive properties.

For pyrophoric liquids and for substances, which in contact with water emit flammable gases properties, no data were available. Nevertheless, no classification is proposed based on experience in handling of 1,4-DMN,

The DS proposed no classification of 1,4-DMN as an oxidising liquids. No data were available but the substance does not need to be classified according to the CLP criteria as the substance does not contain oxygen, fluorine or chlorine.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees with DS's proposal to not classify 1,4-DMN for physical hazards.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The mammalian toxicity studies of 1,4-dimethylnaphthalene were assessed in the Draft Assessment Report (October 2012) prepared in the context of the approval, under Reg. (EC) 1107/2009. All studies were carried out under GLP unless indicated otherwise. Other than the public literature studies all studies reported in this section were carried out in accordance with OECD guidelines. Minor deviations were noted in some cases but these did not affect the overall reliability of the studies. The deviations are included in the summaries where relevant. For the public literature studies the reliability of the studies were assessed using Klimisch Scores.

Table 10: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Public literature, non-GLP 1,4-dimethylnaphthalene (purity not reported), single dose i.p. at 28 mg/kg bw/day.	Rapid excretion within 72 hours (57% via urine, 40.8% via faeces). No evidence of accumulation Metabolism via: oxygenation of alkyl side chain, ring hydroxylation yielding naphthols and binding of epoxide intermediates to glutathione leading to thionaphthols.	Klimisch score 1	IIA 5.1/01
Partially in accordance with OECD 417 Deviations: 3 instead of 4 animals, absorption and distribution not investigated. 1,4-dimethylnaphthalene, purity 98.28%, single oral dose 28.6 mg/kg	Rapid excretion essentially complete within 48 hours (71.6% via urine) Metabolism via oxygenation of alkyl side chains and ring hydroxylation yielding naphthols. No metabolites were found to confirm the 3 rd pathway (binding of epoxide intermediates to glutathione leading to thionaphthols).	Study was carried out to confirm the results from the public literature study (IIA 5.1/01). The study is considered acceptable.	IIA 5.1/23, DocID 2263W-1
OECD 503 (metabolism in livestock) 1,4-dimethylnaphthalene, purity 97.7%, 7-day oral dose at 12.5 mg/kg bw/day	Rapid absorption (76%). No evidence of accumulation.	Study in goat was used to support absorption after oral exposure.	IIA 6.2/28

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

Some information is available on the oral **absorption** of 1,4-dimethylnaphthalene indicating an oral absorption of at least 72% (based on % found in urine). A study in the goat indicates that oral absorption in goats is at least 76%. Some public literature information was available for structurally similar naphthalenes (see Annex I for details). Oral absorption of 2-methylnaphthalene was at least 72% in guinea pigs. For 2-isopropylnaphthalene, oral absorption was at least 95% in rats, whereas in another rat study the oral absorption of monoisopropylnaphthalene was at least 78%. The oral absorption of 2,6-diisopropylnaphthalene was 85% in rats. On the basis of the results of the study with 1,4-dimethylnaphthalene in rats and goats and from the studies with 2-methylnaphthalene and mono- and di-isopropylnaphthalenes it can be assumed that the oral absorption of 1,4-dimethylnaphthalene will be high (>80%).

No information is available on the **distribution** of 1,4-dimethylnaphthalene after oral administration. After intraperitoneal administration, 1,4-dimethylnaphthalene and its metabolites were widely and rapidly distributed in rats. The highest concentrations were observed in adipose tissue, followed by liver, kidneys, spleen and adrenals. The skin was not examined. After 72 h tissue levels, including in adipose tissue, were low indicating that 1,4-dimethylnaphthalene or its metabolites do not accumulate. Similar patterns of distribution were found for the alkylated naphthalenes 1,2-dimethylnaphthalene and 1,6-dimethylnaphthalene after intraperitoneal administration (see Annex I for details). After oral administration of the structurally related 2-methylnaphthalene to guinea pigs, distribution was also rapid with the highest levels of parent and/or metabolites in the gallbladder, followed by the kidneys,

liver and lung. Adipose tissue was not examined, but after intraperitoneal administration of 2-methylnaphthalene to mice this tissue revealed the highest concentration. 2-Methylnaphthalene is the only compound for which both an oral and an intraperitoneal study was available, but the intraperitoneal study was too limited to conclude on route- or species-specific differences in distribution. Monoisopropylnaphthalenes and 2,6-diisopropylnaphthalene were also rapidly and widely distributed after single and repeated oral administration, with the highest levels of parent compound and/or metabolites in the adipose tissue, followed by the skin. Repeated administration of 2-isopropylnaphthalene to rats did not result in higher levels of parent compound in the various tissues.

Information is available on the **excretion** of 1,4-dimethylnaphthalene after oral administration: after a single oral dose of 1,4-dimethylnaphthalene, excretion is rapid and essentially complete after 48 hours, with urine being the primary route of excretion (71.6%). The amount found in faeces and cage wash after 48 hours was 27.5 and 5.6% respectively. The parent molecule was detected in trace amounts in urine. After intraperitoneal administration, 1,4-dimethylnaphthalene and its metabolites were excreted mainly via urine (56.5% within 72 h) but also via faeces (40.8% within 72 h, reflecting excretion via bile since the administration was intraperitoneally).

The metabolites found in rat urine after oral administration of 1,4-dimethylnaphthalene indicate that the **metabolism** of 1,4-dimethylnaphthalene proceeds via at least two different routes:

- a) side chain oxidation resulting ultimately in the carboxylic acid 4-methyl-1-naphthoic acid (ca 12% of the dose),
- b) ring hydroxylation resulting in 1,4-dimethylnaphthols.

The metabolites found in rat urine after intraperitoneal administration of 1,4-dimethylnaphthalene indicate that the metabolism of 1,4-dimethylnaphthalene proceeds via at least three different routes:

- a) side chain oxidation resulting ultimately in the carboxylic acid 4-methyl-1-naphthoic acid (20% of the dose),
- b) ring hydroxylation resulting in 1,4-dimethylnaphthols (ca 10% of the dose) via reactive epoxide with
- c) subsequent binding of epoxide intermediates to glutathione via conjugation leading ultimately to 1,4-dimethyl-methylthionaphthalene (1.4% of the dose, indicating a minor pathway).

This 3rd pathway described for 1,4-dimethylnaphthalene, namely the binding of epoxide intermediates to glutathione leading to thionaphthols, excreted in urine as mercapturates involved the forming of 1,4-dimethyl-methylthionaphthalene; this thiomethyl deriviate of 1,4-DMN was reported in small amounts after i.p administration, but is not detected after oral administration.

Metabolites of 1,4-dimethylnaphthalene are also excreted via bile and faeces, but biliary and faecal metabolites have not been identified. Compared to the structurally related alkylated naphthalenes 1,2-dimethylnaphthalene and 1,6-dimethylnaphthalene, the side chain oxidation route is more important and ring oxidation with subsequent glutathione conjugation route is less important for 1,4-dimethylnaphthalene.

For 2-methylnaphthalene, most metabolites identified in guinea pig urine resulted from side chain oxidation, but also metabolites that may involve ring epoxidation were found. All identified metabolites of mono- and diisopropylnaphthalenes in urine and bile of rats resulted from the side chain oxidation pathway.

After a single oral or intraperitoneal dose to mammals, alkylnaphthalenes demonstrate very similar profiles for absorption and excretion with rapid absorption and essentially complete excretion within 72 hours. All alkylnaphthalenes are extensively metabolised in rat, mouse and guinea pig. Rat and mouse urine contains metabolites formed by three different pathways (side chain oxidation, ring oxidation to naphthols and mercapturates via reactive epoxide with subsequent conjugation to glutathione). This similarity in mechanism supports the use of alkylnaphthalene ADME data in the assessment of 1,4-DMN.

In addition, the relative toxicity profiles for alkylnaphthalenes can be linked to the proportion of metabolism which occurs by side chain or ring oxidation. For 1,4- dimethylnaphthalene, the primary route of metabolism has been shown to be via side chain oxidation and therefore this compound is one of the least toxic alkylnaphthalenes. This provides further support for the use of data from the alkylnaphthalene chemical group as it provides a worst case assessment for the hazard to humans.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 11: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD ₅₀	Reference
FIFRA 152-10 (in line with OECD 401) Deviations: none	Rat, Sprague-Dawley (CrI:CD@BR) 5/sex/dose	1,4-dimethylnaphthalene (Batch H5510, purity 96.4%)	Single dose, gavage at 750, 1000, 1300, 1700, 2000, 2100, 2300 and 2500 mg/kg bw	2730 mg/kg bw	IIA 5.2/01, Doc ID L08456, study 6,7,8

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

Five rat/sex/dose were exposed to 1,4-dimethylnaphthalene (purity 96.4%) to dose level of 750, 1000 and 2500 mg/kg bw in the first, 1300, 1700 and 2100 mg/kg bw in the second and 2000, 2300 and 2500 mg/kg bw in the third study (IIA 5.2/01). In a previous limit test at 5000 mg/kg bw all animals (10/10) died. The results from this study were included in the LD₅₀ calculations.

Death occurred in group dose with 1700 mg/kg bw and higher (see Table 13). Signs of toxicity were observed in all dose groups. In the lowest dose group, signs were restricted to discolouration around the mouth and red and/or clear nasal discharge. In the other dose groups, clinical signs included hypoactivity, ataxia, coma, irritability, chromodacryorrhea, lacrimation, salivation, redness around the nose and/or eyes, wet/dicoloured inguinal fur, coldness to the touch, hunched posture, diarrhoea, hair loss and discoloured paws. All surviving rats had recovered at 7 days after administration.

The calculated acute oral LD₅₀ for 1,4-dimethylnaphthalene in male and female rats was 2730 mg/kg bw with a confidence interval of 2346-3178 mg/kg bw.

Table 12: Mortality in the acute oral toxicity study

	Part 1	Part 2	Part 3
--	--------	--------	--------

Dose group mg/kg bw	750	1000	2500	1300	1700	2100	2000	2300	2500
Males	0	0	5	0	0	0	1	1	2
Females	0	0	5	0	1	1	0	0	3

10.1.2 Comparison with the CLP criteria

According to the Regulation EC No 1272/2008 a substance should be classified for acute oral toxicity when:

Category 1: $ATE \leq 5$ mg/kg bw

Category 2: $5 < ATE \leq 50$ mg/kg bw

Category 3: $50 < ATE \leq 300$ mg/kg bw

Category 4: $300 < ATE \leq 2000$ mg/kg bw

Based on the LD₅₀ value of 2730 mg/kg bw 1,4-dimethylnaphthalene does not have to be classified for acute oral toxicity.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

No classification is proposed.

10.2 Acute toxicity - dermal route

Table 13: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose duration levels of exposure	Value LD ₅₀	Reference
FIFRA 152-11 (in accordance with OECD 402) Deviations: none	Rabbit, New Zealand/White 5/sex	1,4-dimethylnaphthalene (batch H5510, purity 96.4%)	2000 mg/kg bw Single dose, 24h under occlusion	> 2000 mg/kg bw	IIA 5.2/02, Doc ID L08456, study 4

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

In an acute dermal toxicity study (IIA 5.2/02) the undiluted test substance was administered to rabbit for 24 hours after which the test substance was wiped off with a gauze pad moistened with saline. Rabbits were observed for 14 days after administration.

No mortality occurred throughout the study. Except for 1 rabbit, all animals gained weight during the study. Treatment-related signs of systemic toxicity were not observed. Signs of dermal irritation at the application site (oedema and erythema) were observed in all rabbits immediately following removal of the wrappings. All animals developed eschar formation within five days following unwrapping. In 4 animals, eschar formation persisted until the end of the 14 days observation period. The LD₅₀ was > 2000 mg/kg bw.

10.2.2 Comparison with the CLP criteria

According to the Regulation EC No 1272/2008 a substance should be classified for acute dermal toxicity when:

- Category 1: ATE ≤ 50 mg/kg bw
- Category 2: 50 < ATE ≤ 200 mg/kg bw
- Category 3: 200 < ATE ≤ 1000 mg/kg bw
- Category 4: 1000 < ATE ≤ 2000 mg/kg bw

Based on the LD₅₀ value of >2000 mg/kg bw 1,4-dimethylnaphthalene does not have to be classified for acute dermal toxicity.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

No classification is proposed.

10.3 Acute toxicity - inhalation route

Table 14: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC ₅₀	Reference
FIFRA 152-12 (in line with OECD 403) Deviations: temperature outside of 22 ±2°C range (25-26°C)	Rat, Sprague-Dawley (CrI:CD@BR) 5/sex	1,4-dimethylnaphthalene (batch H5510, purity 96.4%) MMAD: 2.82 µm	5 mg/L (target conc); 4.16 mg/L (achieved conc) Single dose, whole body, 4h	> 4.16 mg/L	IIA 5.2/03, DocID L08456L001

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

In an acute inhalation study (IIA 5.2/03) Sprague-Dawley rats 5 male and 5 female animals were exposed to 4.16 mg/L 1,4-dimethylnaphthalene (achieved concentration). The mean aerosol Mass Median Aerodynamic Diameter (MMAD) of the chamber atmosphere was 2.82 µm (SD 1.67). Rats were observed for 14 days after exposure.

One rat died within 24 hours after administration. Clinical signs were observed in all animals and included dyspnea, prostration, ptosis, hypoactivity, discolouration around the mouth and/or nose and wet inguinal fur. In addition, tremors and red nasal and ocular discharge were also observed. Except for one animal, which showed ptosis on day 5 and 6 following exposure, all of the surviving rats appeared normal 3 days following the exposure. The incidences of the clinical signs are shown in the table below

Observation	Incidence		Timepoint observed
	Males	Females	
Eye discharge –red	1	2	Day 1-2
Ptosis	1	3	Day 5-6
Nasal discharge – red	0	3	Day 1-2
Dyspnea	0	1	Day 0
Hypoactive	1	5	Day 0-1

Prostate	0	3	Day 0-1
Tremors	0	1	Day 1
Discoloration around mouth	2	5	Day 0-1
Discoloration around nose	5	5	Day 0-1
Wet inguinal fur	5	4	Day 0-1

The acute median lethal inhalation dose (LC50) for 1,4-dimethylnaphthalene in adult male and female rats is > 4.16 mg/L.

10.3.2 Comparison with the CLP criteria

According to the Regulation EC No 1272/2008 a substance should be classified for acute inhalation toxicity when:

- Category 1: $ATE \leq 0.05$ mg/L
 Category 2: $0.05 < ATE \leq 0.5$ mg/L
 Category 3: $0.5 < ATE \leq 1.0$ mg/L
 Category 4: $1.0 < ATE \leq 5.0$ mg/L

The achieved dose in the acute inhalation study was below the limit of 5 mg/L. However, it is not expected that an inhalation study in which rats would be exposed to 1,4-dimethylnaphthalene at an actual dose of 5 mg/L would result in a markedly different toxic profile and in a different conclusion with respect to classification and labelling than the results from the current study at 4.16 mg/L. The LC50 was found to be > 4.16 mg/L. Therefore, it is concluded that 1,4-dimethylnaphthalene does not need to be classified for acute inhalation toxicity.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

No classification is proposed.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity - Oral route

For acute oral toxicity, one oral toxicity study in rats was available using doses up to 2500 mg/kg. The study was similar to OECD TG 401 (GLP). A limit dose study conducted earlier than the main study with 5000 mg/kg was also included in LD₅₀ calculation (10 dosed rats died). Death occurred in groups dosed with 1700 mg/kg and higher. The calculated acute oral LD₅₀ was 2 730 mg/kg (95 % confidence interval: 2346-3178 mg/kg bw). On this basis, the DS proposed no classification for 1,4-DMN.

Acute toxicity - Dermal route

No deaths occurred in an acute rabbit dermal toxicity study performed according to OECD

TG 402 (GLP). The acute dermal LD₅₀ was greater than 2000 mg/kg in the study. On this basis, no classification was proposed by the DS.

Acute toxicity - Inhalation

In a study in line with OECD TG 403 (GLP), the LC₅₀ observed in rats exposed to a single dose of 1,4-DMN (aerosol) was found to be greater than 4.16 mg/L. One death out of 10 animals occurred at this dose level. The DS reasoned that, if an actual dose of 5 mg/L was used, would result in no difference in the toxicity outcome and proposed no classification.

Comments received during public consultation

No specific comments were received.

Additional key elements

In the first part (1993) of the *in vivo* micronucleus assay in mice, a dose selection study was performed before the main study. According to the full study report, mice (3/sex) were given one-time 500, 900, 1300, 1625, 2750, 3875 and 5000 mg/kg of 1,4-DMN by gavage (vehicle was corn oil). Mortality observed within 3 days was as indicated in the table below. The cause of death was not given and no necropsy was performed.

Treatment (mg/kg)	Males	Females
500	0/3	0/3
900	0/3	1/3
1300	2/3	0/3
1625	3/3	3/3
2750	3/3	3/3
3875	3/3	3/3
5000	3/3	3/3

Assessment and comparison with the classification criteria

Acute toxicity: oral

The LD₅₀ of 1,4-DMN was above 2000 mg/kg in the available rat acute toxicity study (vehicle was corn oil). Based on the results of the preliminary study performed in the *in vivo* micronucleus assay, mice seemed more sensitive than rats as the LD₅₀ was below 2000 mg/kg. Although this range-finding study was not designed for acute toxicity classification, the study was GLP, well-performed to investigate acute toxicity of 1,4-DMN and used the same vehicle as in the rat study. There is no information in the dossier supporting that mice would not be a relevant species. Therefore, according to the CLP criteria, **a classification as Acute Tox. 4, H302 is warranted** via the oral route.

Regarding the ATE, the default converted value of 500 mg/kg for Acute Tox. 4 may be too conservative as no death occurred at this dose level in the mice study. As the LD₅₀ is according to the study between 1300 and 1650 mg/kg bw, RAC proposed an ATE of

1 300 mg/kg bw.

Acute toxicity: dermal

The LD₅₀ of 1,4-DMN in rabbits was greater than 2000 mg/kg in both sexes. **No classification via the dermal route is warranted** according to the CLP criteria.

Acute toxicity: inhalation

In an OECD TG 403 study, 10 rats (5/sex) were exposed, whole-body, to 4.16 mg/L/4h of 1,4-DMN (aerosol). Only one death occurred at 4.16 mg/L. Thus, the LC₅₀ in rats is expected to be higher than the 5 mg/L cut-off for acute inhalation toxicity classification. Therefore, RAC agrees with the DS **not to classify 1,4-DMN for acute inhalation toxicity**.

10.4 Skin corrosion/irritation

Table 15: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
FIFRA 152-14 (in line with OECD 404) Deviations: none	Rabbit, New Zealand White, 3/sex	1,4-dimethylnaphthalene (batch H5510, purity 94.6%)	0.5 ml, 4h, semi-occluded	Erythema (grade 1-2) and oedema (grade 1-3) from 1 h after exposure Mean scores: Erythema: 1.67; 2; 2; 2; 2; 2 Oedema: 1; 0.33; 0.67; 0.33; 0.33; 0.67 Reversible by day 14.	IIA 5.2/04, DocID L08456-study 2
FIFRA 152-11 (in accordance with OECD 402) Deviations: none	Rabbit, New Zealand/White 5/sex	1,4-dimethylnaphthalene (batch H5510, purity 96.4%)	2000 mg/kg bw Single dose, 24h under occlusion	> 2000 mg/kg bw Eschar formation was observed within 5 days following unwrapping.	IIA 5.2/02, Doc ID L08456, study 4

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

In a skin irritation study (IIA 5.2/04) 0.5 ml of undiluted 1,4-dimethylnaphthalene (purity 96.4%) was applied to the backs of 3 male and 3 female New Zealand White rabbits under semi-occluded conditions. After 4 h the application sites were wiped with gauze and 0.9% saline to remove residual

test substance. Dermal changes were graded according to Draize. The skin was examined up to 14 days after application. Erythema (grade 1-2) and oedema (grade 1-3) was observed in all animals at 1 hour after application (see Table 16). The effects were fully reversible by day 14 of the study. The individual mean scores were 1.67, 2, 2, 2, 2 and 2 for erythema and 1; 0.33, 0.67, 0.33, 0.33 and 0.67 for oedema.

Table 16: Individual skin irritation scores up to 14d after application

	1 h	24 h	48 h	72 h	7d	14d
erythema	1/2/2/1/1/1*	2/2/2/2/2/2	1/2/2/2/2/2	2/2/2/2/2/2	0/0/0/2/2/2	0/0/0/0/0/0
oedema	3/2/1/2/2/1	2/1/2/1/1/1	0/0/0/0/0/0	1/0/0/0/0/1	0/0/0/0/0/0	0/0/0/0/0/0

*m/m/m/f/f/f

In an acute dermal toxicity study (IIA 5.2/02) the undiluted test substance was administered to rabbit for 24 hours after which the test substance was wiped off with a gauze pad moistened with saline. Rabbits were observed for 14 days after administration. Signs of dermal irritation at the application site (oedema and erythema) were observed in all rabbits immediately following removal of the wrappings. All animals developed eschar formation within five days following unwrapping. In 4 animals, eschar formation persisted until the end of the 14 days observation period.

10.4.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) Table 3.2.2 a substance should be classified for skin irritation Category 2 in the case where:

- (1) Mean value of $\geq 2,3 - \leq 4,0$ for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or
- (2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or
- (3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

In an acute dermal toxicity study carried out in accordance with OECD Guideline 402 signs of dermal irritation were observed which persisted until the end of the 14 days observation period. The irreversibility could be considered sufficient for classification as irritating. However, in a dermal irritation study following OECD Guideline 404 the mean erythema and oedema scores were below 2.3 in all animals and considering that the effects were fully reversible by day 14 no classification for skin irritation is required. As the exposure period in the acute dermal toxicity test (24 hours) was much longer than in the acute irritation test (4 hours) and occlusion instead of semi-occlusion was applied, the results of the dermal irritation study are given a higher weight than the results of the acute dermal toxicity study. The criteria for classification as irritant are not fulfilled.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

No classification is proposed.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin irritation potential of 1,4-DMN was investigated in a rabbit study. The study was GLP-compliant and performed according to US EPA TG (FIFRA 152-14) which is similar to OECD TG 404. Test substance was applied undiluted for 4-hour in six rabbits (3/sex). One hour following exposure, skin irritation was slight to moderate (grade 1 to 3). Individual mean scores for 24-72h were 1.7, 2, 2, 2, 2, 2 for erythema and 1, 0.33, 0.67, 0.33, 0.33, 0.67 for oedema. All the findings were reversible by day 14. Based on the available data no classification was proposed by the DS.

In the acute dermal toxicity study (see above), eschar formation was noted within 5 days following unwrapping in all 10 rabbits. The effects were not reversible at the end of the 14-day observation period in 4 rabbits. As the exposure period was 24 hours and occlusion instead of semi-occlusion was used, the DS gave more weight to the results of the dermal irritation study. On this basis, the DS considered that the criteria for classification as skin irritant were not fulfilled.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

RAC agrees with the DS that based on *in vivo* dermal irritation study, in accordance with the Guidance on the Application of the CLP Criteria v5.0, July 2017 ("CLP Guidance") in case of 6 rabbits, no classification is warranted.

RAC agrees that in some cases, acute dermal toxicity data may also be used for classification as skin corrosion/irritation. In the available acute dermal toxicity study also performed in rabbits with undiluted test material, there were no signs of skin corrosion. Eschar formation was noted in all rabbits (n = 10) but no scores were provided. In four rabbits, eschars were not reversible by day 14 but new or repaired skin was evident at the application site of these rabbits suggesting a transient effect. RAC considers that the transient eschar formation observed in this acute toxicity study using worst case conditions does not support classification of 1,4-DMN. RAC agrees to give more weight to the rabbit skin irritation study.

Overall, RAC agrees with the DS that **no classification** for skin corrosion/irritation **is warranted** for 1,4-DMN.

10.5 Serious eye damage/eye irritation

Table 17: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
FIFRA 152-13 (in line with OECD 405) Deviations: none	Rabbit, New Zealand White 3/sex	1,4-dimethylnaphthalene (batch H551, purity 96.4%)	0.1 ml (undiluted), single instillation in conjunctival sac	Conjunctival redness and chemosis from 1 hour after exposure. Mean Score: Corneal opacity: 0 Iris: 0 Conj. Redness: 1.67; 1.33; 1; 1; 1.67; 2 Conj. Chemosis: 1; 2; 2; 2; 2; 3	IIA 5.2/05, Doc ID L08456-study 1

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

In a eye irritation study (IIA 5.2/05) 0.1 ml undiluted 1,4-dimethylnaphthalene (purity 94.6%) was applied to the right eye of 3 male and 3 female New-Zealand White rabbits. Ocular changes were graded according to Draize. The eyes were examined up to 21 days after instillation.

Two rabbits were vocal immediately after administration of the test substance. Circumocular alopecia was observed in 3 animals at 14 days and in 2 animals at 21 days after application. The individual eye irritation scores are reported in table 19. The mean scores were 0 for corneal opacity and iritis. For conjunctival redness the individual mean scores were 1.67, 1.33, 1, 1, 1.67 and 2. For conjunctival redness and chemosis the individual mean scores were 1, 2, 2, 2, 2, 3.

Table 18: Individual eye irritation scores up to 21d after instillation

	1 h	24 h	48 h	72 h	Day 7	Day 14	Day 21
Corneal opacity	0/0/0/0/0*	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0
Corneal area	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0
Iris	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0
Conj. redness	2/2/2/2/2	2/2/2/2/2	2/1/1/2/2	1/1/0/1/2	1/1/0/1/2	0/0/0/1/0	0/0/0/0/0
Conj. chemosis	1/2/3/2/1/2	1/2/2/2/3	1/2/2/2/3	1/2/2/2/3	1/1/1/1/3	0/0/0/1/2	0/0/0/0/0
Conj. discharge	0/0/0/0/0	0/0/0/0/2	0/0/0/1/2	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0	0/0/0/0/0

* m/m/m/f/f/f

10.5.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) Table 3.3.2.1.2 a substance should be classified for Serious eye damage Category 1 or eye irritation Category 2 in the case where:

a. Classification for serious eye damage – Category 1 if:

i. at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or(ii) at least 4 out of 6 rabbits show a mean score per animal of ≥ 3 for corneal opacity and/or > 1.5 for iritis

b. Classification for eye irritation – Category 2 if at least 4 out of 6 rabbits show a mean score per animal of:

i. ≥ 1 for corneal opacity and/or

ii. ≥ 1 for iritis and/or

iii. ≥ 2 conjunctival erythema (redness) and/or

iv. ≥ 2 conjunctival oedema (swelling) (chemosis)

and which fully reverses within an observation period of normally 21 days.

The mean scores for corneal opacity and iritis was 0. For conjunctival redness the individual mean scores were below the criteria of 2 except for in 1 animal (out of 6). For conjunctival oedema the individual mean scores were ≥ 2 in 5 out of 6 animals which fulfils the criteria for category 2. The effects observed were fully reversible at day 21 and therefore classification as eye irritant Category 2 is required.

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

Harmonised classification proposed (Eye irritation Category 2).

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter’s proposal

A reliable GLP-compliant study was performed in six albino rabbits according to US EPA TG (FIFRA 152-13), similar to OECD TG 405. Undiluted substance induced slight to moderate conjunctival erythema and oedema, which was fully reverse after day 21. The mean individual 24-72h scores were as follow:

- 0 for corneal opacity and iritis;
- For conjunctival redness, scores were ≥ 2 in 1 out of 6 animals;
- For conjunctival oedema, scores were ≥ 2 in 5 out of 6 animals.

According to the CLP Guidance in case of 6 rabbits, based on mean individual scores ≥ 2 for conjunctival oedema in 5 out of 6 rabbits, the DS concluded that 1,4-DMN should be classified as Eye Irrit. 2; H319.

Comments received during public consultation

Two member states (MS) agrees with the proposed classification.

Assessment and comparison with the classification criteria

Based on the available reliable eye irritation study in rabbits, RAC agrees with the DS’s proposal to **classify 1,4-DMN as Eye Irrit. 2; H319.**

10.6 Respiratory sensitisation

No data available.

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

No data available.

10.6.2 Comparison with the CLP criteria

Not relevant.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification proposed, data lacking.

RAC evaluation of respiratory sensitisation
<p>Summary of the Dossier Submitter’s proposal</p> <p>There is no data available. No classification is proposed.</p> <p>Comments received during public consultation</p> <p>No comments were received.</p> <p>Assessment and comparison with the classification criteria</p> <p>RAC agrees with the DS proposal for no classification as data are lacking.</p>

10.7 Skin sensitisation

Table 19: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD 429 Deviations: none	mice, CBA/J 5/females/dose	1,4-dimethylnaphthalene (batch 14D06B01-01, purity 98.4%)	0, 25, 50 and 100% topical induction	SI for 25, 50% and 100%: 2.1, 2.4, 2.8	IIA 5.2/07, Doc ID 495316

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

In a LLNA study (IIA 5.2/07) four groups of five female CBA/J mice were treated to the control (acetone/olive oil 4:1 v/v) or to the 1,4-dimethylnaphthalene (purity 98.4%) at 25, 50 or 100% w/w

on three consecutive days by topical application on the ears. Three days after the last exposure, all animals were injected via the tail vein with ³H-methyl thymidine and after five hours the draining (auricular) lymph nodes were excised and pooled for each animal. After precipitating the DNA of the lymph node cells, radioactivity measurements were performed. The activity was expressed as the number of Disintegrations Per Minute (DPM) and a stimulation index (SI) was subsequently calculated for each group.

Enlarged auricular lymph nodes were found in the groups dosed at 50 and 100%. Mean DPM/animal values for the experimental groups treated with test substance concentrations 25, 50 and 100% were 1031, 1167 and 1384 DPM respectively. The mean DPM/animal value for the vehicle control group was 488 DPM. The SI values calculated for the substance concentrations 25, 50 and 100% were 2.1, 2.4 and 2.8, respectively.

10.7.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) substance should be classified for skin sensitisation when the results of a LLNA study show a SI above 3. As this was not the case for 1,4-dimethylnaphthalene no classification for skin sensitisation is needed.

10.7.3 Conclusion on classification and labelling for skin sensitisation

No classification is proposed.

<p>RAC evaluation of skin sensitisation</p>
<p>Summary of the Dossier Submitter’s proposal</p> <p>1,4-DMN was tested in a Local Lymph node assay (LLNA) in mice, conducted according to OECD TG 429 (GLP-compliant). The vehicle used in the study was acetone/olive oil (4:1 v/v). In this study, 1,4-dimethylnaphthalene was not found to be a skin sensitiser since stimulation index were below 3 up to 100 % w/w concentration. On this basis, no classification was proposed by the DS.</p> <p>Comments received during public consultation</p> <p>No comments were received.</p> <p>Assessment and comparison with the classification criteria</p> <p>No human data were available in the dossier.</p> <p>RAC considers the available LLNA study reliable. Although a dose-response was observed in the stimulation index values calculated for the substance concentrations (2.1, 2.4 and 2.8 at 25, 50 and 100 %, respectively), no classification is warranted as the values were below the cut-off criteria of 3 for a positive result in an LLNA study.</p> <p>RAC agrees with the DS that no classification is warranted for 1,4-DMN.</p>

10.8 Germ cell mutagenicity

Table 20: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Ames test, FIFRA 84-2 (in line with OECD 471) Deviations: TA1538 was tested instead of TA 102 or <i>E. coli</i>	1,4-dimethylnaphthalene (batch H5510, purity 96.4%)	Test system: TA98, TA100, TA1535, TA1537, TA1538 Test concentrations: +S9: 0, 10, 50, 100, 250, 500 and 1000 µg/plate -S9: 0, 1, 5, 10, 25, 50 and 250 µg/plate Based on range finding study. Positive controls: 2-aminoanthracene (all strains +S9), 2-nitrofluorene (TA98, TA1538 -S9), sodium azide (TA100, TA1535 -S9) and ICR-191 (TA1537 -S9)	Cytotoxicity: ≥ 333 µg/plate in the presence of S9 and doses ≥ 33.3 µg/plate in the absence of S9 No treatment related increase in revertant colonies.	IIA 5.4/01 Doc ID 15683-0-401
Ames test OECD 471 Deviations: none	1,4-dimethylnaphthalene (batch 14D03M01-02, purity 98.4%)	Test system: TA98, TA100, TA1535, TA1537, TA102 Test concentrations: Exp 1: 0, 0.128, 0.64, 3.2, 16, 80, 400 and 2000 µg/plate Exp 2: 0, 1.638, 4.096, 10.24, 25.6, 64, 160, 400 µg/plate Positive controls were 2-nitrofluorene (TA98, -S9), sodium azide (TA100 and TA1535, -S9), 9-aminoacridine (TA1537, -S9), mytomycin C (TA102, -S9), benzo[a]pyrene (TA98, -S9) and 2-aminoanthracene (all strains, +S9).	Cytotoxicity: ≥ 400 µg/plate in the presence of S9 and doses ≥ 80 µg/plate in the absence of S9 No treatment related increase in revertant colonies.	IIA 5.4/02 Doc ID 2782/2-D6171

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Mammalian gene mutation OECD 476 Deviations: none	1,4-dimethylnaphthalene (batch 14D03M01-02, purity 98.4%)	Test system: L5178Y mouse lymphoma cells Test concentrations: +S9: 50, 75, 100, 150, 170, 200, 210 and 220 µg/ml -S9: 1, 5, 10, 20, 30, 35, 37.5, 40 µg/ml Positive controls: methylmethanesulfonate (- S9) and cyclophosphamide (+S9)	Cytotoxicity: RTG <20% from 40 µg/plate in absence of S9 and from 210 µg/plate in presence of S9 Dose-related increase in mutant frequency outside of historical control in the presence of S9, but not in the absence of metabolic activation.	IIA 5.4/03 Doc ID 424711
Unscheduled DNA synthesis FIFRA 84/2(3) (in line with OECD 482) Deviations: no statistical analysis was carried out.	1,4-dimethylnaphthalene (batch H5510, purity 96.4%)	Test system: rat liver primary cell cultures Test concentrations: 0.250 -10 µg/ml Positive control: 2-acetylaminofluorene	Cytotoxicity: 58.2% survival at 10 µg/ml Gene mutation: equivocal	IIA 5.4/04 Doc ID 15683-0-447

Table 21: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
<i>In vivo</i> micronucleus FIFRA 84.2 (in line with OECD 474) Deviations:	1,4-dimethylnaphthalene (batch H5510, purity 96.4%)	Male and female CD-1 (ICR) mice, 5/sex/dose Dose levels: 0, 225, 450, 900 mg/kg bw/day, single dose Positive controls:	Slightly reduced PCE:NCE in females (statistically significant, but within HCD) No treatment related increase in micronucleated PCE.	IIA 5.4/05 and IIA 5.4/06 Doc ID 15683-0-455 and 7931-100

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
none		cyclophosphamide		
<i>In vivo</i> Unscheduled DNA synthesis OECD 486 Deviations: acclimatisation period of 2 days instead of 5.	1,4-dimethylnaphthalene batch no: 14D03M01-02 (range-finding) and 14D06B01-01 (main study); purity: 98.4-98.6%)	Rats, Sprague Dawley, males (4/group) Dose levels: 0, 500, 1000 mg/kg bw. Positive controls: 2-cetamidofluorene and dimethylnitrosamine	No increase in net grain count.	IIA 5.4/07 Doc ID 1000/31-D6172

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro data:

In a bacterial reverse mutation assay (IIA 5.4/01) 1,4-dimethylnaphthalene (batch H5510, purity 96.4%) was tested ranging from 10-1000 µg/plate in the presence of S9 and from 1-250 µg/plate in the absence of S9 mix. Used doses were based on a range finding study with TA100 with doses up to 5 mg/plate, which resulted in cytotoxicity at doses ≥ 333 µg/plate in the presence of S9 and doses ≥ 33.3 µg/plate in the absence of S9 (Aroclor 1254). The testes strains used in the study were TA98, TA100, TA1535, TA1537 and TA1538. 2-aminoanthracene was used as positive control in the presence of S9 and 2-nitrofluorene (TA98, TA 1538), sodium azide (TA100, TA 1535) and ICR-191 (TA1537) were used as positive controls in the absence of S9.

No increase in the number of revertants per plate were observed for strains TA98, TA100, TA1535 and TA1537. For strain TA1538 there was a 2.7 fold increase in revertants at a concentration of 250 µg/plate in the presence of S9. Although this increase was within the 3-fold range which was used in the study report as criteria for a positive effect it was decided to repeat the test in strain TA1538 in the presence of S9 in two separate experiments. No increase in revertant colonies was observed in these repeat studies. It was therefore concluded that 1,4-dimethylnaphthalene does not induce gene mutations in *S. typhimurium*.

Table 22: Summary table of the bacterial reverse mutation assay in strain TA1538 with S9

Dose level (µg/plate)	TA1538 (exp. 1)	TA1538 (exp. 2)	TA1538 (exp. 3)
0	16	14	18
10	14	14	17
50	16	15	20
100	23	16	20
250	43	20	18
500	2	0	14
1000	0	0	5
Positive control	940	1123	905

A second bacterial reverse mutation assay (IIA 5.4/02) was carried in accordance with OECD 471. 1,4-dimethylnaphthalene (batch no: 14D03M01-02; purity: 98.4%) was assayed for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments. Concentrations used were based on a range finding study with TA100. Appropriate positive controls were included.

In experiment 1, clear evidence of toxicity occurred in all strains at 400 µg/plate and above, in the absence and in the presence of S9, and also at 80 µg/plate in most strains in the absence of S9 and strain TA1535 in the presence of S9. In experiment 2, the dose range was narrowed and evidence of toxicity was observed in all strains at 64 µg/plate and above in the absence of S9 and 160 µg/plate and above in the presence of S9. 1,4-dimethylnaphthalene did not induce a biologically significant increase in revertant colonies. The positive controls induced the expected increases in revertant colonies in all strains. It was concluded that 1,4-dimethylnaphthalene does not induce gene mutations in *S. typhimurium*.

A mammalian gene mutation study (IIA 5.4/03) evaluated the effect of 1,4-dimethylnaphthalene (batch no: 14D03M01-02; purity: 98.8%) on the induction of forward mutation at the thymidine-kinase locus in L5178Y mouse lymphoma cells. Used doses of 1,4-dimethylnaphthalene (purity 98.8%) were based on a range finding study with doses up to 1 mg/mL, which resulted in cytotoxicity at doses ≥ 100 µg/mL in the presence of S9 and doses ≥ 33 µg/mL in the absence of S9. The dose levels selected to measure mutation frequencies at the *tk*-locus were: 1, 5, 10, 20, 30, 35, 37.5 and 40 µg/mL exposure medium in the absence of S9 and 50, 75, 100, 150, 170, 200, 210 and 220 µg/mL exposure medium in the presence of S9. Positive controls were methylmethanesulfonate (MMS) without S9 and cyclophosphamide (CP) with S9.

In the presence of S9, dose-related increases in the mutant frequency were observed (at precipitating dose levels) both in small and large sized colonies. Increases were more than three-fold (3.7-fold for small colonies and 4.0-fold for large colonies) at a dose of 170 µg/mL (survival 38% in exp 1 and 33% in exp 2) and outside the historical control data range. At higher doses of 200-220 µg/ml increases were less than three-fold. At these doses the relative total growth was dose-dependently further reduced to 12% compared to the total growth of the solvent controls.

Based on the results of the study 1,4-dimethylnaphthalene was concluded to be mutagenic in mouse lymphoma L5178Y in the presence of metabolic activation, but not in the absence of metabolic activation. It is noted that the positive effect was observed at precipitating dose levels.

1,4-dimethylnaphthalene (batch no: H5510; purity: 96.4%) was tested in an *in vitro* assay for Unscheduled DNA Synthesis in rat liver primary cell cultures (IIA 5.4/04). Five cultures/treatment were included. Two were used for cytotoxicity measurements, the other 3 for analysis of nuclear labeling. Six treatments, from 0.250 to 10 µg/mL (108.9 to 58.2% survival) were selected for analysis of nuclear labeling. The positive control was 2-acetylaminofluorene. The study report used as evaluation criteria an increase in the mean net nuclear grain count to at least five grains nucleus above the concurrent solvent control (2.32) and/or an increase in the percent of nuclei having five or more grains such that the percentage of these nuclei in test cultures is at least 10% above the percentage observed in the solvent control cultures (12.67%). This differs from the criteria in OECD Guideline 482 (1987) which uses a significant dose-related increase in radiolabel incorporation or a reproducible and statistically significant positive response for at least one of the test points.

At a concentration of 10 µg/mL some toxicity was observed (58.2%). The high dose of 10.0 µg/ml resulted in a slight increase in mean nuclear grains (NNG) and in the percentage of cells with ≥ 5 mean NNG (see Table below). This was within the evaluation criteria used within the study but was slightly outside of historical control range (NNG -0.48 to -2.11, % cells with >5 mean NNG 0.67-9.00). Since no statistical analysis was carried out and since no repeat study was performed to

determine the reproducibility of the finding at 10 µg/ml the result of the study is considered equivocal.

Table 23: Results of the *in vitro* UDS

Concentration µg/ml	Mean Net Nuclear Grains (NNG) ¹	% Cells w/≥ 5 mean NNG ¹	Mean Cytograins	% Survival at 20 hours
0	-2.68	2.67	16.17	100
0.250	-1.65	6.00	13.97	103
0.500	-1.37	3.33	12.45	103.2
1.00	-1.18	8.67	10.82	106.8
2.50	-2.77	3.99	16.36	108.9
5.00	-1.38	7.97	14.06	95.8
10.0	0.01	10.00	9.75	58.2
Positive control	7.35	63.33	12.35	89.4

¹ Average values for triplicate coverslips

In vivo data:

In an *in vivo* micronucleus study (IIA 5.4/05 and IIA 5.4/06) male and female CD-1 (ICR) mice (5/sex/dose) received a single dose of 1,4-dimethylnaphthalene (batch no: H5510; purity: 96.4%) in corn oil, by gavage in doses of 225, 450 or 900 mg/kg bw. Bone marrow was harvested at 24, 48 and 72 hr after treatment and bone marrow slides were made and analysed. Appropriate controls were used (solvent control and cyclophosphamide as positive control). The study was performed in 2 parts. In the first part, reported in 1993, 1000 polychromatic erythrocytes were scored for the incidence of micronucleated polychromatic erythrocytes results of the study. In the second part, reported in 2007, 2000 polychromatic erythrocytes were scored for micronuclei.

Approximately 1 day after dosing, 1 high-dosed female appeared prostrate with dyspnoea. One male and 1 female (high-dose) appeared languid with dyspnoea. Approximately 2 days after dosing, 2 males and 3 females (high-dosed) were found dead. 1,4-dimethylnaphthalene caused decreases in the PCE:NCE ratios at 48 and 72h as compared to 24h (statistically significant in females only), although PCE/NCE ratios for the treated groups were within the historical control range. In view of the mortalities and the above mentioned clinical signs, and the toxicokinetic studies showing that closely related naphthalenes are well absorbed from the gut and rapidly occur in blood following oral dosing, it is assumed that 1,4-dimethylnaphthalene in this study will have reached the bone marrow of the mice.

In the first part of the study (1993), 1,4-dimethylnaphthalene induced a statistically significant increase in micronucleated polychromatic erythrocytes in females at the low dose in the 48-hour harvest group only. This was considered biologically irrelevant, since at higher doses as well as at later sampling points no increases in micronucleated polychromatic erythrocytes were observed. In addition, values were within the historical response range. In the second part of the study (2007) 1,4-dimethylnaphthalene induced statistically significant increases in micronucleated polychromatic erythrocytes in females at the mid-dose in the 24-hour harvest group. This was considered biologically irrelevant, since at higher doses as well as at later sampling points no increases in micronucleated polychromatic erythrocytes were observed and again the values were within historical control range.

1,4-dimethylnaphthalene was concluded to be negative in the mouse bone marrow micronucleus assay.

In an *in vivo* unscheduled DNA synthesis assay (IIA 5.4/07) male Sprague Dawley rats (n=4/group) received a single dose of 1,4-dimethylnaphthalene (batch no: 14D03M01-02 (range-finding) and 14D06B01-01 (main study); purity: 98.4-98.6%) in corn oil, by gavage in doses of 500 or 1000 mg/kg bw. Doses were based on a range finding study in male and female rats with doses of 1000, 1400 and 2000 mg/kg bw, in which toxicity was observed at doses \geq 1400 mg/kg bw. No clinical signs of toxicity were observed in rats dosed with 500 or 1000 mg/kg bw. Positive controls were 75 mg/kg 2-acetamidofluorene and 10 mg/kg dimethylnitrosamine.

1,4-dimethylnaphthalene did not induce an increase net grain count. One of the positive controls failed the acceptance limits for a positive control response. Nevertheless, a marked increase in net nuclear grain (NNG) value was observed compared to the concurrent vehicle control (almost 4-fold increase) with a clear percentage of cells in repair, indicating that an increase in unscheduled DNA synthesis could be detected by the assay. It was concluded that 1,4-dimethylnaphthalene does not produce induction of unscheduled DNA synthesis in rat liver.

Overall, while some *in vitro* tests were positive for gene mutation the *in vivo* study were clearly negative and therefore 1,4-dimethylnaphthalene was concluded to be non-genotoxic.

10.8.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP), Table 3.5.2.2, classification in Category 2 mutagen is based on:

- *Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:*
- *Somatic cell mutagenicity tests in vivo, in mammals; or*
- *Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays*

1,4-dimethylnaphthalene was tested negative for gene mutation in 2 bacterial gene mutation assay, but was tested positively in an *in vitro* mammalian gene mutation study. In addition, an *in vitro* UDS assay was concluded to be equivocal. In contrast, in the available *in vivo* micronucleus and UDS assay 1,4-dimethylnaphthalene was negative. It is therefore concluded that 1,4-dimethylnaphthalene does not fulfil the criteria for classification for mutagenicity.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification is proposed.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

No classification for germ cell mutagenicity was proposed by the DS. A positive result was obtained in an *in vitro* mammalian cell gene mutation test in presence of metabolic activation. Moreover, an equivocal result was observed in an *in vitro* unscheduled DNA synthesis test. Nevertheless, negative results were obtained in two Ames assays, in an *in vivo* unscheduled DNA synthesis assay and in an *in vivo* micronucleus test. Based on the two negative *in vivo* assays, the DS concluded that 1,4-DMN does not fulfil the classification criteria for germ cell mutagenicity.

Comments received during public consultation

One MS and one national authority commented that uncertainties remained on the genotoxic potential of 1,4-DMN. Indeed, the MS pointed out that no relevant *in vivo* follow-up test was available to rule out the positive *in vitro* mammalian gene mutation assay.

Moreover, the national authority provided *in silico* modelling showing positive alerts for *in vitro* gene mutation assay and *in vivo* comet assay in mice (Danish(Q)SAR database). In addition, they reported a positive alert for *in vitro* mutagenicity in VEGA (Q)SAR platform (SARpy model). As mutagenicity cannot be excluded, the national authority proposed classification for germ cell mutagenicity in category 2.

The DS agreed that the positive result obtained in the *in vitro* mammalian gene mutation study was insufficiently addressed and that an appropriate follow-up would be needed for the renewal of the authorisation of the pesticide active substance. Thus, the DS proposed no classification based on **lack of data**.

Assessment and comparison with the classification criteria

In vitro data

Two bacterial gene mutation assays were negative.

The first test was equivalent to OECD TG 471 (GLP-compliant) except that:

- The test did not include *S. typhimurium* TA102 or *E. coli* WP2;
- Only 2-aminoanthracene was used as positive control with metabolic activation whereas at least a second positive control is recommended in the test guideline.

Overall, RAC considered this negative study reliable with limitations.

In the second Ames assay performed according to OECD TG 471 and GLP-compliant, negative results were obtained in 5 strains including *S. typhimurium* TA100 and *E. coli* WP2.

An *in vitro* gene mutation assay in mammalian cells was performed according to OECD TG 476 (GLP-compliant). In comparison with the latest version of the guideline, no statistical analysis was performed. A dose-related three-fold increase in mutant frequency was observed at 170 µg/mL, in presence of metabolic activation, in both independent experiments. At this dose, the mutation frequency was observed above historical control range values. At 170 µg/mL, survival was above 20 %. Precipitation was observed in the study at 150 µg/mL and above. At higher dose levels, mutation frequency was no more increased but observed precipitation may have interfere with the conduct of the test. 1,4-DMN showed up to 4.2 and 2.1 fold increases in the mutation frequency of small and large colonies compared to controls. Overall, RAC considers the study positive in presence of metabolic activation.

An *in vitro* UDS was also reported in the dossier (similar to OECD 482 except that no statistical analysis was performed, GLP unknown status). The study was considered negative based on the evaluation criteria of the study but as the mean net nuclear grains at 10 µg/mL was outside the historical control data, RAC agrees that the study is equivocal. Moreover, RAC notes that no metabolic activation was used in the study.

In vivo data

In vivo, negative results were obtained in a micronucleus test performed in mice up to 900 mg/kg. The study was performed according to OECD 474 in two parts (one reported in 1993 and one in 2007 analysing a higher number of micronucleated PCEs). The top dose was chosen based on mortality observed in a preliminary study at ≥ 900 mg/kg. PCE:NCE ratio was decreased at 48 and 72h compare to 24h. Nevertheless, RAC notes that the decrease was not clearly dose-related. In the first part of the study, a statistically significant increase in micronucleated PCE was observed in female at the low dose only. The increase was within the historical control range. In the second part of the study, an increase in micronucleated PCE was noted in females at 450 mg/kg. The increase was within the historical control range and was due to a single female. Therefore, RAC agrees that the study was negative.

An *in vivo* UDS assay was reported in rat. The test was performed up to 1 000 mg/kg in rats due to toxicity observed at ≥ 1400 mg/kg in a preliminary test. The reliability of the study is questionable. Positive control was not clearly positive as net grain count was below 5. Moreover, corn oil was used as a vehicle (which may have contained antioxidants that could interfere with the test results) and negative control was not clearly negative as net grain count was positive. Due to the above limitations, RAC considered this study unreliable.

In silico data

With regard to structure-activity relationships, the predicted positive results in Ames assay obtained in the SARpy model (VEGA platform) has low weight as the model investigate Ames assay and two negative experimental Ames assay were available with 1,4-DMN. Moreover, VEGA consensus model (based on 4 models) for Ames assay leads to a negative prediction. RAC however notes the consensus alert in the *in vivo* comet assay (Case Ultra, Leadscope, SciQSAR, inside applicability domain).

Comparison with the classification criteria

No classification is warranted based on the CLP classification criteria as negative results were observed in the *in vivo* micronucleus assay. Nevertheless, RAC agrees with the DS that the results in the genotoxicity studies are inconclusive as the equivocal result obtained in the *in vitro* mammalian gene mutation assay was not adequately followed-up *in vivo*. Therefore, RAC agrees with **no classification for germ cell mutagenicity due to lack of data.**

10.9 Carcinogenicity

Table 24: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels of duration exposure	Results	Reference

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels of duration of exposure	Results	Reference
<p>OECD 453</p> <p>Deviations: histopathology report did not differentiate between interim and terminal kill</p> <p>Rat, Sprague-Dawley CD IGS, Crl:CD(SD)</p> <p>Interim: 20/sex/dose</p> <p>Terminal: 65/sex/dose</p>	<p>1,4-dimethylnaphthalene</p> <p>(Batch no: F243A040/14D06B01-01, Purity: 98.7%)</p> <p>Dose levels: 0, 150, 500 and 3750 ppm (equal to 0, 8, 27 and 208 mg/kg bw/day in males and 0, 10, 33 and 247 mg/kg bw/day in females)</p> <p>Interim: 52 week</p> <p>Terminal: 104 week</p>	<p>NOAEL general toxicity: 150 ppm (equal to 10 mg/kg bw/day) based on minimal karyomegaly in kidneys in females.</p> <p>NOAEL carcinogenicity: 3750 ppm (equal to 208 mg/kg bw/day).</p> <p>No increase in neoplastic findings.</p>	<p>IIA 5.3/01 Doc ID 02-154</p>

Table 25: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
<p>Public literature, non-GLP</p> <p>Klimisch Score 2</p>	<p>1-methylnaphthalene (purity >97%)</p>	<p>B6C3F1 mice (50/sex/dose)</p> <p>0, 0.075, 0.15% 1-methylnaphthalene for 81 weeks (average intake 75.1 and 71.6 mg/kg bw/day in the low dose group, and 143.7 and 140.2 mg/kg bw/day in the high dose group for females and males)</p>	<p>Increased pulmonary alveolar proteinosis at both dose groups (46.0 and 34.7% of females and 46.0 and 38.0% of males, respectively).</p> <p>Increased bronchiolar alveolar adenomas in males (26% and 24%).</p> <p>No difference in bronchiolar/alveolar carcinomas.</p>	<p>IIA 5.5/04</p> <p>Cited in ATSDR 2005 (IIA 5.7/02)</p>
<p>Public literature, non-GLP</p> <p>Klimisch Score 2</p>	<p>2-methylnaphthalene (purity >97%)</p>	<p>B6C3F1 mice (50/sex/dose)</p> <p>0, 0.075, 0.15% 1-methylnaphthalene for 81 weeks (average intake 50.3 and 54.3 mg/kg bw/day in the low dose group, and</p>	<p>Increased pulmonary alveolar proteinosis at both dose groups (55.1 and 45.8% in females and 42.9 and 46.9% in males, respectively).</p> <p>Increased bronchiolar/alveolar adenomas and carcinomas in males (20.4% and 12.2%).</p>	<p>IIA 5.5/05</p> <p>Cited in ATSDR 2005 (IIA 5.7/02)</p>

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		107.6 and 113.8 mg/kg bw/day in the high dose group for females and males)		

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

A combined chronic toxicity/carcinogenicity study (IIA 5.3/01) was carried out in accordance with OECD 453 (1981). 1,4-dimethylnaphthalene was applied by diet at dose levels of 0, 150, 500 and 3750 ppm. Relatively low survival was seen in control, low-dose and mid-dose females (28-35%) compared to high dose females (48%). The most common cause of death was pituitary adenomas; the incidence of pituitary adenomas was similar across groups (treated and control) for each sex. For high dose males the survival was acceptable (60%). For high dose females the survival was 48% however as the study started with 65 animals the reduction of the survival still results in a sufficient number of animals surviving at the end of the study. As the high dose groups showed a sufficient survival, the study was considered suitable for evaluation of the carcinogenic properties of 1,4-dimethylnaphthalene. Dietary intake of 1,4-dimethylnaphthalene resulted in actual doses of 8, 27 and 208 mg/kg bw/day in males and 10, 33 and 247 mg/kg bw/day in females.

No treatment-related clinical signs or mortality were observed in the study. At ophthalmic examinations, no eye abnormalities were observed. In high-dose males and females a significant decrease in food intake was observed. At termination of the study, body weight was significantly reduced in high-dose males (14%) and females (25%).

Platelet count was significantly decreased in high dosed males at 3 and 12 months and in high-dosed females at 3 and 6 months. In addition, high-dosed females showed significant decreases in MCV, haemoglobin and MCH at 6 months and significant decreases in MCV, MCH, MCHC, haemoglobin and white blood cell count at 12 months. High dosed males also had a significantly reduced white blood cell count at 12 months. No relevant effects were observed in the other groups. At study termination, high-dosed females showed significant decreases in MCV and MCH. No further toxicological relevant changes in haematology were seen at 18 months and at study termination.

Plasma cholesterol was increased in mid dose females (12 months only), high dose females (3, 6, 12 and 18 months), and in high dose males (18 months only).

Relative liver weight was statistically significantly increased (>10%) at the high dose in both sexes. At the terminal kill, high-dose females showed a statistically significant decrease in absolute kidney weight (17%). Histopathological changes were observed in the kidney. At 52 weeks these changes included an increased incidence in minimal to mild karyomegaly of the cortical tubules in mid and high-dosed females and high-dosed males, and an increased incidence of proteinosis in the tubules of the papilla and minimal papillary necrosis in high-dosed males. At the high dose the incidence of alveolar histiocytosis was increased in animals of both sexes. In males of the high dose group the incidences of lymphoid depletion and erythrocytic accumulation in the mesenteric lymph node were increased. At termination of the study at 104/100 weeks, histopathological changes in the kidneys included increased incidences of renal infarction, papillary mineralization, papillary necrosis, cysts in the cortex, proteinosis, dilatation of the papillary tubules and chronic progressive nephropathy in the high-dose males and/or females.

No increased incidences in neoplastic lesions were noted at study termination. The neoplasms observed in the study were typical of those frequently present in aged Sprague-Dawley rats, and no test material-related changes were noted for the incidence of neoplasms. Therefore, the administration of 1,4-dimethylnaphthalene to male and female Sprague-Dawley rats did not cause carcinogenicity.

Information on other naphthalene compounds from an Agency for Toxic Substances and Disease Registry (ATSDR) evaluation on the toxicological profile for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene showed that 1-methylnaphthalene and 2-methylnaphthalene induced bronchiolar/alveolar adenomas in mice (IIA 5.5/04 and IIA 5.5/05). No significant difference was observed in the incidences of bronchiolar/alveolar carcinomas between 1-MN-treated and control mice. For 2-MN, the incidences of total lung tumours, including bronchiolar/alveolar adenomas and carcinomas, were significantly increased only at the lowest dose males (control 4.1%, mid dose 20.4%, high dose 12.2%). It is noted that no dose-response was observed. There was no effect on adenocarcinoma in males and no effect on lung adenomas of adenocarcinoma in females.

Information from public literature indicate that mice are markedly more susceptible than rats to the pulmonary findings caused by naphthalene. Mice exposed by inhalation to 10 or 30 ppm naphthalene for 2 years showed lung inflammation, but rats exposed to concentrations up to 60 ppm showed no lung inflammation (Abdo et al. 2001 (IIA 5.5/11; NTP 1992 (IIA 5.5/12), NTP 2000 (IIA 5.5/13). The species difference in lung susceptibility to naphthalene has been correlated with higher rates of formation of a specific enantiomeric epoxide (1R,2S-naphthalene oxide) in lung microsomes and isolated dissected airways of mice compared with rats (Buckpitt et al. 1992, 1995 (IIA 5.5/09 and IIA 5.5/14). The highest rates of naphthalene metabolism were observed in mouse lung and liver microsomal incubations. Rat, hamster and monkey lung microsomal preparations metabolized naphthalene at 12, 37, and 1%, respectively, of the rate observed in mouse lung. Rat, hamster, and monkey lung microsomes preferentially formed the 1S,2R-naphthalene oxide enantiomer and showed lower rates of formation of epoxides than mouse lung microsomes with ratios of 0.48, 0.61 and 0.12 in rat, hamster and monkey lung microsomes, respectively compared to mice (Buckpitt et al. 1992 IIA 5.5/09). Microsomes from human lymphoblastoid cells expressing recombinant human CYP2F1 also showed preferential formation of the 1S,2R-naphthalene oxide enantiomer over the opposite enantiomeric 1R,2S-naphthalene oxide (Lanza et al. 1999 (IIA 5.5/15).

1,4-dimethylnaphthalene has a lower formation of highly reactive epoxide intermediates compared to 1-methylnaphthalene and 2-methylnaphthalene (see toxicokinetic information in section 9). The binding of epoxide intermediates to glutathione leading to thionaphthols results in the formation of 1,4-dimethyl-methylthionaphthalene. This thiomethyl derivative of 1,4-DMN was reported in the study IIA 5.1/01 in small amounts after i.p administration (1.39% of total identified compounds), but was not detected after oral administration in the ADME studies. It is therefore considered that systemic pulmonary alveolar proteinosis is unlikely for 1,4-dimethylnaphthalene.

10.9.2 Comparison with the CLP criteria

No information is available regarding carcinogenicity in humans. Therefore category 1A is not applicable.

Classification in category 1B requires “a causal relationship between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites”.

Classification in category 2 requires “the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or

lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs”.

In the carcinogenicity study in rat with 1,4-dimethylnaphthalene carried out in accordance with OECD Guideline 453 no increased incidence in neoplastic lesions were observed.

Information on other naphthalene compounds from public literature for 1-methylnaphthalene, and 2-methylnaphthalene showed that 1-methylnaphthalene and 2-methylnaphthalene induced bronchiolar/alveolar adenomas in mice. However, literature data indicate a clear species difference in susceptibility of this findings with naphthalene compounds due to higher rates of formation of a specific enantiomeric epoxide (1R,2S-naphthalene oxide) in lung microsomes and isolated dissected airways of mice compared with rats. Rat, hamster, and monkey lung microsomes preferentially formed the 1S,2R-naphthalene oxide enantiomer and showed lower rates of formation of epoxides than mouse lung microsomes with ratios of 0.48, 0.61 and 0.12 in rat, hamster and monkey lung microsomes, respectively compared to mice. Microsomes from human lymphoblastoid cells expressing recombinant human CYP2F1 also showed preferential formation of the 1S,2R-naphthalene oxide enantiomer over the opposite enantiomeric 1R,2S-naphthalene oxide. Humans are therefore expected to be less susceptible to this findings compared to mice. In addition, 1,4-dimethylnaphthalene has a lower formation of highly reactive epoxide intermediates compared to 1-methylnaphthalene and 2-methylnaphthalene. The binding of epoxide intermediates to glutathione leading to thionaphthols results in the formation of 1,4-dimethyl-methylthionaphthalene. This thiomethyl derivate of 1,4-DMN was reported in the study IIA 5.1/01 in small amounts after i.p administration (1.39% of total identified compounds), but was not detected after oral administration in the ADME studies. It is therefore considered that systemic pulmonary alveolar proteinosis is unlikely for 1,4-dimethylnaphthalene.

As there were no treatment related effects on tumor formation in the available experimental animal study with 1,4-demethylnaphthalene, classification for carcinogenicity is not warranted.

10.9.3 Conclusion on classification and labelling for carcinogenicity

No classification is proposed.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter’s proposal

In the carcinogenicity study (1981) performed in rats with 1,4-DMN, conducted according to OECD TG 453, no increase in the incidence of neoplastic lesions was observed following dietary exposure. The main limitation of the study was the low survival rate in females (attributed to pituitary adenomas which was of similar incidence in treated and control groups). Due to this low survival rate, the study was terminated at week 100 instead of week 104 in females.

Dose (mg/kg)	Males, n=65				Females, n=65			
	0	8	27	208	0	10	33	247
Survival (%)	60	29	40	60	28	35	29	48

As the high dose groups showed a sufficient survival (48 % and 60 % in female and male, respectfully), the DS considered the study suitable for evaluation of the carcinogenic properties.

The DS also provided information from ATSDR on the alkyl derivatives 1-methylnaphthalene (1-MN) and 2-methylnaphthalene (2-MN). In two published studies, using the same protocols, 1-MN and 2-MN induced bronchioalveolar proteinosis and bronchiolar/alveolar tumours in mice following 81-week dietary exposure (ATSDR, 2005). The postulated mode of action (MoA) is that the tumour formation is mediated by the formation of a highly reactive epoxide. Although evidence of the formation of this reactive epoxide was observed following a single intraperitoneal administration of 1,4-DMN, no evidence was observed following oral administration of 1,4-DMN (single exposure, 28 mg/kg). The DS concluded that the induction of systemic pulmonary alveolar proteinosis and pulmonary tumours was thus unlikely for 1,4-DMN. The DS also pointed out that mice may be more susceptible than rats to this type of lung tumours based on potential differences in metabolism between mice and other species as seen with naphthalene.

Overall, the DS proposed no classification for 1,4-DMN by the oral route. The proposal is based on the negative carcinogenicity study in rats and the low relevance of the lung tumours observed in mice with 1-MN or 2-MN by the oral route.

In addition, the DS proposed no classification for 1,4-DMN by inhalation based on lack of data (see DS's response to comments received during public consultation below).

Comments received during public consultation

Two MS and one National Authority requested further discussion on the potential extrapolation of the carcinogenicity of the structural analogue naphthalene to 1,4-DMN. Naphthalene is classified as Carc. 2 (Annex VI of CLP regulation) based on neuroblastoma of the olfactory epithelium observed in male and female rats following long-term inhalation exposure. One MS noted that data were lacking for 1,4-DMN by inhalation.

The DS agreed that data are lacking on the carcinogenicity potential of 1,4-DMN by inhalation. Moreover, the DS considered the comparison between the results observed with naphthalene and 1,4-DMN difficult to interpret as olfactory tumours were observed in rats following inhalation exposure whereas dietary exposure was used with 1,4-DMN. Toxicokinetic may differ between the two routes of exposure. Therefore, the DS proposed no classification for 1,4-DMN carcinogenicity by the oral route based on the available study and no classification based on lack of data by inhalation route.

The National Authority also requested further discussion on some specific neoplasms observed in the carcinogenicity study performed with 1,4-DMN. Indeed, hepatocellular carcinoma was observed in 2 out of 65 male rats and cervix leiomyosarcoma in 2 out of 62 female rats at the top dose. Trend analysis (performed by the National Authority) was positive for these two tumour types. Moreover, the national authority considered that a genotoxic MoA cannot be entirely ruled out.

Assessment and comparison with the classification criteria

Data on 1,4-DMN

The available 2-year rat carcinogenicity study, performed with 1,4-DMN, had limitations. Indeed, a reduced survival rate was observed in females at all concentration and in males at the low and mid dose. Decreased body weight and food consumption were observed at the top dose in both sexes.

In this study, no neoplastic findings were reported to be treatment-related by the DS. The presence of hepatocellular carcinoma in 2 out of 65 males and cervix leiomyosarcoma in 2 out of 62 females at the high dose was pointed out by a national authority. Both tumour types are rare and malignant in rats.

In regard to hepatocellular carcinoma in males, in the repeated-dose toxicity studies, liver was shown to be a target organ (increased in liver relative weight and cholesterol) but no histopathological findings were observed. As no hyperplasia, pre-neoplastic lesions or adenomas were observed following short-term or long-term exposure in rats, RAC considered the observed hepatocellular carcinoma not treatment-related.

Although no historical control data are available, cervix leiomyosarcoma are usually rare uterine malignant tumour in rats. No evidence of endocrine disrupting potential was observed in the EOGRTS study available in rats and no effects in the uterus were observed in the available studies with 1,4-DMN. RAC considers difficult to make a firm conclusion on the relation to treatment as survival in females was low. Thus, the exact incidence of this tumour type in control or treatment groups remains uncertain. Moreover, as the potential carcinogenicity of 1,4-DMN was not investigated in mice, it would be too speculative to conclude whether this effect would also be seen in a second species. Nevertheless, based on the available data, no classification is warranted as the incidence was low and the uterus was not identified as a target organ in the repeated-dose toxicity studies.

Data on 1-MN and 2-MN

Dietary exposure to 2-MN and 1-MN for 81 weeks was associated with a statistically significant increase of pulmonary alveolar proteinosis in both male and female B6C3F1 mice and lung adenoma in males (Murata *et al.*, 1993 and 1997).

2-MN Dose (mg/kg)	Males			Females		
	0	54	114	0	50	108
Pulmonary alveolar proteinosis	4/49	21/49*	23/49*	5/50	27/49*	22/48*
Lung adenoma	2/49	9/49*	5/49	4/50	4/49	5/48
Lung adenocarcinoma	0/49	1/49	1/49	1/50	0/49	1/48

1-MN Dose (mg/kg)	Males			Females		
	0	75.1	144	0	71.6	140
Pulmonary alveolar proteinosis	4/49	23/50*	19/38*	5/50	23/50*	17/49*
Lung adenoma	2/49	13/50*	12/50*	4/50	2/50	4/49
Lung adenocarcinoma	0/49	0/49	3/50	1/50	0/50	1/49

*p < 0.05

RAC notes several limitations in these studies:

- According to the authors, as all animals were housed in the same room, control may have been exposed by inhalation to 1-MN or 2-MN due to insufficient ventilation (explaining pulmonary alveolar proteinosis in control mice, not seen in controls by the authors in the laboratory);
- The mice were only exposed 81 weeks instead of 18-month recommended in the test guideline.
- Only two concentrations were used in the study.

There is no clear evidence that pulmonary alveolar proteinosis is related to lung adenoma as in females, although this lesion was observed, no tumours were induced. Moreover, no dose-relation was observed for lung adenomas with 1-MN nor with 2-MN. A possible confounding factor is the possible inhalation exposure of the test material by the rats during the study.

Overall, RAC considers the studies no sufficiently reliable to assess the carcinogenic potential of 1,4-DMN. Moreover, there are insufficient data to consider the read-across between 1-MN, 2-MN and 1,4-DMN valid (see Background Document).

Data with naphthalene

Naphthalene induced in rats, following long-term inhalation, neuroblastoma of the nasal olfactory epithelium in both sexes and respiratory epithelium adenomas in males. No lung tumours were observed in rats. In mice, exposed 104-weeks by inhalation, an increase in alveolar/bronchiolar adenomas were observed in females in presence of inflammatory changes. Alveolar/bronchiolar adenoma and carcinoma were also increased in males but within historical control range values. Pulmonary alveolar proteinosis was not reported in mice. Based on the rat nasal cavity, the substance was classified Carc. 2. The relevance of the mouse lung adenomas were considered unlikely due to species differences in pulmonary metabolism (summary from the Substance Evaluation conclusion of UK on naphthalene published in 2018).

Evidence of carcinogenicity of the structurally-related naphthalene has been hypothesised to be due to metabolism *via* CYP-mediated ring epoxidation to reactive metabolites such as the 1,2-epoxide or 1,2-quinone derivatives in the nasal epithelium. The metabolic formation of ring epoxides is a relatively minor pathway for alkyl naphthalene derivatives, whereas it is one of the major pathway for naphthalene (see background document). Moreover, quinone derivatives are not formed with alkylated naphthalene compounds. As major differences in the metabolism of naphthalene and alkylated naphthalene (1-MN, 2-MN or 1,-DMN) exist, the use of naphthalene carcinogenicity data as evidence for 1,4-DMN carcinogenicity is not supported by RAC.

Overall conclusion

Overall, RAC considers that based on the available data **no classification is warranted for 1,4-DMN.**

However, RAC also noted the following deficiencies in the database:

- The potential for carcinogenicity via inhalation has not been sufficiently investigated;
- The carcinogenic potential of the substance was not investigated in a second

species.

Supplemental information - In depth analyses by RAC

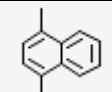
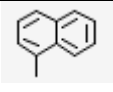
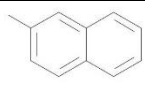
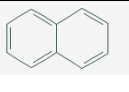
Read-across analysis

1,4-DMN belongs to the chemical family of alkylated naphthalenes. Carcinogenicity studies were reported on 1-MN, 2-MN and naphthalene. The acceptability of read-across between these 3 compounds is discussed below.

Substance identity

The selected supporting substances share structural similarity as all have the naphthalene structure. They only differ by one or two methyl groups. RAC has no information whether naphthalene could be a relevant impurity of 1-MN or 2-MN.

Physicochemical properties (from ATSDR, 2005):

Substance	1,4-DMN	1-MN	2-MN	Naphthalene
CAS no	571-58-4	90-12-0	91-57-6	91-20-3
Molecular weight	156.23	142.2	142.2	128.19
Vapour pressure (Pa)	2.5	7.2	7.3	11
Log K _{ow}	4.37	3.87	3.86	3.29
Solubility (mg/L, 25 °C)	5.1	25.8	24.6	31.7
Structure				

With increasing molecular weight, the solubility and the vapour pressure decreased whereas the Log K_{ow} increases. Nevertheless, no major differences in the physico-chemical properties were observed between the compounds.

Comparative toxicokinetics (metabolism)

No data are available on the toxicokinetic of 1-MN. Therefore, it is not possible to conclude that the metabolism of 1-MN is similar to 2-MN and 1,4-DMN.

Alkylated naphthalene metabolism involved several competing initial steps. There are three main metabolic pathways identified for these compounds: side chain oxidation leading to naphthoic acids, ring oxidation leading to naphthols (directly or through epoxide formation) and binding of epoxide intermediates to glutathione leading to thionaphthols, excreted in urine as mercapturates.

The position of methyl seems to have an impact on the proportion of ring oxidation (leading to reactive epoxide intermediate) vs side chain oxidation (See table 2.1.4-6 of annex I of the background document). Therefore, 2-MN and 1,4-DMN may have differences in their metabolic profile and their toxicity.

RAC disagrees with the DS's statement that based on the available metabolism studies, 1,4-DMN is expected to be less toxic than 2-MN based on a lower epoxide intermediate formation following long-term exposure. Differences in metabolism was noted but quantitative differences and comparison cannot be made as the metabolism data were not comparable:

- Different route of exposure were used;
- Dose used in the studies were not equivalent;
- Only single exposure used in the metabolism studies with different concentration.

With regard to naphthalene, the proposed MoA is the formation of reactive metabolites such as the 1,2-epoxide or 1,2-quinone derivatives in the nasal epithelium following inhalation. No metabolism data in the nasal epithelium are available with 1,4-DMN, 1-MN or 2-MN. Moreover, quinone derivatives are not expected to be form following alkylated naphthalene metabolism.

As major differences in the metabolism of naphthalene and alkylated naphthalene (1-MN, 2-MN or 1,-DMN) exist, the use of naphthalene carcinogenicity data as evidence for 1,4-DMN carcinogenicity is not supported by RAC.

Comparison of toxicological profiles

No comparison of the toxicological profile of 1-MN, 2-MN and 1,4-DMN was provided to RAC.

Based on carcinogenicity studies, a comparison of the toxicity profiles of naphthalene and 1,4-DMN is not appropriate as different routes of exposure were used in rats (oral vs. inhalation).

With regard to 1-MN and 2-MN, differences in toxicokinetics with 1,4-DMN may lead to differences in the carcinogenic potential of the compounds in mice. It may be highlighted that differences in the carcinogenicity potential of 1-MN and 2-MN in mice were observed.

Conclusion on read-across

Overall, RAC does not consider acceptable the read-across with 1-MN due to insufficient data. RAC notes differences in the metabolic profiles of 2-MN and 1,4 DMN and naphthalene and a lack of data on 1-MN. As metabolism is involved in the postulated MoA of tumour formation, RAC considers the read-across between the compounds not acceptable.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 26: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Extended 1-generation OECD 443 Rat, Sprague-Dawley 24/sex/dose	1,4-dimethylnaphthalene Batch no: F243A040/14D06B01-01, purity: 98.4% Doses: 0, 500, 2000, 7500 ppm (equivalent to 40, 160, 510 mg/kg bw/day) 2-week pre-mating through to lactation.	Parental: NOAEL parental: 40 mg/kg bw/day, based on reduced liver weight and increased cholesterol. Developmental NOAEL 160 mg/kg bw/day, based on delayed vaginal patency, preputial separation and reduced body weight Reproductive NOAEL: 510 mg/kg bw/day (highest dose tested)	IIA 5.6/07 (Doc ID 10-593)

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In an extended one-generation study 1,4-dimethylnaphthalene (98.4%) was administered, as an admixture in the diet, at target concentrations of 0, 500, 2000 and 7500 mg/kg diet to 24 parental Sprague-Dawley rats/sex/group for 2 weeks prior to mating, during the 2-week mating period, for up to 10 weeks for the parental males and through gestation, lactation and until scheduled necropsy for the parental females. F1 pups were exposed to 1,4-DMN during the lactation phase of the study and selected F1 offspring were exposed to the same concentration of the diet admixtures as the parental dams from rearing until scheduled sacrifice. The F1 offspring were subdivided into two Cohorts; Cohort 1 was treated until 13 weeks of age and then subjected to a complete necropsy, while Cohort 2 was utilized for immunotoxicity assessment around 8 weeks of age (T-cell dependant antibody response). Thyroid hormones (thyroxine and thyroid stimulating hormone) were determined in P and F1 animals (PND 4 and 26). Food consumption, body weights, body weight gain, reproductive performance, organ weights, clinical pathology and histopathology were evaluated during the study, along with offspring body weights (growth), measurement of anogenital distance, survival and developmental landmarks (vaginal patency and preputial separation for the F1 generation).

No test substance related mortality was seen during the study period, and no treatment-related changes in clinical signs were observed. Food consumption was significantly reduced in the high-dose group in the first days of treatment (approximately 60%), and significantly reduced throughout the study period, although less severe than in the first week. Reduced food consumption was likely due to palatability. In

the mid-dose group, food consumption was slightly reduced, when compared to controls. Body weight (approximately 10% in males and 15% in females) and body weight gain (around 30%) were significantly reduced in the high dose animals, and is considered to be due to the reduced food intake and the related reduced body weight gain in the first week of treatment for males, and the reduced body weight gain throughout the study period for females. Increased absolute and relative liver weights were observed in parental males and the mid and high dose groups (> 10% for both absolute and relative weights at the mid dose, and > 20% in the high dose). In parental males absolute spleen weights were decreased at the high dose (17%), and in parental high dose females absolute and relative spleen weights were decreased (22 and 12%, respectively). In high dose parental males testes relative weights were slightly increased (114%), and in parental females absolute and relative adrenal weights were decreased (25 and 16%, respectively, in mid dose parental females only absolute adrenal weight was decreased (15%).

Reproductive performance and litter viability were unaffected by treatment. No treatment related changes in sperm morphology and oestrus cycle were observed. The high dose group showed the lowest number of implantation sites (10 vs. 11.7) and a reduced incidence of live births (9.6 vs 11.2). These changes were not statistically significant, but may have been a secondary effect to the maternal toxicity observed in this group. No significant differences in the percent loss of fetuses between implantation and delivery were observed. A single high dose dam had a non-viable litter with 100% fetal loss prior to delivery, but at this incidence rate (1/23) the observation was considered within normal biological variation and unrelated to treatment. No statistically significant difference in offspring survival was detected between the treated and control groups. Viability and lactation indices were unaffected by treatment. In addition, there were no changes in sex ratio, and the average number of surviving pups by sex was similar across groups.

Litter body weights were significantly reduced in the high dose group on the day of birth; this trend persisted throughout lactation, increasing in severity, and continued after weaning. From PND 7 to 21, litter body weights were reduced > 40% in both males and females of the high dose. Litter body weights were also reduced at the mid dose for both males (10-14%) and females (15-16%), and for females only at the low dose (-8%) at the end of the lactation period. Anogenital distance as measured in PND 4 was not significantly different in the treated groups when compared to control. Preputial separation was delayed in the mid and high dose group and vaginal patency was delayed in the high dose group. The delayed preputial separation and vaginal patency appeared to be related to the reduced fetal body weight (see Table below). The applicant provided historical control data for preputial separation, generated by the laboratory in the period 2007-2008. Preputial separation has been observed in historical controls to occur on average around PND 44 (range PND 41-50); as such the delay noted in the 2000 mg/kg group was within normal biological variation. Moreover, the values in controls in the study (average PND 37, range PND 35-41) and low dose group (average PND 38, range PND 35-41) appear to be rather low, compared to the historical controls.

Food consumption, body weight and body weight gain of F1 pups selected for Cohort 1 and 2 were significantly reduced in all dose groups during the first three weeks of independent living. This was also considered to be due to palatability. Thyroxine (T4) and TSH were measured in both the parental and F1 generations as well as in PND26 weanlings, and no significant treatment-related changes were observed. In the immunotoxicity assessment in F1 animals, no treatment related changes were observed. At clinical biochemistry, increased in GGT and cholesterol levels were noted in both males and females of the high dose parental and F1 generation. In addition, increased cholesterol was also noted at the mid dose group. Triglycerides were increased in the high dose parental and F1 females, while triglycerides were reduced in high dose F1 males. Creatinin was reduced in mid and high dose parental and F1 females, and in mid dose parental males only. In F1 males absolute and relative liver weight were increased in mid dose (both 118%) and high dose (110 and 138%, respectively). In F1 females relative liver weight was increased in mid dose (113%) and high dose (152%). Several absolute organ weights were decreased in F1 high dose animals (i.e. adrenals, spleen, pituitary), however, these decreases were considered to be associated to the reduced body weight at the high dose. No treatment-related findings were seen at macroscopy.

Based on the minimal changes in liver weight and cholesterol at the mid dose groups, the NOAEL for parental toxicity is set at 500 mg/kg food (equivalent to 40 mg/kg bw/day). The NOAEL for developmental toxicity can be set at 2000 mg/kg food (equal to 160 mg/kg bw/day), based on delayed vaginal patency and preputial separation, and reduced body weight. As no fertility effects were observed, the NOAEL for reproductive toxicity is set at 7500 mg/kg food (equal to 510 mg/kg bw/day).

Table 27: Summary table of effects on delay preputial separation and vaginal patency

Endpoint	Dose (mg/kg food)							
	0		500		2000		7500	
	m	f	m	f	m	f	m	f
Preputial separation (days)	37		38		40*		46*	
Pup bw at preputial separation (g)	161		163		168		144*	
Vaginal patency (days)		34		36		35		43*
Pup bw at vaginal patency (g)		118		122		118		105*

*Statistically significant.

Historical control data

Sprague-Dawley Male rats	Age (days)	Body weight (g)
Mean	44.7	229.5
SD	2.5	2.3
Min	41.0	191.0
Max	50.0	273.0
N	46	46

No adverse effects on the reproductive organs in the available repeated dose toxicity studies was observed (studies summarized in section 10.12). An increase in relative testes weight (+31%) was observed in the 90-day study in rats (IIA 5.6/02) but this was considered to be possible related to the reduced body weight at this dose level and no histopathological findings were observed.

10.10.3 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP), Table 3.7.2.2, classification as for effects on fertility is based on:

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*

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

According to the CLP criteria classification as Repr. 1A is based on human data. No human data are available for 1,4-dimethylnaphthalene and therefore, classification as Repr 1A is not justified.

Since no effect on fertility or sexual function was found in the experimental animal studies the criteria for classification for cat. 1B and cat 2 are not met.

10.10.4 Adverse effects on development

Table 28: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels of duration of exposure	Results	Reference
OECD 414 Deviations: none New Zealand White rabbits 23 females/dose	1,4-dimethylnaphthalene (batch 14D06B01-01, purity 98.4%) GD6-28, gavage Dose levels: 0, 25, 80 and 250 mg/kg bw/day.	NOAEL maternal: 80 mg/kg bw/day based on the decreased body weight and reduced food consumption. NOAEL developmental: 250 mg/kg bw/day (highest dose tested)	IIA 5.6/08, Doc ID 10-601

Table 29: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Public literature, non-GLP Klimisch Score 1.	2,6-di-isopropylnaphthalene (purity not reported)	ICR-JCL mice (10/dose) Gavage treatment GD6-12 Dose: 0, 19.2, 192 mg/kg bw/day	No treatment related effects on developing fetus	IIA 5.6/01
Public literature, non-GLP Klimisch Score 1 (although unclear if 1-methylnaphthalene or 2-methylnaphthalene was	methylnaphthalene (purity not reported)	Wistar rats (22-24/dose) Gavage GD0-19 at 0, 16, 63 and 250 mg/kg bw/day	No treatment related effects on developing fetus	IIA 5.6/06

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
tested)				
EPA evaluation (limited information available on the conduct and the results of the study)	2,6-diisopropylnaphthalene (purity unknown)	rat (strain unknown, groups size unknown) Dose: 0, 50, 150 and 150 mg/kg bw/day	Maternal NOAEL: 50 mg/kg bw/day based on decreased body weight and food consumption Developmental NOAEL: 150 mg/kg bw/day based on a slightly increased incidence of skeletal alteration (fusion of cartilaginous bands in the cervical centra).	II 5.6/05

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

In a developmental toxicity study female New Zealand White rabbits (23 per dose) were exposed to 0, 25, 80 and 250 mg/kg bw/day of 1,4-dimethylnaphthalene (batch 14D06B01-01, purity 98.4%) via gavage from gestation day 6 till 2 (IIA 5.6/08).

One rabbit in the high dose group was found dead on gestation day 21, likely related to the abortion that occurred one day earlier. Abortions occurred in all dose groups, without a dose-related response (incidence 2, 2, 1, 3). No treatment related changes in clinical signs, were observed in maternal animals. Food consumption (-45%) and body weight gain (-51% from GD6-9, -13% from GD9-12) were reduced in high dose maternal animals over gestation days 6-12. Absolute body weights were reduced when compared to controls in the high dose group on gestation days 9-18.

No treatment-related differences in litter viability were detected. A single statistically significant reduction in total implants was seen in the 80 mg/kg/day group. The number of total implants was also lower, but not significantly at 250 mg/kg/day group. The percent pre- and post-implantation loss was highest in the 80 and 250 mg/kg/day groups. Each of these groups had a single doe with a non-viable litter; exclusion of the non-viable litter showed that the post-implantation loss was similar across groups, while the pre-implantation loss remained higher in the 80 and 250 mg/kg/day groups even after exclusion of the non-viable litters. This change is considered toxicologically irrelevant as the exposure period is targeted to occur after implantation.

No treatment-related changes were noted in foetal weight and gross post mortem examination. External, visceral, cephalic and skeletal examinations revealed no treatment related variations or malformations.

The maternal NOAEL for 1,4-dimethylnaphthalene in rabbits was established to be 80 mg/kg bw/d based on decreased body weight gain and reduced food consumption. The NOAEL for developmental toxicity was 250 mg/kg bw/d.

Table 30: Summary table of pre- and postimplantation loss

Endpoint	Dose (mg/kg bw/day)			
	0	25	80	250
Non-viable litters	0	0	1	1
Pre-implantation loss (%)	2.6	3.4	8.3	10.1

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Pre-implantation loss excluding single non-viable litter (%)	2.6	3.4	8.8	7.2
Post-implantation loss (%)	2.9	5.6	8.2*	8.8
Post-implantation loss excluding single non-viable litter (%)	2.9	5.6	3.4	2.3

* statistically significant

Some public literature information was available for other naphthalene compounds which are considered supplementary as a fully compliant guideline study is already available for 1,4-dimethylnaphthalene itself.

In a study from public literature (IIA, 5.6/01) pregnant ICR-JCL mice (four weeks, 18-20g) were treated by gavage from days 6-12 of gestation with 2,6-**di-isopropylnaphthalene** (purity not reported) at doses of 0, 19.2 and 192 mg/kg bw/day. Feed and water was supplied ad libitum. Each group within P₁ consisted of 20 dayms. Of these 10 were killed on day 18 of gestation and organs were examined macroscopically. The number of implantation sites and absorbed dead embryos was examined. Body weights of the pups was measured. The pups were examined for the presence of absence of fetal abnormalities. Skeletal evaluation was carried out using Alizarin red S staining. The ten remaining dams were allowed to deliver and F1 offspring were obtained. The numbers of those alive and dead at birth, the sex, and external abnormalities were observed, and body weights measured. Live pups were kept with the dams during the lactation period. Bodyweight of the pups was measured during this time On Day 21 after birth, touch (Pryer reaction), hearing (Haffner method), mobility (balance, gait), and other functions and senses were tested. Six weeks after weaning five males and five females were killed and major internal organs were observed macroscopically, the masses of their internal organs measured, and histopathological investigations conducted and on another five males and five females per group skeletal formation was examined using super soft X-ray photography. At ten weeks of age another 10 mice were mated and for the offspring the same examination was carried out as for the F1 offspring.

No significant effect on body weights occurred in dams. Through P1 and P2, no significant (p>0.05) differences were observed between the dosed groups and the respective control groups in the average numbers of implants per mouse, numbers of offspring per mouse, numbers of absorbed dead embryos per mouse, or average body weights of the fetuses per mouse, and in addition, no fetal external, intraoral or visceral abnormalities were seen within any group.

The EPA (2003, IIA 5.6/05) has reviewed a prenatal developmental toxicity study of 2,6-diisopropylnaphthalene in rats. The study was conducted at doses of 0, 50, 150, and 500 mg/kg bw/day. The NOAEL for maternal toxicity was considered to be 50 mg/kg bw/day based on decreased body weight and food consumption and the NOAEL for prenatal developmental toxicity was considered to be 150 mg/kg bw/day, based on decreased fetal body weight and a slightly increased incidence of a skeletal alteration (fusion of cartilaginous bands in the cervical centra).

In another study from public literature (Noda et al., 1982, IIA 5.6/06) pregnant Wistar rats (22-24/dose, 11 weeks at start of the study) were treated by gavage from gestation days 0-19 with **methylnaphthalene** (purity not reported, supplied by Tokyo Kasei Kogyo) at 0, 0.016, 0.063 or 0.25 ml/kg bw/day, equal to 0, 16, 63 and 250 mg/kg bw/day, assuming 100% purity and a specific gravity of 1. Olive oil was used as solvent. Dosing was based on a preliminary study in 6 non-pregnant females. It is not clear whether 1-methylnaphthalene or 2-methylnaphthalene was used. Bodyweight and food and water intake was measured on each day and clinical signs were observed. On gestation day 20 the

dams were killed and the effects of treatment with methylnaphthalene on the fetuses were investigated. The numbers of implantation scars, absorbed embryos, dead fetuses, and live fetuses were recorded. Various organs from the chest and stomach areas were dissected. The body weights of the live fetuses were measured, and they were examined for sex and external abnormalities. In addition, the numbers of corpora lutea in the dams' ovaries were examined. Approximately half of the live fetuses from each dam were fixed in 90% ethanol, and their skeletons stained with Alizarin red S. These were examined for degree of ossification and for the presence and absence of skeletal abnormalities and variations. The remaining live fetuses were fixed in Bouin's solution, and were examined using the Wilson⁴) method for the presence or absence of visceral abnormalities.

No effect on bodyweight occurred. A slight reduction in food intake (<10%) was observed in the medium and high dose group dams, but from day 6 of gestation this recovered. Increased water intake was observed throughout gestation in the treatment groups. Since there were no changes in serum levels of urea nitrogen,

Na⁺, or K⁺, or in kidney weights, kidney failure was not considered, and the cause of these increases was not known. No treatment-related effects on the developing fetuses were reported.

The results of these studies do not impact the overall conclusion for 1,4-dimethylnaphthalene.

10.10.6 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP), Table 3.7.2.2, classification as for effects on development is based on:

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 development is considered not to be a secondary non-specific consequence of other toxic effects

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 development is considered not to be a secondary non-specific consequence of the other toxic effects

According to the CLP criteria classification as Repr. 1A is based on human data. No human data are available for 1,4-dimethylnaphthalene and therefore, classification as Repr 1A is not justified.

In the developmental toxicity study in rabbits no relevant adverse effects on development were observed. In the 2-generation study (see 10.10.2) a delay in preputial separation in the mid and high dose males and vaginal opening in the high dose females was observed. The effects occurred in the presence of maternal toxicity in the form of reduced body weight (-15%), reduced body weight gain (-30%), reduced food consumption (-91%), increased cholesterol, gamma-GT and triglycerides, and increased liver, spleen and adrenal weight at the high dose group. In the mid dose maternal toxicity

consisted of reduced food consumption (-11%), changes in liver weight and cholesterol. The delay in preputial separation and vaginal opening therefore appears to be secondary to maternal toxicity which is further supported by the fact that the finding seems to be related to a reduced body weight of the pups. The body weight at preputial separation and vaginal patency was the same or lower than in the control. In addition, there was no effect on anogental distance or other parameters which would be consistent with altered androgenicity or estrogenicity. It is therefore concluded that these findings are considered secondary to the maternal toxicity and do not warrant classification.

10.10.7 Adverse effects on or via lactation

No specific study carried out, see summary of the 2-generation study in section 10.10.2.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

No specific study carried out, see summary of the 2-generation study in section 10.10.2.

10.10.9 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP), Table 3.7.2.2.2, classification for lactation effects is based on:

- (a) human evidence indicating a hazard to babies during the lactation period; and/or*
- (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or*
- c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.*

In the 2-generation study the severity of pup weight decreases increased throughout the lactation period. At birth pup weight was only 8% decreased in the high dose compared to control with no significant effect occurring in the mid dose. At PND 4 pup weight was already reduced by 21-22% in the high dose while at PND 14 pup body weight was reduced by 45-56% in the high dose and also in the mid dose by 14-16%. However, it is noted that this effect was observed in the presence of maternal toxicity (i.e. reduced food consumption, increased liver weight, increased cholesterol). Therefore, these effects on pup growth are considered secondary to the maternal toxicity and are not further taking into account for classification for adverse effects on or via lactation.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

No classification proposed.

RAC evaluation of reproductive toxicity
<p>Summary of the Dossier Submitter’s proposal</p> <p><i>Sexual function and fertility/effects on or via lactation</i></p> <p>The potential of 1,4-DMN to adversely affect sexual function and fertility was investigated in an extended one-generation reproduction toxicity (EOGRTS) study in rats (OECD TG 443, GLP). In this study, test material was administered via the diet. Male and female</p>

reproductive parameters and organs were not affected by treatment.

The F1 pup body weight and food consumption were statistically significantly decreased during the first three weeks of living. Litter body weights were also significantly reduced in the high dose group on the day of birth and persisted throughout lactation and continued after weaning.

Preputial separation was delayed in the mid and high dose group in male pups and vaginal patency was delayed at the top dose in female pups.

The DS concluded that the effects on pup growth were secondary to maternal toxicity and that 1,4-DMN does not meet the criteria for classification for sexual function and fertility or adverse effect on or *via* lactation.

Developmental toxicity

The developmental toxicity of 1,4-DMN was investigated in rabbits. No developmental toxicity was observed in the study. Additionally, three prenatal developmental toxicity studies on methylnaphthalene and 2,6-diisopropylnaphthalene were provided in rats. These studies had some limitations but no relevant developmental findings for classification were observed.

Overall, the DS concluded that no classification for developmental toxicity was warranted.

Comments received during public consultation

One MS requested discussion regarding classification for effects on sexual function and fertility. The MS requested more information on maternal toxicity observed in the EOGRTS study at the time of preputial separation and vaginal patency. Moreover, the MS considered that the reduced male pup weight (11 %) at the time of preputial separation was insufficient to explain the delayed puberty at the top dose. The DS further noted that the historical controls were limited as only 46 males were evaluated and that the concurrent controls should thus be given a higher weight. The MS noted that historical control data were not provided for vaginal patency.

The MS also raised a concern on the severe litter weight reduction throughout the lactation period and requested more information on maternal toxicity throughout lactation period and provided information on potential mortality in the litters.

The DS provided detailed information on maternal toxicity throughout the study (body weight, body weight gain, food consumption). The DS considered that no classification was warranted for this endpoint as the observed effects were secondary to maternal toxicity.

Considering developmental toxicity, no comments were received.

Assessment and comparison with the classification criteria

Sexual function and fertility

No treatment-related effects on fertility parameters were observed in the EOGRTS study in rats. In the available 90-day study, no effects on reproductive organs were seen in rats.

In F1 male pups, preputial separation was statistically significantly delayed at both the

mid and high dose levels (more than 1 week vs in controls at top dose). Compare to controls, body weight in F1 males was statistically significantly reduced in the mid and high dose groups (around 10 % in the mid dose group and 50 % in the high dose group). At the day of measurement, male pup body weight was only statistically significantly decrease at the top dose. Although historical controls were provided, they are not considered acceptable. Indeed, the concurrent control range values were lower than the minimum observed in the historical control values provided. Moreover, historical controls were based on insufficient number of animals (n=46 males), most probably from only one study.

In F1 female pups, a marked delayed in the day of vaginal opening was also noted at the top dose. At the day of measurement, the body weight of F1 female pups was statistically significantly decreased. No historical controls were provided.

Parameter	Delay of the onset of puberty			
Dose (mg/kg)	0	40	160	510
Vaginal patency				
Days	34	36	35	43*
Body weight at criterion (g)	118	122	118	105*
Preputial separation				
Days	37	38	40*	46*
Body weight at criterion (g)	161	163	168	144*

* $p \leq 0.05$

As effects were observed in both males and females and as developmental delays were noted in both groups, RAC considers that the delays in the onset of puberty may be due to a generalised growth delay. Parental toxicity observed at the top dose consisted of decrease body weight gain, decreased food consumption, increased relative liver weight (38 % in males and 57.5 % in females) and cholesterol. At the mid dose, a decrease in food consumption was seen at several time points in females and increased liver relative weight (16 %) and cholesterol were already noted (see tables below).

Study day Dose (mg/kg)	Body weight in females (g)			
	0	40	160	510
Pre-mating				
0	212	212	215	212
3	216	218	216	195* (-9.8 %)
7	225	225	223	204* (-9.3 %)
14	233	233	230	217* (-6.9 %)
Gestation				
0	234	234	228	214* (-8.5 %)
7	257	258	250	228* (-11.2 %)
14	282	285	278	248* (-12.1 %)
21	363	366	353	307* (-15.4 %)
Lactation				
0	278	275	271	240* (-13.7 %)
4	287	284	276	229* (-20.2 %)
7	299	297	288	233* (-22.1 %)
14	307	308	299	246* (-19.9 %)
21	299	299	296	253* (-16.4 %)

Study day	Food consumption in females (g)			
-----------	---------------------------------	--	--	--

Dose (mg/kg)	0	40	160	510
Pre-mating				
3	46	48	41	18* (-60.9 %)
7	73	76	68	54* (-26.0 %)
14	124	117	122	122
Gestation				
7	134	142	123	101* (-24.6 %)
14	138	148	130	116* (-15.9 %)
21	164	151	141* (-14.1 %)	122* (-25.6 %)
Lactation				
4	152	141	128	69* (-54.6 %)
7	152	155	134	73* (-52.0 %)
14	392	391	382	218* (-44.4 %)
21	629	575	502* (-20.2 %)	288* (-54.2 %)

*Significantly different from control $p \leq 0.05$

Overall, RAC agrees that the effects were due to a general growth delay and not a direct effect of 1,4-DMN. Moreover, no other findings were observed in the EOGRTS in endocrine sensitive organs (uterus, testis, seminal vesicles) or on anogenital distance. RAC concludes that **no classification is warranted for sexual function and fertility.**

Developmental toxicity

Based on the absence of effect in the available prenatal developmental toxicity study performed in rabbits, no classification is required.

There are also three poorly reported published developmental toxicity studies performed in mice or rats with methylnaphthalene (exact substance unknown) or 2,6-diisopropylnaphthalene. As there are existing data on 1,4-DMN and as potential toxicity differences between 1,4-DMN and 2,6-diisopropylnaphthalene have not been investigated (no data on the toxicity profile of 2,6-diisopropylnaphthalene), these data are not further considered.

RAC agrees with the DS that **no classification is warranted for developmental toxicity.**

Adverse effects on or via lactation

In the extended one-generation study in rats, dietary treatment with 1,4-DMN resulted in a treatment-related reduction in male and female pup body weights during the first three weeks. The decrease was more pronounced throughout the lactation period: 8 % at birth, 21-22 % at PND4, 45-56 % at PND14 at the top dose and 14-16% at the mid dose at PND14. Food consumption was also reduced in F1 pups from week 1.

A significant reduction in the litter body weight was found at birth, throughout the lactation period (> 40 % from PND 7 to 21) and continued after weaning. At the mid dose, litter body weight were also reduced for both males (10-14 %) and females (15-16 %) and also at the low dose in females (8-13 %) from day 7 to PND 21.

Maternal toxicity during lactation consisted of reduced body weight gain during lactation, decreased food consumption (around 50 % throughout the lactation period) and increased liver weight and cholesterol.

No information is available on the quantity or quality of the milk produced by the dams, nor was the rat milk analysed for the presence of 1,4-DMN or its metabolites.

Nevertheless, the substance was found in the milk of goat (metabolism study in livestock). Moreover, the substance is lipophilic and preferentially distributed to fat. Therefore, it is likely that the substance could be transferred to milk of rats.

Nevertheless, as the findings were observed in the presence of maternal toxicity and marked decreased in food consumption, RAC agrees with the DS that the observed pup delayed growth was not a direct effect of the substance and thus **no classification is warranted for lactation.**

10.11 Specific target organ toxicity-single exposure

Table 31: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
FIFRA 152-10 (in line with OECD 401) Deviations: none Rat, Sprague-Dawley (CrI:CD®BR) 5/sex/dose	1,4-dimethylnaphthalene (Batch H5510, purity 96.4%) Single oral gavage dose, Dose levels: Part 1: 750, 1000, 2500 mg/kg bw Part 2: 1300, 1700, 2100 mg/kg bw Part 3: 2000, 2300 and 2500 mg/kg bw	Mortality at 1700 mg/kg bw/day and higher. Clinical signs: hypoactivity, ataxia, coma, irritability, chromodacryorrhea, lacrimation, salivation, redness around the nose and/or eyes, wet/discoloured inguinal fur, coldness to the touch, hunched posture, diarrhoea, hair loss and discoloured paws (see Annex 1 for details).	IIA 5.2/01, Doc ID L08456, study 6,7,8

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

<p>FIFRA 152-11 (in accordance with OECD 402)</p> <p>Deviations: none</p> <p>Rabbit, New Zealand/White</p> <p>5/sex</p>	<p>1,4-dimethylnaphthalene (batch H5510, purity 96.4%)</p> <p>Single dermal dose (occluded)</p>	<p>No mortality</p> <p>No systemic signs of toxicity.</p>	<p>IIA 5.2/02, Doc ID L08456, study 4</p>
<p>FIFRA 152-12 (in line with OECD 403)</p> <p>Deviations: temperature outside of 22 ±2°C range (25-26°C)</p> <p>Rat, Sprague-Dawley (CrI:CD®BR)</p> <p>5/sex</p>	<p>1,4-dimethylnaphthalene (batch H5510, purity 96.4%)</p> <p>Inhalation 4 h, whole-body</p> <p>Dose: 5 mg/L (achieved conc. 4.16 mg/L)</p> <p>MMAD: 2.82 µm</p>	<p>Mortality: 1 out of 5 animals at 4.16 mg/L</p> <p>Clinical signs: dyspnea, prostration, ptosis, hypoactivity, discolouration around the mouth and/or nose and wet inguinal fur, tremors, red nasal and ocular discharge.</p>	<p>IIA 5.2/03, DocID L08456L001</p>

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

See section 10.1 to 10.3 for summaries on the acute studies.

10.11.2 Comparison with the CLP criteria

Comparison with Category 1 and 2:

According to Regulation EC No 1272/2008 (CLP) the following criteria apply for STOT SE Cat 1 and 2:

Category 1:

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.

Category 2:

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

Table 3.8.2 of the CLP Guidance indicates that the limits for category 1 from the animal studies are 300 mg/kg bodyweight for oral exposure, 1000 mg/kg bodyweight for dermal exposure and 1.0 mg/L/4h for

inhalation exposure. For category 2 the limits are 2000 mg/kg bodyweight for oral and dermal exposure and 5.0 mg/L/4h for inhalation exposure. While clinical signs such as hypoactivity and salivation were observed in the acute oral toxicity at dose levels <2000 mg/kg bw/day there were no signs of specific target toxicity. Therefore, classification with Category 1 or 2 is not required.

Comparison with Category 3:

According to Regulation EC No 1272/2008 (CLP) the following criteria apply for STOT SE Cat 3:

Transient target organ effects

This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above.

In the acute inhalation study dyspnoea was observed on day 0 only and nasal discharge was observed during 1-2 after treatment. The effects were not considered severe enough to warrant classification with STOT SE 3. In the acute oral toxicity study ataxia was observed at a high dose level of 2500 mg/kg bw/day. Since there were no other sign of narcotic effects classification with STOT SE 3 is not warranted.

10.11.3 Conclusion on classification and labelling for STOT SE

No classification proposed.

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

Summary of the Dossier Submitter’s proposal

The DS concluded that no signs of specific target organ toxicity was observed in the available acute toxicity studies and that there is no basis for STOT SE category 1 or 2 classification.

Moreover, dyspnoea and nasal discharge observed in the acute inhalation toxicity study was considered not severe enough for classification as STOT SE 3; H335 for respiratory tract irritation.

In addition, ataxia observed in the acute oral toxicity study was also not considered severe enough for classification as STOT SE 3; H336 for narcosis. Therefore, no classification was proposed by the DS.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

No human data are available in the dossier.

In the acute inhalation toxicity study, at 4.16 mg/L, clinical signs suggestive of respiratory irritation (dyspnea, eye and nasal discharge) were observed in several rats. These clinical signs were reversible by day 2 and no abnormalities were observed at

necropsy (including the rat that died). The substance is not a skin irritant. As no histopathological findings were observed, RAC agrees with the DS's proposal not to classify 1,4-DMN for STOT SE 3; H335 for respiratory tract irritation.

In the available acute oral toxicity study, hunched posture and hypoactivity were consistently observed among treated animals from 1300 mg/kg. Ataxia and coma were only noted in animals exposed to high dose levels \geq 2500 mg/kg i.e. close to the LD₅₀ (calculated to be 2730 mg/kg). As hunch posture and hypoactivity are not specific clinical signs, RAC agrees with the DS that STOT SE 3; H336 for narcotic effects is not warranted.

RAC agrees with the DS that **no classification for STOT SE 1 or 2 is warranted** for 1,4-DMN since no specific target organ toxicity after single exposure was identified.

10.12 Specific target organ toxicity-repeated exposure

Table 32: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

<p>90-day study OECD408</p> <p>Deviations: water consumption not measured, blood clotting potential not analysed</p> <p>Rat, Sprague-Dawley (CRL:CD(SD)I GSBR)</p> <p>10/sex/dose</p>	<p>1,4-dimethylnaphthalene, batch 01C-01, purity 98.8%</p> <p>Doses: 0, 500, 2500, 10000 ppm (equal to 0, 32, 161 and 677 mg/kg bw/d in males and 0, 38, 186 and 747 mg/kg bw/d in females)</p> <p>13-week exposure by diet</p>	<p><u>10,000 ppm (677 mg/kg bw/day for males, 747 mg/kg bw/day for females):</u></p> <ul style="list-style-type: none"> • Clinical signs (alopecia, rough fur, discoloured fur, vaginal discharge) • Reduced body weight gain (-32-34%) • Reduced food consumption (-10-20%) • Reduced grip strength forelimb • Reduced Hb in females (-4%), MCH (-4%) and reduced MCHC in females (-3%) • Increased cholesterol (+37-97%), decreased triglycerides in males (-49%) • Increased relative liver weight in males and females (+26-62%) and absolute liver weight in females (+41%). Increased relative kidney (+18%). Increased relative testes weight (+31%) possible due to reduced body weight. • chronic progressive nephropathy (CPN) in kidneys <p><u>5000 ppm (161 mg/kg bw/day for males, 186 mg/kg bw/day for females)</u></p> <ul style="list-style-type: none"> • Clinical signs (discoloured fur) • Reduced body weight gain in males (-11%) • Reduced food consumption in males (-10%) • Increased cholesterol (+27-31%), decreased triglycerides in males (-29%) • Increased relative liver weight (+20%) <p><u>500 ppm (32 mg/kg bw/day for males, 38 mg/kg bw/day for females)</u></p> <p>No adverse effects.</p>	<p>IIA 5.6/02, Doc ID 02-154</p>
---	---	--	----------------------------------

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

<p>Chronic toxicity/carcinogenicity</p> <p>OECD 453</p> <p>Deviations: histopathology report did not differentiate between interim and terminal kill</p> <p>Rat, Sprague-Dawley CD IGS, Crl:CD(SD)</p> <p>Interim: 20/sex/dose</p> <p>Terminal: 65/sex/dose</p>	<p>1,4-dimethylnaphthalene</p> <p>(Batch no: F243A040/14D06B01-01, Purity: 98.7%)</p> <p>Dose levels: 0, 150, 500 and 3750 ppm (equal to 0, 8, 27 and 208 mg/kg bw/day in males and 0, 10, 33 and 247 mg/kg bw/day in females)</p> <p>Administration via diet</p> <p>Interim: 52 week</p> <p>Terminal: 104 week</p>	<p><u>3750 ppm (208 mg/kg bw/day for males, 247 mg/kg bw/day for females)</u></p> <ul style="list-style-type: none"> • Reduced body weight (-14 to 25%) • Reduced food consumption • Haematological findings (reduced Hb (-5%), reduced MCV (-9%), reduced MCH (-9%), reduced MCHC, decreased platelet count (-21%) • Increased cholesterol (+71%) • Increased relative liver weight (+42%), increased relative kidney weight (+15%) • Kidney nephropathy, papillary necrosis, karyomegaly tubuli, microabces, proteinosis, mineralization. Liver cysts, mesenteric lymph node depletion <p><u>500 ppm (27 mg/kg bw/day for males and 33 mg/kg bw/day for females):</u></p> <ul style="list-style-type: none"> • Increased cholesterol (+30%) • Kidney, karyomegaly tubuli <p><u>150 ppm (8 mg/kg bw/day for males and 10 mg/kg bw/day for females):</u></p> <p>No adverse findings</p>	<p>IIA 5.3/01 Doc ID 02-154</p>
<p>Extended 1-generation</p> <p>OECD 443</p> <p>Rat, Sprague-Dawley</p> <p>24/sex/dose</p>	<p>1,4-dimethylnaphthalene</p> <p>Batch no: F243A040/14D06B01-01, purity: 98.4%</p> <p>Doses: 0, 500, 2000, 7500 ppm (equivalent to 40, 160, 510 mg/kg bw/day)</p> <p>2-week pre-mating through lactation.</p>	<p><u>7500 ppm (510 mg/kg bw/day)</u></p> <ul style="list-style-type: none"> • Reduced body weight (-15%) and body weight gain (-30%) • Reduced food consumption (-61%) • Increased cholesterol (+123%), gamma-GT and triglycerides (+41%) • Increased liver weight (+52%), decreased absolute spleen weight (-22%, P-generation only) increased absolute testes weight (+14%, P-generation only), decreased absolute and relative adrenal weight (-25%, P-generation only) • Moderate kidney karyomegaly in 1 adult male <p><u>2000 ppm (160 mg/kg bw/day)</u></p> <ul style="list-style-type: none"> • Reduced food consumption (-11%) • Increased cholesterol (+45%) • Increased liver weight (>10%) <p><u>500 ppm (40 mg/kg bw/day)</u></p> <p>No treatment related adverse findings.</p>	<p>IIA 5.6/07 (Doc ID 10-593)</p>

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Table 33: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
IIA 5.3/01 Doc ID 02-154	208 mg/kg bw/day	52 weeks (effects on the kidney already occurred in interim sacrifice)	843.6 mg/kg bw/day	No

Rats (10/sex/dose) were given 0, 500, 2500 or 10.000 ppm 1,4-dimethylnaphthalene for 13 weeks in their diet. Two additional groups (control and high dose group, 10/sex) were held for an additional 28 days to evaluate the recovery potential from 1,4-dimethylnaphthalene-induced effects (0 and 10.000 ppm). Full histopathology (including reproductive organs) was confined to controls and high dose animals, except for kidneys which were histopathologically examined in all dose groups.

No treatment-related deaths were observed during the study. No treatment-related clinical signs were observed, except for discoloured inguinal fur. Body weight gain was statistically significantly reduced by 32-34% in both sexes at the high dose. A statistically non significant decrease in body weight gain of 11% was observed in males of the mid-dose group. A dose-related decrease in food intake was observed: high-dosed rats (both sexes) consumed significantly less food throughout the study (10-20% decrease); mid-dosed male rats ate significantly less in the first 10 weeks (10% decrease). Effects on haematological and clinical chemistry parameters were mainly observed in the highest dose group and consisted of changes in haemoglobin, MCH, MCHC, A/G ratio, globulin, BUN, glucose, cholesterol and triglyceride levels. Cholesterol was increased in high-dosed females and in mid- and high-dosed males. The reduction in triglyceride levels in males may be related to the poor nutritional state. Increased relative organ weights (111-163% compared to controls) were observed in both males and females of the highest dose group and in males of the mid-dose group for a number of organs (including testes), which may be, at least partly, related to the observed decreased body weight gain in the highest dose group. No gross pathological findings were observed. Microscopically, changes were observed in the kidneys and included an increased incidence and severity of chronic progressive nephropathy (CPN) in the high-dose group. The effects were more pronounced in males than in females.

For the summaries of the chronic toxicity study and 2-generation study it is referred to section 10.9.1 and 10.10.2. In the chronic toxicity study effects on the kidney, including nephropathy, papillary necrosis, were observed at high dose levels of 208 mg/kg bw/day in males and 247 mg/kg bw/day in females. These effects were already observed in the interim sacrifice. However, they occurred at dose levels not relevant for classification with STOT-RE. In the 2-generation study no effects occurred that are relevant for STOT-RE.

10.12.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP) the following criteria apply for STOT RE:

Category 1 (H372):

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 repeated exposure.

Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: 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.

Equivalent guidance values for different study durations (oral only, since dermal and inhalative studies not relevant in this case):

Rat:

28-day: ≤ 30 mg/kg bw/d

90-day: ≤ 10 mg/kg bw/d

Category 2 (H373)

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 repeated exposure.

Substances are classified in Category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

Equivalent guidance values for different study durations (oral only, since dermal and inhalative studies not relevant in this case):

Rat:

28-day: ≤ 300 mg/kg bw/d

90-day: ≤ 100 mg/kg bw/d

No adverse findings were observed in the repeated dose studies that would trigger classification with STOT-RE. In the 90-day study the LOAEL was 161 mg/kg bw/day for males and 186 mg/kg bw/day and the effects observed at this dose level were not severe. In the chronic toxicity study effects on the kidney were observed, including papillary necrosis, but only at a high dose level of 208 mg/kg bw/day for males and 247 mg/kg bw/day for females which would therefore not trigger classification. In the extended one-generation study no effect was observed that would trigger STOT-RE classification. Similarly in the developmental toxicity study in rabbits only effects on decreased body weight and reduced food consumption was observed. Therefore, it is concluded based on the results of the studies classification with STOT-RE is not warranted for 1,4-dimethylnaphthalene.

10.12.3 Conclusion on classification and labelling for STOT RE

No classification proposed.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter’s proposal

As no adverse findings were observed in the repeated dose toxicity studies at dose

triggering classification STOT RE, the DS proposed no classification.

Comments received during public consultation

One National authority proposed to classify 1,4-DMN as STOT RE 2 based on naphthalene induced haemolytic anaemia, observed in humans (single events of incidental exposure) and in dogs. The national authority further noted that there is a lack of human or dog studies using 1,4-DMN and rodent studies may not be adequate to investigate this potential concern. The DS responded that the classification should be based on data obtained with 1,4-DMN and that, on this basis, no classification is warranted.

Assessment and comparison with the classification criteria

RAC agrees with the DS that based on the available data on 1,4-DMN, **no classification for STOT-RE is warranted**. Moreover, RAC considers that based on potential toxicokinetic differences between naphthalene and 1,4-DMN, the proposed read-across of data from one to the other is not acceptable (see in depth analysis in the carcinogenicity section).

10.13 Aspiration hazard

Table 34: Summary table of evidence for aspiration hazard

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
FIFRA § 63-18 Viscosity	1,4-dimethylnaphthalene	Measured.	Mean viscosity was 6 mPa s at 12 rounds per minute and 6 mPa s at 30 rounds per minute at 25 °C. The kinematic viscosity is 6 mPa.s / 1.014 = 5.9 mm ² /s.	IIA 2.1/02 Doc ID 4373-93-0226-AS

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

The viscosity of 1,4-dimethylnaphthalene was measured at 25°C using a Brookfield viscometer (IIA 2.1/02). No justification was provided on why the study was conducted at 25°C The analysis was repeated with the same sample aliquot. A viscosity standard was also analysed in replicate. Mean viscosity was 6 mPa s at 12 rounds per minute and 6 mPa s at 30 rounds per minute at 25 °C. The kinematic viscosity is 6 mPa.s / 1.014 = 5.9 mm²/s.

10.13.2 Comparison with the CLP criteria

According to Regulation 1272/2008 a substance should be classified for aspiration toxicity when:

Substances known to cause human aspiration toxicity hazards or to be regarded as if they cause human aspiration toxicity hazard

A substance is classified in Category 1:

(a) based on reliable and good quality human evidence or

(b) if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm²/s or less, measured at 40 °C.

The kinematic viscosity of 1,4-dimethylnaphthalene was found to be 5.9 mm²/s. As this is below 20.5 mm²/s classification for aspiration toxicity is required.

10.13.3 Conclusion on classification and labelling for aspiration hazard

Harmonised classification proposed (Asp. Tox 1, H304)

RAC evaluation of aspiration toxicity
<p>Summary of the Dossier Submitter’s proposal</p> <p>Based on the kinematic viscosity of 1,4-DMN of 5.9 mm²/s at 25 °C, , classification as Asp. Tox. 1; H304 was proposed by the DS.</p>
<p>Comments received during public consultation</p> <p>Two MS agreed with the DS's proposal.</p>
<p>Assessment and comparison with the classification criteria</p> <p>According to the criteria, the substance shall be classified if it is a hydrocarbon and its kinematic viscosity is ≤ 20.5 mm²/s at 40 °C.</p> <p>As kinematic viscosity decreases with higher temperature, it is expected that kinematic viscosity of the substance at 40 °C would fulfil the criteria. Therefore, RAC agrees with the DS to classify the substance as Asp. Tox. 1, H304.</p>

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 35: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradability	Degradation: >60% within 28 days).	Non-GLP, public literature study	Study IIA 7.7/01
OECD301C (EEC C.4-F)	Readily biodegradable	MITI (I) test Ri = 2	
1,4-			

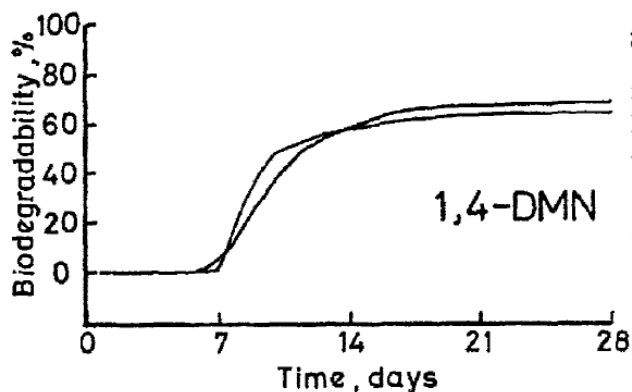
Method	Results	Remarks	Reference
dimethylnaphthalene (purity not reported)			
Water-sediment degradation OECD 308 1,4-dimethylnaphthalene (batch 3501-032, purity 97.1%)	-	Study not acceptable Rapid disappearance from water-sediment due to volatility of 1,4-dimethylnaphthalene Ri = 3	Study IIA 7.8/01 Doc ID 535E-102
Photochemical degradation (QSPR model) Non-guideline	Photolytic half-life 3.2-12.8 h	Non-GLP, public literature study QSAR generated endpoint	Study IIA 7.6/01

11.1.1 Ready biodegradability

In a public literature study the biodegradability of 1,4-dimethylnaphthalene was evaluated using the MITI (I) test method (IIA 7.7/01). Inoculated test medium is dosed with 100 mg/kg of 1,4-DMN as the nominal sole source of organic carbon. Degradation was followed by analysing dissolved oxygen over a 28-day test period at 25°C. Measurements of dissolved oxygen were performed on two test chambers from the control, reference (aniline), and treatment groups on days 7, 14, 21 and 28.

The system used in the MITI test is a closed system under pressure which should prevent volatilisation from the system. The shape of the degradation curve indicates a proper biological degradation.

The results indicate that the inoculum was active by degrading the reference substance within the acceptable range (at least 40% after 7 days and 65% after 14 days). In this study, more than 60% degradation of 1,4-dimethylnaphthalene (60% of theoretical maximum) is achieved. 1,4-dimethylnaphthalene was concluded to be readily biodegradable (please note that the 10-d window does not apply for the OECD 301C test). Although an abiotic control –as laid down in OECD 301 C- is not included. The shape of one of the degradation curves indicates a proper biological degradation (see below). The other curve, where loss due to biodegradation increases steeply after 7 days, may indicate (some) effect of volatilization in the first few days. However, a full biphasic pattern, which would be expected in case volatilization was a major process is not observed. Overall, the study is conducted well and the results can be considered reliable; Klimisch score = 2 (public literature).



11.1.2 BOD₅/COD

No data available.

11.1.3 Hydrolysis

No experimental data are available. The solubility of 1,4-DMN in water is very low (5.1 mg/L at 25°C). Based on the molecular structure, hydrolytic degradation of 1,4-DMN is expected to be very low in aquatic systems. Estimation of a DT50 for hydrolysis is not possible with EPIWeb 4.0 (HydroWin). EUSES 2.1 estimation of DT50 = 5.27×10^5 day (20°C).

This estimation is made based on a model used for other purposes, using QSAR estimates and assumptions and is therefore considered not reliable. Klimisch score = 3.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No further data needed.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Water-Sediment

A water-sediment degradation study was submitted (Study IIA 7.8/01). The study showed poor recoveries and in additional aerobic mineralisation/volatilisation tests it was shown that 1,4-DMN was being lost, potentially to the rubber stopper assemblies used to affix the test chambers to the mineralisation apparatus. In the third additional experiment to the water-sediment study, it was demonstrated that 46% of the radioactivity had escaped to the gaseous phase after 2 days (75% after 23 days). Although the composition of the medium in this additional experiment was not the same as in the main study, rubber stoppering was not used to minimize losses. Therefore, the additional experiments demonstrate that the high loss of radioactivity from the water-sediment phase in the main test was largely due to volatilisation, instead of to adsorption to the rubber stoppers. Therefore, there would be minimal opportunity for retention by sediment followed by metabolite formation, as the extensive volatility would preclude such from happening. The study is considered unreliable for classification purposes (Ri = 3)

Soil

No fully adequate experimental data are available on biodegradation of 1,4-DMN in soil. Some indications based on literature references were submitted (see Annex 1 for details). No reliable DT50 value for 1,4-DMN can be derived from the submitted data.

Some indications based on literature studies were submitted (IIA 7.1/01, IIA 7.1/02 IIA 7.1/03, IIA 7.1/04 and IIA 7.1/05).

In the first study (IIA 7.1/01) the microbial oxidation of 1,4-DMN by isolated bacteria (*Alcaligenes* sp. strain D-59 and *Pseudomonas* sp. strains D-87 and D-186) was investigated. Strain D-87 grew well on (amongst others) 1,4-DMN as the sole source of carbon and energy and accumulated 4-methylnaphthalene-1-carboxylic acid from 1,4-DMN. The information points at the possibility of metabolisation of 1,4-DMN by soil micro-organisms.

In the second study (IIA 7.1/02) evaluated the metabolism of 2,6-DMN by flavobacteria. Flavobacteria that were able to grow on 2,6-dimethylnaphthalene (2,6-DMN) were isolated from soil. Most were able to oxidize a broad range of aromatic hydrocarbons after growth on 2,6-DMN at rates comparable to that of the oxidation of 2,6-DMN itself. One small group was neither able to grow on naphthalene nor able to oxidize this compound after growth on 2,6-DMN, but metabolised 2,6-DMN by a pathway which

converged with that previously described for naphthalene metabolism in *Pseudomonas*. No information on 1,4-DMN was reported in this study.

The third study (IIA 7.1/03) concerned the composting of hazardous waste and hazardous substances. The results demonstrate that composting can be used to decrease the concentration of hazardous organic substances, but that the more volatile constituents may vaporize, rather than degrade. When the effects of volatilization were removed, the half life based solely on biodegradation ranged from 7.0 - 11.6 days for DMN. It was not reported which congener it was. Therefore the results can only be used as indicative values.

A fourth study (IIA 7.1/04) was submitted concerning the laboratory scale in-vessel composter designed for volatile emissions analysis. Volatilisation of DMN is mentioned sideways in this study. The information presented is not considered relevant.

In the fifth study (IIA 7.1/05) pathways of aerobic bacterial degradation of methylated naphthalenes in soil were studied. It was reported that *Paucimobilis* 2322 formed 3,6-dimethylsalicylic methyl ester and 4-methyl-1-naphtoic acid methyl ester from 1,4-DMN. The information points at the possibility of metabolisation of 1,4-DMN by soil micro-organisms, but cannot be used to quantify biodegradation in soil.

From the various references, if taken together, it is evident that 1,4-dimethylnaphthalene will be degraded by soil micro-organisms, that 4-methylnaphthalene-1-carboxylic acid is the most important degradation product in soil, and that volatility is a major route of disappearance from soil.

11.1.4.4 Photochemical degradation

Photolysis in water

In a public literature study Weterings (2004, IIA 7.6/01) used a QSPR model to predict the photolytic half-life of 1,4-DMN, based on quantum chemical descriptors. The developer of the model (Chen et al., 2000, 2001) developed the model based on 13 closely related polyhydrocarbons. Inclusion of 1,4 DMN in the model resulted in a photolytic half-life of 6.4 hours.

In addition, a literature study that aimed to describe the development of the QSPR model used in the Weterings study was submitted. This study was based on the observed and predicted photolytic half-lives for 1-methylnaphthalene and 2-methylnaphthalene. The study shows that the model predicts the photolytic half-life to structural analogies of 1,4-DMN with a uncertainty factor of 2. In addition, a study by Fukada *et al.* (1988) showed that the degradation rates of of alkylnaphthalenes from monomethylnaphthalene to diisopropylnaphthalene are actually very similar. Based on this, the RMS concludes that the photolytic half-life of 1,4-DMN in water ranges from 3.2 – 12.8 hours. Photolysis studies may be used to evaluate whether under field conditions metabolites may be formed that are not detected in water/sediment studies, which are performed in the dark.

Based on the above and considering the Guidance given in QSAR methods for degradability is presented in IR&CSA, Chapter R.7.9.3.1. and the document 'Practical report – How to use and report (Q)SARa 3.1 (July, 2016), the evaluator notes that the available information is not sufficient to judge the algorithm nor the statistics of this QSAR. Taking this, and the Guidance in consideration the above QSAR may be useful as supplementary data but **cannot be the key endpoint for classification**.

11.2 Environmental transformation of metals or inorganic metals compounds

11.2.1 Summary of data/information on environmental transformation

Not relevant for this dossier.

11.3 Environmental fate and other relevant information

Vapor pressure:

1,4-dimethylnaphthalene has a high vapour pressure of 2.50 Pa at 25°C (see Table 7) and therefore concluded to be volatile.

Adsorption/desorption from soil:

In a public literature study (Klimisch score = 2) the soil sorption coefficient for 336 hydrocarbon and organic chemicals in water were reported (IIA 7.4/01). The non-linear group contribution method was used to estimate the soil sorption coefficient when experimental Koc values were not available. A comparison of calculated and experimental values for the soil sorption coefficient shows general agreement of calculated and experimental values for different organic chemicals. The Koc value for 1,4-DMN was estimated to be 4230 L/kg.

In addition the Koc was estimated to be 4139 L/kg using EPIWIN (EPIWeb 4.0), using Molecular Connectivity Indices Method. The Koc using the experimental log Kow of 4.37 is 6199 L/kg. Based on this information the results from the study are in line with the calculated values and the Koc of 4230 L/kg.

11.4 Bioaccumulation

Table 36: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
No guideline reported. <i>Cyprinodon variegatus</i> (sheepshead minnows) 36-day continuous flow 1,3-dimethylnaphthalene (purity not reported)	BCF: 5751 L/kg	non-GLP, public literature study Study carried out with 1,3-dimethylnaphthalene which was considered comparable to 1,4 DMN. Ri = 2	IIA 8.2/07

11.4.1 Estimated bioaccumulation

No data.

11.4.2 Measured partition coefficient and bioaccumulation test data

Adult sheep head minnows (*Cyprinodon variegatus*; 158 females and 129 males, mean weight 2.47 ± 1.23 g and mean length 4.7 ± 0.8 cm at sampling times) were exposed to two concentrations of PAH mixtures in seawater (a stock solution was prepared in acetone) in a continuous-flow system (IIA 8.2/07). The fish were exposed for a period of 36 days, followed by 8 days of depuration. The PAHs studied were pure naphthalene, 2-methylnaphthalene, 1,3-dimethylnaphthalene, 2-isopropylnaphthalene, phenanthrene, pyrene, 9-methylphenanthrene, and 9-ethylphenanthrene. The 1,3-dimethylnaphthalene concentrations were 2.74 ± 0.73 µg/L and 26.67 ± 9.19 µg/L. Bioconcentration factors (BCFs) for individual PAHs were estimated from the ratio of k_1 to k_2 by use of a first-order, one-compartment kinetics model and also directly from PAH concentrations in fish tissue and water samples (from sampling days 4, 7 and 36).

Naphthalene and its three alkylated isomers revealed peak levels within 4 d in both treatments except for the low-exposure concentration of C2 - and C3 -alkylated naphthalene, which reached maximum at day 7. Uptake rate constants (k_1) for the naphthalenes increased with increasing degree of alkylation and log value of the octanol/ water partition coefficient (K_{ow}), whereas k_1 values for three- and four-ring PAHs were lower despite their high log K_{ow} values. Elimination rate constants (k_2) for the homologue series of naphthalenes and phenanthrenes generally increased with decreasing degree of alkylation and log K_{ow} values. However, the depuration time did not directly correlate with the molecular size for nonalkylated PAHs.

A significant positive correlation was determined between log BCFs and log K_{ow} values for the series of C0- through C3-naphthalenes at both low ($r^2 = 0.985$, $p = 0.0077$) and high ($r^2 = 0.956$, $p = 0.022$) exposures. The two exposure levels revealed minor variations in k_1 and k_2 values for parent PAHs and in the temporal pattern of PAH metabolite concentrations in bile. The present results indicate that the presence and nature of alkyl groups have a significant influence on the kinetics and metabolism of PAHs in fish.

For 1,3-dimethylnaphthalene, a wet weight-based bioconcentration factor of 4000 L/kg was determined at a concentration of 2.74 $\mu\text{g/L}$ and 8000 L/kg at 26.7 $\mu\text{g/L}$. The geometric mean of the BCF values for both concentrations results in an overall BCF value for 1,3-dimethylnaphthalene of 5751 L/kg. Normalized BCF values were not reported.

The study is conducted well and the results can be considered reliable; Klimisch score = 2 (public literature).

No specific study was carried with 1,4-dimethylnaphthalene. However, study IIA 8.2/07 showed that there is a correlation between the log K_{ow} and BCF value. The log K_{ow} values of 1,3-dimethylnaphthalene and 1,4-dimethylnaphthalene are very similar. A published literature study (Dimitrou-Christides, 2003) which on the basis of an accepted methodology (GC and HPLC retention time comparison for estimating several physical-chemical characteristics) established several partition coefficients for a number of methylated naphthalenes, including 1,3-DMN and 1,4-DMN. The estimated log K_{ow} values for 1,3-DMN and 1,4-DMN were 4.27 and 4.22 resp. The article also stated that Log K_{ow} increases with the number of methyl groups (1,3-DMN and 1,4-DMN have an equal number of methyl-groups). Since the Log K_{ow} is a driving potential for the BCF value it is concluded that the BCF value of 5751 can be extrapolated to 1,4-dimethylnaphthalene.

11.5 Acute aquatic hazard

The acute aquatic toxicity studies of 1,-4-dimethylnaphthalene were assessed in the Draft Assessment Report (October 2012) prepared in the context of the approval, under Reg. (EC) 1107/2009. Studies considered valid in the and DAR (reliability score of 1 or 2) have been included in this report. All studies were carried out under GLP unless indicated otherwise and in accordance with OECD guidelines. Minor deviations were noted in one study but these did not affect the overall reliability of the studies. The deviations are included in the summaries were relevant.

Table 37: Summary of relevant information on acute aquatic toxicity

Method	Species	Test duration	Test material	Results ¹	Remarks	Reference
FIFRA 72-1, OECD 203, EEC C.1	Rainbow trout (<i>Oncorhynchus mykiss</i>)	96 h	1,4-dimethylnaphthalene, batch H5510, purity 96.4% Dose levels: 0, 0.32,	LC ₅₀ = 0.67 mg/L Mean measured	-	IIA 8.2/01, DocID 93-10-4955

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

			0.54, 0.90, 1.5 and 2.5 mg a.s./L			
OPPTS 850.1075 (= OECD 203, EEC C.1) Deviations: concentrations of the solvent are not reported.	Fathead minnow (<i>Pimephales promelas</i>)	96 h	1,4-dimethylnaphthalene, batch 01C-01, purity 98.8% Dose levels: 0, 0.25, 0.50, 1.0, 2.0, 4.0 and 8.0 mg a.s./L	LC ₅₀ = 1.4 mg a.s./L Mean measured.	-	IIA 8.2/02 DocID 535A-101A
FIFRA 154-9, OECD 202, EEC C.2	<i>Daphnia magna</i>	48 h	1,4-dimethylnaphthalene, batch H5510, purity 96.4% Dose levels: First test: 0.16, 0.26, 0.43, 0.72, and 1.2 mg as/L Second test: 0.65, 1.1, 1.8, 3.0, and 5.0 mg as/L	EC ₅₀ = 0.54 mg a.s./L Mean measured	-	IIA 8.3.1/01 DocID 93-104951
OECD 221	<i>Lemma gibba</i>	7 d	1,4-dimethylnaphthalene, batch 01C-01, purity 98.8% Concentration: 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L	I _r C ₅₀ 1.1 mg/L Mean measured. Mean measured.	-	IIA 8.6/01 DocID 535A-103
OPPTS 850.5400, OECD 201, EEC C.3	<i>Pseudokirchneriella subcapitata</i>	96 h	1,4-dimethylnaphthalene, batch 01C-01, purity 98.8% Concentration: 0.028, 0.056, 0.11, 0.23, 0.45 and 0.90 mg a.s./L	72-h E _b C ₅₀ : 0.32 mg a.s./L 72-h E _r C ₅₀ : 0.62 mg a.s./L Mean measured.	The validity criteria of OECD 201 were not met with respect to control growth.	IIA 8.4/01 DocID 535A-102

¹ Indicate if the results are based on the measured or on the nominal concentration

11.5.1 Acute (short-term) toxicity to fish

Rainbow trout (*Oncorhynchus mykiss*), mean wet weight 1.5 g (range 0.80 to 2.84 g); mean total length 50 mm (range 42 to 63 mm), were exposed to a concentration range of 1,4-dimethylnaphthalene, batch H5510, purity 96.4%, in a flow-through test (6.6 volume replacements/aquarium every 24 h) (IIA 8.2/01). The study was carried out in accordance with OECD 203. A stock solution of the test substance was prepared in acetone. Nominal test concentrations 0.32, 0.54, 0.90, 1.5, and 2.5 mg as/L a control and a solvent control (0.5 mL/L acetone) Test concentrations were based on the results of a range-finding test. Two replicates with ten fish each, 15 L water per test aquarium.

Mean measured concentrations were 0.19, 0.22, 0.41, 0.75 and 1.2 mg/L. Measured test concentrations ranged from 40% to 58% of nominal test concentrations, but were constant during the test. At test

termination (96 hours), mortality of 100% was observed among rainbow trout exposed to the highest mean measured concentration (1.2 mg/L). Mortality of 5%, 5% and 45% was observed among organisms exposed to the 0.22, 0.41 and 0.75 mg/L test concentrations, respectively. In addition, sublethal effects (e.g., loss of equilibrium) were observed among all of the surviving fish exposed to these test concentrations.

The 96-hour LC₅₀ was calculated to be 0.67 mg/L with a 95% confidence interval of 0.57 to 0.80 mg/L using the moving average angle method. The 48-h LC₅₀ was 1.0 mg as/L, the 72-h LC₅₀ was 0.89 mg as/L. The LC₅₀ for 48 and 72 hours of exposure was based on probit analysis and nonlinear interpolation, respectively. The No-Observed-Effect Concentration (NOEC) was determined to be 0.19 mg/L. LC₅₀ and NOEC values are based on mean measured test concentrations. Study is considered reliable and the endpoint is acceptable for classification.

Fathead minnow (*Pimephales promelas*), mean wet weight 0.062 g (range 0.035 to 0.12 g); mean total length 2.0 cm (range 1.7 to 2.5 cm), were exposed to a concentration range of 1,4-dimethylnaphthalene, batch 01C-01, purity 98.8%, in a static renewal test (daily renewal) (IIA 8.2/02). The study was carried out in accordance with OECD 203 with the exception that the concentration of the solvent was not reported. Nominal test concentrations were: 0.25, 0.50, 1.0, 2.0, 4.0 and 8.0 mg as/L, a control and a solvent control (DMF). Two replicates with 10 fish per concentration. 4 L test solution per vessel and each vessel was closed by caps. Observations: 5, 24, 48, 72 and 96 hours after test initiation.

Mean measured concentrations were 0.22, 0.43, 0.86, 1.7, 3.2 and 5.4 mg/L (68-88% of nominal). Measured test concentrations from freshly prepared test concentrations ranged from 77 – 101% of nominal and the measured test concentrations from the 24 h old solutions ranged from 52 – 92% of nominal. Fathead minnows in the 0.22 and 0.43 mg/L treatment groups appeared normal, with no treatment-related mortalities or overt signs of toxicity noted during the test. While no mortalities occurred in the 0.86 mg/L treatment group, all of the fish exhibited signs of toxicity by test termination. Percent mortality in the 1.7, 3.2 and 5.4 mg/L treatment groups at test termination was 80, 100 and 100%, respectively. Symptoms included surfacing, erratic swimming, lethargy, and loss of equilibrium.

24 hours LC₅₀ of 2.3 mg as/L, 48-hours LC₅₀ of 1.6 mg as/L, 72-hours LC₅₀ of 1.4 mg as/L and 96-hours LC₅₀ of 1.4 mg as/L (95% CL 0.86 – 1.7 mg as/L), based on mean measured test concentrations. All LC₅₀ values were calculated using binomial probability method. Study is considered reliable and the endpoint is acceptable for classification.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Twenty daphnids (*Daphnia magna*, ≤24 hours old) were exposed to a range of concentrations of 1,4-dimethylnaphthalene (batch H5510, purity 96.4%) (IIA 8.3.1/01) under flow-through conditions. The study was carried out in accordance with OECD 202. The study was conducted in two parts with the following nominal test concentrations: 0.16, 0.26, 0.43, 0.72, and 1.2 mg as/L (test 1) and 0.65, 1.1, 1.8, 3.0, and 5.0 mg as/L (test 2). Two replicates with 10 daphnids per treatment were carried out. Observations of immobility and abnormal behaviour of appearance were performed after 24 and 48 hours of exposure.

In the first test the mean measured test concentrations were 0.056, 0.10, 0.18, 0.31, and 0.48 mg as/L. Immobility occurred at concentrations of 0.18 mg/L and above. At the highest test concentration of 0.48 mg as/L, 15% immobilised daphnids were observed. Other symptoms included lethargy, erratic movement and paleness. All daphnids at the highest test concentration were pale and erratic. EC₅₀ of the first test is reported to be > 0.48 mg as/L, based on mean measured test concentrations.

In the second test the measured test concentrations were 0.21, 0.33, 0.56, 0.94 and 2.2 mg as/L. Immobility was found at 0.33 mg as/L (5%) and increased dose-related to 100% at 2.2 mg as/L. The EC₅₀ of the second test is calculated at 0.54 mg as/L (95% CL 0.33 – 0.94 mg as/L), based on mean measured test concentrations. Study is considered reliable and the endpoint is acceptable for classification.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

See long-term aquatic hazard Section 11.6.3.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No additional data.

11.6 Long-term aquatic hazard

The long-term aquatic toxicity studies of 1,-4-dimethylnaphthalene were assessed in the Draft Assessment Report (October 2012) prepared in the context of the approval, under Reg. (EC) 1107/2009. Studies considered valid in the and DAR (reliability score of 1 or 2) have been included in this report. Other than the public literature data all studies were carried out under GLP and in accordance with OECD guidelines. Minor deviations were noted in one study but in general these did not affect the overall reliability of the studies. The deviations are included in the summaries were relevant. For the public literature studies the reliability of the studies were also assessed using Klimisch Scores.

Table 38: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test duration	Test material	Results ¹	Remarks	Reference
OECD 215	Rainbow trout (<i>Oncorhynchus mykiss</i>)	28 d	1,4-dimethylnaphthalene (lot number 14D03M01-02, purity 98.8%) Concentration: 0, 20, 46, 106, 243 and 560 µg a.s./L	NOEC _{growth} : 0.09 mg a.s./L Mean measured.	-	IIA 8.2/03, DocID 535A-105
Partly in accordance with early life stage test, OECD 210	<i>Gadus morhua</i>	4 d	1,4-dimethylnaphthalene, purity 98% Concentration: 0, 3.09, 2.92, 2.06, 1.03, 0.95 mg/L	NOEC <0.67 mg a.s./L Mean measured	Non GLP, public literature study. Klimisch score: 2 Mortality and sublethal effects were reported together therefore NOEC reflect mortality and sublethal effects together.	IIA 8.2/04
OECD 211	<i>Daphnia magna</i>	21 d	1,4-dimethylnaphthalene (batch 14D03M01-02, purity 98.8%)	NOEC: 0.16 mg a.s./L Mean measured.	-	IIA 8.3/02 DocID 535A-104

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

			Concentration: 25, 50, 100, 200 and 400 µg/L			
Partly in accordance with OECD 210	Sea urchin eggs (<i>Strongylocentrotus droebachiensis</i>)	4 d	1,4-dimethylnaphthalene, purity 98% Concentration: nominal conc. 2.92 and 0.95 mg as/L in the first test and 3.09, 2.06 and 1.03 mg as/L in the second test	NOEC: 0.54 mg a.s./L (test 1) and 0.31 mg/L (test 2) Mean measured.	Non GLP, public literature study. Klmisch score: 1	IIA 8.2/04
OECD 221	<i>Lemna gibba</i>	7 d	1,4-dimethylnaphthalene, batch 01C-01, purity 98.8% Concentration: 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L	NOEC 0.15 mg a.s./L Mean measured.	-	IIA 8.6/01 DocID 535A-103
OPPTS 850.5400, OECD 201, EEC C.3	<i>Pseudokirchneriella subcapitata</i>	96 h	1,4-dimethylnaphthalene, batch 01C-01, purity 98.8% Concentration: 0.028, 0.056, 0.11, 0.23, 0.45 and 0.90 mg a.s./L	72h NOEC 0.03 mg a.s./L Mean measured.	The validity criteria of OECD 201 were not met with respect to control growth.	IIA 8.4/01 DocID 535A-102

¹ Indicate if the results are based on the measured or on the nominal concentration

11.6.1 Chronic toxicity to fish

Rainbow trout were exposed to 1,4-dimethylnaphthalene under flow-through conditions. Nominal test concentrations of 1,4-dimethylnaphthalene (lot number 14D03M01-02, purity 98.8%) selected were control, solvent control (0.10 mL dimethylformamide/L), 20, 46, 106, 243 and 560 µg as/L (IIA 8.2/03). The study was carried out in accordance with OECD 215. Mean fish weight at test initiation was 1.1 g (range 1.0 – 1.3 g). Four replicates per treatment with 10 fish each.

Mean measured test concentrations, determined from samples of test water collected at test initiation and at approximately weekly intervals thereafter, were 18, 40, 90, 205 and 488 µg/L, which represented 90, 87, 85, 84 and 87% of nominal concentrations, respectively.

At test termination, there were no statistically significant differences ($p > 0.05$) in survival between treatment groups and the pooled control. Consequently, the NOEC for survival was 488 µg/L and the LOEC was > 488 µg/L. No significant differences between the negative and solvent control groups in growth rate. The differences between the pooled control and 205 and 488 µg/L, the two highest treatment groups, were statistically significant ($p \leq 0.05$). Consequently, the NOEC for growth rate was 90 µg/L and the LOEC was 205 µg/L. The NOEC was based on mean measured test concentration. Study is considered reliable and the endpoint is acceptable for classification.

In a non-GLP public literature study 1,4-dimethylnaphthalene, purity 98%, was added to seawater with fertilised Cod fish eggs (*Gadus morhua*) in a duplicate static test (IIA 8.2/04). The studies was partially in accordance with OECD 210 with the main difference being that mortality and sublethal effects were not reported separately. Approximately 50 cod eggs were placed in test beakers containing 100 ml of seawater mixed with the test substance. Two experiments were performed, the first test with concentrations at 2.92 and 0.95 mg/L and a control, the second at 3.09, 2.06 and 1.03 mg/L and a control. Observations of mortality and abnormal embryos were performed after 6 h, 1 day, 2 days and 4 days.

Analysed concentrations at t= 4 d in the first test were 1.75 and 0.54 mg/L, 60% and 57% of nominal, respectively (2.92 and 0.95 mg/L at t= 0h resp). Analysed concentrations at t= 4 d in the second test were and 1.08, 0.56 and 0.31 mg/L, 35%, 27% and 30%, respectively (3.09, 2.06 and 1.03 mg/L at t=0 h resp). At nominal concentrations of 2.92, 3.09 and 2.06 mg/L, the substance caused mortality or impairment of normal embryonic development. At 0.95 and 1.03 mg/L, a slight effect on development to the gastrula stage was observed. Therefore, the NOEC is lower than the lowest test concentration, which was 1.03 mg/L at t=0 h and 0.31 mg/L at t=4d. Mortality and sub-lethal effects are taken together in the results. Therefore, a NOEC based on mortality cannot be determined. A NOEC for mortality and sub-lethal effects together can be set at <0.67 mg/L, based on mean measured concentrations. Study is considered reliable (Klimisch Score 2) and the endpoint is acceptable for classification.

11.6.2 Chronic toxicity to aquatic invertebrates

In a *Daphnia magna* study daphnids (< 24-hours of age) were exposed during 21 days to a geometric series of five test concentrations 1,4-dimethylnaphthalene, a negative (dilution water) control and a solvent control (dimethylformamide 0.1 mL/L) under flow-through conditions (IIA 8.3/02). The study was carried out in accordance with OECD 211. Nominal test concentrations of 1,4-dimethylnaphthalene (lot number 14D03M01-02, purity 98.8%) selected for the test were 25, 50, 100, 200 and 400 µg/L. Two replicate test chambers were tested for each treatment and control. Each replicate contained two compartments with five daphnids, resulting in a total of 20 *Daphnia magna* in each treatment and control group. Test compartments were covered with Petri dish to minimise volatilisation. Daily observations of survival, clinical signs and reproduction. Body length and dry weight of surviving daphnids (F0) were measured.

Mean measured test concentrations were 24, 48, 89, 160 and 339 µg/L. Clinical effects of lethargy, discoloration and a smaller size of the daphnids were observed at 339 µg as/L.

NOEC for reproduction, length, and dry weight of the F0 generation is reported as 160 µg as/L. A 21-day EC₅₀ for mortality is reported to be > 339 µg as/L and the 21-day EC₅₀ for reproduction is 275 µg as/L (mean measured). An EC₁₀ values were not reported. Study is considered reliable and the endpoint is acceptable for classification.

In a non-GLP public literature study 1,4-dimethylnaphthalene, purity 97.5%, was added to seawater with fertilised sea urchin eggs (*Strongylocentrotus droebachiensis*) in a duplicate static test (IIA 8.2/04). Nominal concentrations of 2.92 and 0.95 mg as/L in the first test and 3.09, 2.06 and 1.03 mg as/L in the second test. At fixed time intervals (6 h, 1 day, 2 days and 4 days) the number of abnormal and dead embryos noted. Test concentration was measured by ultraviolet spectrophotometry at test start and termination.

Mean measured concentrations were 1.75 and 0.54 mg/L, 60% and 57% of nominal, respectively, in the first test and 1.08, 0.56 and 0.31 mg/L, 35%, 27% and 30%, respectively, in the second test. In the first test, the no effect level was 0.54 mg/L (mean measured concentration). In the second test the no effect

level was 0.31 mg/L (mean measured concentration. Study is considered reliable and the endpoint is acceptable for classification.

11.6.3 Chronic toxicity to algae or other aquatic plants

The green alga, *Pseudokirchneriella subcapitata*, was exposed to a geometric series of six concentrations of 1,4-dimethylnaphthalene, batch 01C-01, purity 98.8%, a blank control and a solvent control (dimethylformamide 0.1 mL/L) under static conditions for 96 hours (IIA 8.4/01). The study was carried out in accordance with OECD 201. Twelve replicate test chambers were maintained in each treatment and control group. Nominal test concentrations selected were 0.028, 0.056, 0.11, 0.23, 0.45 and 0.90 mg/L. Initial cell density 5000 cells/mL. Cell densities were counted after 24, 48, 72 and 96 hours using and electronic particle counter.

Mean measured test concentrations over test duration were 0.030, 0.053, 0.11, 0.21, 0.44 and 0.86 mg as/L (95-107% of nominal). Cells appeared normal except at 0.86 mg as/L after 96 hours of exposure. Those cells were noted to be enlarged. Growth rate was inhibited by 3.6% at 0.053 mg as/L (significant) and was reduced with 77% at 0.86 mg as/L, the highest concentration, after 72 hours. Biomass was also significantly reduced at 0.053 mg as/L (8.7%) and further reduced by 95% at 0.86 mg as/L after 72 hours. Both reductions were dose-related. E_bC_{50} is reported as 0.32 mg as/L (95% CL: 0.30 – 0.35 mg as/L) and E_rC_{50} as 0.62 mg as/L (95% CL 0.60 – 0.64 mg as/L) after 72 hours of exposure. The E_bC_{50} and E_rC_{50} values after 96 hours were 0.33 and 0.60 mg as/L, respectively. EC_{10s} were not reported. NOE_bC and NOE_rC after 72 and 96 hours are both 0.030 mg as/L. All values based on mean measured test concentrations and compared with solvent control.

The validity criteria of OECD 201 (2001) are not met with respect to control growth. No exponential growth rate in the control. The mean variance of daily growth rate in the control was > 35% (mean value 79%). However, the study did meet the criteria of OECD 201 adopted July 1984 which was in effect at the time of the conduct of the study. In addition, due to volatility of the test substance and a desire to maintain test concentration throughout the study the test was done in a closed system. Due to the lack of gas exchange in this testing environment, algal growth is limited. The algae growth was exponential during the first 48 hours but quickly outgrew the carrying capacity of the media. Growth peaked very quickly so the algae were past logarithmic growth phase by 72 hours. Moreover, the observed effects of 1,4-DMN on green algal were evident and consistent at 48, 72 and 96 hours. These effects were observed during the early portion of the test when exponential growth was occurring as well as at 72 and 96-hours, after cell growth had peaked. The failure to have exponential growth between 48 and 72 hours did not appear to change the conclusion of the test. Study is considered reliable and the endpoint is acceptable for classification.

Fronds of duckweed, *Lemna gibba* G3, were exposed to six concentrations of 1,4-dimethylnaphthalene, batch 01C-01, purity 98.8%, a negative control (culture medium) and a solvent control (DMF 0.1 mL/L) under static-renewal conditions for seven days (IIA 8.4/01). The study was carried out in accordance with OECD 201. Nominal test concentrations selected were 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L. Three replicates for each test treatments and controls and one replicate without duckweed at the highest test concentration of 5.0 mg as/L. Renewal of test solutions on days 3 and 5 of the test. Four plants with in total 12 fronds were added to each replicate. Frond numbers were determined on days 3, 5 and 7. Observations of chlorosis, necrosis, break-up of duckweed colonies, death and any other abnormalities in plant or frond appearance were also performed on days 3, 5 and 7.

Mean measured test concentrations were 0.15, 0.31, 0.61, 1.2, 2.3 and 4.4 mg/L. Doubling time of frond number in the controls is about 3 days. Mean number of fronds after 7 days was 73 in the negative control and 75 in the solvent control and decreased treatment-related. Frond numbers after 7 days were 79, 67, 53, 35, 18, and 14 at 0.15, 0.31, 0.61, 1.2, 2.3, and 4.4 mg as/L. Percent inhibition increased from 8.6% to 81% at 0.31 to 4.4 mg as/L, at 0.15 mg as/L a stimulation of growth was noted of 6.8%. Frond numbers and percent inhibition were significant reduced at 0.31 mg as/L and onwards. Chlorotic

and necrotic fronds were observed at 0.31 mg as/L and onwards. 7-days I_rC_{50} for frond number is reported to be 1.1 mg as/L (95% CI 1.1 – 1.2 mg as/L), NOEC of 0.15 mg as/L both based on measured concentration. Study is considered reliable and the endpoint is acceptable for classification.

11.6.4 Chronic toxicity to other aquatic organisms

No additional data.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

The criteria for Category Acute 1 in line with Table 4.1.0 from the Guidance on the Application of the CLP Criteria are:

96 hr LC ₅₀ (for fish)	≤ 1 mg/l and/or
48 hr EC ₅₀ (for crustacea)	≤ 1 mg/l and/or
72 or 96 hr ErC ₅₀ (for algae or other aquatic plants)	≤ 1 mg/l.

The lowest LC₅₀ in fish (Rainbow trout) was 0.67 mg/L. For aquatic invertebrates (*Daphnia magna*) the LC₅₀ was 0.54 mg a.s./L. The 72 h ErC₅₀ in *Pseudokirchneriella subcapitata* was 0.62 mg a.s./L. Based on the observed LC₅₀ values with the lowest LC₅₀ value being 0.54 mg a.s./L 1,4-dimethylnaphthalene should be classified with Category Acute 1 with an M-factor of 1 ($0.1 < L(EC)C_{50} < 1 \text{ mg/L}$).

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

In the OECD 301C ready biodegradability study 1,4-dimethylnaphthalene did fulfil the 60% criteria at the end of the study. The other available degradation data cannot confirm a rapid biodegradability. The substance is considered rapidly degradable (RD) according to the criteria outlined in section 4.1.2.9 of the CLP Regulation (EC) 1272/2008 where is indicated that the percentage degradation reached after 28 days in ready biodegradability tests may be used directly for the assessment of ‘rapid degradability’ if the data were derived with the MITI 1 test (OECD 301C). The criteria for Category Chronic 1, 2 and 3 the CLP Regulation (EC) 1272/2008 for rapidly degradable substances for which adequate chronic toxicity data are available are:

Category Chronic 1:

Chronic NOEC or ECx (for fish)	≤ 0.01 mg/l and/or
Chronic NOEC or ECx (for crustacea)	≤ 0.01 mg/l and/or
Chronic NOEC or ECx (for algae or other aquatic plants)	≤ 0.01 mg/l

Category Chronic 2:

Chronic NOEC or ECx (for fish)	≤ 0.1 mg/l and/or
Chronic NOEC or ECx (for crustacea)	≤ 0.1 mg/l and/or
Chronic NOEC or ECx (for algae or other aquatic plants)	≤ 0.1 mg/l

Category Chronic 3:

Chronic NOEC or ECx (for fish)	≤ 1 mg/l and/or
Chronic NOEC or ECx (for crustacea)	≤ 1 mg/l and/or
Chronic NOEC or ECx (for algae or other aquatic plants)	≤ 1 mg/l

Chronic toxicity studies are available for fish, invertebrates, algae and higher plants. The lowest chronic NOEC value in fish (Rainbow trout) was 0.09 mg a.s./L. For aquatic invertebrates (*Daphnia magna*) the lowest NOEC was 0.16 mg a.s./L. The lowest NOEC for aquatic plants (*Pseudokirchneriella subcapitata*) was 0.03 mg a.s./L. Based on the lowest NOEC of 0.03 mg a.s./L for *Pseudokirchneriella subcapitata* Category Chronic 2 applies according to Table 4.1.0 of the CLP Regulation (EC) 1272/2008 (RD and $0.01 < \text{NOEC} \leq 0.1 \text{ mg/L}$).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Acute (short-term) aquatic hazard: category Acute 1, M-factor: 1.

Long-term aquatic hazard: category Chronic 2.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

1,4-dimethylnaphthalene (1,4-DMN) currently has no classification in Annex VI to CLP. The DS's proposal for consideration by RAC was to classify 1,4-DMN as Aquatic Acute 1 (M=1) and Aquatic Chronic 2. This was based on the lowest EC₅₀ value of 0.54 mg/L for *Daphnia magna* and the lowest NOEC value of 0.030 mg/L for *Pseudokirchneriella subcapitata* and the substance being rapidly degradable for classification purposes.

Degradation

No experimental information on hydrolysis is available and it is indicated that HydroWin QSAR (EPIWeb 4.0) cannot be used to estimate the hydrolysis half-life for 1,4-DMN. A hydrolysis DT₅₀ of 5.27×10^5 day (20°C) estimated by EUSES 2.1 is reported in the dossier but it is considered not reliable by the DS. Based on the structure of the substance, hydrolysis is expected to be very slow.

The dossier includes QSAR estimations on the photolytic half-life in water of 1,4-DMN ranging from 3.2 to 12.8 hours. However, due to the limited information available on these QSAR models, the DS concludes that they can only be used as supplemental information.

A biodegradation screening study following OECD TG 301C (not GLP) is available for 1,4-DMN. In the study, the test substance at initial concentration of 100 mg/kg was incubated at 25°C in duplicate test flasks containing activated sludge. Inoculum control and reference substance test flasks were also incubated whereas abiotic control was not included even though it is required by the guideline. The degradation was followed by dissolved oxygen measurements on days 7, 14, 21 and 28. The degradation of the reference substance reached the pass levels indicated in the guideline. The test substance reached 66% degradation after 28 days based on the theoretical oxygen demand. It is not clear whether the 10-day window criteria were met but this is not required in OECD TG 301C. It is indicated that some volatilisation of the test substance cannot be excluded. However, the DS concluded that the degradation curves indicate that proper biodegradation occurred and that the study and its results can be considered reliable.

The dossier also includes a water-sediment simulation study with the substance, which,

however, is not considered reliable by the DS because the test substance was quickly lost from the system due to volatilisation and adsorption to rubber stoppers used in the test vessels. No DT₅₀ values could be calculated in the study.

No soil simulation studies are available for the substance. However, information from public literature studies on degradation of 1,4-DMN or other similar PAHs by soil or compost microorganisms is included in the dossier. In three of these studies, isolated bacteria were able to degrade 1,4-DMN or 2,6-DMN. In two of the studies, unspecified DMNs were exposed to compost microorganisms and biodegradation and/or volatilisation of DMNs were observed. According to the DS, no reliable DT₅₀ values can be obtained from these studies, but the studies indicate that soil microorganisms can degrade 1,4-DMN.

Based on the available information, the DS concluded that the substance is rapidly degradable for classification purposes.

Bioaccumulation

A measured log K_{ow} of 4.37 (at 22.5°C) is reported for 1,4-DMN in the section on physico-chemical properties of the CLH dossier. This value comes from a study in which log K_{ow} values were determined for 12 naphthalene homologues using a shake flask method with fluorometric measurements. In addition, in the section on bioaccumulation, a log K_{ow} of 4.22 determined in another published study using HPLC method (Dimitrou-Christides, 2003) is reported for 1,4-DMN.

No bioaccumulation study is available for 1,4-DMN. A study that assessed the bioaccumulation of 1,3-dimethylnaphthalene (1,3-DMN), among other PAHs, in fish is included and used for the bioaccumulation assessment of 1,4-DMN. In the study, adult sheepshead minnows (*Cyprinodon variegatus*) were exposed to two concentrations of PAH mixture in seawater during 36 days, followed by an 8-day depuration period, under flow-through conditions. The PAHs studied were pure naphthalene, 2-methylnaphthalene, 1,3-dimethylnaphthalene, 2-isopropylnaphthalene, phenanthrene, pyrene, 9-methylphenanthrene, and 9-ethylphenanthrene. The 1,3-DMN concentrations in water were $2.74 \pm 0.73 \mu\text{g/L}$ and $26.67 \pm 9.19 \mu\text{g/L}$.

Naphthalene and its three alkylated isomers revealed peak levels within 4 days in both treatments except for the low-exposure concentration of C2 - and C3 -alkylated naphthalene, which reached maximum at day 7. Uptake rate constants (k_1) for the naphthalenes increased with increasing degree of alkylation and log K_{ow} value, whereas the elimination rate constants (k_2) generally increased with decreasing degree of alkylation and log K_{ow} values. A significant positive correlation was determined between log BCFs and log K_{ow} values for the series of C0-C3 naphthalenes at both low ($r^2 = 0.985$, $p = 0.0077$) and high ($r^2 = 0.956$, $p = 0.022$) exposures.

According to the dossier, BCFs of 4,000 L/kg and 8,000 L/kg were determined for 1,3-DMN at low and high exposure concentrations, respectively. The DS calculated a geometric mean of these values resulting in a BCF of 5,751 L/kg. The BCFs are not lipid-normalised.

The DS concludes that the study is reliable and that the results of 1,3-DMN can be read-across to 1,4-DMN. According to the DS, this is justified because in the BCF study a correlation between log BCFs and log K_{ow} values was found for alkylated naphthalenes, and because the log K_{ow} values of 1,3-DMN and 1,4-DMN are very similar; 4.27 and 4.22, respectively (Dimitrou-Christides *et al.* 2003).

Aquatic toxicity

Acute and chronic toxicity studies are available for fish, aquatic invertebrates, algae and aquatic plants. The studies were performed in accordance with GLP, unless stated otherwise.

Acute toxicity

Two acute toxicity studies on fish, performed following OECD TG 203, are available for 1,4-DMN. In one of the studies, a 96-h LC₅₀ value of 0.67 mg/L (95% CL of 0.57 to 0.80 mg/L) based on mean measured concentrations was determined for *Oncorhynchus mykiss* under flow-through conditions. In the other, study, a 96-h LC₅₀ value of 1.4 mg/L (95% CL 0.86 – 1.7 mg/L) is reported for *Pimephelas promelas*, based on mean measured concentrations. It is noted that in the *Pimephelas promelas* study, DMF was used as a solvent but its concentration is not reported. Both studies met the validity criteria indicated in OECD TG 203 and are considered reliable by the DS.

The dossier includes an acute toxicity study with *Daphnia magna* performed under flow-through conditions and following OECD TG 202. The study consisted of two tests. In the first test, only 15% immobilisation was observed at the highest concentration, and hence, an EC₅₀ value of > 0.48 mg/L was reported based on mean measured concentrations. In the second test, higher concentrations were used and a dose-related increase of immobilisation, up to 100% immobilisation at the highest concentration, was observed. A 48-h EC₅₀ value of 0.54 mg/L (95% CL 0.33 – 0.94 mg as/L) based on mean measured test concentrations was determined. The mean measured concentrations were 30-45% of the nominal concentrations but they were constant during the tests. The validity criteria of the OECD TG 202 were met.

One algal toxicity study following OECD TG 201 is available for 1,4-DMN. *Pseudokirchneriella subcapitata* algae were exposed to the test substance in closed test vessels with minimal headspace during 96 hours under static conditions. An E_rC₅₀ value of 0.62 mg/L (95% CL 0.60 – 0.64 mg as/L) is reported after 72 hours of exposure and an E_rC₅₀ of 0.60 mg/L after 96 hours. It is stated that constant exponential growth was not observed in the controls throughout the study; the growth peaked at 48 hours after which it slowed down. The cell number increased by a factor 16 within the first 48 hours, and hence, the first validity criterion of the current OECD TG 201 (2006) was met. This was the only validity criterion regarding control growth indicated in the OECD TG 201 (1984) that was in use at the time when the study was conducted (year 2002). However, the second validity criterion of the current OECD TG 201 (i.e. the mean coefficient of variance for section-by-section specific growth rates in controls should be ≤ 35%) was not met. According to the annex of the CLH report, the mean variance of daily growth rate in the control was 41% at 48 hours, 58% at 72 hours and 79% at 96 hours.

In the CLH dossier, it is stated that the effects in growth rate in the treatments were observed during the early portion of the test when exponential growth was occurring, as well as at 72 and 96-hours, after cell growth had peaked. Furthermore, it is mentioned that the results of the test showed a clear dose-response relationship between algal growth and mean measured concentrations of 1,4-DMN, which were maintained between 90 and 110% of nominal concentrations throughout the test. The DS concluded that the results of the study are reliable.

The dossier also includes a semi-static study assessing the toxicity of 1,4-DMN to the aquatic plant *Lemna gibba*, performed in accordance with OECD TG 221 (Draft). A 7-d IC₅₀ value for frond number is reported to be 1.1 mg/L (95% CI 1.1 – 1.2 mg/L), based on mean measured

concentrations. According to the DS, the water quality parameters were within accepted limits and the results of the study are considered acceptable for classification.

In conclusion, the DS proposed a classification as Aquatic Acute 1 (M=1), based on an overall lowest EC50 value of 0.54 mg/L for *Daphnia magna*.

Chronic toxicity

A fish juvenile growth test (OECD TG 215) is available for 1,4-DMN. Juvenile rainbow trout were exposed to the substance under flow-through conditions during 28 days. A 28-d NOEC value of 0.09 mg/L for growth rate was determined based on mean measured concentrations. The study met the validity criteria of the OECD TG 215.

A study assessing the effects of the substance in fertilised cod (*Gadus morhua*) eggs is also included. The non-GLP study is stated to be partly in accordance with the OECD TG 210. The study consisted of two tests in which the eggs were exposed to different concentrations of the substance in seawater during 4 days under static conditions. Observations of mortality and abnormal embryos were performed after 6 hours and after 1, 2 and 4 days. At the highest test concentrations the substance caused mortality or impairment of embryonic development, and at the lowest concentrations a slight effect on the development of gastrula stage was observed. Therefore, the NOEC for embryo development was determined to be lower than the lowest tested concentration, i.e., NOEC < 0.67 mg/L based on mean measured concentrations.

The chronic toxicity of 1,4-DMN to *Daphnia magna* was assessed in a flow-through study following OECD TG 211. A 21-d NOEC of 0.16 mg/L for reproduction, length, and dry weight is reported based on mean measured concentrations. The study met the validity criteria of OECD TG 211. However, it is noted that the dissolved oxygen (DO) concentration dropped below 60% saturation level on days 10 and 12, which seemed to be caused by microbial growth in the test chambers. The daphnids were transferred to new test vessels and the flow rate was increased, after which the DO levels increased to > 89% saturation. According to the DS this deviation did not affect the results and the study can be considered reliable.

The dossier also includes a non-GLP study, performed partly in accordance with OECD TG 210, assessing the effects of 1,4-DMN on fertilised sea urchin eggs (*Strongylocentrotus droebachiensis*). The eggs were exposed, in two separate tests, to 5 different concentrations of the substance in seawater during 4 days under static conditions. Observations of mortality and abnormal embryos were performed after 6 hours and after 1, 2 and 4 days. A NOEC of 0.57 mg/L for embryo development is reported based on mean measured concentrations.

The chronic toxicity values determined in the available algal and aquatic plant studies (see the section on acute toxicity above for more information) were a 72-h and 96-h NOEC of 0.030 mg/L for *Pseudokirchneriella subcapitata* and a 7-d NOEC of 0.15 mg/L (frond number) for *Lemna gibba*. The results are based on mean measured concentrations.

In conclusion, the DS proposed a classification as Aquatic Chronic 2, based on a lowest NOEC value of 0.03 mg/L for *Pseudokirchneriella subcapitata*.

Comments received during public consultation

One member state stated that they agree with the proposed environmental classification during the public consultation. Another MS also supported the proposed classification but they indicated that the study on algae should not be considered reliable as the validity criteria are not met. Nevertheless, the MS noted that the available fish chronic value is of the same order of magnitude as the algal NOEC.

A third member state commented on the uncertainties regarding the available OECD 301C test asking whether further information supporting the conclusion on rapid degradation is available. Furthermore, they stated that the algae study should not be used for classification and asked whether information on the analogue substance 1,3-DMN would be available for algal toxicity. The MS also noted that the chronic fish data comes from an OECD TG 215 study but that growth may not be the most sensitive endpoint, and hence, the use of the surrogate approach should be considered for determining the chronic classification.

A company/manufacturer submitted a comment indicating that according to the CLP Guidance, in the chronic studies EC₁₀ values (when available) should be preferred over NOEC values and provided an attachment with new calculations and statistical analyses for EC₁₀ and NOEC values for the available OECD TG 201 algal study and OECD TG 215 fish study. This resulted in E_rC₁₀ = 0.357 mg a.s./L for algae, and EC₁₀ = 0.200 mg a.s./L for fish, which would lead to a classification as Aquatic Chronic 3. The DS agreed with the comment and modified their proposal for environmental classification accordingly.

Assessment and comparison with the classification criteria

Degradation

No experimental information on abiotic degradation is available for 1,4-DMN, and the estimated hydrolytic and photolytic half-lives are not fully reliable.

In the available ready biodegradation screening test (OECD TG 301C), 66% degradation of 1,4-DMN was observed after 28 days based on O₂ consumption. It is not clear whether the 10-d window was met but it does not apply to tests performed according to OECD TG 301C. Regarding the lack of abiotic control, RAC notes that if part of the test substance was lost from the test vessels by volatilisation, it would mean that the BOD, and consequently, the degradation of the test substance, were underestimated because the BOD was calculated considering the initial test substance concentration. Therefore, since the degradation of the test substance reached the pass level, the lack of abiotic control does not affect the conclusion of the study, and hence, RAC considers that the study can be considered acceptable for classification.

The available water-sediment simulation study is not relevant for classification as the test substance was lost from the test system through volatilisation and adsorption, and no DT₅₀ values were determined. The five studies regarding degradation of the substance or similar substances by soil or compost microorganisms are not considered relevant for classification purposes. This is because based on the available information in the CLH report, in two of the studies the microorganisms were pre-exposed to 2,6-DMN and in other two studies compost microorganisms were used, and hence, pre-exposure to DMNs or other similar PAHs cannot be excluded. In the fifth study, it is not clear whether the used soil bacteria might have been pre-

exposed to similar substances but no information on biodegradation rate is reported.

RAC agrees with the DS's proposal that based on the available ready biodegradation screening study, 1,4-DMN is rapidly degradable for classification purposes.

Bioaccumulation

The measured log K_{ow} values of 1,4-DMN are 4.37 and 4.22. The values come from studies that were not performed in accordance with standard guidelines but the methods are to some extent similar with the shake flask or HPLC methods described in OECD TGs 107 and 117. There are some uncertainties regarding the studies, e.g. in the study using the shake flask method, the concentration of the substance was only measured in the water phase, and in the HPLC study the used reference substances were not fully representative as they were all unsubstituted PAHs. RAC ran the KOWWIN (v1.68) QSAR model for the substance, which resulted in a predicted log K_{ow} of 4.26. This is in good agreement with the measured values.

RAC notes that based on the surface tension value of 36.88 mN/m reported in the CLH dossier, the substance could have surface active properties, which could result in uncertainties regarding the measured and predicted log K_{ow} values. However, the reported surface tension value of 1,4-DMN comes from a study and according to the DS there is not enough information to evaluate its reliability. Based on the structure of the substance, no surface active properties are expected, and hence, no uncertainties in the log K_{ow} values are considered based on these properties. In conclusion, considering that all the measured and predicted log K_{ow} values of the substance are very similar and above the cut-off value of 4 indicated in CLP, RAC considers that they indicate a bioaccumulation potential for classification purposes.

No experimental BCF value is available for 1,4-DMN. The DS calculated a mean BCF of 5751 L/kg for the similar substance 1,3-DMN based on BCFs reported for the substance in a study (Jonsson *et al.* 2004) assessing the bioconcentration of a mixture of PAHs in sheepshead minnows (*Cyprinodon variegatus*). RAC notes that in the CLH dossier it is mentioned that both BCFs were determined based on uptake and depuration rate constants (kinetic BCF) and based on the concentration of the substance in fish and water (steady-state BCF) but which type of BCFs values are reported is not stated. Furthermore, the BCFs are not lipid normalised. Based on the information found in Jonsson *et al.* (2004), the whole fish kinetic BCFs for 1,3-DMN were 3,979 and 8,313 L/kg and the steady-state BCFs were 3,426 and 4,761 L/kg at the low and high exposure concentrations, respectively. The kinetic BCF values are preferred as it seems that steady-state may not have been reached in the study, at least in the high exposure group. Based on the lipid weight based BCF_k values (41,020 and 85,696 L/kg for the low and high exposure groups) reported, the mean lipid content of the fish was 9.7%. RAC normalised the whole fish BCFs to 5% lipid content, which lead to BCF_k values of 2,051, and 4,285 L/kg, for the low and high exposure concentrations, respectively.

RAC agrees with the DS that the BCF values determined for 1,3-DMN can be considered reliable. Structural similarity and relevant physico-chemical properties that influence bioavailability and uptake potential (e.g. water solubility, adsorption, lipophilicity) have been considered (AE 2.2 Assessment elements for Bioaccumulation potential of the ECHA's Read-Across Assessment Framework) when assessing whether read-across from 1,3-DMN to 1,4-DMN can be accepted in the bioaccumulation assessment. Based on their similar structures (only differing in the position of one of the methyl groups) and log K_{ow} values, these two substances can be expected to have similar bioaccumulation potential, although it is noted that the different positions of the methyl groups in naphthalene could potentially cause some differences in the metabolism, and consequently in the BCF values, of the two substances.

However, since the BCF values of 1,3-DMN are well above the cut-off value of 500 L/kg indicated in CLP, RAC agrees with the DS's proposal that 1,4-DMN has a bioaccumulation potential for classification purposes.

Acute toxicity

The available acute toxicity studies on 1,4-DMN with fish and aquatic invertebrates are valid and resulted in 96-h LC₅₀ values of 0.67 mg/L for *Oncorhynchus mykiss* and 1.4 mg/L for *Pimephelas promelas* and in a 48-h EC₅₀ value of 0.54 mg/L for *Daphnia magna*.

In the available algal toxicity study 72-h and 96-h E_rC₅₀ values of 0.62 and 0.60 mg/L were determined for *Pseudokirchneriella subcapitata*. As stated in the CLH report, constant exponential growth was not observed in the control groups, and hence, the study does not fulfil the validity criteria of OECD TG 201. Furthermore, it is noted that the pH of the test mediums had increased by more than 1.5 units after 4 days in the controls and all treatment groups except in the highest test concentrations. According to ECHA Guidance R.7b, if in an algal growth inhibition test the exponential growth ceased in the control before the end of the test period, only data from the part of the test where exponential growth occurs and the validity criteria for the controls are fulfilled, should be used.

RAC notes that, based on the information in the full study report, the cell density of each replicate was not measured daily during the 4 days test period but instead at each sampling (every 24 hours during 4 days) 3 replicates per treatment and control group were used and their cell densities measured. Therefore, it is not possible to determine section-by-section growth rates for each replicate and the mean coefficient of variance for those growth rates as indicated in OECD TG 201. It is not clear to RAC how the "mean variance values for daily growth rates" mentioned in the CLH report were calculated. To be able to assess whether the second validity criterion of OECD TG 201 was met, RAC calculated the section-by-section growth rates based on the mean cell densities measured for the 3 replicates per control group at each sampling, and determined the coefficient of variation (CV) for these growth rates. When considering only the first 48 hours of the test, the resulting CV values were 26% and 31% for blank and solvent controls, respectively. When considering 72 hours test duration, the resulting CV value was 47% for both controls. Hence, based on these values, at 72 hours the validity criterion of the guideline is not met, whereas at 48 hours it was met.

In conclusion, after up to 48 hours of exposure, the growth in the control groups met the validity criteria, as the cell number had already increased by a factor of 16 and the coefficient of variance for section-by-section specific growth rates was $\leq 35\%$. Furthermore, based on the available information in the full study report, the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was $\leq 7\%$. Therefore, RAC considers that only the results up to 48 hours of exposure should be considered valid for classification. In the full study report, a 48-h E_rC₅₀ of 0.58 mg/L (95% CL 0.55-0.61 mg/L) is reported.

In addition, a 7-d IC₅₀ of 1.1 mg/L for *Lemna gibba* is reported in a study following OECD TG 221 (draft). RAC notes that according to the current OECD TG 221, for the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d⁻¹. Based on the information in the CLH report and its annex, the doubling time of frond number in the controls was about 3 days, and after 7 days the mean frond numbers were 73 and 75 in blank and solvent controls, respectively. RAC calculated mean growth rates based on these values and considering that the initial frond number was indicated to be 12. (Growth

rates could not be calculated for each replicate as indicated in the guideline due to lack of data.) This resulted in mean growth rates of 0.258 d^{-1} and 0.262 d^{-1} for the blank and solvent controls, respectively. Hence, the growth in the control groups was slightly below the validity criteria of the current OECD TG 221. Furthermore, it seems that the reported IC₅₀ is for frond number instead of for growth rate, which is the preferred endpoint. Therefore, RAC considers that the study and its reported results are not fully acceptable but they can be used as supporting information for classification, as lower acute values are available for other organisms.

In conclusion, RAC agrees with the DS's proposal that the lowest acute value of 1,4-DMN is the 48-h EC₅₀ of 0.54 mg/L for *Daphnia magna*. This is below the classification threshold of 1 mg/L for Aquatic Acute 1 and in the range of $0.1 < \text{L(E)C}_{50} \leq 1 \text{ mg/L}$ leading to an acute M-factor of 1. The lowest LC₅₀/EC₅₀ values determined for fish and algae support this classification, as they are in the same order of magnitude.

Chronic toxicity

The available chronic toxicity tests with fish and aquatic invertebrates following OECD TGs 215 and 211, respectively, are valid and resulted in a 28-d NOEC of 0.09 mg/L for *Oncorhynchus mykiss* based on growth rate and a 21-d NOEC of 0.16 mg/L *Daphnia magna* based on reproduction, length and dry weight. During the public consultation, a company/manufacturer provided a document containing new statistical analyses performed for the raw data of the OECD TG 215 study with *Oncorhynchus mykiss*, and a 28-d EC₁₀ of 0.200 mg/L (95% CL 0.159-0.251 mg/L) for growth rate (based on mean measured concentrations) is reported.

The dossier includes also studies on the effects of 1,4-DMN in fertilised cod and sea urchin eggs, performed partly in accordance with OECD TG 210. A NOEC value of $< 0.67 \text{ mg/L}$ is reported for cod and a NOEC value of 0.57 mg/L for sea urchin based on embryonic development. RAC notes that there is not enough information (e.g. regarding number of replicates, water quality parameters) to assess their reliability. Considering that the tests were performed under static conditions and in closed vessels, it would be informative to have information on the dissolved oxygen levels. Furthermore, the test duration was only 4 days, which is a limited time, especially for the development of cod eggs as the time to hatching at the test temperature (5°C) is usually above 10 days (Geffen *et al.* 2006). OECD TG 210 tests are normally initiated with fertilised eggs and continued for a species appropriate time period that is necessary for the control fish to reach a juvenile life-stage. Therefore, RAC considers that, due to the limited test duration and lack of detailed data, these studies can only be used as supporting information for classification.

The chronic toxicity value reported for algae in the CLH dossier is the 72-h and 96-h NOE_rC of 0.030 mg/L for *Pseudokirchneriella subcapitata*. However, as stated above, in RAC's opinion, in the *Pseudokirchneriella subcapitata* study only results up to 48 hours of exposure are acceptable for classification. 48-h NOEC values were not determined in the original study. However, as stated above, during the public consultation, a company/manufacturer provided a document with new statistical analyses on the raw data of the study including E_rC₁₀ and NOE_rC values for different study periods. RAC considers the new analysis reliable. The 48-h E_rC₁₀ reported in the new document is 0.232 mg/L (95% CL 0.208-0.259 mg/L). Regarding the 48-h NOE_rC, a statistically significant difference in growth rates when compared to pooled controls was determined at concentrations of 0.11 mg/L and above. Furthermore, at the concentration of 0.053 mg/L the growth rate did not differ statistically significantly from the pooled control while at the lowest test concentration of 0.030 mg/L it did. RAC notes that the inhibition in growth rate at the lowest concentration was only 2.7% after 48 hours. Furthermore, based on

the provided new information, the NOEC after 24 hours of exposure would be 0.053 mg/L. Therefore, since in the second lowest concentration (0.053 mg/L) no statistically significant inhibition was observed at 48 hours, it is not clear whether the observed low inhibition at 0.030 mg/L was caused by the test substance. In conclusion, RAC is of the opinion that a 48-h NOEC of 0.053 mg/L (based on mean measured concentration) can be considered reliable.

In the *Lemna gibba* study, a 7-d NOEC of 0.15 mg/L was determined for frond number. As indicated above, this can only be used as supporting information.

RAC agrees with the company/manufacturer's comment submitted during the public consultation and the DS's response to that comment: according to the current CLP Guidance, when EC₁₀ values are available these are preferred over NOEC values in chronic toxicity studies. RAC notes that this applies in cases where EC₁₀ and NOEC values are available for the same endpoint and the same study. RAC considers it more appropriate to use EC₁₀ values for aquatic chronic classification because NOEC values strongly depend on the experimental design (number of doses, width of the inter-dose interval, etc.), whereas EC₁₀ values are derived from the whole concentration-response curve. Therefore, in RAC's opinion, the relevant chronic values available for fish and algae are the 28d-EC₁₀ of 0.200 mg/L for *Oncorhynchus mykiss* and the 48h-EC₁₀ of 0.232 mg/L for *Pseudokirchneriella subcapitata*, respectively, even though these are significantly higher than the respective NOEC values. For the available *Daphnia magna* study only NOEC values are reported, and hence, they are used for classification.

In conclusion, based on the available chronic toxicity data on 1,4-DMN for three trophic levels, RAC considers that the lowest valid chronic toxicity value is the 21-d NOEC of 0.16 mg/L for *Daphnia magna*, which is below the classification threshold of 1.0 mg/L for Aquatic Chronic 3 for rapidly degradable substances.

Conclusion on Classification

Based on the above assessment, RAC concludes that 1,4-dimethylnaphthalene meets the classification criteria for **Aquatic Acute 1 (H400)** with an **acute M-factor of 1** and **Aquatic Chronic 3 (H412)**. This is in agreement with the DS's proposal as modified after considering the additional information provided during the public consultation.

Supplemental information - In depth analyses by RAC

As explained above, in the available OECD TG 201 test with *Pseudokirchneriella subcapitata* the algae in the control groups did not grow exponentially during the entire study period (see figure 1). Hence, the validity criterion of the current version of OECD TG 201 regarding section-by-section specific growth rates in controls was not met after 72 and 96 hours of exposure. However, based on RAC's analysis on the raw data available in the full study report, the growth in the controls met the validity criteria of the guideline after 48 hours of exposure (see table 1).

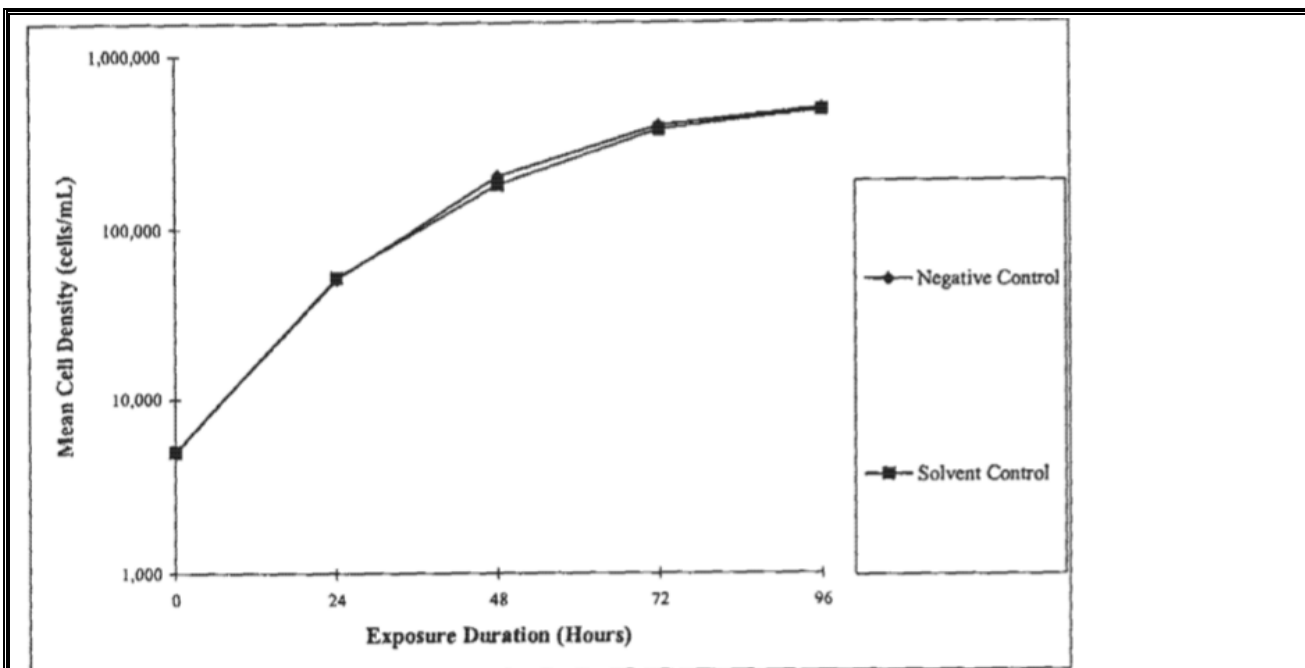


Figure. Mean cell densities in the negative and solvent control groups during the 96h test period in the OECD TG 201 study with *Pseudokirchneriella subcapitata*.

Table A) Section-by-section growth rates for controls, calculated based on the mean cell densities of the three replicates sacrificed and measured at each sampling time, and B) the coefficient of variation of the section-by-section growth rates.

A)

	Section-by-section growth rate (day ⁻¹)		
	0-24h	24-48h	48-72h
Negative control	2.332	1.359	0.668
Solvent control	2.352	1.231	0.722

B)

	Negative control		Solvent control	
	0-48h	0-72h	0-48h	0-72h
mean of section-by-section growth rates	1.846	1.453	1.792	1.435
STD section-by-section growth rates	0.487	0.683	0.560	0.681
CV% section-by-section growth rates	26.358	46.997	31.275	47.430

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

No data.

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

No data.

12.1.2 Comparison with the CLP criteria

No data.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

No data.

13 ADDITIONAL LABELLING

None.

14 REFERENCES

A full reference list of the available studies is included in Annex 1.

Additional references

Geffen, A.J., Fox, C, J. and Nash, D. M. 2006. Temperature-dependent development rates of cod *Gadus morhua* eggs. *Journal of Fish Biology*, 69: 1060–1080.

OECD (1998) - Report on the OECD workshop on statistical analysis of aquatic toxicity data. Series on testing and assessment, N° 10. Environmental Health and Safety Publications. Series on testing and Assessment. ENV/MC/CHEM(98)18.

15 ANNEXES

The study summaries from the DAR of 1,4-dimethylnaphthalene have been included in Annex I.

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

1,4-dimethylnaphthalene

EC Number: 209-335-9

CAS Number: 571-58-4

Index Number: -

Contact details for dossier submitter:

**Bureau REACH
National Institute for Public Health and the Environment (RIVM)
The Netherlands
bureau-reach@rivm.nl**

Version number: 2.0

Date: July 2018

CONTENTS

1	PHYSICAL HAZARDS	4
1.1	EXPLOSIVES	4
1.1.1	<i>Study 1</i>	4
1.2	FLAMMABLE GASES (INCLUDING CHEMICALLY UNSTABLE GASES).....	4
1.3	OXIDISING GASES	4
1.4	GASES UNDER PRESSURE.....	4
1.5	FLAMMABLE LIQUID.....	4
1.5.1	<i>Study 1 - Flashpoint</i>	4
1.5.2	<i>Study 2 – Boiling point</i>	4
1.6	FLAMMABLE SOLID	5
1.7	SELF-REACTIVE SUBSTANCES.....	5
1.8	PYROPHORIC LIQUIDS.....	5
1.9	PYROPHORIC SOLID	5
1.10	SELF-HEATING SUBSTANCES.....	5
1.11	SUBSTANCES WHICH IN CONTACT WITH WATER EMIT FLAMMABLE GASES.....	5
1.12	OXIDISING LIQUIDS.....	5
1.13	OXIDISING SOLIDS	5
1.14	ORGANIC PEROXIDES.....	5
1.15	CORROSIVE TO METALS	5
2	TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)	5
2.1.1	<i>STUDY 1</i>	5
2.1.2	<i>Study 2</i>	8
2.1.3	<i>Study 3</i>	11
2.1.4	<i>Study 4 – Kinetic information for other naphthalenes</i>	14
3	HEALTH HAZARDS	27
3.1	ACUTE TOXICITY - ORAL ROUTE.....	27
3.1.1	<i>Animal data</i>	27
3.1.1.1	<i>Study 1</i>	27
3.2	ACUTE TOXICITY - DERMAL ROUTE	29
3.2.1	<i>Animal data</i>	29
3.2.1.1	<i>Study 1</i>	29
3.3	ACUTE TOXICITY - INHALATION ROUTE.....	30
3.3.1	<i>Animal data</i>	30
3.3.1.1	<i>Study 1</i>	30
3.4	SKIN CORROSION/IRRITATION.....	31
3.4.1	<i>Animal data</i>	32
3.4.1.1	<i>Study 1</i>	32
3.5	SERIOUS EYE DAMAGE/EYE IRRITATION	32
3.5.1	<i>Animal data</i>	32
3.5.1.1	<i>Study 1</i>	33
3.6	RESPIRATORY SENSITISATION	34
3.7	SKIN SENSITISATION.....	34
3.7.1	<i>Animal data</i>	34
3.7.1.1	<i>Study 1 – LLNA</i>	34
3.8	GERM CELL MUTAGENICITY	35
3.8.1	<i>In vitro data</i>	35

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

3.8.1.1	Study 1 – Bacterial reverse mutation assay	35
3.8.1.2	Study 2 – Bacterial reverse mutation assay	37
3.8.1.3	Study 3 – mammalian gene mutation test	38
3.8.1.4	Study 4 – Unscheduled DNA synthesis	39
3.8.2	<i>Animal data</i>	41
3.8.2.1	Study 1 – <i>in vivo</i> micronucleus	41
3.8.2.2	Study 2 – <i>in vivo</i> unscheduled DNA synthesis assay	42
3.9	CARCINOGENICITY	44
3.9.1	<i>Animal data</i>	44
3.9.1.1	Study 1 – Combined chronic toxicity/carcinogenicity study, rat	44
3.9.1.2	Study 2 – carcinogenicity information on other naphthalenes	48
3.10	REPRODUCTIVE TOXICITY	51
3.10.1	<i>Animal data</i>	51
3.10.1.1	Study 1 – Extended one-generation study	51
3.10.1.2	Study 2 – Developmental toxicity, rabbits	56
3.10.1.3	Study 3 – Public literature information	59
3.11	SPECIFIC TARGET ORGAN TOXICITY – SINGLE EXPOSURE	60
3.11.1	<i>Animal data</i>	60
3.12	SPECIFIC TARGET ORGAN TOXICITY – REPEATED EXPOSURE	60
3.12.1	<i>Animal data</i>	60
3.12.1.1	Study 1 – 13 week rat	60
3.13	ASPIRATION HAZARD	64
4	ENVIRONMENTAL HAZARDS	64
4.1	DEGRADATION	64
4.1.1	<i>Ready biodegradability (screening studies)</i>	64
4.1.2	<i>BOD₅/COD</i>	66
4.1.3	<i>Aquatic simulation tests</i>	66
4.1.4	<i>Other degradability studies</i>	66
4.1.4.1	Hydrolysis	66
4.1.4.2	Water-sediment degradation	66
4.1.4.3	Soil degradation	69
4.1.4.4	Photochemical degradation	70
	STUDY IIA, 7.6/01	70
	STUDY IIA, 7.6/03 – CHEN ET AL, 2001.	71
4.2	BIOACCUMULATION	72
4.2.1	<i>Bioaccumulation test on fish</i>	73
4.2.2	<i>Bioaccumulation test with other organisms</i>	75
4.3	ACUTE TOXICITY	75
4.3.1	<i>Short-term toxicity to fish</i>	75
4.3.2	<i>Short-term toxicity to aquatic invertebrates</i>	78
4.3.3	<i>Algal growth inhibition tests</i>	79
4.3.4	<i>Lemna sp. growth inhibition test</i>	81
4.4	CHRONIC TOXICITY	81
4.4.1	<i>Chronic toxicity fish</i>	81
4.4.2	<i>Fish early-life stage (FELS) toxicity test</i>	83
4.4.3	<i>Chronic toxicity to aquatic invertebrates</i>	84
4.4.4	<i>Chronic toxicity to algae or aquatic plants</i>	87
5	REFERENCE LIST	88

1 PHYSICAL HAZARDS

1.1 Explosives

1.1.1 Study 1

Study 1 reference: Pelton 1993, IIA 2.1/02 Doc ID 4373-93-0226-AS

Test type: FIFRA § 63-16 Impact apparatus (GLP)

Material and methods

A study was conducted to determine the impact explosability of 1,4-DMN batch H5510 at 25°C using the Burea of Explosives impact apparatus. The minimum explosive drop height was 31 1/4 inches (maximum drop height). Three replicates were carried out.

Results

No explosive behaviour was observed in the three trials.

1.2 Flammable gases (including chemically unstable gases)

Hazard class not applicable.

1.3 Oxidising gases

Hazard class not applicable.

1.4 Gases under pressure

Hazard class not applicable.

1.5 Flammable liquid

1.5.1 Study 1 - Flashpoint

Study 1 reference: Pelton 1993, IIA 2.1/02 Doc ID 4373-93-0226-AS

Test type: FIFRA § 63-15 (equivalent to EEC A.9), GLP compliant

Material and methods

A study was conducted to determine the flammability of 1,4-DMN (batch H5510). The study was conducted by the Pensky-Martens Closed Tester. The sample was heated at a slow constant rate with stirring. A small flame was introduced at regular temperature intervals. The flash point was reported as the lowest temperature at which the test flame cause the vapor above the sample to ignite. The analysis was conducted in duplicate and the average results reported.

Results

The mean flash point corrected to a barometric pressure of 760 mm Hg was 122°C.

1.5.2 Study 2 – Boiling point

Study 1 reference: Pelton 1993, IIA 2.1/02 Doc ID 4373-93-0226-AS

Test type: FIFRA § 63-6 (ASTM D 1120-89, ebulliometric method) equivalent to OECD TG103 and EEC A2. (GLP compliant)

Material and methods

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

An experiment was conducted to determine the boiling point of 1,4-DMN (batch H5510). Approximately 60 ml of the sample and a few boiling chips were transferred into a flask. The condenser was attached to the flask and the thermometer was inserted so that the tip was approximately 1/4 inch from the flask bottom. The condenser water was started and heat applied so that the sample reached its boiling point within 30 minutes. Once the sample started to reflux the heat was adjusted so that the reflux rate was 1 to 2 drops per second. The temperature was recorded after 2 minutes at the specified reflux rate. The test was run in duplicate. The test was repeated with a standard material (ethylene glycol) of known boiling point. The atmospheric pressure was recorded in mm Hg at the conclusion of each test.

Results

The mean boiling point was 264°C at 744 mm Hg.

1.6 Flammable solid

Hazard class not applicable (1,4-DMN is not a solid).

1.7 Self-reactive substances

No study available.

1.8 Pyrophoric liquids

No study available.

1.9 Pyrophoric solid

Hazard class not applicable.

1.10 Self-heating substances

Hazard class not applicable.

1.11 Substances which in contact with water emit flammable gases

No data.

1.12 Oxidising liquids

No data.

1.13 Oxidising solids

Hazard class not applicable.

1.14 Organic peroxides

Hazard class not applicable.

1.15 Corrosive to metals

No data.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 STUDY 1

Characteristics

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Reference	: IIA5.1/01	Exposure	: single dose
Type of study	: absorption, distribution, metabolism, excretion	Doses	: 28 mg/kg bw
Year of execution	: 2000	Vehicle	: olive oil
Test substance	: [ring-U- ³ H]-1,4-dimethylnaphthalene (purity not reported, specific activity about 751 MBq/g)	GLP statement	: No
Route	: Intraperitoneal	Guideline	: none
Species	: rat (male outbred Imp:WIST rats, 200-220 g bw)	Acceptability	: acceptable
Group size	: 5/time point		

Study design

In a study from public literature, male rats received a single intraperitoneal dose of 28 mg/kg bw of [ring-U-³H]-1,4-dimethylnaphthalene. Immediately after administration, the rats were placed in individual metabolism cages. Urine and faeces were collected. Blood samples were collected from the tail vein. Rats were decapitated under light-ether narcosis at different time intervals (4, 8, 24, 48 and 72 h after administration; 5 rats/time point). Several tissues were removed for determination of radioactivity. The total balance of tritium was reported for 0-24 h, 0-48 h and 0-72 h in urine, faeces, blood cells + plasma, liver, adipose tissue, muscles and remaining tissues. The specific activity of tritium was reported in sciatic nerve, adrenals, liver, kidneys, spleen, brain, lung, muscles, plasma, blood cells and adipose tissue at 4, 8, 24, 48 and 72 h after administration. To study the metabolism of 1,4-dimethylnaphthalene, samples of urine (2 ml) collected during the first 24 h were acidified (pH = 1) and extracted directly with ethyl ether. The samples of ether extract (1 µl) were analyzed using GC-MS. Where peaks differing in retention time showed essentially identical mass spectra, the presence of isomers was assumed. The isomeric structure of the metabolites could not be identified due to lack of synthetic standard substances. The amount (%) of the identified metabolites was examined by comparing the peak surfaces presented in the chromatographic diagram.

Results

The maximum level of tritium in plasma and in all examined tissues was observed 4 h after compound administration (first measurement). Tritium was widely distributed; after 4 h the highest concentrations of tritium were observed in adipose tissue, followed by liver, kidneys, spleen and adrenals. After 72 h, excretion was essentially complete, since 56.5% of the tritium was excreted via urine and 40.8% via faeces (after 24 h, 41.3% was excreted via urine and 21.9% via faeces and after 48 h, 52.8% was excreted via urine and 36.3% via faeces). Half-life in blood was about 8 h. Since administration was by the intraperitoneal route, faecal excretion represented excretion via bile. At 72 h after intraperitoneal administration tissue levels, including in adipose tissue, were low (see table 2.1.1-1), indicating that 1,4-dimethylnaphthalene or its metabolites do not accumulate.

Table 2.1.1-1 Total balance of tritium following a single intraperitoneal administration of [ring-U-³H]-1,4-dimethylnaphthalene.

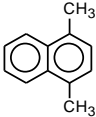
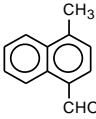
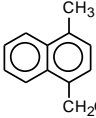
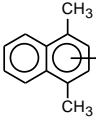
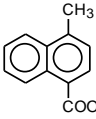
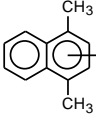
Matrix	% -age of administered dose (mean ± S.D. of 5 animals)		
	0-24h	0-48h	0-72h
Urine	41.3 ± 10.5	52.8 ± 5.4	56.5 ± 4.0
Faeces	21.9 ± 7.3	36.3 ± 4.4	40.8 ± 3.2
Blood cells + plasma	1.3 ± 0.0	0.9 ± 0.0	0.8 ± 0.0
Liver	0.7 ± 0.0	0.5 ± 0.0	0.2 ± 0.0
Adipose tissue	13.9 ± 0.2	2.7 ± 0.1	0.6 ± 0.0
Muscles	3.8 ± 0.0	3.5 ± 0.0	1.4 ± 0.0

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Remaining tissues	0.3	0.2	0.2
Total	83	97	100

In urine the following substances were identified and quantified by GC peak areas: unchanged 1,4-dimethylnaphthalene (35%), 4-methyl-1-naphthoic aldehyde (4%), 1-hydroxymethyl-4-methylnaphthalene (29%), 1-methyl-4-naphthoic acid (20%), 1,4-dimethylnaphthol (2 isomers, 11%), and 1,4-dimethyl-methylthionaphthalene (1%).

Table 2.1.1-2 Contribution of identified urinary substances calculated by peak area of chromatogram

Chemical name	Suggested chemical structure	% of total area
1,4-dimethylnaphthalene		34.84
4-methyl-1-naphthoic aldehyde		3.75
1-hydroxymethyl-4-methylnaphthalene		29.43
1,4-dimethyl-naphthol (2 isomers)		5.35 + 5.24
4-methyl-1-naphthoic acid		20.00
1,4-dimethyl-methylthionaphthalene		1.39

Acceptability

Although the study was not performed according to GLP the study is considered acceptable for the overall toxicological evaluation.

Conclusion

After a single intraperitoneal dose (28 mg/kg bw), 1,4-dimethylnaphthalene is rapidly absorbed ($T_{max} = 4$ h) and widely distributed in the rat. Excretion is rapid and essentially complete after 72 hours, 56.5% with urine and 40.8% with faeces. There is no evidence of accumulation in fat tissue. Because of the fact that administration of 1,4-dimethylnaphthalene in this study was by the intraperitoneal route, faecal excretion constituted entirely of excretion with bile.

Unchanged parent compound is a minor component in urine or faeces. Apart from the unchanged substance, the rat urine contains metabolites formed by three different pathways: oxygenation of the alkyl side chains

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

through an initial hydroxylation to the corresponding carboxylic acids, ring hydroxylation yielding naphthols (such reaction could proceed either directly or through epoxide intermediates), and binding of epoxide intermediates to glutathione leading to thionaphthols, excreted in urine as mercapturates.

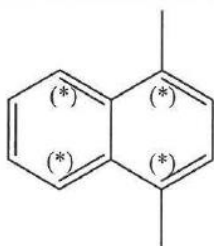
2.1.2 Study 2

Characteristics

Reference	: IIA 5.1/23, Doc ID 2263W-1	Exposure	: single dose
Type of study	: metabolism, excretion	Doses	: 28.6 mg/kg bw
Year of execution	: 2012	Vehicle	: corn oil
Test substance	: [¹⁴ C]-1,4-dimethylnaphthalene (purity 98.28%, specific activity about 12.6 MBq/mg)	GLP statement	: Yes
Route	: oral	Guideline	: Partially in accordance with OECD 417
Species	: rat (male Sprague Dawley rats, 385-389 g bw)	Acceptability	: acceptable
Group size	: 3		

Study design

A study of the metabolism of [¹⁴C]1,4-dimethylnaphthalene after administration of a single oral dose was conducted in the male Sprague-Dawley rat of appr. 12 weeks of age. The objectives of this study were to partially fulfil the data requirements of OECD 417 (22 July, 2010) and to confirm the formation of a 1-hydroxymethyl-4-methylnaphthalene metabolite.



(*) Indicates possible positions of the ¹⁴C label.

Each molecule has only one ¹⁴C.

In this GLP study, 3 male rats received a single oral dose of 28 mg/kg bw of [¹⁴C]-1,4-dimethylnaphthalene. Immediately after administration, the rats were placed in individual metabolism cages for 48 hours. All animals received water and food ad libitum throughout the study. Urine, cage wash and faeces were collected at 24 hr and 48 hr after dose administration. Rats were sacrificed at 48 hr after dosing. Excretion and metabolism were studied.

Results

Urinary excretion was the primary route of elimination of 1,4-DMN after oral administration (average 71.6% of the administered dose)

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

The nature of the urinary ¹⁴C was examined by HPLC/MS and GC/MS, and the identified metabolites are shown in Table 2.1.2-1. The parent molecule was not detected in urine by HPLC. But trace amounts were confirmed to be present by GC/MS. The complex metabolism of 1,4-DMN is the result of two primary metabolic pathways: side chain oxidation and ring hydroxylation yielding naphthols that are excreted in urine unchanged or conjugated with glucuronide or sulphate. There is a 3rd pathway described for alkylnaphthalenes, namely the binding of epoxide intermediates to glutathione leading to thionaphthols, excreted in urine as mercapturates. However, this pathway involved the forming of 1,4-dimethylmethylthionaphthalene; this thiomethyl derivate of 1,4-DMN was reported in Kilanowicz et al (see study 1) in small amounts after i.p administration, but is not detected after oral administration in the present study.

The metabolite indicating the 2nd pathway (ring hydroxylation yielding naphthols) is confirmed by the presence of 4-methyl-1-naphthoic acid (metabolite 4 in B.6.1.3).

Table 2.1.2-1a Individual balance of tritium following a single oral administration of [¹⁴C]-1,4-dimethylnaphthalene.

Sample	Rat 1	Rat 2	Rat 3	Average
0-24hr Urine	58.1%	60.1%	59.8%	59.3%
0-24hr Cage Wash	5.0%	5.9%	4.1%	5.0%
24-48hr Urine	6.0%	4.9%	9.1%	6.7%
24-48hr Cage Wash	0.6%	0.8%	0.4%	0.6%
<i>subtotal</i>	<i>69.7%</i>	<i>71.7%</i>	<i>73.4%</i>	<i>71.6%</i>
0-24 hr Feces	16.5%	10.6%	13.2%	13.4
24-48hr Feces	14.3%	20.5%	7.5%	14.1
<i>subtotal</i>	<i>30.8%</i>	<i>31.1%</i>	<i>20.7%</i>	<i>27.5%</i>
Total	100.5%	102.8%	94.1%	99.1%

Table 2.1.2-1b Cumulative total balance of tritium following a single oral administration of [¹⁴C]-1,4-dimethylnaphthalene.

Matrix	%age of administered dose (mean ± S.D. of 5 animals)	
	0-24h	0-48h
Urine	59.3	66.0
Faeces	13.4	27.5
Cage wash	5.0	5.6
Total	77.7	99.1

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Table 2.1.2-2 Metabolites of 1,4- dimethylnaphthalene in urine identified by HPLC/MS

Metabolite	% of Dose
Thiomethyl-hydroxymethyl-1-naphthoic acid (isomer)	4.6%
Hydroxylated 4-methyl-1 naphthoic acid (isomers) containing some Thiomethyl-hydroxymethyl-1-naphthoic acid (isomer)	13.2%
<i>N</i> -Acetylcysteine-4-methyl-1-naphthoic acid (isomer) Hydroxy- <i>N</i> -acetylcysteine-dihydro-1,4-dimethylnaphthalene (isomer)	2.5%
<i>N</i> -Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.2%
<i>N</i> -Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.5%
Hydroxy- <i>N</i> -acetylcysteine-dihydro-1,4-dimethylnaphthalene (isomer)	3.2%
Hydroxy- <i>N</i> -acetylcysteine-dihydro-1,4-dimethylnaphthalene (isomer)	1.8%
4-Methyl-1-naphthoic acid glucuronide (Isomer)	2.0%
4-Methyl-1-naphthoic acid glucuronide (Isomer)	4.1%
<i>N</i> -Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.3%
<i>N</i> -Acetylcysteine-1,4-dimethylnaphthalene	2.6%
4-Methyl-1-naphthoic acid	6.0%
1-Hydroxymethyl-4-methylnaphthalene	Trace ^A
4-Methylnaphthoic aldehyde	Trace ^A
1,4-DMN	Trace ^A
Total Identified	44.0%

^A Not detected by HPLC, but trace amount found by GC/MS.

Acceptability

The objectives of this study were to partially fulfil the data requirements of OECD 417 (22 July, 2010) and to confirm the formation of a 1-hydroxymethyl-4-methylnaphthalene metabolite. Deviation of OECD 417 were e.g. the use of only 3 male rats, instead of 4. Moreover, absorption and distribution were not investigated. This is acceptable, since the study confirms the results of the more complete ADME, yet non GLP study, described under study 1 (Kilanowicz et al., 2000).

The study is considered acceptable for the overall toxicological evaluation.

Conclusion

After a single oral dose of 1,4-dimethylnaphthalene (28.6 mg/kg bw), excretion is rapid and essentially complete after 48 hours, with urine being the primary route of excretion (71.6%).

Unchanged parent compound is only found in trace amounts in urine. The rat urine contains mainly metabolites formed by two different pathways: oxygenation of the alkyl side chains through an initial hydroxylation to the corresponding carboxylic acids, and ring hydroxylation yielding naphthols (such

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

reaction could proceed either directly or through epoxide intermediates). No metabolites were found to confirm the existence of the 3rd pathway described for alkylnaphthalenes (binding of epoxide intermediates to glutathione leading to thionaphthols, excreted in urine as mercapturates).

2.1.3 Study 3

Reference/notifier	: IIA, 6.2/28	GLP statement	: yes
Type of study	: Metabolism study in livestock	Guideline	: OECD 503
Year of execution	: 2007	Acceptability	: acceptable
Test substance	: 1,4-dimethylnaphthalene, batch 162-169-052, radio purity 97.7%, specific activity 52 mCi/mmol		

Materials and methods

1,4-dimethyl-[1,4,5,8-¹⁴C]-naphthalene (Lot No. 162-169-052, radiochemical purity 97.7%, specific activity 52 mCi/mmol), mixed with unlabelled 1,4-dimethylnaphthalene (batch no: 14D06B01-01, purity 98.7%) was administered orally in gelatin capsules to a single lactating goat (British Saanen, bw: 55.1 kg), once daily in a gelatin capsule for 7 consecutive days at an actual mean dose level of 12.5 mg/kg dry weight of diet (0.39 mg/kg bw/day), in excess of the minimum dose level required by the guidelines (10 mg/kg feed) and 1.2N compared to the actual estimated dietary intake.

Urine and faeces were collected prior to dose administration and at 24 h intervals thereafter until the time of sacrifice. The goat was milked twice daily and immediately prior to sacrifice. Blood was taken from the jugular vein prior to first dose administration and one hour post dose during the dosing period. Following the final dose administration, a blood sample was collected at 1, 2, 4, 8 and 16 h post dose in order to determine T_{max} .

Approximately 16 h post final dose, the goat was sacrificed and tissues, bile and blood were isolated. The cage was washed with a minimal amount of acetonitrile:water (1:1 v/v) and the rinsings were retained. Total radioactivity residues (TRR) of all collected samples was measured with LSC-analysis. Tissues (liver, kidney, composite muscle and composite fat) were extracted with a combination of acetonitrile and aqueous methanol, which yielded *ca* 50-80% TRR. Residues remaining in liver following solvent extract were further investigated using digestive enzymes (pepsin and protease) and acid hydrolysis. The combined hydrolysis experiments resulted in a further 28% TRR being liberated from the liver, however, attempts to clean up and lyse these samples by HPLC was not possible due to very low tissue levels and losses incurred during sample concentration.

Composite (day 1-7) milk was extracted using a combination of potassium oxalate, ethanol, diethyl ether and hexane. The majority of radioactivity remained in the aqueous phase following removal of the organic layer and precipitate. Urine and bile were lysed by HPLC and LC-MS to aid identification of the major residues in milk and tissues and to try to define the biotransformation pathway of the test item.

Results

Analysis of plasma and excreta at various intervals throughout the dosing period indicated that gastrointestinal absorption and excretion was rapid and essentially complete. A T_{max} of 4 hours was estimated. About 75% of the administered dose was eliminated in urine while approximately 14% of the administered dose was recovered from the faeces in the 24 h period following dose administration.

Recovery of the total administered dose in excreta, milk and tissues was 92% (see **Table 2.1.3-1**). Urine, faeces and cage wash contained 76%, 14% and 1.5% of the total administered dose respectively. Liver,

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

kidney, and milk contained 0.18%, 0.02% and 0.36% of the administered dose, respectively. The TRR in liver, kidney, muscle (average) and fat (average) was 0.24, 0.28, 0.017 and 0.018 mg eq/kg, respectively.

Radioactive residues in milk reached a plateau after 48 h (0.034 mg eq/kg), with daily averages remaining between 0.033 mg eq/kg and 0.038 mg eq/kg for the remaining 24 h collection periods. TRR in milk at 16 h post final dose (time of sacrifice) was 0.027 mg eq/kg.

The majority of radioactivity of the composite milk remained in the aqueous phase. 51% TRR (0.017 mg/kg) was submitted for HPLC analysis. Further investigation of precipitate, using pepsin and protease enzymes liberated a further 14% and 2.1% respectively, which however could not be submitted to chromatographic analysis for investigation.

Tissues (liver, kidney, fat and muscle) were extracted with a combination of acetonitrile and aqueous methanol, which yielded 50-80% TRR. Residues remaining in liver following solvent extract were further investigated using pepsin, protease and acid hydrolysis, liberating a further 28% TRR.

Characterization of chromatographic fractions of the various matrices and identification of major components in these fractions is summarised in Table 2.1.3-2. Two major components were detected in the HPLC profile of milk, of which the most significant component (18% TRR; 0.006 mg eq/kg) also occurred in kidney (17% TRR; 0.046 mg eq/kg) and bile, and was chromatographically similar to a component identified as a glycine conjugate of 4-methyl-1-naphthoic acid (P7) in urine; the other peak (P1) was not identified. Other components found in urine chromatographed in a similar region with the reference standards dihydroxy-1-naphthoic acid (P4) and 4-methyl-1-naphthoic acid (P8). In kidney, dihydroxy-1-naphthoic acid (P4) and 4-methyl-1-naphthoic acid (P8) were identified in equal amounts (1.3% TRR, 0.004 mg eq/kg). One other peak above 5% TRR was found (16%, 0.044 mg eq/kg, P3) in kidney, but could not be identified. Due to the extremely concentrated nature of the tissue extracts,

it was not possible to perform meaningful LC-MS analysis on the peaks in these extracts. Based on comparisons in retention time it is concluded that P3 is more polar than the glycine conjugate of 4-methyl-1-naphthoic acid and both P4 and 3,7-dihydroxy-1-naphthoic acid. The chromatographic properties of P3 suggest that it is likely to be a hydroxylated 4-methyl-1-naphthoic acid, where ring

and/or methyl hydroxylation has occurred. Alternatively, P3 may be a conjugate (other than glucuronide or sulphate, eg amino acid) of one of these structures.

Peaks found in liver extract, of which the most significant was 7% TRR (0.018 mg eq/kg, P5), could not be identified with LC-MS. In muscle, the only identified substances were 1,4-dimethylnaphthalene (parent compound, P9) and dihydroxy-1-naphthoic acid (P4), both in 0.001 mg eq/kg (4.0 and 4.7% TRR respectively). Fat extract could not be lysed by HPLC due to very low residue levels and loss of radioactivity during clean-up.

Table 2.1.3-1 Total Radioactive Residues (TRR) in urine, faeces, bile, blood, plasma, milk, cage wash and tissues following daily oral administration of [¹⁴C]-1,4-dimethylnaphthalene to a lactating goat for 7 consecutive days

Sample	TRR (mg eq/kg)	% Administered Dose Recovered
Urine	NA	76
Faeces	NA	14
Cage Wash	NA	1.5
Milk	0.034	0.36
Whole Blood (160 h)	0.15	NA
Plasma (160 h)	0.21	NA
Bile	0.60	NA
Kidneys	0.28	0.02
Liver	0.24	0.18
Forequarter Muscle	0.016	NA

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Sample	TRR (mg eq/kg)	% Administered Dose Recovered
Hindquarter Muscle	0.017	NA
Tenderloin Muscle	0.017	NA
Mean Muscle Concentration ^(a)	0.017	NA
Omental Fat	0.018	NA
Renal Fat	0.016	NA
Subcutaneous Fat	0.019	NA
Mean Fat Concentration ^(b)	0.018	NA
Total Recovery	NA	92

NA = Not Analysed

(a) = Mean of forequarter, hindquarter and tenderloin muscle

(b) = Mean of omental, renal and subcutaneous fat

Table 2.1.3-2 Radioactive residues identified in matrices of the goat dosed with [¹⁴C]-1,4-DMN for 7 consecutive days

Component	Total Radioactive Residues						
	% Dose	mg eq/kg					
	Urine	Bile	Milk	Liver	Kidney	Muscle	Fat
TRR (Direct) ^(a)	76	0.60	0.034	0.24	0.28	0.017	0.018
TRR (Extraction) ^(b)	NA	NA	0.034	0.25	0.28	0.028	0.017
Extracted	67	0.60	0.017	0.15	0.22	0.021	0.008
Pepsin digest + concentrated digest	NA	NA	0.005	0.043	0.025	NA	NA
Protease digest	NA	NA	0.001	0.016	NA	NA	NA
6M HCl digest	NA	NA	NA	0.024	NA	NA	NA
Remaining lipophilic fractions	NA	NA	0.002	NA	NA	NA	NA
Unextracted	NA	NA	0.009	0.032	0.019	0.007	0.009
Polar	ND	ND	0.001	0.010	ND	0.001	NA
P1	ND	ND	0.004	ND	0.004	ND	NA
P2	7.5	0.060	ND	ND	0.007	ND	NA
P3	ND	ND	ND	0.007	0.044	ND	NA
P4: dihydroxy-1-naphthoic acid	4.4	ND	ND	ND	0.004	0.001	NA
P5	2.2	ND	ND	0.018	ND	ND	NA
P6	6.2	ND	ND	ND	0.006	ND	NA
P7: 4-methyl-1-naphthoic acid, glycine conjugate	38	0.24	0.006	ND	0.046	ND	NA
P8: 4-methyl-1-naphthoic acid	3.8	ND	ND	ND	0.004	ND	NA
P9 (parent): 1,4-dimethylnaphthalene	ND	ND	ND	ND	ND	0.001	NA
Assigned	62	0.30	0.011	0.035	0.12	0.003	NA
Unassigned	4.8	0.29	-	-	-	-	NA
Unaccounted ^(c)	0.01	0.00	0.006	0.11	0.10	0.018	NA

(a) = Value obtained by direct LSC analysis

(b) = Value obtained by summation of TRR in solvent extractable and PES fractions initial extract, the digests and non-extracted residue.

(c) = Components <LOQ

ND = Not Detected

NA = Not Analysed (because of too low a volume)

Conclusions

1,4-dimethylnaphthalene is rapidly absorbed and widely distributed in the goat. Excretion is rapid, more in urine than in faeces. There is no evidence of accumulation in fat tissue. Metabolism of 1,4-dimethylnaphthalene in the goat involved carboxylation of one of the methyl groups, followed by

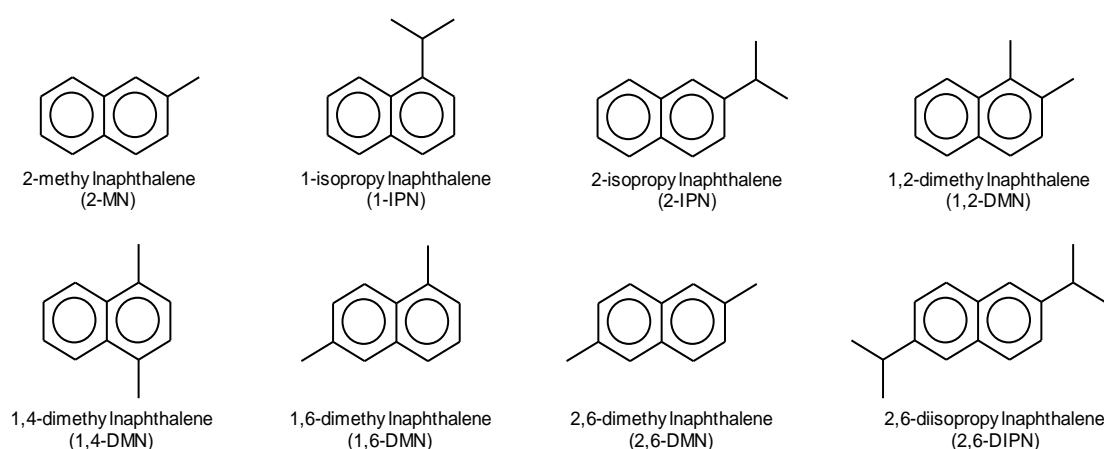
ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

conjugation with glycine or hydroxylation of the ring. None of the residue components detected accounted for >0.05 mg/kg or >10% TRR in any of the edible matrices.

2.1.4 Study 4 – Kinetic information for other naphthalenes

1,4-dimethylnaphthalene belongs to the chemical family of alkylated naphthalenes. Additional health effects information is also provided through extrapolation of findings in toxicological studies with structurally related substances to 1,4-dimethylnaphthalene. The substances to be discussed in relation to 1,4-dimethylnaphthalene are depicted in Figure 2.1.4-1. Physical-chemical properties of these alkylated naphthalenes are listed in Table 2.1.4-1.

Figure 2.1.4-1 Structural formulas of selected alkylated naphthalenes



The short names in brackets are used to identify the alkylated naphthalene being discussed.

Table 2.1.4-1 Physical-chemical properties of selected alkylated naphthalenes source: EPI Suite (except for 1,4-dimethylnaphthalene)

	CAS #	MW	M.P. (°C)	B.P. (°C)	Water Sol. (mg/L at 25°C)	VP (Pa)	Log Pow
2-methylnaphthalene	91-57-6	142.20	34	241	24.6	7.3	3.86
1,2-dimethylnaphthalene	573-98-8	156.23	2	267	18.14	1.69	4.31
1,4-dimethylnaphthalene	571-58-4	156.23	1	264	5.1	2.5	4.37
1,6-dimethylnaphthalene	575-43-9	156.23	-17	264	11.5	1.95	4.26
2,6-dimethylnaphthalene	581-42-0	156.23	97	262	2.0	0.57	4.31
1-isopropylnaphthalene	6158-45-8	170.26	-16	268	8.9	0.81	4.63
2-isopropylnaphthalene	2027-17-0	170.26	15	268	8.9	0.69	4.63
2,6-diisopropylnaphthalene	24157-81-1	212.33	59	310	0.11	0.07	6.08

Source (except for 1,4-DMN): EPI Suite v3.20 (February 2007). United States EPA Office of Pollution Prevention Toxics and Syracuse Research Corporation. Experimentally determined values supersede estimated parameters.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

With increasing molecular weight, the octanol-water partition coefficient increases, whereas the vapour pressure and the solubility in water decrease. The position of the methyl groups in the four dimethylnaphthalene isomers has no significant effect on boiling point, vapour pressure and log Pow. There are small differences in the water solubility of the dimethylnaphthalenes but this may be due to experimental variation (e.g., the EPI Suite database lists an experimentally derived water solubility for 1,4-dimethylnaphthalene of 11.4 mg/L).

1,2-DIMETHYLNAPHTHALENE (1,2-DMN) AND 1,6-DIMETHYLNAPHTHALENE (1,6-DMN)

In two similar intraperitoneal studies with radiolabelled 1,2-dimethylnaphthalene and 1,6-dimethylnaphthalene, respectively, the absorption, distribution, metabolism and excretion patterns were comparable to the results obtained with 1,4-dimethylnaphthalene, with the same corresponding metabolites found in urine. Additional urinary metabolites were 1,2-dimethylnaphthalene-thionaphthol and 1,6-dimethylnaphthalene-thionaphthol, respectively. In addition, for 1,6-dimethylnaphthalene, also monomethylnaphthols (1-methyl-hydroxynaphthalene and 6-methyl-hydroxynaphthalene) were identified. These monomethylnaphthols probably resulted from decarboxylation followed by hydroxylation of the naphthalene ring. In contrast to the study with 1,4-dimethylnaphthalene, the metabolite 1-methyl-2-naphthoic aldehyde was not found in the experiment with 1,2-dimethylnaphthalene. Compared to 1,2-dimethylnaphthalene and 1,6-dimethylnaphthalene, the side chain oxidation route is more important and glutathione conjugation route is less important for 1,4-dimethylnaphthalene (Kilanowicz and Sapota, 1998 (IIA 5.1/06); Kilanowicz et al., 2002 (IIA 5.1/07)).

2-METHYLNAPHTHALENE (2-MN)

In a study from public literature, male guinea pigs received a single oral dose of 2-[³H]-methylnaphthalene in olive oil at 10 mg/kg bw. Three animals per time point were dissected at 3, 6, 24 and 48 h after administration and blood, organs and tissues were collected. Urine was collected in the first 24 h following administration. Some guinea pigs were given 500 mg/kg bw non-radioactive 2-methylnaphthalene orally and the urine was collected similarly. The radioactivity reached a maximum level in the blood, gallbladder and spleen 3 h after administration and in the other examined organs and tissues 6 h after administration. The highest maximum levels were observed in the gallbladder, followed by the kidney, liver and lung. Adipose tissue was not examined. After 48 h, 72% of the radioactivity was excreted via the urine and 12% via the faeces, and only 0.05% of the radioactivity was detected in internal organs (total recovery was 85%). There were no indications for accumulation of 2-methylnaphthalene. In urine, the following metabolites were identified: 2-naphthuric acid (61% of total urinary radioactivity), 2-naphthoic acid and its glucuronide (together 15% of total urinary radioactivity), 7-methyl-1-naphthol and its glucuronic acid and sulphate conjugates and S-(7-methyl-1-naphthyl)-cystein (Teshima et al., 1983 (IIA 5.1/02)).

In a limited distribution study from public literature, male mice were administered 400 mg/kg bw [¹⁴C]-2-methylnaphthalene in corn oil intraperitoneally and radioactivity was measured in the liver, lung, kidney and fat surrounding the testes of four animals per time interval. Maximum levels of radioactivity were reached in the examined organs after 2 to 4 h after administration. Maximum levels were highest in fat, followed by liver and kidneys. There were no indications for accumulation of 2-methylnaphthalene (Griffin et al., 1982 (IIA 5.1/03)).

2-ISOPROPYLNAPHTHALENE (2-IPN)

In a study from public literature, male rats (3-5 animals/time point) were administered 2-isopropyl-naphthalene in olive oil at 100 mg/kg bw via a stomach tube. Also three rats with a bile duct cannula received this dose. After 24 h, only about 2.5% of the dose was excreted unchanged in the faeces and less than 0.01% of the dose was excreted unchanged via the bile, indicating that more than 95% of the

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

dose was absorbed from the gastrointestinal tract. Less than 0.01% of the dose was excreted unchanged in the urine. Maximum levels of unchanged 2-isopropyl-naphthalene in the blood, liver, kidneys, brain, heart, spleen and muscle were observed at 2 h after the administration (first time point). Disappearance of 2-isopropyl-naphthalene from blood was biphasic with half-lives of about 1.6 and 6.0 h. Maximum levels in adipose tissue and skin were higher and were reached later (6 h after administration, second time point). Also the disappearance of unchanged 2-isopropyl-naphthalene from these tissues was slower than from the other examined tissues. No information is provided on the distribution and excretion of metabolites of 2-isopropyl-naphthalene (Kojima and Maruyama, 1979 (IIA 5.1/04)).

In the same study, groups of male and female rats (4/sex/group) were given 0.1% 2-isopropyl-naphthalene in the diet for 14 or 28 days (reported to be equal to 13-18 mg/kg bw/day). Again, the highest 2-isopropyl-naphthalene concentration was measured in the adipose tissue, but the maximum concentration was about five-fold lower than the levels observed after single administration of a dose of 100 mg/kg bw. In rats (2/sex/time point) that received control diet for 1 - 21 days after exposure to 0.1% 2-isopropyl-naphthalene in the diet during 28 days, the 2-isopropyl-naphthalene content of blood and skin decreased to about one-third and the 2-isopropyl-naphthalene content of the adipose tissue decreased to about 50% within the first 24 h after withdrawal, and then decreased more slowly. Disappearance of 2-isopropyl-naphthalene from the adipose tissue was biphasic with half-lives of about 31 and 113 h in females and 34 and 110 h in males. Although 2-isopropyl-naphthalene levels in the skin were initially about 5 times lower than in the adipose tissue, skin levels were higher than adipose levels between days 7 and 14 after cessation of the exposure. This could be the result of slower disappearance of 2-isopropyl-naphthalene from the skin or from contamination of the skin via urine and/or faeces. No information was provided on the distribution and excretion of metabolites (Kojima and Maruyama, 1979 (IIA 5.1/05)).

In rats orally administered 2-isopropyl-naphthalene at 1 g/kg bw daily for 7 days, all 5 urinary metabolites identified resulted from oxidation of the isopropyl side chain. Two additional metabolites were not elucidated (Kojima et al., 1980 (IIA 5.1/17)). Also in rats with bile duct cannula that were administered 2-isopropyl-naphthalene at 100 mg/kg bw as an olive oil solution, all metabolites identified in 24 h bile and urine resulted from side chain oxidation. The identified metabolites of 2-isopropyl-naphthalene excreted in urine and bile amounted to, respectively, 23% and 18% of the dose (Kojima et al., 1984 (IIA 5.1/18)). The excretion pattern of urinary metabolites of 2-isopropyl-naphthalene was almost the same as that of biliary metabolites. Since the study of Kojima et al., (1981 (IIA 5.1/04)) indicated that considerable enterohepatic circulation takes place, it is likely that some further metabolites other than the metabolites identified are present in urine and bile of 2-isopropyl-naphthalene-treated rats (Kojima et al., 1984 (IIA 5.1/18)).

1-ISOPROPYLNAPHTHALENE (1-IPN)

In a study from public literature, groups of male rats (3 animals/time point) were administered by stomach tube a single dose of monoisopropyl-[¹⁴C]naphthalene (6.25 µCi/kg bw ~ 1 mg/kg bw) plus 100 mg/kg bw non-labelled monoisopropyl-naphthalene, both consisting of 60% 2-isopropyl-naphthalene and 40% 1-isopropyl-naphthalene, in olive oil. Other groups of male rats (3 animals/time point) received 100 mg/kg bw unchanged 1-isopropyl-naphthalene in olive oil by stomach tube. The tissue concentration patterns of unchanged 1-isopropyl-naphthalene resembled those of 2-isopropyl-naphthalene described above. The decline in radioactivity (metabolites) from blood and several tissues was slower than the decline in parent compound. Within 96 h of administration, 78% of the total radioactivity was excreted via the urine, most of which (50%) was excreted during the first 24 h. Only about 14% of the total dose was excreted via the faeces. A biliary excretion study showed that approximately 60% of the total dose was excreted via the bile during the first 24 h. Given the low excretion via faeces, enterohepatic circulation, i.e. absorption of biliary metabolites and/or parent compound from the intestine has occurred (Kojima et al., 1981 (IIA 5.1/04)).

2,6-DIISOPROPYLNAPHTHALENE (2,6-DIPN)

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

In a study from public literature, male rats were given 2,6-diisopropylnaphthalene at 100 mg/kg bw as olive oil solution by stomach tube. Faeces and urine were collected 2, 4, 6, 8, 14, 24 and 48 h after administration. Groups of rats (3-5/time point) were sacrificed at the same time points. The amount of unchanged 2,6-diisopropylnaphthalene was determined in faeces, urine, blood, liver, kidney, spleen, heart, brain, muscle, skin and adipose tissue. Absorption was rapid with maximum blood levels within 2 h after administration. In total 85% of the dose was absorbed and 15% was excreted unchanged in the faeces. In urine, only < 0.01% of the dose was excreted as unchanged 2,6-diisopropylnaphthalene. The maximum levels of unchanged 2,6-diisopropylnaphthalene in the liver, kidney, heart, spleen, brain and muscle were observed within 4 h after administration and then decreased with time, whereas maximum levels in skin and adipose tissue were reached approximately 10 h after administration. The major sites for the distribution during the early period after administration appeared to be the liver, muscle, adipose tissue and skin. At the longer time, 2,6-diisopropylnaphthalene was mainly deposited in the adipose tissue (8% of the dose after 24 hr) and skin (1.6% of the dose after 24 h). No information is provided on the distribution and excretion of metabolites (Kojima et al., 1978 (IIA 5.1/10)).

In a study from public literature, groups of male and female rats (3-4/sex/group) were given 0.1% or 0.2% 2,6-diisopropylnaphthalene in the diet for 17 or 31 days. In the second set of experiments, groups of male and female rats (3-4/sex/group) were treated in the same way and were then fed control diet for 24 h. The doses were reported to be equal to 18-24 or 34-46 mg/rat/day, respectively. In the third set of experiments, groups of male rats (4/group) were fed a diet containing 0.1% 2,6-diisopropylnaphthalene for 14 days. In all experiments one group of rats was killed and examined immediately; the other groups were sacrificed after 7, 14, 21, 28 or 35 days, respectively. Blood, liver, kidneys and adipose tissue were examined, and in the latter experiment also the skin was examined. The 2,6-diisopropylnaphthalene content of the blood was increased in proportion to the dose, but was not affected by the duration of the administration periods (17 or 31 days). The 2,6-diisopropylnaphthalene content in adipose tissue was much greater than in the other tissues and also greater than the levels found in the adipose tissue of rats receiving a single oral dose of 100 mg/kg bw in the study described above. Concentrations in blood, liver and kidneys were greatly reduced after 24 h of withdrawal, whereas levels in adipose tissue were only decreased with 10 – 30%. After 7 days, 2,6-diisopropylnaphthalene was not detectable anymore in blood, liver and kidneys. Although the skin content of 2,6-diisopropylnaphthalene initially was about 8 times lower than in the adipose tissue, 28 days after halting the exposure, skin levels were higher than adipose levels. This could be the result of slower disappearance of 2-isopropylnaphthalene from the skin or from contamination of the skin via urine and/or faeces. No information was provided on the distribution and excretion of metabolites (Kojima et al., 1979 (IIA 5.1/05)).

A study in mice with ³H-labeled diisopropylnaphthalene was available in the Chinese language. Based on abstract, figure legends and tables (in English), radioactivity reached maximum levels in various organs at 2 to 4 h after oral administration (dose unknown). The only exception was the adipose tissue in which the maximum value was reached 8 hr after administration. A large portion of the dose was taken up by the gallbladder. In total, 26% of the dose was excreted via the urine and 71% was excreted via the faeces, of which 95% was excreted within the first 24 h, indicating that there was hardly any accumulation (Iwahara, 1974 (IIA 5.1/22)).

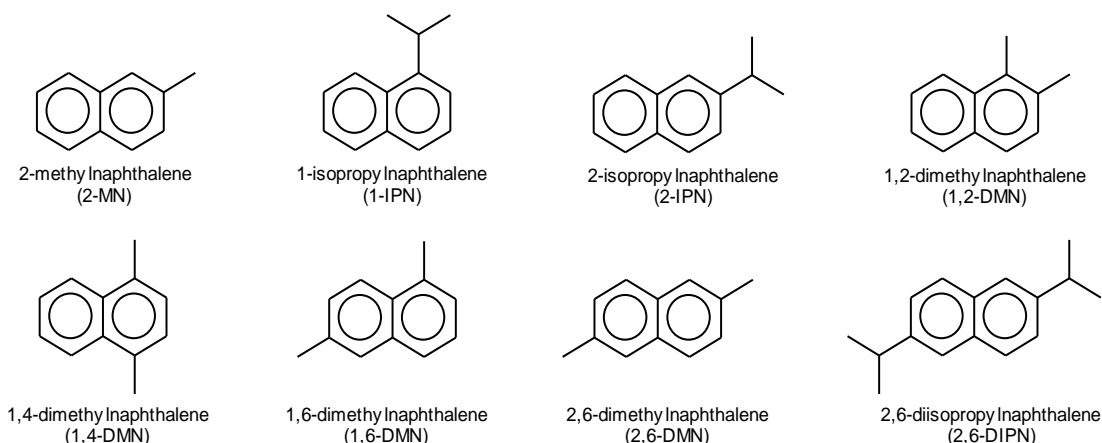
In male rats the urinary metabolites that were found after oral administration of 2,6-diisopropylnaphthalene in olive oil at 240 mg/kg bw/d for 15 days all resulted from oxidation of the isopropyl chain of the molecule. The total urinary excretion of these metabolites and the parent compound in 24 h after administration was about 23% of the dose (Kojima et al., 1982), and was comparable to the total urinary excretion of 1,4-dimethylnaphthalene and its metabolites in the study of Iwahara (1974 (IIA 5.1/22)). Also the biliary metabolites that were found in male rats after oral administration of 2,6-diisopropylnaphthalene at 100 mg/kg bw in olive oil were exclusively the result of oxidation of the side chain. The total biliary excretion of these metabolites and the parent compound in 24 h after administration was about 17% of the dose (Kojima et al., 1985 (IIA 5.1/12)).

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

JUSTIFICATION FOR EXTRAPOLATION

1,4-DMN belongs to the chemical family of alkylated naphthalenes. The substances to be discussed in relation to 1,4-DMN are depicted in Figure 2.1.4-2.

Figure 2.1.4-2. Structural formulas of selected alkylated naphthalenes



After a single oral or intraperitoneal dose to mammals, alkyl naphthalenes demonstrate very similar rates for absorption and excretion. Excretion is essentially complete within 72 hours.

Table 2.1.4-2 24-Hour absorption/excretion and distribution in rats^a after a single administration of selected alkyl naphthalenes

	Route	Tmax blood	% Excreted in 24h in			Distribution	Reference
2-MN	oral ^b		79 ^d	11	N.A.		Teshima et al. 1983
	i.p. ^c					4 h: adipose tissue > kidney > liver > lung 24 h: kidney > adipose tissue, liver, lung	Griffin et al. 1982
1-IPN/2-IPN	Oral	2 ³	55	2.5	60	parent: adipose tissue > skin > kidney > liver, brain > spleen > muscle > blood metabolites: liver > kidney > adipose tissue > blood > skin > spleen, brain, muscle	Kojima et al. 1981, Kojima & Maruyama 1979
1,2-DMN	i.p.	4	30	35	35 ^e	24 h: muscle > adipose tissue, blood	Kilanowicz & Sapota
1,4-DMN	i.p.	4	41	22	22 ^e	24 h: adipose tissue > muscle > blood > liver 72 h: muscle > blood, muscle > liver	Kilanowicz et al. 2000
1,4-DMN	oral	N.A.	59	13	N.A.	N.A.	Dohn et al. 2012

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

1,6-DMN	i.p.	2	39	34	34 ^e	24 h: adipose tissue > muscle > blood 72 h: muscle > adipose tissue > blood	Kilanowicz et al. 2002
2,6-DMN	Intraduo- denal, bile cannula-	N.A.	18- 20	N.A.	30- 32	N.A.	Rahman et al. 1986
2,6-DIPN	Oral	2	23	7	17	parent: adipose tissue > skin > muscle > liver > kidney, spleen, heart, brain	Kojima et al. 1978, 1982, 1985

N.A. - no information available

a Except when stated differently in footnotes.

b Guinea pig

c Mouse

d In a subcutaneous study in rats, 55% of the administered dose was found in urine (Melancon et al. 1982).

e Biliary excretion was not determined but since administration was intraperitoneally, excretion with bile is assumed to be equal to faecal excretion.

The maximum concentration in blood is reached within 2 to 4 hours after dosing, implying that the rate of absorption was independent of the degree of alkylation. However, the extent of absorption was influenced by the degree of alkylation. Based on excretion in urine and bile over the first 24 hours after dosing, absorption from the gastrointestinal tract was inversely related to the degree and complexity of alkylation: monoalkylated naphthalenes were completely absorbed, the absorption of dimethylnaphthalenes reached 60 to 70 percent, while 50 percent of the administered dose of 2,6-DIPN was absorbed.

Alkylnaphthalenes are widely distributed through the body with a preference for adipose tissue and skin. Concentration in fat was highest for 1,4-DMN (14% of the dose at 24 hours after i.p. administration, see Table 2.1.4-3).

Table 2.1.4-3. Concentrations in fat and muscle at 24 h after administration (%-age of dose)

	Adipose tissue	Muscle	Reference
2-MN	N.A. ^a	N.A.	Griffin et al. 1982
2-IPN	6.6 ^b	N.D.	Kojima and Maruyama 1979
1,2-DMN	0.34	2.3	Kilanowicz and Sapota 1998
1,4-DMN	13.9	3.8	Kilanowicz et al. 2000
1,6-DMN	4.3	2.3	Kilanowicz et al. 2002
2,6-DIPN	9.9 ^b	0.14	Kojima et al. 1978

N.A. no information available

N.D. not detectable

a only presented graphically.

b including skin

After administration, all alkylnaphthalenes disappeared rapidly from most tissues except from adipose tissue and skin. With the exception of 2-MN, the disappearance curves were biphasic (see Table 2.1.4-4). At 72 hours after administration, the amount remaining in blood, tissues and organs was 1.5% for 1,2-DMN, 3.2% for 1,4-DMN and 3.9% for 1,6-DMN.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Table 2.1.4-4 Disappearance half-lives of selected alkylnaphthalenes from blood

	T ½ - I (h)	T ½ - II (h)	Reference
2-MN	10.4 / 3		Teshima et al. 1983 / Griffin et al. 1982
2-IPN	1.6	6.0	Kojima and Maruyama 1979
1,2-DMN	0.7	19	Kilanowicz and Sapota 1998
1,4-DMN	0.53	8	Kilanowicz et al. 2000
1,6-DMN	2	70	Kilanowicz et al. 2002
2,6-DIPN	2.2	22.4	Kojima et al. 1978

Distribution and disappearance of alkylnaphthalenes after repeated administration was only investigated with 2,6-DIPN. After daily oral administration of rats for 17 or 31 days (Kojima et al. 1979), adipose tissue and skin contained higher concentrations of 2,6-DIPN in comparison to the single dose study (Kojima et al. 1978). 2,6-DIPN concentrations in blood, liver and kidneys corresponded to those in these tissues observed at 6-8 hours after a single oral administration. Disappearance from all organs and tissues except from fat was rapid after cessation of dosing (see Table 2.1.4-5 from Kojima et al. 1979). Disappearance from fat was biphasic with half-lives of 55 and 270 hours. Excretion of 2,6-DIPN from skin proceeded slower than from adipose tissue.

Table 2.1.4-5 Disappearance of 2,6-DIPN from various tissues of male rats fed on an experimental diet containing 0.1% 2,6-DIPN for 14 days

Day after halting 2,6-DIPN administration	2,6-DIPN (average for 3 or 4 animals)				
	Blood (µg/ml)	Liver (µg/g)	Kidneys (µg/g)	Skin (µg/g)	Adipose tissue (µg/g)
0	0.25	2.46	1.77	23.41	198.48
7	0	0	0	-	23.97
14	-	-	-	-	4.09
21	-	-	-	-	0.35
28	-	-	-	2.73	0.23
35	-	-	-	-	0.14

Metabolism

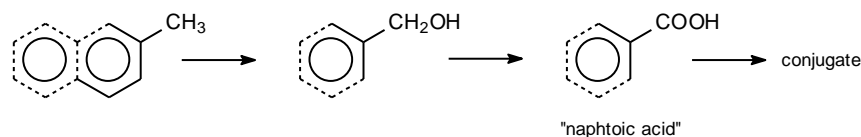
All alkylnaphthalenes are extensively metabolised in rat, mouse and Guinea pig; unchanged parent compound is a minor component in urine or bile. Apart from the unchanged substance, the rat urine contains metabolites formed by three different pathways (see Figure 2.1.4-3):

1. oxygenation of the alkyl side chains through an initial hydroxylation to the corresponding carboxylic acids,
2. ring hydroxylation yielding naphthols that are excreted in urine unchanged or conjugated with glucuronide or sulphate. Such reaction could proceed either directly or through epoxide intermediates,
3. binding of epoxide intermediates to glutathione leading to thionaphthols, excreted in urine as mercapturates.

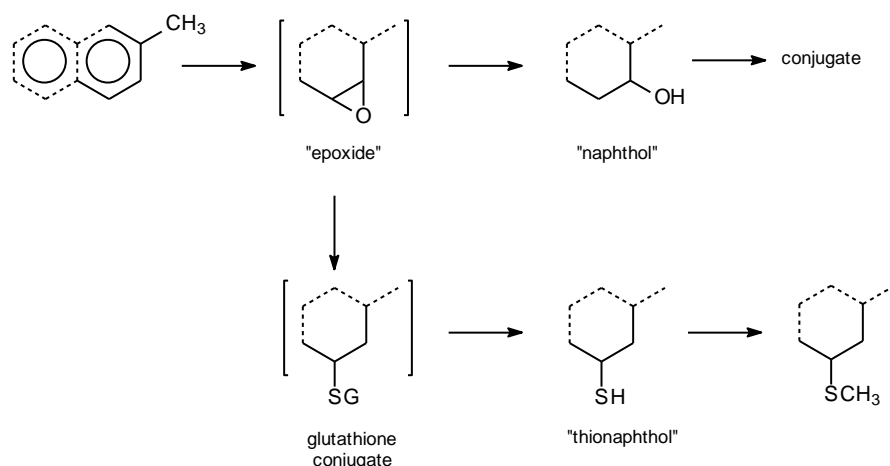
Figure 2.1.4-3 Mammalian metabolic pathways of alkylnaphthalenes

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Side chain oxidation



Ring oxidation



The relative toxicity profiles of alkylated naphthalenes have been associated with the differences in the excretion of mercapturates and/or naphthols with urine (Höke and Zellerhoff, 1998, IIA 5.1/14). Naphthalene and 1- and 2-MN produce highly selective injury to the nonciliated bronchiolar epithelial cell (Clara cell) of the mouse (for a review, see ATSDR 2003 (IIA 5.1/15)). The lung toxicity appears to be related to P-450 monooxygenase-dependent formation of reactive metabolites (Shultz et al., 2001 (IIA 5.1/16)) and glutathione is an important intracellular protectant. In the mouse, epoxidation of naphthalene occurs at high rates. Epoxide-formed glutathione adducts are excreted as sulfur containing metabolites in the urine (pathway 2 and 3). The amount of sulfur-containing metabolites therefore reflects the amount of highly reactive epoxide intermediates formed and consequently, the toxicity of the parent compounds.

The amount of sulfur containing metabolites excreted with urine differed significantly between naphthalene (5.5%), 1,2-DMN (35%), 1,4-DMN (0-1.5%) and 1,6-DMN (22%) (Kilanowicz and Sapota, 1998 (IIA 5.1/21); Kilanowicz et al., 2000 (IIA 5.1/01), 2002 (IIA 5.1/07), IIA 5.1/23). Since there is a very low amount of glutathione conjugate formed from the metabolism of 1,4-DMN, it is concluded that very little reactive epoxide metabolites are formed.

This relationship can be further examined by comparing the relative proportions of urinary alkyl-naphthalene metabolites, derived from ring and side chain oxidation (Table 2.1.4-6). The relative concentration of ring-oxidized compounds is lowest for 1,4-DMN and 2,6-DIPN.

Table 2.1.4-6 Metabolism of selected alkyl-naphthalenes in the rat (excreted in urine)

	Ring oxidation (%)	Parent + side chain oxidation (%)	Reference
2-MN ^a	42-53	42-56	Melancon et al. 1982
2-IPN	54 ^b	46	Kojima et al. 1980, Kojima et al. 1984
1,2-DMN	50	22 ^c	Kilanowicz and Sapota 1998

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

1,4-DMN	12	88 ^d	Kilanowicz et al. 2000
1,6-DMN	43	57 ^e	Kilanowicz et al. 2002
2,6-DIPN	12 ^f	88	Kojima et al. 1982

a Metabolites in rat urine after subcutaneous administration. In Guinea pigs, side-chain oxidation was the preferred metabolic route (Teshima et al. 1983). The parent compound and side-chain oxidation products accounted for 76% of the urinary metabolites; 18% was identified as ring-oxidation products.

b Parent and side chain metabolites accounted for 23 percent of the administered dose. In view of the total 24-h urinary excretion amounting to 50% of the dose and the almost complete absence of parent compound (<0.05% of dose), the authors conclude that further metabolites should be present. It is conservatively assumed that these represent ring-oxidized metabolites (no such compounds were identified in the study).

c Unchanged parent compound amounted to 29% of the urinary radioactivity.

d Unchanged parent compound amounted to 35% of the urinary radioactivity.

e Unchanged parent compound amounted to 31% of the urinary radioactivity.

f The identified metabolites in urine (parent and side chain oxidation products) accounted for 23 percent of the administered dose. Iwahara 1974 reported that 26% of the dose was excreted in urine. Consequently, approximately 12 percent of the dose, excreted in urine, is unaccounted for. It is conservatively assumed that this fraction represents ring-oxidized metabolites.

Thus, among the dimethylnaphthalenes, 1,2-DMN would be most toxic and 1,4-DMN least toxic.

An alternative approach can also be used to compare the toxicity of alkylnaphthalenes. This involves the potential for glutathione depletion.

Glutathione plays a protective role against the toxicity of many xenobiotics by reacting, either spontaneously or in enzyme catalyzed reactions, with electrophilic metabolites to form nontoxic conjugates, which are readily excreted in the urine. By comparing histological changes in mouse bronchioles with the level of intracellular glutathione, Plopper et al. 2001 (IIA 5.1/19) identified glutathione depletion as an early event preceding irreversible cell injury after exposure to naphthalene. Glutathione depletion after administration of alkylated naphthalenes was observed in several studies in which high doses of a substituted naphthalene were administered. Griffin et al. 1982 (IIA 5.1/03) reported that pulmonary glutathione in the mouse is decreased after intraperitoneal administration of 2-MN (400 mg/kg bw). Honda et al. 1990 also observed a considerable depletion of pulmonary glutathione within 6h after intraperitoneal administration of 2-MN (400 mg/kg bw) while administration of 2-IPN or 2,6-DIPN (3,000 mg/kg bw) only caused a slight depletion of pulmonary glutathione.

Kilanowicz et al. 2003 (IIA 5.1/21) studied glutathione depletion in liver and lung tissue of rats after intraperitoneal administration of 1,2-DMN, 1,3-DMN or 1,4-DMN (600 mg/kg bw, 50% of the LD₅₀). Eight hours after treatment, liver glutathione was decreased at the same magnitude for all isomers, but recovery was much faster for 1,3-DMN and 1,4-DMN in comparison to 1,2-DMN. Pulmonary glutathione was only decreased after treatment with 1,2-DMN; at the same dose level, no change in lung glutathione content was observed for 1,3-DMN and 1,4-DMN.

These studies show two important interrelated factors, namely that very high doses of the naphthalenes are required to substantially decrease glutathione levels, and that essentially full depletion is necessary to cause sufficient levels of reactive metabolite to be present to induce tissue damage. It is important to recognize that the doses used to deplete glutathione levels are very high. From the studies with naphthalene and monomethylnaphthalenes (1-MN and 2-MN) it has become apparent that tissue damage occurs when the metabolic detoxifying capacity (i.e. the tissue glutathione pool) becomes exhausted.

Of the many alkylated naphthalenes discussed in this document, 1,4-DMN and 2,6-DIPN revealed an equally low cytotoxicity and an equally low rate of formation of reactive metabolite. The correspondence is quite evident. It is therefore concluded that their mammalian toxicity is also equivalent.

Conclusion

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

After a single oral or intraperitoneal dose to mammals, alkylnaphthalenes demonstrate very similar profiles for absorption and excretion with rapid absorption and essentially complete excretion within 72 hours. All alkylnaphthalenes are extensively metabolised in rat, mouse and Guinea pig. Rat and mouse urine contain metabolites formed by three different pathways (side chain oxidation, ring oxidation to naphthols and mercapturates via reactive epoxide with subsequent conjugation to glutathione). This similarity in mechanism supports the use of alkylnaphthalene ADME data in the assessment of 1,4-DMN.

In addition, the relative toxicity profiles for alkylnaphthalenes can be linked to the proportion of metabolism which occurs by side chain or ring oxidation. For 1,4-DMN, the primary route of metabolism has been shown to be via side chain oxidation and therefore this compound is one of the least toxic alkylnaphthalenes. This provides further support for the use of data from the alkylnaphthalene chemical group as it provides a worst case assessment for the risk to consumers and operators.

List of identified compounds following oral administration of 1,4-dimethylnaphthalene in rats.

Metabolite	% of Dose
Thiomethyl-hydroxymethyl-1-naphthoic acid (isomer)	4.6%
Hydroxylated 4-methyl-1 naphthoic acid (isomers) containing some Thiomethyl-hydroxymethyl-1-naphthoic acid (isomer)	13.2%
<i>N</i> -Acetylcysteine-4-methyl-1-naphthoic acid (isomer) Hydroxy- <i>N</i> -acetylcysteine-dihydro-1,4-dimethylnaphthalene (isomer)	2.5%
<i>N</i> -Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.2%
<i>N</i> -Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.5%
Hydroxy- <i>N</i> -acetylcysteine-dihydro-1,4-dimethylnaphthalene (isomer)	3.2%
Hydroxy- <i>N</i> -acetylcysteine-dihydro-1,4-dimethylnaphthalene (isomer)	1.8%
4-Methyl-1-naphthoic acid glucuronide (Isomer)	2.0%
4-Methyl-1-naphthoic acid glucuronide (Isomer)	4.1%
<i>N</i> -Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.3%
<i>N</i> -Acetylcysteine-1,4-dimethylnaphthalene	2.6%
4-Methyl-1-naphthoic acid	6.0%
1-Hydroxymethyl-4-methylnaphthalene	Trace ^A
4-Methylnaphthoic aldehyde	Trace ^A
1,4-DMN	Trace ^A
Total Identified	44.0%

^A Not detected by HPLC, but trace amount found by GC/MS.

List of identified compounds following intraperitoneal administration of 1,4-dimethylnaphthalene in rats.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

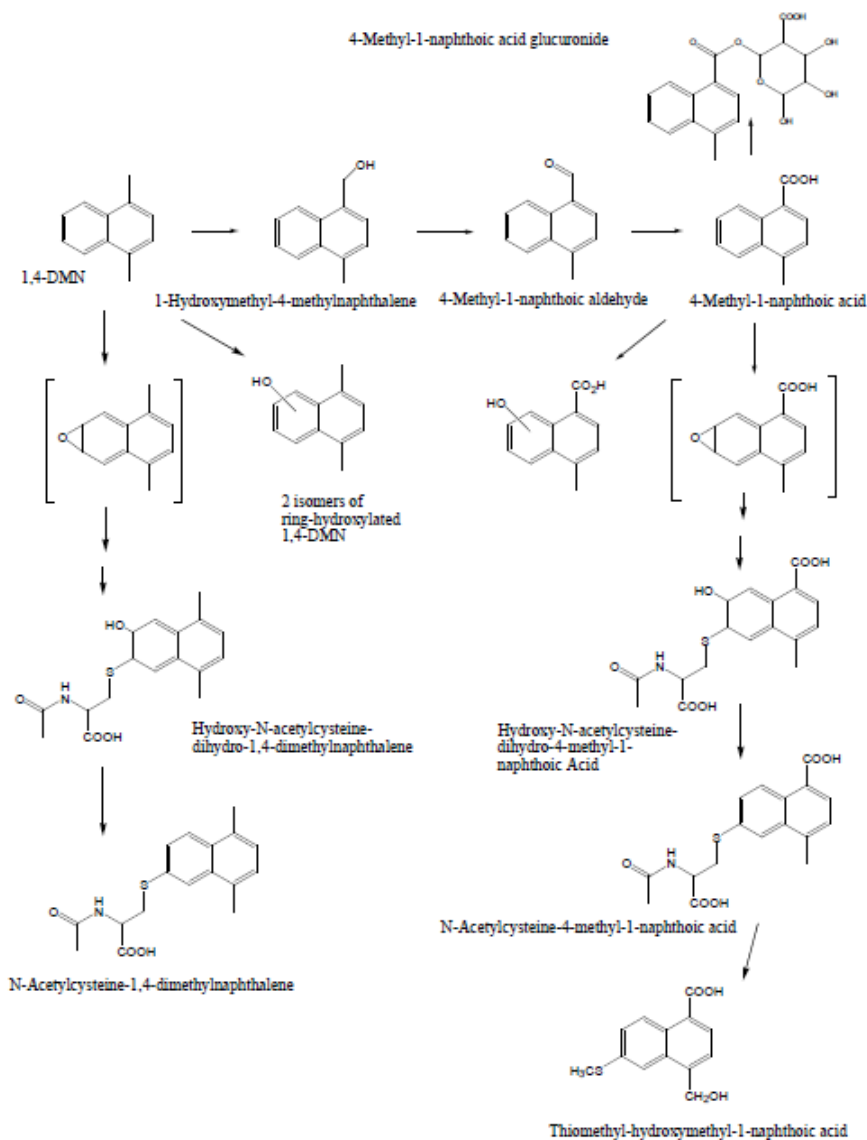
Code	Compound	Occurrence*	% of total area
1	1,4-dimethylnaphthalene	U	35
2	4-methyl-1-naphthoic aldehyde	U	4
3	1-hydroxymethyl-4-methylnaphthalene	U	29
4	4-methyl-1-naphthoic acid	U	20
7	1,4-dimethyl-methylthionaphthalene	U	1
8	1,4-dimethyl-naphthol (2 isomers)	U	11

* F=faeces, U=urine, B=bile

PROPOSED METABOLIC PATHWAY OF 1,4-DIMETHYLNAPHTHALENE IN RAT

Proposed metabolic pathway after oral administration:

Only one of the possible positions of oxidation of the naphthalene ring is shown.



ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

After a single oral dose of 1,4-dimethylnaphthalene, excretion is rapid and essentially complete after 48 hours, with urine being the primary route of excretion (71.6%).

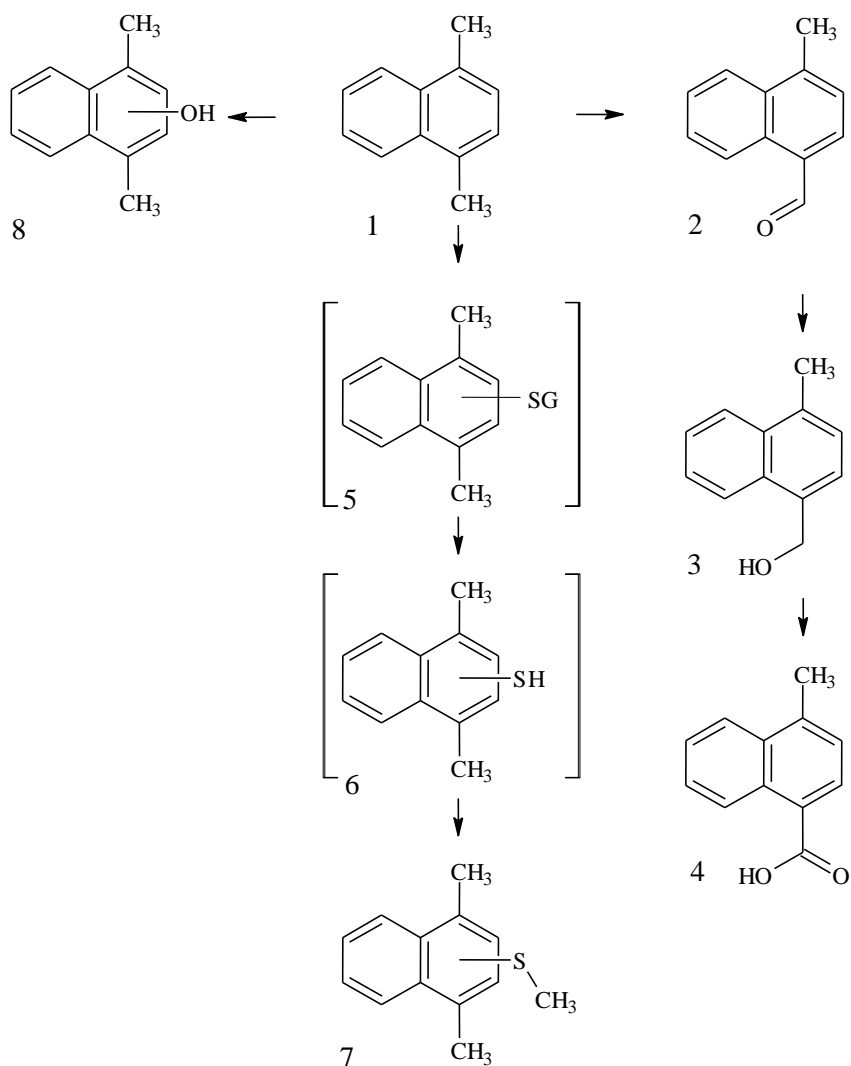
Unchanged parent compound is only found in trace amounts in urine. The rat urine contains mainly metabolites formed by two different pathways:

- 1) oxygenation of the alkyl side chains through an initial hydroxylation to the corresponding carboxylic acids: for 1,4-DMN this was initiated by hydroxylation on a methyl group, to form 1-hydroxymethyl-4-methylnaphthalene, that was further oxidised to 4-methyl-1-naphthoic acid (metabolite 4) (6.0% in urine). Another portion (equivalent to 6.1% of the dose) of the 4-methyl-1-naphthoic acid was conjugated with glucuronic acid.
- 2) and ring hydroxylation yielding naphthols (such reaction could proceed either directly or through epoxide intermediates). These epoxides reacted with glutathione to form vicinal hydroxyl thioether. This was further metabolised as follows:
 - hydroxylation of the glutathione conjugates to form the corresponding cysteine conjugates.
 - N-acetylation of the cysteine conjugates (6.4% of the dose)
 - dehydration to re-form the aromatic naphthalene system, producing N-acetylcysteine-1,4-dimethylnaphthalene derivatives (2.6% of the dose)

No metabolites were found to confirm the existence of the 3rd pathway described for alkylnaphthalenes (binding of epoxide intermediates to glutathione leading to thionaphthols, excreted in urine as mercapturates).

A similar pathway was proposed after intraperitoneal injection:

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE



After intraperitoneal injection of 1,4-dimethylnaphthalene, in rat urine the parent compound 1 and the metabolites 2, 3, 4, 7 and 8 were found. The data indicate that the metabolism of 1,4-dimethylnaphthalene proceeds via at least three different routes:

- side chain oxidation resulting ultimately in the carboxylic acid 4-methyl-1-naphthoic acid (4),
- ring hydroxylation resulting in 1,4-dimethylnaphthols (2 isomers, (8)) via reactive epoxide with
- subsequent binding of epoxide intermediates to glutathione via conjugation leading ultimately to 1,4-dimethyl-methylthionaphthalene (7).

Pathway c probably involves metabolite 6, given that corresponding metabolites have been found after intraperitoneal administration of 1,2-dimethylnaphthalene and 1,6-dimethylnaphthalene. It has to be noted that metabolites of 1,4-dimethylnaphthalene are also excreted via bile and faeces, but that the biliary and faecal metabolites have not been identified.

Compared to 1,2-dimethylnaphthalene and 1,6-dimethylnaphthalene, the side chain oxidation route is more important and ring oxidation with subsequent glutathione conjugation route is less important for 1,4-dimethylnaphthalene.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

The main metabolites of 2-methylnaphthalene that were identified in the urine of guinea pigs were naphthoic and naphthuric acid, resulting from side chain oxidation. Furthermore, 7-methyl-1-naphthol and S-(7-methyl-1-naphthyl)-cystein were identified. The latter compound could result from direct coupling with cystein or by further metabolism of S-(7-methyl-1-naphthyl)glutathione hydroxylation. Epoxide formation may be involved in the formation of 7-methyl-1-naphthol and S-(7-methyl-1-naphthyl)-cystein.

All metabolites of 2-isopropylnaphthalene that were identified in the urine and bile of rats resulted from oxidation of the isopropyl side chain. In addition, two unknown metabolites were detected, and it is likely that some more metabolites may be formed. The metabolite patterns in urine and bile were comparable. Also for 2,6-diisopropylnaphthalene, all identified metabolites in rat urine and bile were the result of side chain oxidation.

3 HEALTH HAZARDS

3.1 Acute toxicity - oral route

3.1.1 Animal data

3.1.1.1 Study 1

Characteristics

Reference/notifier	: IIA, 5.2/01, Docu ID L08456, study 6,7,8	Exposure	: Single dose, gavage, after 18 h fast
Type of study	: Acute toxicity	Dose	: 750, 1000, 1300, 1700, 2000, 2100, 2300 and 2500 mg/kg bw
Year of execution	: 1993	Vehicle	: Corn oil
Test substance	: 1,4-dimethylnaphthalene, liquid batch no.: H5510 purity: 96.4%*	GLP statement	: Yes
Route	: oral	Guideline	: FIFRA 152-10
Species	: Rat, Sprague Dawley (CrI:CD@BR)	Acceptability	: Acceptable
Group size	: 5/sex/dose	LD50 rats	: 2730 mg/kg bw conf. interval: 2346-3178 mg/kg bw

* purity was not reported in the study report. Purity data are obtained from studies on genotoxicity.

Study design

The guideline resembles OECD 401. Five rats/sex/dose were tested. Three successive studies were performed, with doses of 750, 1000 and 2500 mg/kg bw in the first, 1300, 1700 and 2100 mg/kg bw in the second and 2000, 2300 and 2500 mg/kg bw in the third study. Results of a previously performed limit test in which rats (5/sex) received a dose of 5000 mg/kg bw (undiluted test substance) were included in the LD50 calculations.

The test substance was administered in corn oil, at constant dosing volumes of 10 mL/kg bw. Rats were observed for 14 days after administration.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Results

Mortality: Death occurred in groups dosed with 1700 mg/kg bw and higher. All deaths occurred within 3 days after dosing. In the earlier performed limit study with 5000 mg/kg bw, all 10 dosed rats died.

Table 3.1.1.1-1. Mortality in the acute oral toxicity study

Dose group mg/kg bw	Part 1			Part 2			Part 3		
	750	1000	2500	1300	1700	2100	2000	2300	2500
Males	0	0	5	0	0	0	1	1	2
Females	0	0	5	0	1	1	0	0	3

Table 3.1.1.1-2. Clinical signs in the acute oral toxicity study

	Part 1		
Dose group mg/kg bw	750	1000	2500
	Salivation, discoloration around mouth, redness around nose fur	Hunched posture, lacrimation, salivation, discoloration around mouth, clear nasal discharge, redness around nose fur, wet inguinal fur	Coma, ataxia, hypoactivity, hunched posture, lacrimation, salivation, discoloration around mouth, red nasal discharge, clear nasal discharge, redness around nose fur, redness around eyes, wete inguinal fur, discoloured paws
	Part 2		
Dose group mg/kg bw	1300	1700	2100
	Hypoactivity, hunched posture, lacrimation, salivation, discoloration around mouth, red nasal discharge, redness around nose, redness around eyes	Hypoactivity, hunched posture, lacrimation, salivation, discoloration around mouth, red nasal discharge, redness around nose, redness around eyes, red discolored inguinal fur	Hypoactivity, hunched posture, lacrimation, salivation, discoloration around mouth, red nasal discharge, redness around nose, redness around eyes, red discolored inguinal fur, diarrhea
	Part 3		
Dose group mg/kg bw	2000	2300	2500
	Hypoactivity, hunched posture, lacrimation, salivation, discoloration around mounts, clear nasal discharge, redness around nose, discolored inguinal fur, wet inguinal fur, hair loss	Cold to touch, hypoactivity, hunched posture, lacrimation, salivation, discoloration around mounts, redness around nose, discolored inguinal fur	Hypoactivity, hunched posture, lacrimation, salivation, discoloration around mounts, clear nasal discharge, redness around nose, discolored inguinal fur, hair loss

Symptoms of toxicity: Signs of toxicity were observed in all dose groups. In the lowest dose group, signs were restricted to discolouration around the mouth and red and/or clear nasal discharge.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

In the other dose groups, clinical signs included hypoactivity, ataxia, coma, irritability, chromodacryorrhea, lacrimation, salivation, redness around the nose and/or eyes, wet/discoloured inguinal fur, coldness to the touch, hunched posture, diarrhoea, hair loss and discoloured paws. All surviving rats had recovered at 7 days after administration.

Body weight: All surviving animals gained weight during the study.

Pathology: No gross necropsy findings related to the test substance were observed.

Acceptability

Acceptable

Conclusions

Based on the results of this study and the results of an earlier limit study with 5000 mg/kg bw, the calculated acute oral LD50 for 1,4-dimethylnaphthalene in male and female rats was 2730 mg/kg bw with a confidence interval of 2346-3178 mg/kg bw.

3.2 Acute toxicity - dermal route

3.2.1 Animal data

3.2.1.1 Study 1

Characteristics

Reference/notifier	: IIA, 5.2/02, DocID L08459-study 4	Exposure	: Single dose, 24 h, under occlusion
Type of study	: Acute toxicity limit test	Dose	: 2000 mg/kg bw, undiluted
Year of execution	: 1993	Vehicle	: -
Test substance	: 1,4-dimethylnaphthalene, liquid batch no.: H5510 purity 96.4%*	GLP statement	: yes
Route	: dermal	Guideline	: FIFRA guideline 152-11
Species	: Rabbit, New Zealand White	Acceptability	: Acceptable
Group size	: 5/sex	LD50 rabbits	: > 2000 mg/kg bw

* purity was not reported in the study report. Purity data are obtained from studies on genotoxicity.

Study design

The study is performed in accordance with OECD 402. The undiluted test substance was administered to rabbits on the shaved back whereafter the test site was wrapped. After 24 hours, any residual test substance was wiped off with a gauze pad moistened with saline. Rabbits were observed for 14 days after administration.

Results

Mortality: None of the animals died during the study.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Symptoms of toxicity: Treatment-related signs of systemic toxicity were not observed. Signs of dermal irritation at the application site (oedema and erythema) were observed in all rabbits immediately following removal of the wrappings. All animals developed eschar formation within five days following unwrapping. In 4 animals, eschar formation persisted until the end of the 14 days observation period. New or repaired skin was evident at the application site of all rabbits during the study.

Body weight: Except for 1 rabbit, all animals gained weight during the study.

Gross necropsy: At necropsy in 2 rabbits eschar formation at the application site was observed. In addition, in three rabbits kidneys were pale.

Acceptability

The study is considered acceptable.

Conclusions

The acute median lethal dermal dose (LD50) for 1,4-dimethylnaphthalene in adult male and female rabbits is > 2000 mg/kg bw.

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

3.3.1.1 Study 1

STUDY 1

Characteristics

Reference/notifier	: IIA, 5.2/03 Doc ID	Exposure	: single dose, whole-body, 4h
	L08456L001		
Type of study	: Acute toxicity, limit test	Dose	: 5 mg/L (target conc); 4.16 mg/L (achieved conc)
Year of execution	: 1993	Vehicle	: -
Test substance	: 1,4-dimethylnaphthalene, liquid batch no.: H5510 purity: 96.4%*	GLP statement	: yes
Route	: Inhalation	Guideline	: FIFRA 152-12
Species	: Rat, Sprague Dawley (CrI:CD®BR)	Acceptability	: Acceptable
Group size	: 5/sex	LD50 rats	: > 4.16 mg/L

* purity was not reported in the study report. Purity data are obtained from studies on genotoxicity.

Study design

The study resembles OECD 403. The temperature of the chamber during exposure was between 25 and 26°C instead of 22 ±2°C. The target concentration was 5 mg/L, whereas the nominal exposure concentration, based on the total amount of test substance consumed, was 5.76 mg/L and the mean actual concentration of

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

1,4-dimethylnaphthalene determined by chemical analysis of the filter collected samples was 4.16 mg/L. The mean aerosol Mass Median Aerodynamic Diameter (MMAD) of the chamber atmosphere was 2.82 µm (SD 1.67). Rats were observed for 14 days after exposure.

Results

Mortality: One rat died within 24 hours after administration.

Symptoms of toxicity: Following exposure, clinical signs were observed in all animals. These included dyspnea, prostration, ptosis, hypoactivity, discolouration around the mouth and/or nose and wet inguinal fur. One day after exposure, most of the animals still exhibited these signs. In addition, tremors and red nasal and ocular discharge were also observed. Except for one animal, which showed ptosis on day 5 and 6 following exposure, all of the surviving rats appeared normal 3 days following the exposure.

The incidences of the clinical signs are shown in the table below

Observation	Incidence		Timepoint observed
	Males	Females	
Eye discharge –red	1	2	Day 1-2
Ptosis	1	3	Day 5-6
Nasal discharge – red	0	3	Day 1-2
Dyspnea	0	1	Day 0
Hypoactive	1	5	Day 0-1
Prostate	0	3	Day 0-1
Tremors	0	1	Day 1
Discoloration around mouth	2	5	Day 0-1
Discoloration around nose	5	5	Day 0-1
Wet inguinal fur	5	4	Day 0-1

Body weight: All surviving animals gained weight during the study.

Pathology: No gross necropsy findings related to the test substance were observed, including the rat that died.

Acceptability

It is noted that the actual dose of 4.16 mg/L is below the limit concentration of 5 mg/L. However, it is not expected that an inhalation study in which rats would be exposed to 1,4-dimethylnaphthalene at an actual dose of 5 mg/L would result in a markedly different toxic profile. Therefore, the study is considered acceptable.

Conclusions

The acute median lethal inhalation dose (LC50) for 1,4-dimethylnaphthalene in adult male and female rats is > 4.16 mg/L.

3.4 Skin corrosion/irritation

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

3.4.1 Animal data

3.4.1.1 Study 1

Characteristics

Reference/notifier	: IIA, 5.2/04, Doc ID L08456-study 2	Exposure	: 4h, semi-occluded
Type of study	: Skin irritation/corrosivity study	Dose	: 0.5 mL (undiluted)
Year of execution	: 1993	Vehicle	: -
Test substance	: 1,4-dimethylnaphthalene, liquid batch no.: H5510 purity: 96.4%*	GLP statement	: yes
Route	: dermal	Guideline	: FIFRA 152-14
Species	: Rabbit, New Zealand White	Acceptability	: Acceptable
Group size	: 3/sex	Effect	: Slightly to moderately irritating to skin.

* purity was not reported in the study report. Purity data are obtained from studies on genotoxicity (Lawlor, 1993; Ham, 1993; Murli, 1993).

Study design

The study is in accordance with OECD 404. Undiluted test substance (0.5 mL) was applied to the back of the rabbits (clipped fur) and covered with a cotton gauze patch covered with porous tape. After 4 h the application sites were wiped with gauze and 0.9% saline to remove residual test substance.

Dermal changes were graded according to Draize. The skin was examined up to 14 days after application.

Results

Irritation scores are presented in table 3.4.1.1-1

Table 3.4.1.1-1. Individual skin irritation scores up to 14d after application

	1 h	24 h	48 h	72 h	7d	14d
erythema	1/2/2/1/1/1*	2/2/2/2/2/2	1/2/2/2/2/2	2/2/2/2/2/2	0/0/0/2/2/2	0/0/0/0/0/0
oedema	3/2/1/2/2/1	2/1/2/1/1/1	0/0/0/0/0/0	1/0/0/0/0/1	0/0/0/0/0/0	0/0/0/0/0/0

*m/m/m/f/f/f

Acceptability

The study is considered acceptable.

Conclusions

1,4-dimethylnaphthalene induced slight to moderate skin irritation in albino rabbits.

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

3.5.1.1 Study 1

Characteristics

Reference/notifier	: IIA, 5.2/05, DocID L08456-study 1	Exposure	: Single instillation in conjunctival sac
Type of study	: Eye irritation study	Dose	: 0.1 mL (undiluted)
Year of execution	: 1993	Vehicle	: -
Test substance	: 1,4-dimethylnaphthalene, liquid batch no.: H5510 purity: 96.4%*	GLP statement	: yes
Route	: ocular	Guideline	: FIFRA 152-13
Species	: Rabbit, New Zealand White	Acceptability	: acceptable
Group size	: 3/sex	Effect	: Irritating to eyes

* purity was not reported in the study report. Purity data are obtained from studies on genotoxicity.

Study design

The study is in accordance with OECD 405. Undiluted test substance (0.1 mL) was applied to the right eye of the rabbits. The left eye was untreated and served as control.

Ocular changes were graded according to Draize. The eyes were examined up to 21 days after instillation.

Results

Two rabbits were vocal immediately after administration of the test substance. Signs of ocular irritation consisted of conjunctivitis. Circumocular alopecia was observed in 3 animals at 14 days and in 2 animals at 21 days after application.

The results up to day 21 are summarized in Table 6.2.2.2-1.

Table 6.2.2.2-1. Individual eye irritation scores in unwashed eyes up to 21 days after instillation

	1 h	24 h	48 h	72 h	Day 7	Day 14	Day 21
Corneal opacity	0/0/0/0/0/0*	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0
Corneal area	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0
Iris	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0
Conj. redness	2/2/2/2/2/2	2/2/2/2/2/2	2/1/1/1/2/2	1/1/0/0/1/2	1/1/0/0/1/2	0/0/0/0/1/0	0/0/0/0/0/0
Conj. chemosis	1/2/3/2/1/2	1/2/2/2/2/3	1/2/2/2/2/3	1/2/2/2/2/3	1/1/1/1/1/3	0/0/0/0/1/2	0/0/0/0/0/0
Conj. discharge	0/0/0/0/0/0	0/0/0/0/0/2	0/0/0/0/1/2	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0

* m/m/m/f/f/f

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Acceptability

The study is considered acceptable.

Conclusions

According to the authors 1,4-dimethylnaphthalene is not irritating to the eye of albino rabbits. However, the mean score for conjunctival chemosis at 24, 48 and 72 hours is 2.

3.6 Respiratory sensitisation

No data available.

3.7 Skin sensitisation

3.7.1 Animal data

3.7.1.1 Study 1 – LLNA

Characteristics

reference	: IIA, 5.2/07, Doc ID 495316	exposure	: Topical induction (25 µl/ear)
type of study	: Skin sensitization study (LLNA)	doses	: 0, 25, 50 and 100% w/w for topical induction
year of execution	: 2011	vehicle	: Acetone/olive oil (4:1 v/v)
test substance	: 1,4-dimethylnaphthalene, liquid batch no.: 14D06B01-01 Purity: 98.4%	GLP statement	: Yes
route	: Dermal	guideline	: In accordance with OECD 429
species	: CBA/J mice	acceptability	: Acceptable
group size	: 5 animals per group, 1 control and 3 test groups (females only)	Effect	: Not sensitising to skin

Study design

The study was performed in accordance with OECD 429. Three experimental groups of five female CBA/J mice were treated with test substance concentrations of 25, 50 or 100% w/w on three consecutive days, by open application on the ears (test substance concentrations were selected based on the results of a pre-screen study). Five vehicle control animals were similarly treated, but with vehicle alone (acetone/olive oil (4:1 v/v)).

Three days after the last exposure, all animals were injected via the tail vein with ³H-methyl thymidine and after five hours the draining (auricular) lymph nodes were excised and pooled for each animal. After precipitating the DNA of the lymph node cells, radioactivity measurements were performed. The activity

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

was expressed as the number of Disintegrations Per Minute (DPM) and a stimulation index (SI) was subsequently calculated for each group.

Results

Enlarged auricular lymph nodes were found in the groups dosed at 50 and 100%. Mean DPM/animal values for the experimental groups treated with test substance concentrations 25, 50 and 100% were 1031, 1167 and 1384 DPM respectively. The mean DPM/animal value for the vehicle control group was 488 DPM. The SI values calculated for the substance concentrations 25, 50 and 100% were 2.1, 2.4 and 2.8, respectively.

Acceptability

The study is considered acceptable.

Conclusions

Since there was no indication that the test substance elicits an $SI \geq 3$ when tested up to 100%, 1,4-dimethylnaphthalene was considered to be a non skin sensitiser.

3.8 Germ cell mutagenicity

3.8.1 In vitro data

3.8.1.1 Study 1 – Bacterial reverse mutation assay

Reference: IIA 5.4/01, Doc ID 15683-0-401

GLP: yes

Guideline: The study was carried out under FIFRA 84-2 which resembles OECD 471. Deviations: *S. typhimurium* strain TA1538 was used instead of strain TA102 or an appropriate *E. coli* strain; as positive control for the efficacy of the S9 mix, only 2-aminoanthracene was used.

Study design:

The test strains used in the study were TA98, TA100, TA1535, TA1537 and TA1538. The assay was conducted using three replicate per dose level both in the presence and absence of S9 mix. Six doses of 1,4-dimethylnaphthalene (batch H5510, purity 96.4%) was tested ranging from 10-1000 µg/plate in the presence of S9 and from 1-250 µg/plate in the absence of S9 mix. Used doses were based on a range finding study with TA100 with doses up to 5 mg/plate, which resulted in cytotoxicity at doses ≥ 333 µg/plate in the presence of S9 and doses ≥ 33.3 µg/plate in the absence of S9 (Aroclor 1254).

2-aminoanthracene was used as positive control in the presence of S9 and 2-nitrofluorene (TA98, TA 1538), sodium azide (TA100, TA 1535) and ICR-191 (TA1537) were used as positive controls in the absence of S9.

Results:

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

The results of the study are summarized in Table 3.8.1.1-1. With tester strain TA1538 in the presence of S9, a 2.7-fold increase in the number of revertants per plate was observed. It was decided to repeat this test twice in the presence of S9. The result of these repeat experiments were all negative (Table 3.8.1.1-2).

The positive controls induced the expected increases in revertant colonies in all strains.

Table 3.8.1.1-1: Summary of the bacterial reverse mutation assay

Dose level (µg/plate)	S9	TA98	TA100	TA1535	TA1537	TA1538
0	+	33	133	14	10	16
10	+	26	173	13	6	14
50	+	24	184	12	9	16
100	+	26	190	11	9	23
250	+	30	142	11	5	43
500	+	31	93	13	2	2
1000	+	9	13	3	0	0
Positive control	+	677	776	129	96	940
0	-	12	97	11	4	14
1	-	12	89	8	7	15
5	-	14	144	10	5	12
10	-	13	107	12	5	11
25	-	11	93	4	3	11
50	-	11	90	9	5	11
250	-	5	54	5	4	1
Positive control	-	138	452	323	234	241

Table 3.8.1.1-1: Summary of the repeat assay with strain TA1538

Dose level (µg/plate)	S9	TA1538 (exp. 2)	TA1538 (exp. 3)
0	+	14	18
10	+	14	17
50	+	15	20
100	+	16	20
250	+	20	18
500	+	0	14
1000	+	0	5
Positive control	+	1123	905

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, 1,4-dimethylnaphthalene did not induce revertants in *S. typhimurium*.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

3.8.1.2 Study 2 – Bacterial reverse mutation assay

Reference: IIA 5.4/01, Doc ID 2782/2-D6171

GLP: yes

Guideline: OECD 471

Study design:

1,4-dimethylnaphthalene (batch no: 14D03M01-02; purity: 98.4%) was assayed for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments.

Used doses of 1,4-dimethylnaphthalene were based on a range finding study with TA100 with doses up to 5 mg/plate, which resulted in cytotoxicity at doses ≥ 1000 $\mu\text{g}/\text{plate}$ in the presence of S9 and doses ≥ 200 $\mu\text{g}/\text{plate}$ in the absence of S9.

Experiment 1 were performed using the final concentrations of 0.128, 0.64, 3.2, 16, 80, 400 and 2000 $\mu\text{g}/\text{plate}$, plus negative and positive controls. In experiment 2 the maximum concentration in the presence of S9 was reduced to 400 $\mu\text{g}/\text{plate}$ due to the toxicity at higher concentration levels. In experiment 2, treatments in the presence of S9 were further modified by the inclusion of a pre-incubation step.

The positive controls were 2-nitrofluorene (TA98, -S9), sodium azide (TA100 and TA1535, -S9), 9-aminoacridine (TA1537, -S9), mytomyacin C (TA102, -S9), benzo[a]pyrene (TA98, -S9) and 2-aminoanthracene (all strains, +S9).

Results:

In experiment 1, clear evidence of toxicity occurred in all strains at 400 $\mu\text{g}/\text{plate}$ and above, in the absence and in the presence of S9, and also at 80 $\mu\text{g}/\text{plate}$ in most strains in the absence of S9 and strain TA1535 in the presence of S9. In experiment 2, the dose range was narrowed and evidence of toxicity was observed in all strains at 64 $\mu\text{g}/\text{plate}$ and above in the absence of S9 and 160 $\mu\text{g}/\text{plate}$ and above in the presence of S9.

1,4-dimethylnaphthalene did not induce a biologically significant increase in revertant colonies. The positive controls induced the expected increases in revertant colonies in all strains.

Table 3.8.1.2-1: Summary of the bacterial reverse mutation assay

Dose level ($\mu\text{g}/\text{plate}$)	TA98		TA100		TA1535		TA1537		TA102	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Experiment 1										
0	26	40	97	158	19	21	13	15	293	219
0.128	26	46	109	146	17	16	11	17	263	225
0.64	27	49	104	149	12	13	10	21	283	218
3.2	20	39	90	136	14	15	8	18	299	218
16	27	39	106	153	14	15	11	17	287	220
80	18	39	93	145	14	11	8	14	178	211
400	17	44	78	120	9	16	7	11	163	189
2000	16	37	- ¹	- ¹	7	9	5	- ¹	- ¹	- ¹
Postive control	1999	476	1251	2437	906	346	245	245	937	1417
Experiment 2										

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

0	29	37	127	130	19	20	19	17	256	215
1.638	23	37	117	116	22	20	17	15	273	222
4.096	26	48	112	142	23	17	14	22	275	238
10.24	29	35	111	120	13	18	16	15	266	212
25.6	21	27	96	126	19	20	19	14	249	213
64	13	35	92	121	12	16	10	15	165	215
160	23	43	43	126	6	13	4	8	152	168
400	17	45	- ¹	107	- ¹	9	- ¹	16	148	133
Positive control	1235	547	912	1815	706	292	297	149	740	1467

¹Toxic, no revertant colonies.

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, 1,4-dimethylnaphthalene did not induce revertants in *S. typhimurium*.

3.8.1.3 Study 3 – mammalian gene mutation test

Reference: IIA 5.4/03, Doc ID 424711

GLP: yes

Guideline: OECD 476

Study design:

The study evaluated the effect of 1,4-dimethylnaphthalene (batch no: 14D03M01-02; purity: 98.8%) on the induction of forward mutation at the thymidine-kinase locus in L5178Y mouse lymphoma cells.

Used doses of 1,4-dimethylnaphthalene (purity 98.8%) were based on a range finding study with doses up to 1 mg/mL, which resulted in cytotoxicity at doses ≥ 100 $\mu\text{g/mL}$ in the presence of S9 and doses ≥ 33 $\mu\text{g/mL}$ in the absence of S9. 1,4-dimethylnaphthalene was tested beyond the limit of solubility to obtain adequate mutagenicity data. The dose levels selected to measure mutation frequencies at the *tk*-locus were: 1, 5, 10, 20, 30, 35, 37.5 and 40 $\mu\text{g/mL}$ exposure medium in the absence of S9 and 50, 75, 100, 150, 170, 200, 210 and 220 $\mu\text{g/mL}$ exposure medium in the presence of S9. Positive controls were methylmethanesulfonate (MMS) without S9 and cyclophosphamide (CP) with S9.

In the first experiment the incubation time was 3 hours. A second experiment was carried out to verify the results from the first experiment.

Results:

In the presence of S9, dose-related increases in the mutant frequency were observed (at precipitating dose levels) both in small and large sized colonies. Increases were more than three-fold (3.7-fold for small colonies and 4.0-fold for large colonies) at a dose of 170 $\mu\text{g/mL}$ (survival 38% in exp 1 and 33% in exp 2) and outside the historical control data range. At higher doses of 200-220 $\mu\text{g/mL}$ increases were less than three-fold. At these doses the relative total growth was dose-dependently further reduced to 12% compared to the total growth of the solvent controls.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Table 3.8.1.3-1 Results from an *in vitro* mammalian cell gene mutation test

Dose (µg/ml)	S9	RSG	CEday2	RSday2	RTG	Mutation frequency
SC1	-	100	113	100	100	59
SC2	-	-	91	-	--	58
1	-	102	88	86	88	53
5	-	95	116	114	108	38
10	-	92	94	92	85	50
20	-	87	91	89	77	54
30	-	66	107	105	69	45
35	-	41	107	105	43	56
37.5	-	27	118	116	31	48
40	-	12	116	114	14	54
MMS	-	69	76	75	52	677
SC1	+	100	102	100	100	71
SC2	+	-	86	-	-	68
50	+	82	110	117	96	83
75	+	70	98	104	73	122
100	+	65	107	114	74	125
150	+	61	79	84	51	179
170	+	42	85	90	38	220
200	+	35	102	109	38	93
210	+	17	104	111	19	70
220	+	14	81	86	12	66
CP	+	67	53	56	36	1647

RSG = relative suspension growth, CE = cloning efficiency, RS = relative survival, RTG = relative total growth, SC = solvent control, MMS = methylmethanesulfonate, CP = cyclophosphamide

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, 1,4-dimethylnaphthalene is mutagenic in the mouse lymphoma L5178Y in the presence of metabolic activation, but not in the absence of metabolic activation. It is noted that the positive effect was observed at precipitating dose levels.

3.8.1.4 Study 4 – Unscheduled DNA synthesis

Reference: IIA 5.4/04 – Doc ID 15683-0-447

Guideline: FIFRA 84/2(3). The study resembles OECD guideline 482. Deviations: no statistical analysis was carried out.

Study design

1,4-dimethylnaphthalene (batch no: H5510; purity: 96.4%) was tested in an *in vitro* assay for Unscheduled DNA Synthesis in rat liver primary cell cultures. A primary rat liver cell culture was established from hepatocytes obtained from a single adult male Fischer 344 rat. Concentrations ranged from 0.0025 µg/ml to 100 µg/ml in the presence of 10 µCi/ml ³HTdR (46 Ci/mMole). The test material was soluble at and below

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

concentrations of 10 µg/ml and formed a cloudy suspension at higher concentrations. Five cultures/treatment were included. Two were used for cytotoxicity measurements, the other 3 for analysis of nuclear labeling. At a concentration of 10 µg/mL moderate toxicity resulting in poor cellular morphology was observed (58.2% survival). Higher doses were more toxic, whereas at dose levels ≤ 5 µg/mL, toxicity was not observed. Therefore, six treatments, from 0.250 to 10 µg/mL (108.9 to 58.2% survival) were selected for analysis of nuclear labeling. The positive control was 2-acetylaminofluorene.

Results:

The results of the study are reported in Table 3.1.8.4-1.

The study report used as evaluation criteria an increase in the mean net nuclear grain count to at least five grains nucleus above the concurrent solvent control (2.32) and/or an increase in the percent of nuclei having five or more grains such that the percentage of these nuclei in test cultures is at least 10% above the percentage observed in the solvent control cultures (12.67%). This differs from the criteria in OECD Guideline 482 (1987) which uses a significant dose-related increase in radiolabel incorporation or a reproducible and statistically significant positive response for at least one of the test points.

The study report makes no mention of any statistical analysis being carried out and only the average grain counts on triplicate coverslips are reported. It is noted that although the study was considered negative based on the evaluation criteria used in the study the mean net nuclear grain and the % cells with >5 mean NNG do exceed the historical control data slightly at the high dose of 10 µg/ml (Table 3.1.8.4-2). Since no statistical analysis was carried out and since no repeat study was performed to determine the reproducibility of the finding at 10 µg/ml the result of the study is considered equivocal.

The positive control provided a positive response which was within historical control range, albeit somewhat at the lower end of the range (NNG: 6.70-29.45, mean 15.53; % Cells with >5 mean NNG 60.67-100.0, mean 87.3).

Table 3.1.8.4-1: Results of the *in vitro* UDS

Concentration µg/ml	Mean Net Nuclear Grains (NNG) ¹	% Cells w/≥ 5 mean NNG ¹	Mean Cytograins	% Survival at 20 hours
0	-2.68	2.67	16.17	100
0.250	-1.65	6.00	13.97	103
0.500	-1.37	3.33	12.45	103.2
1.00	-1.18	8.67	10.82	106.8
2.50	-2.77	3.99	16.36	108.9
5.00	-1.38	7.97	14.06	95.8
10.0	0.01	10.00	9.75	58.2
Positive control	7.35	63.33	12.35	89.4

¹ Average values for triplicate coverslips

Table 3.1.8.4-1: Historical control data (data from Januari 1992 to May 1993 from the same lab, study conducted in 1993)

Mean Net Nuclear Grains (NNG) ¹	% Cells w/≥ 5 Mean NNG	Mean Cyto Grains ¹
-0.70	8.67	12.15
-1.16	3.33	10.57
-1.43	0.67	9.26
-1.68	6.67	11.02
-2.11	5.33	13.99
-1.87	9.33	18.80

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

-1.54	6.00	19.57
-0.48	9.00	18.25
-1.54	6.00	19.57

¹ Average values for triplicate coverslips

Acceptability

The study is considered acceptable.

Conclusions

The results of the study are considered to be equivocal.

3.8.2 Animal data

3.8.2.1 Study 1 – *in vivo* micronucleus

Reference: IIA 5.4/05 and IIA 5.4/06 (Doc ID 15683-0-455 and 7931-100)

Guideline: FIFRA guideline 84-2. The study resembles OECD guideline 474.

Study design:

The study was performed in 2 parts. In the first part, reported in 1993, 1000 polychromatic erythrocytes were scored for the incidence of micronucleated polychromatic erythrocytes results of the study. In the second part, reported in 2007, 2000 polychromatic erythrocytes were scored for micronuclei.

Male and female CD-1 (ICR) mice (5/sex/dose) received a single dose of 1,4-dimethylnaphthalene (batch no: H5510; purity: 96.4%) in corn oil, by gavage in doses of 225, 450 or 900 mg/kg bw. Bone marrow was harvested at 24, 48 and 72 hr after treatment and bone marrow slides were made and analysed. Appropriate controls were used (solvent control and cyclophosphamide as positive control).

Results:

Approximately 1 day after dosing, 1 high-dosed female appeared prostrate with dyspnoea. One male and 1 female (high-dose) appeared languid with dyspnoea. Approximately 2 days after dosing, 2 males and 3 females (high-dosed) were found dead. All other animals appeared normal at this time and remained healthy until their appropriate harvest times.

1,4-dimethylnaphthalene caused decreases in the PCE:NCE ratios at 48 and 72h as compared to 24h (statistically significant in females only), although PCE/NCE ratios for the treated groups were within the historical control range. In view of the mortalities and the above mentioned clinical signs at 900 mg/kg bw, which are indicative of systemic toxicity, and the toxicokinetic studies showing that closely related naphthalenes are well absorbed from the gut and rapidly occur in blood following oral dosing, it is assumed that 1,4-dimethylnaphthalene in this study will have reached the bone marrow of the mice.

In the first part of the study (1993), 1,4-dimethylnaphthalene induced a statistically significant increase in micronucleated polychromatic erythrocytes in females at the low dose in the 48-hour harvest group only. This was considered biologically irrelevant, since at higher doses as well as at later sampling points no increases in micronucleated polychromatic erythrocytes were observed. In addition, values were within the historical response range.

In the second part of the study (2007) 1,4-dimethylnaphthalene induced statistically significant increases in micronucleated polychromatic erythrocytes in females at the mid-dose in the 24-hour harvest group. This was considered biologically irrelevant, since at higher doses as well as at later sampling points no increases in micronucleated polychromatic erythrocytes were observed.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Table 3.8.2.1-1: Summary of the results – 1993 evaluation

Dose	Harvest time (hr)	Micronucleated PCE (mean of 1000)			Ratio PCE:NCE	
		Males	Females	Total	Males	Females
Control	24	0.02	0.04	0.03	0.77	0.92
225	24	0.12	0.02	0.07	0.71	1.00
	48	0.06	0.20*	0.13	0.54	0.48
	72	0.10	0.02	0.06	0.58	0.32
450	24	0.04	0.04	0.04	0.84	0.92
	48	0.06	0.06	0.06	0.54	0.37
	72	0.06	0.00	0.03	0.54	0.45
900	24	0.14	0.00	0.07	0.87	0.94
	48	0.10	0.02	0.06	0.56	0.44
	72	0.04	0.02	0.03	0.48	0.38
Positive control	24	0.82*	1.16*	0.99*	0.96	0.97

*Statistically significant from control

Table 3.8.2.1-2: Summary of the results – 2007 evaluation

Dose	Harvest time (hr)	Micronucleated PCE (mean of 2000)		Ratio PCE:NCE	
		Males	Females	Males	Females
Control	24	0.09	0.05	0.70	0.98
225	24	0.08	0.02	0.72	0.91
	48	0.09	0.12	0.065	0.53*
	72	0.04	0.06	0.59	0.41*
450	24	0.02	0.13*	0.72	0.74
	48	0.07	0.08	0.57	0.34*
	72	0.04	0.05	0.72	0.49*
900	24	0.07	0.03	0.90	0.84
	48	0.05	0.05	0.65	0.61*
	72	0.12	0.10	0.49	0.41*
Positive control	24	0.39*	0.66*	0.82	0.91

*Statistically significant from control

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, 1,4-dimethylnaphthalene is negative in the mouse bone marrow micronucleus assay.

3.8.2.2 Study 2 – *in vivo* unscheduled DNA synthesis assay

Reference: IIA 5.4/07, Doc ID 1000/31-D6172

Guideline: OECD 486, Minor deviation: Animals from the range finding study were acclimatized for at least two days prior to dosing instead of 5 days.

Study design

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Male Sprague Dawley rats (n=4/group) received a single dose of 1,4-dimethylnaphthalene (batch no: 14D03M01-02 (range-finding) and 14D06B01-01 (main study); purity: 98.4-98.6%) in corn oil, by gavage in doses of 500 or 1000 mg/kg bw. Doses were based on a range finding study in male and female rats with doses of 1000, 1400 and 2000 mg/kg bw, in which toxicity was observed at doses \geq 1400 mg/kg bw. No clinical signs of toxicity were observed in rats dosed with 500 or 1000 mg/kg bw. Positive controls were 75 mg/kg 2-acetamidofluorene and 10 mg/kg dimethylnitrosamine. Hepatocyte cultures were prepared from 3 rats/group at 2-4 and 12-14 hours after dosing.

Results

One of the positive controls failed the acceptance limits for a positive control response, probably due to the autoradiographic emulsion. Nevertheless, a marked increase in net nuclear grain (NNG) value was observed compared to the concurrent vehicle control (almost 4-fold increase) with a clear percentage of cells in repair, indicating that an increase in unscheduled DNA synthesis could be detected by the assay. It is therefore considered that this minor deviation did not influence the results of the assay. Results with 1,4-dimethylnaphthalene were negative at both time points.

Table 1 1,4-dimethylnaphthalene: group mean net nuclear grain count values, 12-14 hour experiment

Dose (mg/kg)	Compound	Net grain count (NNG)		Percent of cells in repair (NNG \geq 5)		Net grain count of cells in repair	
		mean	SD	mean	SD	mean	SD
0	corn oil	0.6	0.1	-	-	-	-
500	1,4-dimethylnaphthalene	0.6	0.1	-	-	-	-
1000	1,4-dimethylnaphthalene	0.6	0.1	-	-	-	-
75	2-AAF	2.3	0.7	13.7	9.0	6.4	0.5

Table 2 1,4-dimethylnaphthalene: group mean net nuclear grain count values, 2-4 hour experiment

Dose (mg/kg)	Compound	Net grain count (NNG)		Percent of cells in repair (NNG \geq 5)		Net grain count of cells in repair	
		mean	SD	mean	SD	mean	SD
0	corn oil	0.4	0.1	-	-	-	-
500	1,4-dimethylnaphthalene	0.5	0.1	-	-	-	-
1000	1,4-dimethylnaphthalene	0.3	0.1	-	-	-	-
10	DMN	5.6	3.5	52.3	41.9	7.8	2.0

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, 1,4-dimethylnaphthalene does not produce induction of unscheduled DNA synthesis in rat liver.

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 Study 1 – Combined chronic toxicity/carcinogenicity study, rat

STUDY 1

Characteristics

Reference/notifier	: IIA, 5.3/01, study report 02-154	Exposure	: Repeated by diet, 52 weeks (chronic phase), 104 week carcinogenicity phase)
Type of study	: 24 months combined chronic toxicity/carcinogenicity study	Doses	: 0, 150, 500 and 3750 ppm ^a
Year of execution	: 2007-2010	Vehicle	: -
Test substance	: 1,4-dimethylnaphthalene Batch no: F243A040/14D06B01-01, Purity: 98.7% in 2007 and confirmed in 2009	GLP statement	: yes
Route	: Oral	Guideline	: OECD 453
Species	: Rat, Sprague Dawley [CD IGS; CrI:CD(SD)]	Acceptability	: Acceptable
Group size	: 20/sex/dose (chronic phase) 65/sex/dose (carcinogenicity phase)	NOAEL	: 10 mg/kg bw/day

^a equal to mean actual doses of 0, 8, 27 and 208 mg/kg bw/day in males and 0, 10, 33 and 247 mg/kg bw/day in females over the 104-week period

Study design

The study performance was performed in accordance with OECD guideline 453 (1981).

In the carcinogenicity phase, surviving males were terminated at week 104; due to decreased survival rate females were terminated at week 100.

Results

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Table 3.9.1.1-1 Results from a 2-year oral toxicity study in rats

Dose (ppm)	0		150		500		3750		dr
Sex	m	f	m	f	m	f	m	f	
Survival (%) (week 52)	95	100	90	95	100	95	95	100	
Survival (%) (week 104/100)	60	28	29	35	40	29	60	48	
Clinical signs	No toxicologically relevant effects								
Body weight (week 52)	655	369	670	393	679	349	597*	307*	
Body weight (week 104/100)	656	440	673	429	631	417	563*	330*	
Food consumption (week 52)						ds (inciden -tally)	ds wk 1-21	ds	
Food consumption (week 104/100)							ds wk 1-25	ds	
Water consumption	Not performed								
Ophthalmoscopy	No treatment-related findings								
Haematology (week 52)									
-white blood cell (10 ³ cells/ μ L)	12.94	8.64	12.91	8.19	11.96	8.04	11.1*	7.24*	(m,f)
-haemoglobin (g/dL)	15.6	14.6	15.5	14.3	15.6	14.6	15.4	13.8*	
-MCV (fL)	54.7	58.1	56.1	57.9	55.0	58.0	54.9	55.4*	
-MCH (pg)	16.5	18.2	17.0	18.1	16.6	18.1	16.6	16.9*	
-MCHC (g/dL)	30.3	31.4	30.4	31.3	30.2	31.2	30.3	30.6*	(m)
-platelets (10 ³ cells/ μ L)	1203	850	1099	927	1073	858	954*	821	
Haematology (week 104/100)									
-white blood cell (10 ³ cells/ μ L)	8.68	10.69	10.26	8.29	10.40	8.57	9.26	6.81	
-haemoglobin (g/dL)	14.2	11.9	13.9	13.2	13.8	13.6	13.1	13.0	(f)
-MCV (fL)	55.4	60.8	55.5	60.1	54.5	57.7	53.9	55.5*	(f)
-MCH (pg)	17.5	19.7	17.3	19.6	17.0	18.8	16.8	18.0*	
-MCHC (g/dL)	31.6	32.4	31.1	32.6	31.1	32.6	31.1	32.5	
-platelets (10 ³ cells/ μ L)	1004	1082	1234	9.25	1256	1019	1292	932	
Clinical Chemistry (week 52)									
-total bilirubin (mg/dL)	0.2	0.20	0.2	0.23	0.2	0.21	0.2	0.25*	
-blood urea nitrogen (mg/dL)	13	13	12	14	12	13	12	10*	
-Cholesterol (mg/dL)	122	110	114	131	134	143*	140	165*	f
Clinical Chemistry (18 months)									
-Cholesterol (mg/dl)	129	100	124	133	132	132	149	171*	
Clinical Chemistry (week 104/100)									
-Cholesterol (mg/dl)	133	103	127	133	120	112	143	159*	
Urinalysis (week 52, week 104/100)	No toxicologically relevant effects								
Organ weights (week 52)									
-Liver (g)	14.30	8.74	15.01	9.74	15.71*	9.15	15.75*	10.49*	m
-Spleen (g)	0.931	0.657	0.992	0.698	0.960	0.630	0.902	0.542*	
-Liver (% bw)	2.2287	2.52	2.344	2.61	2.444	2.76	2.770*	3.59*	m/f
-Brain (% bw)	0.355	0.58	0.348	0.55	0.345	0.61	0.386*	0.69*	
-Heart (% bw)	0.288	0.319	0.280	0.313	0.278	0.334	0.303	0.371*	(f)
-Kidneys (% bw)	0.545	0.67	0.550	0.67	0.555	0.71	0.619	0.77*	(f)
-Uterus (% bw)		0.274		0.284		0.297		0.360*	f
Organs weights (week 104/100)									
-Adrenals (g)	0.082	0.201	0.071	0.112*	0.246	0.134	0.091	0.082*	
-Kidneys (g)	3.97	3.094	4.32	2.667	4.11	3.236	4.22	2.559*	

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Dose (ppm)	0		150		500		3750		dr
Sex	m	f	m	f	m	f	m	f	
Pathology									
<i>Macroscopy (week 52, 100/104)</i>	Not toxicologically relevant effects								
<i>Microscopy (week 52)</i>									
-kidney, proteinosis	0	0	0	0	0	0	13	0	
-kidney, papillary necrosis	0	0	0	0	0	0	5	0	
-kidney, karyomegaly tubuli	0	0	0	0	0	4	4	11	
-liver, multiple cyst	0	0	-	-	-	-	0	3	
-lung, alveolar histiocytosis	6	1	-	-	-	-	10	6	
-mesenteric lymph node									
-lymphoid depletion	1	0	-	-	-	-	6	0	
-erythrocytic accumulation	0	0	-	-	-	-	4	0	
<i>Microscopy (week 100/104)</i>									
Neoplastic lesions	Not toxicologically relevant effects								
<i>Microscopy (week 100/104)</i>									
Non-neoplastic lesions									
Kidneys (n=65)									
-kidney, chronic progressive nephropathy	46	26	48	21	38	12	59	33	
-kidney, infarction	5	3	8	2	8	1	30	1	
-kidney, mineralization, papillary	6	13	8	9	5	5	40	27	
-kidney, cyst, cortex	3	1	3	0	1	1	10	1	
-kidney, necrosis, papillary	0	0	2	1	0	1	38	6	
-kidney, microabces	0	1	1	0	0	0	12	1	
-kidney, proteinosis	28	16	21	9	16	13	51	32	
-kidney, dilatation papillary tubules	1	3	2	1	0	4	35	6	
Lungs (n=65)									
- infiltrate, alveolar macrophage	8	13	4	4	8	4	14	15	

* significantly different from control group ($p \leq 0.05$) dr = dose related; ds = decreased significantly

- not examined

Dietary intake of 1,4-dimethylnaphthalene resulted in actual doses of 8, 27 and 208 mg/kg bw/day in males and 10, 33 and 247 mg/kg bw/day in females. No treatment-related clinical signs or mortality were observed in the study. At ophthalmic examinations, no eye abnormalities were observed.

In high-dose males a significant decrease in food intake was observed from week 1-25. In high-dosed females, food intake was significantly decreased during the entire exposure period. In mid-dosed females food intake was only incidentally decreased significantly. At the end of the 52-weeks exposure period body weight was significantly reduced in the high-dose males (9%) and females (17%). At termination of the study, body weight was significantly reduced in high-dose males (14%) and females (25%). At the high dose body weight gain and food consumption were in particular reduced during the first few weeks of treatment.

Platelet count was significantly decreased in high dosed males at 3 and 12 months and in high-dosed females at 3 and 6 months. In addition, high-dosed females showed significant decreases in MCV, haemoglobin and MCH at 6 months and significant decreases in MCV, MCH, MCHC, haemoglobin and white blood cell count at 12 months. High dosed males also had a significantly reduced white blood cell count at 12 months. No relevant effects were observed in the other groups. At study termination, high-dosed females showed significant decreases in MCV and MCH. No further toxicological relevant changes in haematology were seen at 18 months and at study termination.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

At 3, 6 and 12 months, a significant decrease in BUN was observed in high-dosed females; since it is not observed in males nor at later time points and taking into account that a decrease in BUN is not considered toxicologically relevant, this finding is considered not test-substance related. An effect was observed in plasma cholesterol in female rats, significant in low and high-dose females at 3 and 6 months and in mid and high-dose females at 12 months (dose-related). At 18-months and at study termination, both high dose males and females showed an increase in cholesterol (statistically significant in females only). No further, toxicological relevant changes in blood biochemistry were observed at these time points.

After 52 weeks, an increase in absolute liver weight was observed (significant in mid and high-dosed males and high-dosed females). Relative liver weight was statistically significantly increased at the high dose in both sexes. Statistically non-significant increases in relative liver weight (<10%) observed at the mid-dose were considered not biologically relevant.

At the terminal kill, high-dose females showed a statistically significant decrease in absolute kidney weight (17%). A statistically significant change in adrenal weight was not considered toxicologically relevant since no dose-response was observed and no histopathological counterpart was noted.

All gross lesions reported were considered incidental background lesions in rats.

Although no treatment-related histopathological effects were observed in the liver, significant and dose related increases in plasma cholesterol were observed in females, throughout the study period. Histopathological changes were observed in the kidney. At 52 weeks these changes included an increased incidence in minimal to mild karyomegaly of the cortical tubules in mid and high-dosed females and high-dosed males, and an increased incidence of proteinosis in the tubules of the papilla and minimal papillary necrosis in high-dosed males. At the high dose the incidence of alveolar histiocytosis was increased in animals of both sexes. In males of the high dose group the incidences of lymphoid depletion and erythrocytic accumulation in the mesenteric lymph node were increased.

At termination of the study at 104/100 weeks, histopathological changes in the kidneys included increased incidences of renal infarction, papillary mineralization, papillary necrosis, cysts in the cortex, proteinosis, dilatation of the papillary tubules and chronic progressive nephropathy in the high-dose males and/or females.

As no nephrotoxicity was seen at the mid dose level at study termination, the toxicological significance of the increased incidence of karyomegaly of the cortical tubules in the mid-dose is unclear.

Chronic progressive nephropathy, which is a spontaneous change in the kidney of ageing rats, was present at a frequency which was considered consistent with normal background and the incidence found was not considered increased by 1,4-dimethylnaphthalene treatment. Several other histopathological changes were considered not toxicologically relevant.

No increased incidences in neoplastic lesions were noted at study termination. The most common neoplasms noted in the study were pituitary adenomas, mammary adenomas, fibroadenomas, and adenocarcinomas, and C-cell tumours of the thyroids. The incidence of other neoplasms was low. The neoplasms observed in the study were typical of those frequently present in aged Sprague-Dawley rats, and no test material-related changes were noted for the incidence of neoplasms. Therefore, the administration of 1,4-dimethylnaphthalene to male and female Sprague-Dawley rats did not cause carcinogenicity.

Acceptability

A reduced survival was seen in control, low-dose and mid-dose females (28-35%). The most frequent cause of death was pituitary adenomas. The other causes of death observed in the study were typical of those frequently seen in chronic studies in rats. A cause of death was not determined microscopically for 42 males and 11 females. Test material related renal changes noted microscopically did not increase the incidence of early deaths.

As the high dose females showed a sufficient survival, the study was considered suitable for evaluation of the carcinogenic properties of 1,4-dimethylnaphthalene.

In the histopathology report of the carcinogenicity phase, data of all animals were summarized (n=65). No separation was made between animals terminated at 52 weeks, at 104 weeks and animals died or killed in extremis during the study. For evaluation of the neoplastic lesions, it is important to make an evaluation of

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

the data of animals terminated at 104 weeks separately. However, as the survival rate was highest at the high dose level, a separate examination of histopathology data is not considered necessary. The study is considered acceptable.

Conclusions

At the high dose, reduced body weights, increased serum cholesterol, histopathological changes in the kidneys, including dilatation of the papillary tubules, papillary necrosis and mineralization, pelvic proteinosis and renal cortical infarction were seen. Based on the minimal karyomegaly noted in the kidneys of females at 500 and 3750 ppm, the NOAEL in this chronic toxicity and carcinogenicity study is set at 150 ppm (equal to 10 mg/kg bw/day in females). No increased incidences of neoplasms were noted at study termination.

3.9.1.2 Study 2 – carcinogenicity information on other naphthalenes

The following data are derived from ATSDR (2005) (IIA 5.7/02).

In a 24-month carcinogenicity study, Wistar rats were given a mixture of diisopropylnaphthalene isomers in the diet (0, 96, 240, 600 and 1500 ppm). A NOAEL of 240 ppm, equivalent to 12 mg/kg bw/day was derived based on the effects on body weight and hepatic dysfunction. There was no significant difference in the incidence of tumors between the control and experimental groups. Nevertheless, it should be noted that survival rates were low (21-72% per dose group) due to pneumonia (IIA 5.5/03Kawai 1977, non-GLP).

Note: due to limited information (Klimisch score 4) the study is considered to be supplementary only.

In a public literature study (Murata, 1993 IIA 5.5/04, non-GLP) B6C3F1 mice (50/sex/dose) were provided with diets containing 0, 0.075, or 0.15% 1-methylnaphthalene (1-MN, purity >97%) for 81 weeks. Average intake of 1-MN was 75.1 and 71.6 mg/kg bw/day in the low dose group, and 143.7 and 140.2 mg/kg bw/day in the high dose group for females and males, respectively. No effects on mortality or body weight were observed. Both treatment groups developed pulmonary alveolar proteinosis at high incidence, with 46.0 and 34.7% of females and 46.0 and 38.0% of males, respectively, being affected, compared to 10 and 8.2% in female and male controls. Total lipid and phospholipid levels in sera and monocytes in peripheral blood were also significantly increased in 1-MN-treated mice. A dose related increase was observed in male relative brain weight (significant at both doses). In addition, a dose related increase was observed in female relative salivary gland weight, male and female relative heart weight (significant at both doses) and female pancreas weight (significant at high dose). The incidences of bronchiolar/alveolar adenomas in the lungs of male mice (but not females) were significantly (but not dose-dependently) increased compared to the controls (4.1, 26.0 and 24.0%, for controls, 0.075 or 0.15% 1-MN, respectively, see Table below). No significant difference was observed in the incidences of bronchiolar/alveolar carcinomas between 1-MN-treated and control mice (Murata et al., 1993).

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

TABLE 2
Incidences of Tumors in B6C3F1 Mice Administered 1-Methylnaphthalene in the Diet for 81 Weeks

	Incidence (%)					
	Female			Male		
Dose (%)	0	0.075	0.15	0	0.075	0.15
No. of mice	50	50	49	49	50	50
	Tumors					
Lung						
Adenoma	4 (8.0)	2 (4.0)	4 (8.2)	2 (4.1)	13 (26.0)*	12 (24.0)*
Adenocarcinoma	1 (2.0)	0	1 (2.0)	0	0	3 (6.0)
Total	5 (10.0)	2 (4.0)	5 (10.2)	2 (4.1)	13 (26.0)*	15 (30.0)*
Liver						
Adenoma	0	2 (4.0)	4 (8.2)	9 (18.0)	1 (2.0)	1 (2.0)
Hepatocellular carcinoma	0	0	1 (2.0)	11 (22.4)	6 (12.0)	3 (6.0)
Hemangioma	0	0	0	0	1 (2.0)	0
Lymphoma/leukemia	4 (8.0)	2 (4.0)	3 (6.1)	1 (2.0)	0	1 (2.0)
Harderian gland tumor	3 (6.0)	2 (4.0)	1 (2.0)	0	0	0
Pancreas: Islet cell tumor	0	0	1 (2.0)	1 (2.0)	0	0
Skin						
Keratoacanthoma	0	0	0	1 (2.0)	0	0
Fibroma	0	0	0	0	0	1 (2.0)
Ovary						
Granulosa cell tumor	0	1 (2.0)	0	0	0	0
Theca cell tumor	1 (2.0)	0	0	0	0	0
Mammary gland: Adenocarcinoma	1 (2.0)	0	0	0	0	0
Urethra: Papilloma	0	0	0	1 (2.0)	0	0
Spleen: Hemangioma	0	0	1 (2.0)	0	0	0
Osteosarcoma	0	0	1 (2.0)	0	0	0

* Significantly different from the control value by χ^2 test at $p < 0.05$

Simultaneously with the previous study, another public literature study (Murata, 1997 IIA 5.5/05 non-GLP) provided B6C3F1 mice (50/sex/dose) with diets containing 0, 0.075, or 0.15% 2-methylnaphthalene (2-MN, purity >97%) for 81 weeks. Similar effects were observed. Average intake of 2-MN was 50.3 and 54.3 mg/kg bw/day in the 0.075% group, and 107.6 and 113.8 mg/kg bw/day in the 0.15% group for females and males, respectively. No effects on mortality were observed. Body weight gain was slightly reduced in the 0.15% dose group (4.5 and 7.5% in females and males, respectively). Both doses induced pulmonary alveolar proteinosis at high incidence: 55.1 and 45.8% in females and 42.9 and 46.9% in males, respectively, compared to 10 and 8.2% in female and male controls. The relative brain and kidney weights of treated male mice were significantly increased. The incidences of total lung tumours, including bronchiolar/alveolar adenomas and carcinomas, were 20.4 and 12.2% in male mice given 0.075 and 0.15% 2-MN, respectively, compared with the 4.1% in control males; significantly increased only at the lowest dose (see Table below). No such effect was observed in females or in the respective incidences of the adenomas and carcinomas (Murata, 1997).

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

TABLE 1
Incidences of Tumors in B6C3F₁ Mice Given 2-Methylnaphthalene in the Diet for 81 Weeks

Lesions	Female			Male		
	Dose (%): No. of mice:	0 50	0.075 49	0.15 48	0 49	0.075 49
	Tumor incidences (%)					
Lung						
Adenoma	4 (8.0)	4 (8.2)	5 (10.4)	2 (4.1)	9 (18.4)	5 (10.2)
Adenocarcinoma	1 (2.0)	0	1 (2.1)	0	1 (2.1)	1 (2.0)
Total	5 (10.0)	4 (8.2)	6 (12.5)	2 (4.1)	10 (20.4)*	6 (12.2)
Liver						
Hepatocellular adenoma	0	1 (2.0)	0	9 (18.4)	2 (4.1)	2 (4.1)
Hepatocellular carcinoma	0	0	0	10 (20.4)	6 (12.2)	6 (12.2)
Lymphoma/leukemia	4 (8.0)	1 (2.0)	5 (10.4)	1 (2.0)	0	1 (2.0)
Harderian gland tumor	3 (6.0)	0	0	0	3 (6.1)	1 (2.0)
Pancreas, islet adenoma	0	0	0	1 (2.0)	2 (4.1)	0
Skin, keratoacanthoma	0	0	0	1 (2.0)	0	0
Ovary, granulosa cell tumor	1 (2.0)	4 (8.2)	1 (2.1)	—	—	—
Mammary gland, adenocarcinoma	1 (2.0)	0	0	—	—	—
Cervix, leiomyosarcoma	0	1 (2.0)	0	—	—	—
Urethra, papilloma	0	0	0	1 (2.0)	0	0
Spleen, hemangioendothelioma	0	0	1 (2.1)	0	0	0
Malignant fibrous histiocytoma	0	1 (2.0)	0	0	0	0
Total tumor number	14	12	13	25	23	16
Tumor-bearing mice	12 (24.0)	9 (18.4)	11 (22.9)	21 (42.8)	17 (34.7)	13 (26.5)

* Significantly different from the control value by Fisher's exact test at $p < 0.03$.

The fact that nonneoplastic lesions are not observed in other lung regions or in other tissues indicates that the alveolar region of the lung is a specific toxicity target of chronic oral exposure to methylnaphthalene or 2-methylnaphthalene. Increased incidences of pulmonary alveolar proteinosis are observed following exposure of mice to 1-methylnaphthalene or 2-methylnaphthalene in the diet for 81 weeks (Murata 1993, 1997), as well as after dermal exposure of mice (dermally exposed to 30 or 119 mg/kg bw/day of methylnaphthalene for 30–61 weeks) to a 2:1 mixture of 2-methylnaphthalene and 1-methylnaphthalene (Emi and Konishi 1985; Murata et al. 1992). Subchronic exposure (13-week exposure to concentrations as high as 1.33% 2-MN) however, did not induce alveolar proteinosis (Murata, 1997).

Type II pneumocytes are enriched in cytochrome P-450 monooxygenases (Castranova et al. 1988, IIA 5.5/08), which are involved in metabolizing 2-MN (Shultz et al. 2001 IIA 5.1/16). It is hypothesized that, in response to 1-methylnaphthalene or 2-methylnaphthalene, type II pneumocytes produce increased amounts of lamellar bodies due to hyperplasia and hypertrophy, and eventually transform into balloon cells. Balloon cell rupture has been hypothesized to lead to the accumulation of the myelinoid structures in the alveolar lumen. It is unknown whether the parent compounds or metabolites are responsible for the development of methylnaphthalene-induced pulmonary alveolar proteinosis.

Whereas chronic oral exposure targets alveolar type II pneumocytes, acute intraperitoneal injection of 2-methylnaphthalene into mice targets bronchiolar Clara cells, inducing Clara cell abnormalities, focal or complete sloughing of Clara cells, or complete sloughing of the entire bronchiolar lining. Studies with naphthalene have indicated that mice are markedly more susceptible than rats to acute naphthalene induced Clara cell injury (Buckpitt et al. 1992 (IIA 5.5/09); West et al. 2001 (IIA 5.5/10)). Mice exposed by inhalation to 10 or 30 ppm naphthalene for 2 years showed lung inflammation, but rats exposed to concentrations up to 60 ppm showed no lung inflammation (Abdo et al. 2001 (IIA 5.5/11); NTP 1992 (IIA 5.5/12), NTP 2000 (IIA 5.5/13)). The species difference in lung susceptibility to naphthalene has been correlated with higher rates of formation of a specific enantiomeric epoxide (1R,2S-naphthalene oxide) in lung microsomes and isolated dissected airways of mice compared with rats (Buckpitt et al. 1992, 1995 (IIA 5.5/09 and IIA 5.5/14)).

Rat, hamster, and monkey lung microsomes preferentially formed the 1S,2R-naphthalene oxide enantiomer and showed lower rates of formation of epoxides than mouse lung microsomes (Buckpitt et al. 1992 IIA

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

5.5/09). *Microsomes from human lymphoblastoid cells expressing recombinant human CYP2F1 also showed preferential formation of the 1S,2R-naphthalene oxide enantiomer (Lanza et al. 1999 (IIA 5.5/15)), providing some evidence that human transformation of naphthalene and presumably also of alkylated naphthalenes to reactive epoxides in lung tissue may be more like rats than mice.*

The formation of the highly reactive epoxide intermediates from 2,6-DIPN or 1,4-dimethylnaphthalene is much lower than from 1- or 2-MN, which implies that systemic pulmonary alveolar proteinosis is unlikely with these compounds.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Study 1 – Extended one-generation study

Characteristics

reference	: IIA 5.6/07 (Study No, 10-593)	exposure	: continuously through the study period
type of study	: Extended one-generation study	doses	: 0, 500, 2000 or 7500 mg/kg food ¹
year of execution	: 2010-2011	vehicle	: None
test substance	: 1,4-dimethylnaphthalene Batch no: F243A040/14D06B01-01, purity: 98.4%	GLP statement	: Yes
route	: oral	guideline	: in accordance with OECD draft guideline for extended one-generation study, March 2010
species	: rat, Sprague-Dawley	acceptability	: Acceptable
group size	: 24/sex/dose	NOAELpar	: 40 mg/kg bw/day
		NOAELdev	: 160 mg/kg bw/day
		reproductive effects	: 510 mg/kg bw/day

¹ Equivalent to 0, 40, 160, 510 mg/kg bw/day for both males and females (based on average daily intake of parental animals, F1 animals gave slightly higher values).

Study design

The study was performed in accordance with the OECD guideline for extended one generation studies (OECD 443). The study design was based on the draft guideline of 25 March 2010. Cohort 2 for developmental neurotoxicity testing was not included in view of the absence of neurotoxicity in any other study, including a 90-day study with extensive neurobehavioural examinations.

1,4-dimethylnaphthalene was administered, as an admixture in the diet, at target concentrations of 0, 500, 2000 and 7500 mg/kg diet to 24 parental Sprague-Dawley rats/sex/group for 2 weeks prior to mating, during the 2-week mating period, for up to 10 weeks for the parental males and through gestation, lactation and until scheduled necropsy for the parental females. F1 pups were exposed to 1,4-DMN during the lactation phase of the study and selected F1 offspring were exposed to the same concentration of the diet admixtures as the

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

parental dams from rearing until scheduled sacrifice. F1 offspring were culled to 10 pups (5/sex), when feasible. Approximately 30 pups/sex/group were selected for additional evaluation.

The F1 offspring were subdivided into two Cohorts; Cohort 1 was treated until 13 weeks of age and then subjected to a complete necropsy, while Cohort 2 was utilized for immunotoxicity assessment around 8 weeks of age (T-cell dependant antibody response). Thyroid hormones (thyroxine and thyroid stimulating hormone) were determined in P and F1 animals (PND 4 and 26).

Food consumption, body weights, body weight gain, reproductive performance, organ weights, clinical pathology and histopathology were evaluated during the study, along with offspring body weights (growth), measurement of anogenital distance, survival and developmental landmarks (vaginal patency and preputial separation for the F1 generation).

Results

Table 3.10.1.1-1:

Dose (mg/kg food)	0		500		2000		7500	
	m	f	m	f	m	f	m	f
<u>P animals</u>								
Mortality	No treatment related findings							
Clinical signs	No treatment related findings							
Food consumption (day 3)					-11%*	-11%	-57%*	-61%*
Body weight gain							-25%*	-30%*
Body weight							-10%*	-15%*
Clinical chemistry								
cholesterol	53	42	62	51	77*	68*	84*	94*
gamma-GT	0.0	0.2	0.0	0.0	0.1	0.4	0.3*	3.1*
triglycerides	48	36	48	46	48	53*	34	51*
Haematology								
PT (sec)	10.7	10.6	10.5*	10.6	10.4*	10.6	10.2*	10.5
aPTT (sec)	18.6	18.1	18.3	17.9	17.9	17.3*	18.3	16.7*
Urinalysis	No treatment related findings							
Immunotoxicity	No treatment related findings							
TDAR, splenic lymphocyte subpopulations, lymph node weights	No treatment related findings							
Oestrus cycle								
- frequency (days)		3.7		3.6		3.6		3.5
Sperm evaluation	No treatment related findings							
Organ weight								
liver					ic ^{ar}	ic ^r	ic ^{ar}	ic ^{ar}
spleen							dc ^a	dc ^a
adrenals						dc ^a		dc ^{ar}
testes							ic ^r	
Pathology								
<u>Macroscopy</u>	No treatment-related findings							
<u>Microscopy</u>	No treatment-related findings							

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Dose (mg/kg food)	0		500		2000		7500	
	m	f	m	f	m	f	m	f
Reproduction parameters								
Mating	No treatment-related findings							
Fertility - fertility index	100		100		100		100	
Gestation - length of gestation (days) - gestation index	22.1 100		22.0 100		21.9 100		22.1 95.8	
Number of implantation sites	11.7		13.3		12.7		10.0	
F₁ generation								
Living pups at first litter check	11.2		12.9		12.1		9.6	
Dead pups at first litter check	0.04		0.04		0.04		0.09	
Postimplantation loss (%)	4.2		2.9		4.9		11.3	
Postnatal loss (%)	0.4		0.3		0.4		1.8	
Viability index (PND 0-4)	93.4		94.5		99.3		90.9	
Lactation index (PND 4-21)	95.9		99.5		99.6		92.7	
Sex ratio	No treatment-related findings							
Clinical signs	No treatment related findings							
Litter body weight -PND 4 -PND 14			-8%*		-14%* -16%*		-22%* -21%* -45%* -56%*	
Pup body weight -at preputial separation (g) -at vaginal patency (g)	161 118		163 122		168 118		144* 105*	
Sexual maturation - anogenital distance/litter (cm) - preputial separation/litter (days) - vaginal patency (days) - onset of 1st oestrus (days)	6.6/3.2 37 34 2		6.4/3.1 38 36 2		6.3/3.2 40* 35 2		6.1/3.1 46* 43* 1	
F1 Food consumption					-17%* -10%		-50%* -38%*	
F1 body weights					-11%* -11%*		-53%* -46%*	
Haematology PT (sec) aPTT (sec)	10.6 17.6		10.3 17.0		10.3* 17.3		10.3 16.7	
	10.3 16.8		10.2 16.9		10.1* 16.9		10.1 15.5*	
Clinical chemistry Cholesterol (mg/dL) gamma-GT creatinin triglycerides	58 0.0 0.34 58		50 0.3 0.35 30		66 0.0 0.30 50		51 0.4 0.32 33	
	73* 0.0 0.29 44*		73* 0.7 0.29* 40		80* 1.9* 0.31 35*		123* 9.2* 0.29* 43*	
Urinalysis	No treatment related findings							

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Dose (mg/kg food)	0		500		2000		7500	
	m	f	m	f	m	f	m	f
Immunotoxicity TDAR, splenic lymphocyte subpopulations, lymph node weights	No treatment related findings							
Sperm evaluation	No treatment related findings							
Oestrus cycle - frequency (days)	3.2		2.8		3.0		3.0	
Organ weight liver spleen adrenals kidneys thyroid pituitary					ic ^{ar}	ic ^r	ic ^r dc ^a dc ^a dc ^a	ic ^r dc ^a dc ^a dc ^a dc ^a
Pathology								
<u>Macroscopy</u>	No treatment-related findings							
<u>Microscopy</u> kidney, mod. tubular karyomegaly							1/10	

- dr dose related
- dc/ic statistically significantly decreased/increased compared to the controls
- d/i decreased/increased, but not statistically significantly compared to the controls
- a/r absolute/relative organ weight
- t transiently
- * significantly different from control (p≤0.05)
- ** significantly different from control (p<0.01)

No test substance related mortality was seen during the study period, and no treatment-related changes in clinical signs were observed.

Food consumption was significantly reduced in the high-dose group in the first days of treatment (approximately 60%), and reduced significantly reduced throughout the study period, although less severe than in the first week. Reduced food consumption was likely due to palatability.

In the mid-dose group, food consumption was slightly reduced, when compared to controls.

Body weight (approximately 10% in males and 15% in females) and body weight gain (around 30%) were significantly reduced in the high dose animals, and is considered to be due to the reduced food intake and the related reduced body weight gain in the first week of treatment for males, and the reduced body weight gain throughout the study period for females.

Reproductive performance and litter viability were unaffected by treatment. No treatment related changes in sperm morphology and oestrus cycle were observed. The number of implantation sites and total born alive were similar across groups; however, the high dose groups showed the lowest number of implantation sites and a reduced incidence of live births. These changes were not statistically significant, but may have been a secondary effect to the reduced body size (as indicated by reduced body weight) of dams in this group. No significant differences in the percent loss of fetuses between implantation and delivery were observed. A single high dose dam had a non-viable litter with 100% fetal loss prior to delivery, but at this incidence rate (1/23) the observation was considered within normal biological variation and unrelated to treatment.

No statistically significant difference in offspring survival was detected between the treated and control groups. Pup survival was above 90% throughout lactation and was generally similar across groups. Viability

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

and lactation indices were unaffected by treatment. In addition, there were no changes in sex ratio, and the average number of surviving pups by sex was similar across groups.

Litter body weights were significantly reduced in the high dose group on the day of birth; this trend persisted throughout lactation and continued after weaning. From PND 7 to 21, litter body weights were reduced > 40% in both males and females of the high dose. Litter body weights were also reduced at the mid dose for both males (10-14%) and females (15-16%), and for females only at the low dose (8-13%). This latter change occurred from day PND 7 to PND 21. Since it was not accompanied by further changes in development, it was not considered toxicologically relevant.

Anogenital distance as measured in PND 4 was not significantly different in the treated groups when compared to control. Preputial separation was delayed in the mid and high dose group and vaginal patency was delayed in the high dose group. Preputial separation and vaginal patency were delayed in the high dose group which was not unexpected as these developmental landmarks are closely linked to body weight. In addition, preputial separation was delayed in the mid dose group (average PND 40, range PND 35-46). The onset of oestrus following observed patency in selected F1 female offspring (Cohort 1) was not affected by treatment and averaged 1-3 days.

The applicant provided historical control data for preputial separation, generated by the laboratory in the period 2007-2008. Preputial separation has been observed in historical controls to occur on average around PND 44 (range PND 41-50); as such the delay noted in the 2000 mg/kg group was within normal biological variation. Moreover, the values in controls in the study (average PND 37, range PND 35-41) and low dose group (average PND 38, range PND 35-41) appear to be rather low, compared to the historical controls.

Recent In-House Historical Data Generated by Experimur from 2007-2008

Preputial Separation

Sprague-Dawley Male Rats	Age (Days)	Body Weight (g)
Mean	44.7	229.5
SD	2.5	20.3
Min	41.0	191.0
Max	50.0	273.0
N	46	46
2SD	4.9	40.5
Mean+2SD	49.6	270.0
Mean-2SD	39.7	188.9

Food consumption, body weight and body weight gain of F1 pups selected for Cohort 1 and 2 were significantly reduced in all dose groups during the first three weeks of independent living. This was also considered to be due to palatability. In the low dose groups, food consumption was comparable to controls from week 4 in F1 males and week 6 for F1 females. For mid and high dose group F1 males food consumption was reduced from week 1-10, for mid dose F1 females food consumption was reduced only in week 3-5 and for high dose F1 females food consumption was reduced from week 1-9. For low and mid dose group F1 males and females, body weight recovered from week 5.

Thyroxine (T4) and TSH were measured in both the parental and F1 generations as well as in PND26 weanlings, and no significant treatment-related changes were observed.

In the immunotoxicity assessment in F1 animals, no treatment related changes were observed.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Upon haematological examination, slight decreases in coagulation parameters (PT and/or aPTT) were noted in parental and F1 males and females of all dose groups. Other statistically significant changes in haematology (reduced haemoglobin, increased reticulocytes, decreased neutrophils and increased lymphocytes) were slight and not considered toxicologically relevant. Although consistency seen over parental and F1 animals, the changes in PT and aPTT were only slight and therefore of unknown toxicological relevance. As these changes were not observed in the subacute and chronic toxicity study in rats, it was concluded that these changes at the low dose need not further to be considered for setting of the parental NOAEL, as at the low dose no further changes were seen.

At clinical biochemistry, increased in GGT and cholesterol levels were noted in both males and females of the high dose parental and F1 generation. In addition, increased cholesterol was also noted at the mid dose group. Triglycerides were increased in the high dose parental and F1 females, while triglycerides were reduced in high dose F1 males. Creatinin was reduced in mid and high dose parental and F1 females, and in mid dose parental males only.

Increased absolute and relative liver weights were observed in parental males and the mid and high dose groups (> 10% for both absolute and relative weights at the mid dose, and > 20% in the high dose). In parental males absolute spleen weights were decreased at the high dose (17%), and in parental high dose females absolute and relative spleen weights were decreased (22 and 12%, respectively). In high dose parental males testes weights were slightly increased (114%), and in parental females absolute and relative adrenal weights were decreased (25 and 16%, respectively, in mid dose parental females only absolute adrenal weight was decreased (15%). In F1 males absolute and relative liver weight were increased in mid dose (both 118%) and high dose (110 and 138%, respectively). In F1 females relative liver weight was increased in mid dose (113%) and high dose (152%). Several absolute organ weights were decreased in F1 high dose animals (i.e. adrenals, spleen, pituitary), however, these decreases were considered to be associated to the reduced body weight at the high dose.

In F1 weanlings, organ weights at PND 26, particularly the spleen and thymus, were reduced in all dose groups. However, these changes were considered to be due to the reduced food consumption, no histopathological correlates were seen and were reversible in the low and mid dose groups, as these organ weight changes were not seen in the adult F1 rats.

No treatment-related findings were seen at macroscopy.

A single treatment-related finding was seen in the kidney of one adult high dose F1 male. The findings in this animal consisted of moderate karyomegaly which was present in the inner region of the renal cortex of this rat. No other histopathological findings were deemed to be treatment-related and non of the reproductive organs showed evidence of toxicity.

Acceptability

The study is considered acceptable.

Conclusions

Based on the minimal changes in liver weight and cholesterol at the mid dose groups, the NOAEL for parental toxicity is set at 500 mg/kg food (equivalent to 40 mg/kg bw/day).

The NOAEL for developmental toxicity can be set at 2000 mg/kg food (equal to 160 mg/kg bw/day), based on delayed vaginal patency and preputial separation, and reduced body weight.

As no fertility effects were observed, the NOAEL for reproductive toxicity is set at 7500 mg/kg food (equal to 510 mg/kg bw/day).

3.10.1.2 Study 2 – Developmental toxicity, rabbits

STUDY 1

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Characteristics

reference	: IIA 5.6/08, Study No. 10-601	exposure	: days 6-28 of gestation, gavage
type of study	: teratogenicity study	doses	: 25, 80 and 250 mg/kg bw/d
year of execution	: 2010-2011	vehicle	: Corn oil
test substance	: 1,4-dimethylnaphthalene Batch no: 14D06B01-01, purity: 98.4%*	GLP statement	: yes
route	: Oral	guideline	: in accordance with OECD 414
species	: New Zealand White rabbits	acceptability	: acceptable
group size	: 23 females/dose	NOAEL _{mat}	: 80 mg/kg bw/d
		NOAEL _{dev}	: 250 mg/kg bw/d
		teratogenic effects	: not observed

* purity not provided in study report, but since the batch numbers was equivalent to the batch number of the substance in the extended one generation study, purity was considered equivalent.

Study design

The study was performed in accordance with OECD 414.

Results

Table 6.6.2.1

Dose (mg/kg bw/day)	0	25	80	250	dr
<u>Maternal effects</u>					
Mortality (n=23)	0	0	0	1	
Clinical signs	No treatment-related findings				
Non-pregnant animals	2	3	2	5	
Aborted animals	2	2	1	3	
Pregnant animals	19	18	20	15	
Body weight gain					
post-coitum day 6-9	12	1	-9	-51*	
post-coitum day 9-12	22	6	30	-13*	
Food consumption					
post-coitum day 6-9	480	403	456	309*	
post-coitum day 9-12	499	379	466	275*	
Body weight					
post-coitum day 9-18				d	
Pathology					
macroscopy	No treatment-related findings				
<u>Litter response</u>					
Number of dams examined	19	18	20	15	
Corpora lutea/dam	10.4	10.2	9.1	9.7	
Pre-implantation loss (%)					
-viable and non-viable litters	2.6	3.4	8.3	10.1	
-viable litters	2.6	3.4	8.8	7.2	
Post implantation loss (%)					
-viable and non-viable litters	2.9	5.6	8.2	8.8	
-viable litters	2.9	5.6	3.4	2.3	

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Implantations/dam	10.1	9.8	8.2*	8.9
Number of resorptions/dam				
- early	0.2	0.2	0.4	0.3
- late	0.0	0.1	0.0	0.0
Non-viable litters	0	0	1	1
Dams with live foetuses	19	18	19	14
Live foetuses/dam	9.8	9.3	7.8	8.7
Foetal weight				
male	36.5	39.5	38.6	36.3
female	35.4	37.3	37.2	33.0
Sex ratio	No treatment-related findings			
<u>Examination of the foetuses</u>				
External observations	No treatment-related findings			
Skeletal findings	No treatment-related findings			
Visceral findings	No treatment-related findings			

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

* significantly different from control (p<0.05)

** significantly different from control (p<0.01)

Test substance concentrations were within 7% of the target concentrations.

One rabbit in the high dose group was found dead on gestation day 21, likely related to the abortion that occurred one day earlier. Abortions occurred in all dose groups, without a dose-related response. No treatment related changes in clinical signs, were observed in maternal animals. Food consumption and corollary body weight gain were reduced in high dose maternal animals over gestation days 6-12. Absolute body weights were reduced when compared to controls in the high dose group on gestation days 9-18. Alterations in uterus weight were not observed nor were changes seen in maternal body weight or body weight gain when corrected for uterus weight. As such, the changes seen early on in gestational body weight gain were considered to be solely associated with maternal toxicity.

Overall, no treatment-related differences in litter viability were detected. A single statistically significant reduction in total implants was seen in the 80 mg/kg/day group. The number of total implants was also lower, but not significantly at 250 mg/kg/day group. The lower number of total implants may have been associated with a lower number of corpora lutea, as the 80 and 250 mg/kg/day groups also had on average fewer corpora lutea (again not significantly lower). The average number of non-live implants (resorptions and deaths) was similar across groups and did not appear to be affected by treatment with 1,4-dimethylnaphthalene. The percent pre- and post-implantation loss was highest in the 80 and 250 mg/kg/day groups. Each of these groups had a single doe with a non-viable litter; exclusion of the non-viable litter showed that the post-implantation loss was similar across groups, while the pre-implantation loss remained higher in the 80 and 250 mg/kg/day groups even after exclusion of the non-viable litters. This change is considered toxicologically irrelevant as the exposure period is targeted to occur after implantation.

No treatment-related changes were noted in foetal weight and gross post mortem examination. External, visceral, cephalic and skeletal examinations revealed no treatment related variations or malformations.

Acceptability

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

The study is considered acceptable.

Conclusions

The maternal NOAEL for 1,4-dimethylnaphthalene in rabbits was established to be 80 mg/kg bw/d based on decreased body weight gain and reduced food consumption. The NOAEL for developmental toxicity was 250 mg/kg bw/d. No irreversible structural changes were observed in foetuses.

3.10.1.3 Study 3 – Public literature information

In a study from public literature (Kawai et al, 1977, IIA, 5.6/01) pregnant ICR-JCL mice (four weeks, 18-20g) were treated by gavage from days 6-12 of gestation with **2,6-di-isopropylnaphthalene** (purity not reported) at doses of 0, 19.2 and 192 mg/kg bw/day. Feed and water was supplied ad libitum. Each group within P₁ consisted of 20 days. Of these 10 were killed on day 18 of gestation and organs were examined macroscopically. The number of implantation sites and absorbed dead embryos was examined. Body weights of the pups was measured. The pups were examined for the presence of absence of fetal abnormalities. Skeletal evaluation was carried out using Alizarin red S staining. The ten remaining dams were allowed to deliver and F1 offspring were obtained. The numbers of those alive and dead at birth, the sex, and external abnormalities were observed, and body weights measured. Live pups were kept with the dams during the lactation period. Bodyweight of the pups was measured during this time On Day 21 after birth, touch (Pryer reaction), hearing (Haffner method), mobility (balance, gait), and other functions and senses were tested. Six weeks after weaning five males and five females were killed and major internal organs were observed macroscopically, the masses of their internal organs measured, and histopathological investigations conducted and on another five males and five females per group skeletal formation was examined using super soft X-ray photography. At ten weeks of age another 10 mice were mated and for the offspring the same examination was carried out as for the F1 offspring.

No significant effect on body weights occurred in dams. Through P1 and P2, no significant ($p > 0.05$) differences were observed between the dosed groups and the respective control groups in the average numbers of implants per mouse, numbers of offspring per mouse, numbers of absorbed dead embryos per mouse, or average body weights of the fetuses per mouse, and in addition, no fetal external, intraoral or visceral abnormalities were seen within any group.

The EPA (2003, IIA 5.6/05) has reviewed a prenatal developmental toxicity study of **2,6-diisopropylnaphthalene** in rats. The study was conducted at doses of 0, 50, 150, and 500 mg/kg bw/day. Decreased fetal body weight and a slightly increased incidence of a skeletal alteration (fusion of cartilaginous bands in the cervical centra) was observed at the highest dose level, in the presence of maternal toxicity. The NOAEL for maternal toxicity was considered to be 50 mg/kg bw/day based on decreased body weight and food consumption and the NOAEL for prenatal developmental toxicity was considered to be 150 mg/kg bw/day based on an slightly increased incidence of skeletal alteration (fusion of cartilaginous bands in the cervical centra).

In a study from public literature (Noda et al., 1982, IIA 5.6/06) pregnant Wistar rats (22-24/dose, 11 weeks at start of the study) were treated by gavage from gestation days 0-19 with **methylnaphthalene** (purity not reported, supplied by Tokyo Kasei Kogyo) at 0, 0.016, 0.063 or 0.25 ml/kg bw/day, equal to 0, 16, 63 and 250 mg/kg bw/day, assuming 100% purity and a specific gravity of 1. Olive oil was used as solvent. Dosing was based on a preliminary study in 6 non-pregnant females. It is not clear whether 1-methylnaphthalene or 2-methylnaphthalene was used. Bodyweight and food and water intake was measured on each day and clinical signs were observed. On gestation day 20 the dams were killed and the effects of treatment with methylnaphthalene on the fetuses were investigated. The numbers of implantation scars, absorbed embryos, dead fetuses, and live fetuses were recorded. Various organs from the chest and stomach areas were dissected. The body weights of the live fetuses were measured, and they were examined for sex and external abnormalities. In addition, the numbers of corpora lutea in the dams' ovaries were examined. Approximately half of the live fetuses from each dam were fixed in 90% ethanol, and their skeletons stained with Alizarin red S. These were examined for degree of ossification and for the presence and absence of skelate

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

abnormalities and variations. The remaining live fetuses were fixed in Bouin's solution, and were examined using the Wilson⁴) method for the presence or absence of visceral abnormalities.

No effect on bodyweight occurred. A slight reduction in food intake (<10%) was observed in the medium and high dose group dams, but from day 6 of gestation this recovered. Increased water intake was observed throughout gestation in the treatment groups. Since there were no changes in serum levels of urea nitrogen, Na⁺, or K⁺, or in kidney weights, kidney failure was not considered, and the cause of these increases was not known. No treatment-related effects on the developing fetuses were reported.

3.11 Specific target organ toxicity – single exposure

3.11.1 Animal data

See summaries in sections 3.1-3.3.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Study 1 – 13 week rat

Characteristics

Reference/notifier	: IIA 5.6/02, Doc ID 02-154	Exposure	: Repeated by diet, 13 weeks
Type of study	: 13-week toxicity study in rat	Doses	: 0, 500, 2500 and 10.000 ppm**
Year of execution	: 2002	Vehicle	: -
Test substance	: 1,4-dimethylnaphthalene, liquid batch no.: 01C-01 purity: not reported in the study report*	GLP statement	: yes
Route	: oral	Guideline	: OECD 408
Species	: Rat, Sprague-Dawley (CRL :CD(SD)IGSBR)	Acceptability	: Acceptable
Group size	: 10/sex/dose	NOAEL	: 32 mg/kg bw/day

* Purity 98.8% according to batch analysis volume 4

** Equivalent to 0, 32, 161 and 677 mg/kg bw/d in males and 0, 38, 186 and 747 mg/kg bw/d in females

Study design

The study is in accordance with OECD 408, water consumption was not measured and blood clotting potential was not analyzed.

Rats (10/sex/dose) were given 0, 500, 2500 or 10.000 ppm 1,4-dimethylnaphthalene for 13 weeks in their diet. Two additional groups (control and high dose group, 10/sex) were held for an additional 28 days to evaluate the recovery potential from 1,4-dimethylnaphthalene-induced effects (0 and 10.000 ppm). Full histopathology (including reproductive organs) was confined to controls and high dose animals, except for kidneys which were histopathologically examined in all dose groups.

Results

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Table 3.12.1.1-1 Results from a 13-week oral toxicity study in rats

Dose (mg/kg food)	0		500		2500		10,000		dr
Sex	m	f	m	f	m	f	m	f	
Mortality	0/20	0/20	0/10	0/10	0/10	0/10	1/20	0/20	
Clinical signs									
-Alopecia Abdomen	0/20	0/20	0/10	0/10	0/10	0/10	0/20	2/20	
-Alopecia Ventral	0/20	0/20	0/10	0/10	0/10	0/10	0/20	4/20	
-Rough fur coat	0/20	4/20	0/10	1/10	0/10	1/10	1/20	12/20	
-Discoloured inguinal fur	0/20	0/20	0/10	0/10	1/10	0/10	12/20	1/20	
-Vaginal discharge		0/20		0/10		0/10		1/20	
Body weight gain (g) (% of control)	323	126	303 (94)	132 (105)	289 (89)	121 (96)	214* (66)	86* (68)	m
Food consumption					ds (10%)		ds (10- 20%)	ds (10- 20%)	m
Water consumption	Not performed								
Ophthalmoscopy	No toxicologically relevant effects								
FOB									
-Grip strength forelimb	1.27	0.9	1.06	0.93	1.05	0.84	0.95*	0.97	m
Haematology									
-HGB (g/dL)	15.3	15.0	14.9	14.8	15.3	14.7	14.5	14.4*	(f)
-MCH (pg)	18.2	18.7	17.4*	18.6	17.8	18.3	17.5*	18.0*	(f)
-MCHC (g/dL)	35.9	36	35.6	36	35.5	36	35.4	35*	
-Eos (x10 ³ cells/ μ L)	0.08	0.06	0.05*	0.07	0.09	0.05	0.08	0.06	
Clinical Chemistry									
-A/G ratio	0.9	1.1	0.9	1.0	0.9	1.0	1.0*	1.0*	
-Blood urea nitrogen (mg/dL)	13	17.4	16*	16.8	14	15.5*	15	13.8*	f m/ f
-Cholesterol (mg/dL)	54	59	63	74	69*	77	74*	116*	f
-Globulin (g/dL)	3.2	3.2	3.4	3.2	3.1	3.3	3.0*	3.3	
-Glucose (mg/dL)	148	116.2	150	113.2	141	120.1	132*	142.8	
-Triglycerides (mg/dL)	59	23	48	28	42	28	30*	21	m
Urinalysis	Not performed								
Absolute organ weights									
-heart (g)	1.59	0.880	1.43	0.914	1.53	0.933	1.34*	0.848	
-liver (g)	13.17	6.82	12.96	7.11	14.38	8.32*	13.29	9.64*	f
-spleen (g)	0.886	0.571	0.794	0.519	0.776	0.552	0.654*	0.476*	m
Relative organ weights									
-brain	0.413	0.729	0.444	0.703	0.458*	0.721	0.528*	0.843*	m
-heart	0.309	0.336	0.299	0.341	0.327	0.352	0.343*	0.373*	(f)
-kidneys	0.629	0.690	0.633	0.681	0.715*	0.734	0.785*	0.815*	m/ f
-liver	2.55	2.60	2.69	2.65	3.07*	3.13	3.32*	4.23*	f
-testes	0.714		0.775		0.717		0.941*		m/ f
Pathology									
macroscopy	No toxicologically relevant effects								
microscopy									
-CPN kidneys	1/10	0/10	1/10	0/10	1/10	0/10	5/10	1/10	
-basophilic tubuli kidneys	2/10		2/10		6/10		0/10		
-infiltration MNC kidneys	1/10	2/10	1/10	3/10	3/10	0/10	1/10	1/10	

* significantly different from controls (p<0.05), ds: significantly decreased

No treatment-related deaths were observed during the study. No treatment-related clinical signs were observed, except for discoloured inguinal fur, which was seen in 10% of the mid-dose males and in 60% of the high-dose males, and rough fur coat which was observed in 60% of the high-dose females. Body weight

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

gain was statistically significantly reduced by 32-34% in both sexes at the high dose. A statistically non significant decrease in body weight gain of 11% was observed in males of the mid-dose group. A dose-related decrease in food intake was observed: high-dosed rats (both sexes) consumed significantly less food throughout the study (10-20% decrease); mid-dosed male rats ate significantly less in the first 10 weeks (10% decrease). Effects on haematological and clinical chemistry parameters were mainly observed in the highest dose group and consisted of changes in haemoglobin, MCH, MCHC, A/G ratio, globulin, BUN, glucose, cholesterol and triglyceride levels. Cholesterol was increased in high-dosed females and in mid- and high-dosed males. The reduction in triglyceride levels in males may be related to the poor nutritional state. Although an increase in BUN is considered to reflect kidney toxicity, a reduction in BUN is not considered to be adverse. All other changes were small and concluded to be the result of biological variability instead of treatment-related. Increased relative organ weights (111-163% compared to controls) were observed in both males and females of the highest dose group and in males of the mid-dose group for a number of organs (including testes), which may be, at least partly, related to the observed decreased body weight gain in the highest dose group.

No gross pathological findings were observed. Microscopically, changes were observed in the kidneys and included an increased incidence and severity of chronic progressive nephropathy (CPN) in the high-dose group. The effects were more pronounced in males than in females. In addition, in mid-dose males the incidence of basophilic tubules was increased, and infiltration in mononuclear cells was observed.

Table B.6.3.3-2 Results from a 4-week recovery period

Dose (mg/kg food)	0		10,000	
	m	f	m	f
Mortality	0	0	1	0
Clinical signs				
-Alopecia Forelimbs	2/10	3/10	1/10	
-Alopecia Ventral	1/10			
-Red material around eyes	1/10			
-Discoloured inguinal fur			1/10	
Body weight gain (g)	30	10	53*	28*
Food consumption	No toxicologically relevant effects			
Water consumption	Not performed			
Ophthalmoscopy	Not performed			
FOB	No toxicologically relevant effects			
Haematology				
-MCHC (g/dL)	35.4	35.7	34.6*	35.4
-mono (%)	2.2	1.5	1.4*	1.3
-mono (x10 ³ cells/ μ L)	0.15	0.08	0.08*	0.07
Clinical Chemistry				
-Total bilirubin (mg/dL)	0.2	0.2	0.1*	0.2
-Chloride (mmol/L)	104	105	104	104*
-GLB (g/dL)	2.7	2.5	2.5*	2.7*

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Dose (mg/kg food)	0		10,000	
Sex	m	f	m	f
-Phosphate (mg/dL)	5.7	6.1	6.5*	6.1
-Total protein (g/dL)	5.8	5.9	5.6*	6.2*
-Sodium (mmol/L)	143	143	143	142*
Urinalysis	Not performed			
Absolute organ weights				
-adrenals (g)	0.071	0.068	0.054*	0.069
-heart (g)	1.59	0.94	1.44*	0.95
Relative organ weights				
-brain	0.401	0.699	0.463*	0.761*
-kidneys	0.606	0.660	0.651*	0.701
-liver	2.44	2.52	2.62*	2.85*
-testes	0.661		0.775*	
-ovaries		0.046		0.059*
Pathology				
macroscopy	No toxicologically relevant effects			
microscopy				
-CPN kidneys	1/10	0/10	7/10	3/10
-basophilic tubuli kidneys	2/10		2/10	
-infiltration MNC kidneys	3/10	5/10	2/10	2/10

* significantly different from controls (p<0.05)

During the 4 week recovery period, no differences were observed in food intake when compared to controls. Body weight gain was higher than in control rats, however, at the end of the recovery period, body weight of the high-dose groups was still significantly less than controls. Several changes were noted in haematology and clinical chemistry parameters of the recovery groups, but they are small and probably not treatment-related. Increases in relative organ weights were still observed for a number of organs (including testes), but effects were smaller than before recovery (106-115%). In addition, a small change in relative ovary weight was observed (128%), in contrast to directly after the exposure period. Chronic progressive nephropathy was still observed after the recovery period. The incidence of basophilic tubuli and infiltration of mononuclear cells was comparable to controls.

Acceptability

The study is considered acceptable.

Conclusions

In this 13-week dietary toxicity study in rats discoloured inguinal fur was observed in males at the mid and high dose group, while females at the high dose group showed rough fur coat. 1,4-dimethylnaphthalene induced dose-related reductions in body weight gain and food consumption in males at the mid dose group and in both sexes at the high dose group. Cholesterol was increased in females at the high dose group and in males at the mid and high dose group, while triglyceride levels in males were decreased at these dose levels. Increases in relative organ weights in the mid and high dose groups were probably at least partly related to

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

the lower body weights of these animals. However, increased relative liver weights (20% in both sexes at the mid dose group; 30% in males and 63% females at the high dose group) indicate that 1,4-dimethylnaphthalene induces an increase in liver weight, in particular in females. 1,4-dimethylnaphthalene at the high dose group resulted in an increased incidence of chronic progressive nephropathy, especially in males. However, chronic progressive nephropathy is a kidney alteration that occurs spontaneously in aging rats and is not considered relevant for humans¹.

During a 4 week recovery period the decreased body weight gain and increased relative organ weights in the high dose animals partly recovered.

In this study, the NOAEL was 500 mg/kg food, equivalent to 32 mg/kg bw/day based on clinical signs, an increase in cholesterol levels (males only), increased relative liver weight (males and females) and reduced body weight gain, food consumption and triglyceride levels (males only) observed at the mid dose group of 2500 ppm (161 mg/kg bw/d).

3.13 Aspiration hazard

Study 1 reference: Pelton 1993, IIA 2.1/02 Doc ID 4373-93-0226-AS

Material and methods

The viscosity of 1,4-dimethylnaphthalene was measured at 25°C using a Brookfield viscometer. The analysis was repeated with the same sample aliquot. A viscosity standard was also analysed in replicate.

Results

The mean viscosity was 6 mPA at 12 rounds per minute and 6 mPA at 30 rounds per minute. The kinematic viscosity is $6 \text{ mPa}\cdot\text{s} / 1.014 = 5.9 \text{ mm}^2/\text{s}$.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

STUDY 1

STUDY IIA, 7.7/01

Reference/notifier	: Yoshida, K. et al. (1983)	GLP statement	: No
Type of study	: MITI (I) test	Guideline	: OECD 301C = EEC C.4-F
Year of execution	: 1983	Acceptability	: acceptable
Test substance	: methylnaphthalenes		

Substance	Water type	T	pH	Duration	Transformation	Classification
-----------	------------	---	----	----------	----------------	----------------

¹ GC Hard and KN Khan. Invited Review: A Contemporary Overview of Chronic Progressive Nephropathy in the Laboratory Rat, and Its Significance for Human Risk Assessment *Toxicol Pathol* 2004; 32; 171

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

		[°C]	[d]	at end [%]	
1,4-DMN	test medium	25	n.r. 28	66	readily biodegradable

Description

The biodegradability of 1,4-DMN was evaluated using the MITI (I) test method. Inoculated test medium is dosed with 100 mg/kg of 1,4-DMN as the nominal sole source of organic carbon. Degradation is followed by the analysis of dissolved oxygen over a 28-day test period at 25°C. Evolved carbon dioxide is absorbed by soda lime. The dissolved oxygen uptake of the test solution (corrected for uptake of blank inoculum) is expressed as a percentage of the theoretical oxygen demand (ThOD) of the test substance (3.077 mg O₂/mg). The test contained an inoculum control group (30 mg/kg activated sludge from CITI, Japan), a reference group, and a treatment group (100 mg/kg). The inoculum control was used to measure the oxygen uptake of the inoculum and was not dosed with a carbon source. The reference chambers were dosed with aniline, a substance known to be biodegradable. Measurements of dissolved oxygen were performed on two test chambers from the control, reference, and treatment groups on days 7, 14, 21 and 28.

Results

The results indicate that the inoculum was active by degrading the reference substance within the acceptable range (at least 40% after 7 days and 65% after 14 days). The test substance, 1,4-dimethylnaphthalene, reached 66% degradation after 28 days. Moreover, the critical level of 60% degradation was reached within 10 days from the time that 10% degradation was achieved. The notifier states that 1,4-DMN is readily biodegradable under the conditions of this test.

The notifier states that the volatility of 1,4-DMN may have influenced the result.

Remarks

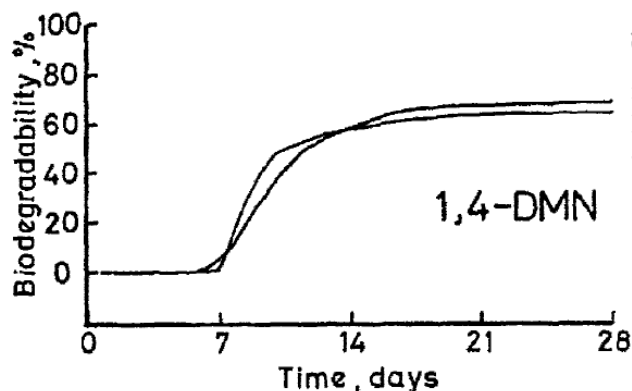
It is not fully clear from the study results that the the critical level of 60% degradation was reached within 10 days from the time that 10% degradation was achieved. However, based on the results it is very likely tht this criterium will be met 60% degradation will likely be met at day 17 and/or 10% degradation will not be met before day 8/9 (see below).

Test Substances	Acclimatization Period (days)	Degradation Level (%)			
		7 days	14 days	21 days	28 days
1,4-dimethylnaphthalene	6	6	58	67	68
	7	0	58	63	64

Therefore, it is very likely that a 60% degradation (60% of theoretical maximum) is achieved within 10 days of the start of degradation (taken as the time point where 10% is degraded).

The statement of the notifier considering the influence of the volatility on the results of the test is not clear to the RMS. The system used in the MITI test is a closed system under pressure which should prevent volatilisation from the system. Unfortunately, an abiotic control –as laid down in OECD 301 C- is not included in the test. This could have provided more information on the impact of volatilization. The shape of one of the degradation curves indicates a proper biological degradation (see below). The other curve, where loss dueto biodegradation increases steeply after 7 days, may indicate (some) effect of volatilization in the first few days. However, a full biphasic pattern, which would be expected in case volatiazation was a major process is not observed. Therefore the test is considered to be acceptable. Based on the result of the study it is concluded that 1,4-DMN is readily biodegradable.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE



4.1.2 BOD₅/COD

No data available.

4.1.3 Aquatic simulation tests

No further data needed for classification purposes.

4.1.4 Other degradability studies

4.1.4.1 Hydrolysis

No experimental data are available. The solubility of 1,4-DMN in water is very low (5.1 mg/L at 25°C). Based on the molecular structure, hydrolytic degradation of 1,4-DMN is expected to be very low in aquatic systems. Estimation of a DT₅₀ for hydrolysis is not possible with EPIWeb 4.0 (HydroWin). EUSES 2.1 estimation of DT₅₀ = 5.27 x 10⁵ day (20°C).

4.1.4.2 Water-sediment degradation

STUDY IIA 7.8/01

Reference/notifier	: DocID 535E-102	GLP statement	: Yes
Type of study	: biodegradation	Guideline	: OECD 308
Year of execution	: 2004	Acceptability	: not acceptable
Test substance	: [ring-U- ¹⁴ C]-1,4-DMN, lot # 3501-032, radiochemical purity 97.1%		

Substance	Soil type	Ratio sediment	T	pH	OM	Duration	Degradation at end	DT ₅₀ water	DT ₅₀ sediment	DT ₅₀ system
-----------	-----------	----------------	---	----	----	----------	--------------------	------------------------	---------------------------	-------------------------

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

		water	[°C]	[%]	[d]	[%]	[d]	[d]	[d]
		[g dwt/mL]							
[ring-U- ¹⁴ C]-1,4-DMN	silt loam aerobe	3:1	19- 20	6.9	3.6	56	-	-	-
[ring-U- ¹⁴ C]-1,4-DMN	silt loam anaerobe	3:1	20- 21	6.9	3.5	56	-	-	-

Description

The fate of [ring-U-¹⁴C]-1,4-DMN was examined in a water/sediment study. Sediment samples were taken from the upper 5-10 cm thickness layer of the underwater sediment layer of a creek. Aerobic sediment samples were collected in an area of the creek at which the depth of the water measured approximately 5 to 10 centimetres at the time of sediment collection. Anaerobic sediment samples were collected from the sediment layer of the creek covered by approximately 0.46 to 0.53 meters of water at the time of collection. Anaerobic sediment collection containers were filled and sealed while submerged to exclude atmospheric oxygen. The waters collected for use in the study were obtained from the same location and at the same time as that of the respective sediment. Anaerobic water collection containers were filled and sealed under the water's surface. Temperature, pH and dissolved oxygen measurements of the waters were taken at collection. The aerobic water measured 19.5°C, with a pH of 7.1 and a dissolved oxygen concentration of 9.5 mg O₂/L. Water collected from above the site of anaerobic sediment collection measured 19.2°C, with a pH of 7.0 and a dissolved oxygen concentration of 9.4 mg O₂/L.

Application of test substance

Appropriate amounts of sediment and water were added to each test chamber to achieve a water: sediment volume ratio of approximately 3:1 and a minimum sediment layer height of 2 cm.

All mineralization and transformation test chambers were fortified with the test substance by applying the radio-labelled dosing solution to the water layer of each chamber. Following the addition of the test substance, the water layer was gently mixed with minimal disturbance to the underlying sediment. The initial nominal concentration of test substance in all dosed test chambers was 1.5 mg/kg dry-weight equivalent sediment.

Sampling frequency

The mineralization apparatus CO₂ traps were sampled on days 14, 25 and 42. The 1st CO₂ trap of each set (before and after the combustion apparatus) was removed and three replicate 1 mL aliquots of each trap were analyzed for radioactivity by LSC. The 2nd trap in each set was moved to the 1st position and a new trap was placed in the 2nd trap spot. On day 56 all remaining traps attached to the abiotic mineralization test chambers were analyzed by LSC. Additionally, 0.1N KOH was added to each spacing bottle in these gas collection systems. Each was then analyzed by LSC to measure any residual radioactivity that may have been present in these bottles. The abiotic mineralization test chambers were then removed from their respective mineralization apparatus and a mass balance determination procedure was performed.

Sample processing and analysis

The day 0 transformation sediment/water systems allocated for analysis were centrifuged and the aqueous portion of each sample was removed. The aqueous volume separated from each test chamber was measured and triplicate aliquots of each aqueous phase were analyzed for radioactivity by LSC. Additional aliquots of each aqueous phase were analyzed for parent material using a HPLC with UV (220 nm) and radiometric

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

detection. Remaining sediments were extracted twice using methanol and analyzed for radioactivity by LSC and by HPLC/UV/beta-RAM. Sub-samples of the extracted solids were subsequently combusted and analyzed by LSC to determine the total remaining radioactivity. Transformation sediment/water systems analyzed on day 14 were processed in an identical manner.

Additional studies

To evaluate potential causes of the failure to adequately recover (^{14}C)-1,4-DMN from the mineralization system, three additional aerobic mineralization/volatilization tests were conducted. In the three tests, the sediment-water test system was replaced by a biologically active aqueous media to provide a less complex matrix for evaluation of the test substance and the mineralization apparatus. The additional tests utilized inocula associated with biodegradation tests of readily biodegradability. Yoshida et al. 1984 (IIA, 7.7/01) have reported the ready biodegradability of 1,4-DMN in MITI (I) method tests utilizing comparable media. Results from the additional tests that utilized a test chamber configuration identical to the one used in this study confirmed (^{14}C)-1,4-DMN was being lost to the rubber stopper assemblies used to affix the test chambers to the mineralization apparatus. Recovery results markedly improved with the elimination of the rubber stopper assemblies. The test chambers specifically designed for assessing aerobic aquatic biodegradation offered the best solution for evaluating the potential biodegradation of (^{14}C)-1,4-DMN. Preliminary work with this test system demonstrated the potential of (^{14}C)-1,4-DMN to undergo rapid degradation and/or volatilization in an inoculated mineral media test system. The various systems used to determine biodegradation and/or volatilization of the test substance consistently showed that 1,4-DMN rapidly disappeared by either partial or full degradation or by volatilization, with very small amounts remaining bound to the sediment. According to the authors the many analyses did not show the formation of degradation products.

Results

Percent moisture determinations performed on the aerobic biotic, aerobic abiotic, anaerobic biotic and anaerobic abiotic sediments immediately prior to chamber preparation yielded results of 60.7%, 53.3%, 76.6% and 68.1%, respectively. The microbial biomass of the aerobic and anaerobic sediment after handling measured 1108 $\mu\text{g/g}$ and 518 $\mu\text{g/g}$, respectively, or approximately 3.1% and 1.5% of the respective sediment total organic carbon.

Mean recoveries (of the duplicate chambers with standard deviations) for the aerobic/biotic and anaerobic/biotic chambers at day 14 were 8.8% and 19.1% of applied radioactivity, respectively. Less than 10% of the dosed radioactivity remained in either the aqueous or sediment layers (extract and combustion combined) of the aerobic/biotic test chamber replicates at day 14. Methanol extracts of the anaerobic/biotic transformation chamber sediments were the only component of these systems containing more than 10% of the initial radioactivity dosed (mean of 12.9%). Matrix fortification samples yielded mean total recoveries more than 90%. HPLC/beta-RAM analysis of the aqueous layers and methanol extracts of the sediment layers yielded problematic results based on the low concentration of radioactivity present in the samples. Although inconclusive, metabolite formation was not apparent in the day 14 samples. On day 22, a determination of the total radioactivity extractable from a representative aerobic/biotic and anaerobic/biotic transformation chamber was performed to confirm the rapid loss of test material demonstrated with the day 14 test chambers. The entire content of each vessel was repeatedly extracted with methanol and aliquots of the resulting extract were assayed by LSC. The total radioactivity recovered (as a percent of dosed radioactivity) was 7.6% and 8.2% for the aerobic/biotic and anaerobic/biotic chamber, respectively.

Although a precise quantification of the disappearance of the test substance could not be made, the author concludes that it is shown that the half-life of 1,4-DMN in a water/sediment system was less than 14 days. In a response of the testing laboratory it is stated:

Wildlife conducted a number of additional experiments to determine the possible causes of the loss of ^{14}C -1,4-DMN observed during the study. Based on the data, it would appear that both volatility and binding of the substance to the materials of the gas sampling manifold contributed to the observed low recoveries. After

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

considerable effort, reasonably acceptable recoveries were obtained by using specialized glassware connected directly to an oxidation furnace. While the test was not able to differentiate between evolved ^{14}C and volatilized radiolabeled organic material, it clearly demonstrated a relatively rapid partitioning from the liquid to the gaseous phase. Radiolabeled material recovered from the gaseous phase accounted for approximately 50% of dose (total radioactivity added) after 2 days of incubation. Therefore, the data indicate that the absence of ^{14}C -1,4-DMN (or potential metabolites/mineralization product) in the test system was due to its volatility. In addition, less than 10% of the dose was observed in solution after 7 days of incubation. While the results of the water sediment and adjunct studies may not fully satisfy regulatory requirements, they are scientifically sound and indicate that significant amounts of metabolites would not be found in aquatic sediment systems should 1,4-DMN enter the aqueous environment. Based on the observed partitioning from the liquid to the gaseous phase, 1,4-DMN or potential metabolites would be predicted to volatilize from the aqueous compartment and not remain in the sediment.

Remarks

^{14}C -1,4-DMN rapidly disappeared from the sediment-water test systems. No degradation products or metabolite formation were evident from the HPLC/beta-RAM analyses performed. The mineralization test system used in the study proved inadequate relative to the recovery of evolved ^{14}C -1,4-DMN. Poor recoveries in the closed mineralization systems necessitated termination of data collection. The percent of dosed radioactivity recovered from the aerobic/biotic and anaerobic/biotic chambers at day 14 were 8.8% (\pm 3.5%) and 19.1% (\pm 0.3%), respectively. Since the disappearance of the substance may have been associated with the use of the rubber stoppers the results of the study can not be relied upon.

In the third additional experiment to the water-sediment study, it was demonstrated that 46% of the radioactivity had escaped to the gaseous phase after 2 days (75% after 23 days). Although the composition of the medium in this additional experiment was not the same as in the main study, rubber stoppering was not used to minimize losses. Therefore, the additional experiments demonstrate that the high loss of radioactivity from the water-sediment phase in the main test was largely due to volatilisation, instead of to adsorption to the rubber stoppers. Therefore, there would be minimal opportunity for retention by sediment followed by metabolite formation, as the extensive volatility would preclude such from happening.

4.1.4.3 Soil degradation

No studies on the route and rate of degradation of 1,4-DMN have been submitted. Some indications based on literature studies are available as shown below.

STUDY IIA, 7.1/01 - Miyachi et al, 1993.

A literature reference was submitted concerning microbial oxidation of DMN isomers by isolated bacteria. Three bacterial strains, identified as *Alcaligenes* sp. strain D-59 and *Pseudomonas* sp. strains D-87 and D-186, capable of growing on 2,6-dimethylnaphthalene (2,6-DMN) as the sole source of carbon and energy were isolated from soil samples. Strain D-87 grew well on (amongst others) 1,4-DMN as the sole source of carbon and energy and accumulated 4-methylnaphthalene-1-carboxylic acid from 1,4-DMN. The information points at the possibility of metabolisation of 1,4-DMN by soil micro-organisms.

STUDY IIA, 7.1/02 – Barnsley, 1988.

A literature reference was submitted concerning the metabolism of 2,6-DMN by flavobacteria. Flavobacteria that were able to grow on 2,6-dimethylnaphthalene (2,6-DMN) were isolated from soil. Most were able to oxidize a broad range of aromatic hydrocarbons after growth on 2,6-DMN at rates comparable to that of the oxidation of 2,6-DMN itself. One small group was neither able to grow on naphthalene nor able to oxidize this compound after growth on 2,6-DMN, but metabolised 2,6-DMN by a pathway which converged with that previously described for naphthalene metabolism in *Pseudomonas*. These organisms could also grow on

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

salicylate or methylsalicylate, and in so doing, early enzymes for 2,6-DMN metabolism were induced. No information on 1,4-DMN was reported in this study.

STUDY IIA, 7.1/03 – Brown, 1998.

A literature reference was submitted concerning the composting of hazardous waste and hazardous substances. A series of research projects have been conducted on the fate of volatile organic chemicals found in municipal and hazardous wastes, and the fate of polynuclear aromatics and explosives during composting. The results demonstrate that composting can be used to decrease the concentration of hazardous organic substances, but that the more volatile constituents may vaporize, rather than degrade. When the effects of volatilization were removed, the half life based solely on biodegradation ranged from 7.0 - 11.6 days for DMN. It was not reported which congener it was. Therefore the results can only be used as indicative values.

STUDY IIA, 7.1/04 – Spongberg et al., 1996.

A literature reference was submitted concerning the laboratory scale in-vessel composter designed for volatile emissions analysis. Volatilisation of DMN is mentioned sideways in this study. The information presented is not considered relevant.

STUDY IIA, 7.1/05 – Dutta et al, 1998.

A literature reference was submitted concerning the oxidation of methyl-substituted naphthalenes. The pathways of aerobic bacterial degradation of methylated naphthalenes in soil were studied. It was reported that *Paucimobilis* 2322 formed 3,6-dimethylsalicylic methyl ester and 4-methyl-1-naphtoic acid methyl ester from 1,4-DMN. The information points at the possibility of metabolisation of 1,4-DMN by soil micro-organisms, but cannot be used to quantify biodegradation in soil.

4.1.4.4 Photochemical degradation

STUDY IIA, 7.6/01

Reference/notifier	: Weterings, P.J.J.M.	GLP statement	: no
Type of study	: photolysis in water	Guideline	: no
Year of execution	: 2004	Acceptability	: Acceptable
Test substance	: -		

Description

The direct photolytic behaviour of 1,4-DMN was estimated using Quantitative Structure-Property Relationship (QSPR) models developed by Chen et al. (2000, 2001) for 13 closely related polycyclic hydrocarbons. The structure of 1,4-DMN is closely related to several of these compounds, allowing use of the models for predicting the direct photolytical degradation of 1,4-DMN. The photolysis QSPR models as developed by Chen et al. are based on quantum chemical descriptors of the compounds. These quantum chemical descriptors were calculated by means of computation software MOPAC version 6.0 using the PM3 Hamiltonian.

Results

Using the quantum chemical descriptors for 1,4-DMN, also calculated by means of MOPAC 6.0 using the PM3 Hamiltonian, and the pseudo-regression coefficients determined by Chen et al. 2001, the photolysis half-life of 1,4-DMN under midsummer, midday sunlight irradiation in water at 40°N latitude was calculated as 6.4 hours.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Using a mercury lamp, Fukuda et al. 1988 experimentally determined the direct photolysis half-life in water of a closely related compound, 2,6-DMN, as 15.5 hours (see IIA 7.6/04). The photolytic half-life for this compound, predicted by the QSPR model, was 6.5 hours. Since the predicted half-lives for 1,4-DMN and 2,6-DMN are identical, the experimentally determined half-life of 2,6-DMN can be extrapolated to 1,4-DMN resulting in a direct photolysis half-life of 15 hours under the experimental conditions employed.

Remarks

The author concludes that based on the fact that the predicted half-lives for 1,4-DMN and 2,6-DMN are identical, the experimentally determined half-life of 2,6-DMN can be extrapolated to 1,4-dimethylnaphthalene. However, results for other naphthalenes with structural similarity to 1,4-DMN show that the uncertainty associated with predicted photolytic behaviour of structural analogues is a factor of 2 (see study IIA, 7.6/03 below). This results in a photolytic half-life range for 1,4-DMN of 3.2 - 12.8 h, considering the predicted half-life of 6.4 hours.

STUDY IIA, 7.6/02 – Chen et al, 2000.

A literature study was submitted concerning QSPR for direct photolysis quantum yields of selected polycyclic aromatic hydrocarbons. Direct photolysis quantum yields were predicted for PAHs for which experimental quantum yield values were lacking. Based on the QSPR models, significant PAH molecular characters governing their direct photolysis quantum yields were identified. Predicted and observed photolytical quantum yields for 1-methylnaphthalene and 2-methylnaphthalene are reported.

STUDY IIA, 7.6/03 – Chen et al, 2001.

A literature study was submitted concerning the possibility to develop a QSPR model for direct photolysis half-lives of PAHs under irradiation of sunlight. The observed and predicted photolytic half-lives for 1-methylnaphthalene and 2-methylnaphthalene are reported in this study. No information on 1,4-DMN was reported in this study. The study shows that the model predicts the photolytic half-life for structural analogues of 1,4-DMN with an uncertainty of a factor of 2.

STUDY IIA, 7.6/04

Reference/notifier	: Fukuda K, Inagaki Y, Maruyama T, Kojima HI, Yoshida T; 1988	GLP statement	: no
Type of study	: photolysis in water	Guideline	: no
Year of execution	: 1988	Acceptability	: Acceptable as supportive information
Test substance	: 2,6-dimethylnaphthalene		

Substance	Water type	T [°C]	pH	Light Source	Wavelength [nm]	Duration [h]	Quantum yield	Transformation at end [%]	DT ₅₀ photo [h]
2,6-dimethylnaphthalene	distilled water	20 ± 1	-	mercury lamp	-	96	-	-	15.5

Description

The photodegradability of 2,6-DMN and related substances was investigated in a one-litre cooled round glass photo-reaction flask (Pyrex), equipped with a high-pressure mercury lamp to imitate natural daylight. The test chemicals were dissolved in acetonitrile to approximately 1 mM, and kept in a refrigerator as stock solution. Ten mL of the stock solution which contained ca. 10 µM of the test chemicals was injected into the reaction flask which was filled with 1 L of distilled water or artificial seawater. The reaction flask was wrapped with aluminium foil. After irradiation of 1 to 96 hour(s), the reaction medium was extracted twice with 300 mL of dichloromethane. The dichloromethane layer was condensed with a rotary evaporator in a water bath at 30°C, and dried by a nitrogen gas jet. The residue was dissolved in methanol and made up to

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

10.0 mL exactly. Chemical analysis was mainly performed by reversed-phase HPLC with UV detector. A GC-MS was also used for identification of the photolysis products. The rate constant and half-life of photodegradation were estimated.

Results

Recovery through this procedure without irradiation was 92-100%, depending on the properties of chemicals tested. Photodegradation followed first order kinetics. Calculated degradation constants and half-lives of several alkylated naphthalenes in distilled water are shown in Table 7.6.1.

The photolysis rates in seawater and in distilled water were compared for a few alkylated naphthalenes showing that photodegradation in seawater was higher. Sodium chloride was shown to be the cause of the enhancement, because the rate increased nearly proportionally to the concentration of NaCl in the system.

An estimation of the photolysis products of 2-isopropyl naphthalene was performed by HPLC. The analytical results suggested the photolysis products were 2-(2-naphthyl)-2-propanol and phthalide. Further purification of the resulting products of photodegradation of 2-IPN was performed using silica gel thin-layer chromatography. Here, benzoic acid and phthalic acid were identified after trimethylsilylation on GC-MS. Therefore, a possible mechanism of the photolysis of alkylated naphthalenes was suggested to be through alkylated benzenes in its course to mineralization.

Table 7.6.1 Photodegradation of alkylated naphthalenes in distilled water

Substance	Half-life (h)
2-Methylnaphthalene	16.4
2-Ethylnaphthalene	18.4
2-Isopropyl naphthalene	22.3
2,6-Dimethylnaphthalene	15.5
2,6-Diethylnaphthalene	13.1
2,6-Diisopropyl naphthalene	16.0
2,7-Diisopropyl naphthalene	6.4

The author states that in view of the similar degradation rates for comparable alkylated naphthalenes, the photodegradation rate constant for 2,6-DMN may be used for 1,4-DMN.

Remarks

The notifier did not present any information to back-up the statement that in view of the similar degradation rates for comparable alkylated naphthalenes, the photodegradation rate constant for 2,6-DMN may be used for 1,4-DMN. Therefore, no definite photodegradation half-lives for 1,4-DMN could be derived. Nevertheless table 7.6.1. shows that the degradation rates of of alkyl naphthalenes from monomethylnaphthalene to diisopropyl naphthalene are actually very similar. These results supports the value as derived in study Weterings (2004); IIA, 7.6/01.

4.1.5 Adsorption and desorption

STUDY IIA, 7.4/01

Reference/notifier	: IIA 7.4/01	GLP statement	: no
Type of study	: estimation of soil sorption coefficients	Guideline	: no
Year of execution	: 1992	Acceptability	: acceptable
Test substance	: 1, 4-dimethylnaphthalene		

Description

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Soil sorption coefficients for 336 hydrocarbon and organic chemicals in water are reported. A database was compiled from many literature sources. The non-linear group contribution method was used to estimate the soil sorption coefficient when experimental Koc values were not available. A comparison of calculated and experimental values for the soil sorption coefficient shows general agreement of calculated and experimental values for different organic chemicals. The Koc value for 1,4-DMN was estimated.

Results

For 1,4- DMN the estimated Koc at 20°C is 4230 L/kg.

Remarks

The reported Koc value is an estimated value. An estimation by the RMS of the Koc using EPIWIN (EPIWeb 4.0), using Molecular Connectivity Indices Method, is 4139 L/kg. The Koc using the experimental log Kow of 4.37 is 6199 L/kg. Based on this information the results from the study are in line with the calculated values and the Koc of 4230 L/kg can be used for risk assessment. The number of sorption data delivered is not enough for a proper risk assessment. However, as the value given above is relatively high, additional sorption tests are not expected to differ significantly. Therefore, it is proposed to consider the data on sorption to be sufficient for risk assessment.

4.2 Bioaccumulation

4.2.1 Bioaccumulation test on fish

STUDY 1

Reference/notifier	: IIA 8.2/07	Exposure duration	: 36 days, followed by 8 days depuration
Year of execution	: 2004	Nominal conc	: 2.74 and 26.67 µg/L
GLP statement	: No	Dosing method	: Continuous flow
Guideline	: -	Acceptability	: Acceptable with remarks
Test substance	: 1,3-dimethylnaphthalene	BCF	: 5751 L/kg
Purity	: -		
Species	: sheepshead minnows (<i>Cyprinodon variegatus</i>)		
Water solubility	: 5.1 mg/L		

Substance	Species	Duration [d]	Method	Exposure conc [µg/L]	BCF [L/kg wwt]	Based on
1,3-dimethylnaphthalene	<i>Cyprinodon variegatus</i>	36 + 8	Continuous flow	2.74	3979	k ₁ /k ₂
1,3-dimethylnaphthalene	<i>Cyprinodon variegatus</i>	36 + 8	Continuous flow	26.67	8313	k ₁ /k ₂

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Description

Adult sheep head minnows (*Cyprinodon variegatus*; 158 females and 129 males, mean weight 2.47 ± 1.23 g and mean length 4.7 ± 0.8 cm at sampling times) were exposed to two concentrations of PAH mixtures in seawater (a stock solution was prepared in acetone) in a continuous-flow system. The fish were exposed for a period of 36 days, followed by 8 days of depuration. The PAHs studied were pure naphthalene, 2-methylnaphthalene, 1,3-dimethylnaphthalene, 2-isopropylnaphthalene, phenanthrene, pyrene, 9-methylphenanthrene, and 9-ethylphenanthrene. The 1,3-dimethylnaphthalene concentrations were 2.74 ± 0.73 µg/L and 26.67 ± 9.19 µg/L.

Seawater samples were collected in 1-L glass bottles containing hydrochloric acid to ensure low pH (<2) and prepared for analysis within 48 h after sampling. Water was sampled from the middle of the exposure chambers by use of a Teflon siphon connected to a piece of glass tubing. Three deuterated PAHs were added as quantitative internal standards to the sample before liquid-liquid extraction with cyclohexane (three times, 50 ml each time). The combined extracts were dried with anhydrous sodium sulphate and filtered, and the volume was then reduced to 0.5 ml. Two deuterated PAHs, acting as recovery internal standards, were added before GC-MS analysis. All sample concentrations were recovery corrected by use of quantitative internal standards in the calculation procedure.

The fish were dissected with whole gallbladders removed and stored at -80°C until analysis. Homogenised fish tissue was weighed, and three quantitative internal standards were added before saponification with methanolic sodium hydroxide under reflux for 2 h. The digest was filtered and extracted three times with cyclohexane. The combined extracts were purified by normal-phase, solid-phase extraction and concentrated to 0.5 ml for GC-MS analysis.

Gallbladders were thawed and disrupted, and the total bile volume for individual fish was measured by use of capillary pipettes. Only fish with a bile volume exceeding 15 µl were used for analysis of hydroxylated PAH after the generation of trimethylsilyl derivatives.

Polycyclic aromatic hydrocarbons in seawater and fish tissue and hydroxylated PAHs in bile were analyzed by GC-MS in selected ion mode.

Bioconcentration factors (BCFs) for individual PAHs were estimated from the ratio of k_1 to k_2 by use of a first-order, one-compartment kinetics model and also directly from PAH concentrations in fish tissue and water samples (from sampling days 4, 7 and 36).

Results

Naphthalene and its three alkylated isomers revealed peak levels within 4 d in both treatments except for the low-exposure concentration of C2 - and C3 -alkylated naphthalene, which reached maximum at day 7. Uptake rate constants (k_1) for the naphthalenes increased with increasing degree of alkylation and log value of the octanol/ water partition coefficient (K_{ow}), whereas k_1 values for three- and four-ring PAHs were lower despite their high log K_{ow} values. Elimination rate constants (k_2) for the homologue series of naphthalenes and phenanthrenes generally increased with decreasing degree of alkylation and log K_{ow} values. However, the depuration time did not directly correlate with the molecular size for nonalkylated PAHs.

A significant positive correlation was determined between log BCFs and log K_{ow} values for the series of C0- through C3-naphthalenes at both low ($r^2 = 0.985$, $p = 0.0077$) and high ($r^2 = 0.956$, $p = 0.022$) exposures. The two exposure levels revealed minor variations in k_1 and k_2 values for parent PAHs and in the temporal pattern of PAH metabolite concentrations in bile. The present results indicate that the presence and nature of alkyl groups have a significant influence on the kinetics and metabolism of PAHs in fish.

For 1,3-dimethylnaphthalene, a wet weight-based bioconcentration factor of 4000 L/kg was determined at a concentration of 2.74 µg/L and 8000 L/kg at 26.7 µg/L.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

The time needed to eliminate 95% of the 1,3-dimethylnaphthalene in the fish ($T_{95} = 3.0/k_2$) amounted to 6.4 days (low concentration) and 8.6 days (high concentration).

Remarks by RMS

The geometric mean of the BCF values for both concentrations results in an overall BCF value for 1,3-dimethylnaphthalene of 5751 L/kg. The applicant states that although the concentration of 1,3-dimethylnaphthalene is more than 1000 times the concentration in the surrounding water, the substance is rapidly removed from the fish when it is returned to clean water. The applicant considers that in view of the structural similarity, it is reasonable to assume that the values for bioconcentration and elimination apply to 1,4-dimethylnaphthalene. This assumption was further substantiated by an article from the public literature from Dimitriou-Christides et al (2003), published in a peer-reviewed journal (*Chemosphere* 52 (2003), 869-881). On the basis of an accepted methodology (GC and HPLC retention time comparison for estimating several physical-chemical characteristics), Dimitriou-Christidis et al 2003 established several partition coefficients for a number of methylated naphthalenes, including 1,3-DMN and 1,4-DMN. The estimated logKow values for 1,3-DMN and 1,4-DMN were 4.27 and 4.22 resp. (existing values from public literature were 4.42 and 4.37 resp.). The article also stated that LogKow increases with the number of methyl groups. (1,3-DMN and 1,4-DMN have an equal number of methyl-groups.) Based on this article RMS deems it acceptable to conclude that the bioconcentration potential of 1,3-DMN and 1,4-DMN is comparable, since the LogKow is a driving parameter for this potential. Therefore, the reported measured BCF value of 5751 for 1,3-dimethylnaphthalene is accepted.

4.2.2 Bioaccumulation test with other organisms

No other data.

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

STUDY 1

Reference/notifier	: IIA 8.2/01, DocID 93-10-4955	Exposure duration	: 96 hours
Year of execution	: 1993	Nominal conc	: 0, solvent control, 0.32, 0.54, 0.90, 1.5, and 2.5 mg as/L
GLP statement	: Yes	Dosing method	: Flow-through
Guideline	: FIFRA 72-1 = OECD 203 = EEC C.1	Acceptability	: Acceptable
Test substance	: 1,4-dimethylnaphthalene, batch H5510	LC ₅₀	: 0.67 mg/L
Purity	: 96.4%,		
Species	: Rainbow trout (<i>Oncorhynchus mykiss</i>)		
Water solubility	: 5.1 mg as/L		

Substance	Species	Method	T	pH	Duration	Criterion	Value
			[°C]		[h]		[mg/L]

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

1,4-dimethylnaphthalene	<i>Oncorhynchus mykiss</i>	Flow-through	11 - 12	7.1 – 7.7	96	LC ₅₀	0.67
-------------------------	----------------------------	--------------	---------	-----------	----	------------------	------

Description

Rainbow trout (*Oncorhynchus mykiss*), mean wet weight 1.5 g (range 0.80 to 2.84 g); mean total length 50 mm (range 42 to 63 mm), were exposed to a concentration range of 1,4-dimethylnaphthalene, batch H5510, purity 96.4%, in a flow-through test (6.6 volume replacements/aquarium every 24 h). A stock solution of the test substance was prepared in acetone. Nominal test concentrations 0.32, 0.54, 0.90, 1.5, and 2.5 mg as/L a control and a solvent control (0.5 mL/L acetone) Test concentrations were based on the results of a range-finding test. Two replicates with ten fish each, 15 L water per test aquarium. Daily observations.

Conditions. Temperature of 11 to 12°C, photoperiod of 16 hours light, 8 hours dark, no feeding. Acclimation 14 days.

Chemical analysis. Samples were collected of each test concentration at the start and end of the study and at the high, middle and low concentration prior to test initiation. Analysis by HPLC with UV detection at 220 nm. Direct aqueous injection of the study sample. Mean recovery 102 ± 3%, recovery was 106, 102, and 97.85 at fortifications of 0.1, 1.0 and 5.0 mg as/L. LOQ of 0.0536 mg/L.

Calculations and statistics. LC₅₀ and NOEC value were determined using the program of Stephan (1977, 1982) using the moving average angle analysis.

Results

Mean measured concentrations were 0.19, 0.22, 0.41, 0.75 and 1.2 mg/L. Measured test concentrations ranged from 40% to 58% of nominal test concentrations, but were constant during the test. Mean measured test concentrations were 48% of nominal. Recovery of the quality control samples averaged 97% of nominal.

At test termination (96 hours), mortality of 100% was observed among rainbow trout exposed to the highest mean measured concentration (1.2 mg/L). Mortality of 5%, 5% and 45% was observed among organisms exposed to the 0.22, 0.41 and 0.75 mg/L test concentrations, respectively. In addition, sublethal effects (e.g., loss of equilibrium) were observed among all of the surviving fish exposed to these test concentrations. Symptoms at earlier time points consisted of darkened pigmentation, erratic swimming behaviour, and partial or complete loss of equilibrium. No mortality or other effects were observed among fish exposed to the 0.19 mg/L test concentration.

The 96-hour LC₅₀ was calculated to be 0.67 mg/L with a 95% confidence interval of 0.57 to 0.80 mg/L using the moving average angle method. The 48-h LC₅₀ was 1.0 mg as/L, the 72-h LC₅₀ was 0.89 mg as/L. The LC₅₀ for 48 and 72 hours of exposure was based on probit analysis and nonlinear interpolation, respectively. The No-Observed-Effect Concentration (NOEC) was determined to be 0.19 mg/L. LC₅₀ and NOEC values are based on mean measured test concentrations.

Remarks by RMS

Water quality parameters were within accepted limits. The validity criterion regarding mortality in the controls of the OECD guideline 203 was met as this mortality did not exceed 10 per cent. The result 96-h LC₅₀ of 0.67 mg as/L, based on mean measured test concentrations is accepted.

STUDY 2

Reference/notifier	: IIA 8.2/02, Doc ID 535A-101A	Exposure duration	: 96 hours
Year of execution	: 2002	Nominal conc	: 0, solvent control, 0.25, 0.50, 1.0, 2.0, 4.0, and 8.0 mg as/L
GLP statement	: Yes	Dosing	: Semi-static

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Guideline	: OPPTS 850.1075 = OECD 203 = EEC C.1	method Acceptability	: Acceptable
Test substance	: 1,4-dimethylnaphthalene, batch 01C-01	LC ₅₀	: 1.4 mg as/L
Purity	: 98.8%		
Species	: Fathead minnow (<i>Pimephales promelas</i>)		
Water solubility	: 5.1 mg/L		

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg as/L]
1,4-dimethylnaphthalene	<i>Pimephales promelas</i>	Semi-static	22.1 – 22.7	8.4 – 8.7	96	LC ₅₀	1.4

Description

Fathead minnow (*Pimephales promelas*), mean wet weight 0.062 g (range 0.035 to 0.12 g); mean total length 2.0 cm (range 1.7 to 2.5 cm), were exposed to a concentration range of 1,4-dimethylnaphthalene, batch 01C-01, purity 98.8%, in a static renewal test (daily renewal). Fish were held under test conditions for 14 days and acclimatised for 51 hours prior to testing. A 80 mg/mL stock solution of the test substance was prepared in dimethylformamide. Nominal test concentrations were: 0.25, 0.50, 1.0, 2.0, 4.0 and 8.0 mg as/L, a control and a solvent control (DMF). New stock and test solutions were prepared on days 0, 1, 2, and 3. Dilution with fresh well water. Two replicates with 10 fish per concentration. 4 L test solution per vessel and each vessel was closed by caps. Observations: 5, 24, 48, 72 and 96 hours after test initiation.

Conditions. temperature controlled environmental room at 22 °C, 16:8 h L:D (747 lux with 30 minutes transition period), no aeration and no feeding during the test.

Chemical analysis. Samples were collected at the beginning of the test, at 24 hours prior to and after renewal of the test solutions, and at the end of the test. Analysis by HPLC with fluorescence detection at 225 - 350nm after dilution the sample with acetonitrile. Mean recovery of fortified control samples was 95.6% (n=9) LOQ was 0.02 mg as/L.

Calculations and statistics. LC₅₀ values were determined using the computer program of Stephan (1977) using the binomial probability method, NOEC by visual interpretation.

Results

Mean measured concentrations were 0.22, 0.43, 0.86, 1.7, 3.2 and 5.4 mg/L (68-88% of nominal). Measured test concentrations from freshly prepared test concentrations ranged from 77 – 101% of nominal and the measured test concentrations from the 24 h old solutions ranged from 52 – 92% of nominal. Fathead minnows in the negative and solvent control groups appeared healthy and normal throughout the test. Fathead minnows in the 0.22 and 0.43 mg/L treatment groups also appeared normal, with no treatment-related mortalities or overt signs of toxicity noted during the test. While no mortalities occurred in the 0.86 mg/L treatment group, all of the fish exhibited signs of toxicity by test termination. Percent mortality in the 1.7, 3.2 and 5.4 mg/L treatment groups at test termination was 80, 100 and 100%, respectively. Symptoms included surfacing, erratic swimming, lethargy, and loss of equilibrium.

24 hours LC₅₀ of 2.3 mg as/L, 48-hours LC₅₀ of 1.6 mg as/L, 72-hours LC₅₀ of 1.4 mg as/L and 96-hours LC₅₀ of 1.4 mg as/L (95% CL 0.86 – 1.7 mg as/L), based on mean measured test concentrations. All LC₅₀ values were calculated using binomial probability method.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Remarks by RMS

Water quality parameters were within accepted limits. Concentrations of the solvent are not reported. The result 96-hours LC₅₀ of 1.4 mg as/L, based on mean measured test concentrations is accepted.

4.3.2 Short-term toxicity to aquatic invertebrates

STUDY 1

Reference/notifier	: IIA 8.3.1/01	Exposure duration	: 48 hours
Year of execution	: 1993	Nominal conc	: First test: 0.16, 0.26, 0.43, 0.72, and 1.2 mg as/L Second test: 0.65, 1.1, 1.8, 3.0, and 5.0 mg as/L For both test a control and a solvent control.
GLP statement	: Yes	Dosing method	: Flow-through
Guideline	: FIFRA 154-9 = OECD 202 (Part A) = EEC C.2	Acceptability	: Acceptable
Test substance	: 1,4-dimethylnaphthalene, batch H5510	EC ₅₀	: 0.54 mg as/L
Purity	: 96.4%		
Species	: <i>Daphnia magna</i>		
Water solubility	: 5.1 mg as/L		

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg as/L]
1,4-dimethylnaphthalene	<i>Daphnia magna</i>	Flow-through	19 - 20	8.0 – 8.3	48	EC ₅₀	0.54

Description

Twenty daphnids (*Daphnia magna*, ≤24 hours old) were exposed to a range of concentrations of 1,4-dimethylnaphthalene, batch H5510, purity 96.4%, under flow-through conditions. Six solution volume replacements/day. Filtered well water with a total hardness of 160 – 180 mg/L as CaCO₃, conductivity of 400 – 600 µmhos/cm and a pH of 7.9 – 8.3. The study was conducted in two parts with the following nominal test concentrations: 0.16, 0.26, 0.43, 0.72, and 1.2 mg as/L (test 1) and 0.65, 1.1, 1.8, 3.0, and 5.0 mg as/L (test 2). A control and a solvent control (acetone 0.5 mL/L) were included. Two replicates with 10 daphnids per treatment. Observations of immobility and abnormal behaviour of appearance were performed after 24 and 48 hours of exposure.

Conditions. 16 : 8 h L : D (380 – 560 lux), 19-20°C, no aeration, no feeding.

Chemical analysis. Samples were taken at the start and at the end of the studies. Analysis by HPLC with UV detection at 220 nm. Samples were analysed by direct aqueous injection. Recovery ranged from 98.2 – 106%, LOQ is 0.0536 mg/L. Three quality control samples were also prepared at each sampling interval.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Calculations and statistics. EC50 values was calculated using the program of Stephan (1985) and by nonlinear interpolation.

Results

Both tests: Throughout the exposure period, no undissolved test material (e.g., precipitate) was observed in the diluter system or in the exposure solutions.

First test. Mean measured concentrations ranged from 35.2 – 42.8 % of nominal concentrations. Concentrations were constant during test. The mean measured test concentrations were 0.056, 0.10, 0.18, 0.31, and 0.48 mg as/L.

Immobility occurred at concentrations of 0.18 mg/L and above. At the highest test concentration of 0.48 mg as/L, 15% immobilised daphnids were observed. Other symptoms included lethargy, erratic movement and paleness. All daphnids at the highest test concentration were pale and erratic.

Second test. Mean measured test concentrations ranged from 30.3 – 44.5% of nominal. The measured test concentrations were 0.21, 0.33, 0.56, 0.94 and 2.2 mg as/L. Immobility was found at 0.33 mg as/L (5%) and increased dose-related to 100% at 2.2 mg as/L.

EC₅₀ of the first test is reported to be > 0.48 mg as/L, based on mean measured test concentrations.

The EC₅₀ of the second test is calculated at 0.54 mg as/L (95% CL 0.33 – 0.94 mg as/L), based on mean measured test concentrations.

Remarks by RMS

Water quality parameters were within accepted range. The validity criterion regarding immobilization in the controls of the OECD guideline 202 was met as this immobilisation did not exceed 10 per cent. Mean measured concentrations are constantly < 80% of nominal, probably to the volatile properties of 1,4-dimethylnaphthalene. An EC₅₀ of 0.54 mg as/L, based on mean measured test concentrations was concluded.

4.3.3 Algal growth inhibition tests

STUDY 1

Reference/notifier	: IIA 8.4/01, DocID 535A-102	Exposure duration	: 96 hours
Year of execution	: 2002	Nominal conc	: 0.028, 0.056, 0.11, 0.23, 0.45 and 0.90 mg/L
GLP statement	: Yes	Dosing method	: Static
Guideline	: OPPTS 850.5400 = OECD 201 = EEC C.3	Acceptability	: See conclusion
Test substance	: 1,4-dimethylnaphthalene, batch 01C-01	72-h E _b C ₅₀	: 0.32
Purity	: 98.8%	72-h E _r C ₅₀	: 0.62
Species	: <i>Pseudokirchneriella subcapitata</i>	72-h NOEC	: 0.030
Water solubility	: 5.1 mg as/L		(all values mm)

Substance	Species	Method	T	pH	Duration	Criterion	Value
			[°C]		[h]		[mg]

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

							as/L]
1,4-dimethylnaphthalene	<i>Pseudokirchneriella subcapitata</i> ¹	Static	22.8 – 24.1	7.9 – 10.4	72	E _b C ₅₀	0.32
1,4-dimethylnaphthalene	<i>Pseudokirchneriella subcapitata</i> ¹	Static	22.8 – 24.1	7.9 – 10.4	72	E _r C ₅₀	0.62
1,4-dimethylnaphthalene	<i>Pseudokirchneriella subcapitata</i> ¹	Static	22.8 – 24.1	7.9 – 10.4	72	NOEC	0.030

1 Former name *Selenastrum capricornutum*

Description

The green alga, *Pseudokirchneriella subcapitata*, was exposed to a geometric series of six concentrations of 1,4-dimethylnaphthalene, batch 01C-01, purity 98.8%, a blank control and a solvent control (dimethylformamide 0.1 mL/L) under static conditions for 96 hours. Freshwater algal medium was used, pH 8.0 – 8.1. The test was conducted using closed test chambers with minimal headspace. Twelve replicate test chambers were maintained in each treatment and control group. Nominal test concentrations selected were 0.028, 0.056, 0.11, 0.23, 0.45 and 0.90 mg/L. Initial cell density 5000 cells/mL. Cell densities were counted after 24, 48, 72 and 96 hours using and electronic particle counter.

Conditions. 23 ± 2 °C, continuous light (4300 lux) and continuous shaking at 100 rpm.

Chemical analysis. Samples were taken from the test medium of each treatment and control at 0 and 96 hours of the test. Analysis by HPLC with fluorescence detection. Mean procedural recovery of fortified samples in freshwater algal medium was 99.5%, LOQ 6.25 µg as/L.

Calculations and statistics. Shapiro-Wilks and Levene's test for normality and homogeneity. Treatment groups were compared to the solvent control using Dunnett's test. All calculations with SAS program.

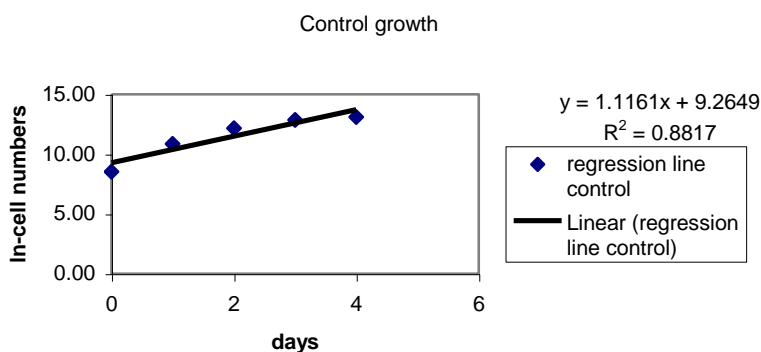
Results

Mean measured test concentrations on day 0 and day 4 were 107% and 87% of nominal. Mean measured test concentrations over test duration were 0.030, 0.053, 0.11, 0.21, 0.44 and 0.86 mg as/L (95-107% of nominal). Percent inhibition was calculated relative to solvent control. Mean cell density was dose-related reduced and statistically significant at 0.053 mg as/L (14% inhibition) and onwards after 72 hours of exposure. Cells appeared normal except at 0.86 mg as/L after 96 hours of exposure. Those cells were noted to be enlarged. Growth rate was inhibited by 3.6% at 0.053 mg as/L (significant) and was reduced with 77% at 0.86 mg as/L, the highest concentration, after 72 hours. Biomass was also significantly reduced at 0.053 mg as/L (8.7%) and increased to 95% at 0.86 mg as/L after 72 hours. Both reductions were dose-related. E_bC₅₀ is reported as 0.32 mg as/L (95% CL: 0.30 – 0.35 mg as/L) and E_rC₅₀ as 0.62 mg as/L (95% CL 0.60 – 0.64 mg as/L) after 72 hours of exposure. The E_bC₅₀ and E_rC₅₀ values after 96 hours were 0.33 and 0.60 mg as/L, respectively. NOE_bC and NOE_rC after 72 and 96 hours are both 0.030 mg as/L. All values based on mean measured test concentrations and compared with solvent control.

Remarks by RMS

pH increased with > 1.5 unit in the control, solvent control and at 0.030, 0.053, 0.11, and 0.21 mg as/L, due to excessive cell growth. This is not considered to have influenced the result. The validity criteria of OECD 201 (2001) are not met with respect to control growth. No exponential growth rate in the control. The mean variance of daily growth rate in the control was > 35% (mean value 79%).

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE



Validity criteria are not met.

The remarks above were addressed by the notifier with the following statement (summarised):

- The test started with 5000 cells/ml and had reached the control criteria (16X or 80,000 cells/ml) by 48 hours and was just short of the 96-hour control criteria (500,000 cells/ml) at both 72 hours and 96 hours. This study was conducted according to the OECD 201, adopted July 1984, in which the validity criteria was that cell concentration in the control cultures should have increased by a factor of at least 16 within three days.
- Due to volatility of the test substance and a desire to maintain test concentrations throughout the study, the test was done in a closed system with limited headspace. Due to the lack of gas exchange in this testing environment, algal growth is limited. The algae growth was exponential during the first 48 hours but quickly outgrew the carrying capacity of the media. Growth peaked very quickly so the algae were past logarithmic growth phase by 72 hours. However, the study did meet the protocol and the OECD 201 growth criteria that were in effect at the time.
- We agree that the mean variance of growth rate in the control was 79% at 96-hours. However, the OECD 201 (2006) guideline recommends 35% at 72-hours. In this study, the mean variance of growth rate was 58% at 72 hours and 41% at 48 hours at the time that the cell growth had peaked.
- The observed effects of 1,4-DMN on green algal were evident and consistent at 48, 72 and 96 hours. These effects were observed during the early portion of the test when exponential growth was occurring as well as at 72 and 96-hours, after cell growth had peaked. The failure to have exponential growth between 48 and 72 hours did not change the conclusion of the test.
- The results of the test showed a clear dose-response relationship between algal growth and mean measured concentrations of 1,4-DMN, which were maintained between 90 and 110% of nominal concentrations throughout the test.

Conclusion RMS: The test result will be used in a weight of evidence approach.

4.3.4 *Lemna* sp. growth inhibition test

No acute study available.

4.4 Chronic toxicity

4.4.1 Chronic toxicity fish

Reference/notifier	: IIA 8.2/03, DocID 535A-105	Exposure duration	: 28 days
Year of execution	: 2006	Nominal conc	: 0, solvent control, 20, 46, 106, 243, and 560 µg as/L.
GLP statement	: Yes	Dosing	: Flow-through

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Guideline	: OECD 215	method	
Test substance	: 1,4-dimethylnaphthalene (lot number 14D03M01-02)	Acceptability	: Acceptable
Purity	: 98.8%	NOEC	: 90 µg as/L
Species	: Rainbow trout (<i>Oncorhynchus mykiss</i>)	growth rate	
Water solubility	: 5.1 mg/L		

Substance	species	method	T [°C]	pH	Duration [d]	Criterion	Value [µg/L]
1,4-dimethylnaphthalene	<i>Oncorhynchus mykiss</i>	Flow-through	12.1 13.5	7.9 – 8.1	28	NOEC growth rate	90

Description

Rainbow trout were exposed to 1,4-dimethylnaphthalene under flow-through conditions. Nominal test concentrations of 1,4-dimethylnaphthalene (lot number 14D03M01-02, purity 98.8%) selected were control, solvent control (0.10 mL dimethylformamide/L), 20, 46, 106, 243 and 560 µg as/L. Fresh well water, hardness 137 mg/L as CaCO₃, conductivity 296 µmhos/cm, pH 8.0. Holding period prior to testing was 14 days. Mean fish weight at test initiation was 1.1 g (range 1.0 – 1.3 g). Four replicates per treatment with 10 fish each. On days 7, 13 and 21, fish were transferred to new test chambers. All test chambers were covered with plexi-glass to minimise volatilisation of the test substance. Each test chamber received 6.4 volume of test solution every 24 hours.

Observations of mortality and other signs of toxicity were made daily during the test. The wet weight of each individual fish was measured at test initiation and at test termination. The weight measurements were then used to calculate tank-average specific growth rates. Effects on survival and growth in the treatment groups were used to estimate the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). EC50 values were determined based on growth rate and survival.

Conditions. 16: 8 h L:D (652 lux) with a 30 minutes transition period, temperature 12 ± 1 °C, no aeration, feeding with a commercial diet.

Chemical analysis. Water samples were taken from control and each test concentration prior to test initiation and on days 0, 7, 13, 21, and 28. Analysis by HPLC with UV detection at 220 nm after dilution of the sample. The mean recovery of the fortification samples was 98.2% (range 92.2 – 103%), LOQ of 10.0 µg as/L.

Calculations and statistics. Growth rates were evaluated for normality and homogeneity of variance using Shapiro-Wilk's test and Barlett's test. Comparison of the negative and solvent control using Student's t-test. Analysis of variance and the Bonferroni t-test was used if growth rate in the treatment groups was significantly reduced compared with control. All calculations were performed using SAS version 8 or TOXSTAT version 3.5.

Results

Mean measured test concentrations, determined from samples of test water collected at test initiation and at approximately weekly intervals thereafter, were 18, 40, 90, 205 and 488 µg/L, which represented 90, 87, 85, 84 and 87% of nominal concentrations, respectively.

At test termination, survival in the 18, 40, 90, 205 and 488 µg/L treatment groups was 100, 100, 100, 100 and 95%, respectively. There were no statistically significant differences ($p > 0.05$) in survival between treatment groups and the pooled control. Consequently, the NOEC for survival was 488 µg/L and the LOEC was > 488 µg/L.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

No significant differences between the negative and solvent control groups in growth rate. The differences between the pooled control and 205 and 488 µg/L, the two highest treatment groups, were statistically significant ($p \leq 0.05$). Consequently, the NOEC for growth rate was 90 µg/L and the LOEC was 205 µg/L.

Remarks by RMS

Water quality parameters were within acceptable limits. The validity criterion regarding the increase of the mean weight of fish in the controls of the OECD guideline 215 was met as the mean weight increased at least the half of their mean initial weight over 28 days. The result NOEC for growth rate of 90 µg as/L, based on mean measured test concentrations is accepted.

4.4.2 Fish early-life stage (FELS) toxicity test

STUDY 1

Reference/notifier	: IIA 8.2/04	Exposure duration	: 4 days
Year of execution	: 1984	Initial conc	: 0, 3.09, 2.92, 2.06, 1.03, 0.95 mg/L and a control
GLP statement	: No	Dosing method	: static
Guideline	: Partly in accordance with early life stage test, OECD 210	Acceptability	: Acceptable
Test Substance	: 1,4-dimethylnaphthalene	NOEC	: < 0.67 mg/L
Purity	: 98%,		
Species	: Cod fish eggs (<i>Gadus morhua</i>)		
Water solubility	: 5.1 mg/L		

Substance	Species	Method	T	Duration	Criterion	Value
			[°C]	[d]		[mg/L]
1,4-dimethylnaphthalene	<i>Gadus morhua</i>	Static	5	4	NOEC	< 0.67

Description

1,4-dimethylnaphthalene, purity 98%, was added to seawater with fertilised Cod fish eggs (*Gadus morhua*) in a duplicate static test. Approximately 50 cod eggs were placed in test beakers containing 100 ml of seawater mixed with the test substance. Two experiments were performed, the first test with concentrations at 2.92 and 0.95 mg/L and a control, the second at 3.09, 2.06 and 1.03 mg/L and a control. The beakers were covered with aluminium foil. Observations of mortality and abnormal embryos were performed after 6 h, 1 day, 2 days and 4 days.

Conditions. All experiments were carried out at ca. 5°C.

Chemical analysis. Samples were taken at the start and at the end of the study. Detection of test substance by UV spectroscopy at 227.9 nm after extracting with hexane.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Results

Analysed concentrations at t= 4 d in the first test were 1.75 and 0.54 mg/L, 60% and 57% of nominal, respectively (2.92 and 0.95 mg/L at t= 0h resp).

Analysed concentrations at t= 4 d in the second test were and 1.08, 0.56 and 0.31 mg/L, 35%, 27% and 30%, respectively (3.09, 2.06 and 1.03 mg/L at t=0 h resp).

The effects of 1,4-dimethylnaphthalene on Cod fish eggs after 6 hours, 2 days and 4 days are shown in Table 4.4.1-1. At nominal concentrations of 2.92, 3.09 and 2.06 mg/L, the substance caused mortality or impairment of normal embryonic development. At 0.95 and 1.03 mg/L, a slight effect on development to the gastrula stage was observed. Therefore, the NOEC is lower than the lowest test concentration, which was 1.03 mg/L at t=0h and 0.31 mg/L at t=4d. The mean measured NOEC therefore amounts <0.67 mg/L.

Table 4.4.1-1. Effects of 1,4-dimethylnaphthalene to Cod fish embryonic development

Substance	Test	Concentration range [mg/L]	Percentage of normal embryos after		
			6 hours	48 hours	96 hours
Control	1	0-0	100	96	91
1,4-dimethylnaphthalene	1	2.92-1.57	0	0*	-
1,4-dimethylnaphthalene	1	0.95-0.54	0	82	38
Control	2	0-0	100	86	86
1,4-dimethylnaphthalene	2	3.09-1.08	98	0	0*
1,4-dimethylnaphthalene	2	2.06-0.56	84	0	0
1,4-dimethylnaphthalene	2	1.03-0.31	100	94	0**

* No surviving test organisms

** Slight inhibition

Remarks by RMS

A NOEC or LOEC value is not reported by the author. Mortality and sub-lethal effects are taken together in the results. Therefore, a NOEC based on mortality cannot be determined. A NOEC for mortality and sub-lethal effects together can be set at <0.67 mg/L, based on mean measured concentrations.

4.4.3 Chronic toxicity to aquatic invertebrates

STUDY 1

Reference/notifier	: IIA 8.3/02, DocID 535A-104	Exposure duration	: 21 days
Year of execution	: 2006	Nominal conc	: 25, 50, 100, 200 and 400 µg/L
GLP statement	: Yes	Dosing method	: Flow-through
Guideline	: OECD 211	Acceptability	: Acceptable
Purity	: 98.8%	NOEC reproduction	: 160 µg as/L
Species	: <i>Daphnia magna</i>		
Water solubility	: 5.1 mg as/L		

Substance	Species	Method	T [°C]	pH	Duration [d]	Criterion	Value [µg as/L]
1,4-dimethylnaphthalene	<i>Daphnia magna</i>	Flow-through	19.2 – 19.9	8.0 – 8.2	21	NOEC reproduction	160

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Description

Daphnids (< 24-hours of age) were exposed during 21 days to a geometric series of five test concentrations 1,4-dimethylnaphthalene, a negative (dilution water) control and a solvent control (dimethylformamide 0.1 mL/L) under flow-through conditions. A flow rate of five volume additions in each test chamber per day. On day 16 a new flow-through system was used, due to drop of DO-value. All daphnids were transferred to the new test chambers. Nominal test concentrations of 1,4-dimethylnaphthalene (lot number 14D03M01-02, purity 98.8%) selected for the test were 25, 50, 100, 200 and 400 µg/L. Two replicate test chambers were tested for each treatment and control. Each replicate contained two compartments with five daphnids, resulting in a total of 20 *Daphnia magna* in each treatment and control group. Test compartments were covered with Petri dish to minimise volatilisation. Fresh well water was used, hardness 136 – 140 mg/L as CaCO₃, conductance 290 – 300 µmhos/cm, pH 8.1. Daily observations of survival, clinical signs and reproduction. Body length and dry weight of surviving daphnids (F0) were measured.

Conditions. 16 : 8 h L : D with a 30 minutes transition period (280 lux), 20 °C. Feeding with a mixture of yeast and trout chow, as well as a suspension of green algae, three times daily.

Chemical analysis. Samples were taken at the start, at weekly intervals and at the end of the test. Analysis by HPLC with UV detection at 220 nm after dilution with freshwater and direct injection on HPLC. Recovery 87.0 – 101% (mean 94.5%), LOQ was 15.0 µg as/L.

Calculations and statistics. Normality using Shapiro-Wilk's test and homogeneity of variance with Levene's test. Statistical significance using ANOVA, Bonferroni's test or Dunnett. All statistical calculations using TOXSTAT or SAS.

Results

Mean measured test concentrations were 24, 48, 89, 160 and 339 µg/L, 96, 96, 89, 80 and 85% of nominal concentrations, respectively. Measured concentrations of the initial diluter system ranged from 82 to 99% of nominal. Samples from the new diluter system on day 14 and 15, ranged from 71 to 89% and 81 to 85% of nominal, respectively.

Significant difference was found in reproduction between the water control and solvent control. Consequently, the solvent control was used for comparison with the treatment groups.

The mean number of offspring per parent animal in the solvent control is 137. The first day of brood production was on day 8, except for two replicates at 339 µg as/L (on day 9), which is not statistically significant.

Table 4.4.2-1 Analytical and biological results.

Nominal concentrations [µg as/L]	Mean measured concentrations [µg as/L]	Mortality on day 21 [%]	Mean number of young produced/reproduction day	Mean length of F0 [nm]	Mean dry weight of F0 [mg]
Control	-	5	14.09	6.3	1.09
Solvent control	-	10	10.32	6.0	1.02
25	24	0	13.48	6.1	1.02
50	48	0	15.09	6.0	1.07
100	89	5	7.94	6.1	1.24
200	160	0	13.74	5.9	1.03
400	339	20	4.06 *	4.9 #	0.44 *

* statistically significantly different compared to solvent control.

statistically significantly different compared to pooled controls.

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Clinical effects of lethargy, discoloration and a smaller size of the daphnids were observed at 339 µg as/L. NOEC for reproduction, length, and dry weight of the F0 generation is reported as 160 µg as/L. A 21-day EC₅₀ for mortality is reported to be > 339 µg as/L and the 21-day EC₅₀ for reproduction is 275 µg as/L.

Remarks by RMS

Water quality parameters were within accepted levels, except for DO on day 10 and 12, which was near 60% at the two highest test concentrations, flow rates were increased and a new diluter system was used. A new identical diluter system was used on days 16 – 21, due to this drop in DO (< 60% saturation), which was attributed to microbial growth in the test chambers. Daphnids were transferred to the new system and DO values increased to > 89% of saturation. This deviation did not influence the results of the study. Validity criteria were met. The result, a NOEC for reproduction of 160 µg as/L, based on mean measured test concentrations is accepted.

STUDY 2

Reference/notifier	: IIA 8.2/04	Exposure duration	: 4 days
Year of execution	: 1984	Nominal conc	: 1 and 3 mg/L
GLP statement	: No	Dosing method	: Static
Guideline	: Partly in accordance with early life stage test, OECD 210	Acceptability	: Acceptable
Test substance	: 1,4-dimethylnaphthalene	NOEC	: 0.57 mg/L
Purity	: 98%,		
Species	: sea urchin eggs (<i>Strongylocentrotus droebachiensis</i>)		
Water solubility	: 5.1 mg/L		

Substance	Species	Method	T [°C]	Duration [d]	Criterion	Value [mg/L]
1,4-dimethylnaphthalene	<i>Strongylocentrotus droebachiensis</i>	Static	5	4	NOEC	0.57

Description

1,4-dimethylnaphthalene, purity 97.5%, was added to seawater with fertilised sea urchin eggs (*Strongylocentrotus droebachiensis*) in a duplicate static test. A single layer of sea urchin eggs was placed in test beakers containing 100 mL of seawater mixed with the test substance in various concentrations (nominal concentrations of 2.92 and 0.95 mg as/L in the first test and 3.09, 2.06 and 1.03 mg as/L in the second test). The beakers were covered with aluminium foil. At fixed time intervals (6 h, 1 day, 2 days and 4 days) the aluminium foil was removed and the number of abnormal and dead embryos noted.

Conditions. Test temperature ca. 5°C.

Chemical analysis. Test concentration was measured by ultraviolet spectrophotometry at test start and termination.

Calculations and statistics. -

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Results

Mean measured concentrations were 1.75 and 0.54 mg/L, 60% and 57% of nominal, respectively, in the first test and 1.08, 0.56 and 0.31 mg/L, 35%, 27% and 30%, respectively, in the second test. Effects of various concentrations are shown in table 4.4.2-2. In the first test, the no effect level was in the range of 0.95-0.54 mg/L, resulting in a NOEC of 0.72 mg/L. In the second test the no effect level was in the range of 1.03-0.31 mg/L, resulting in a NOEC of 0.57 mg/L.

Table 4.4.2-2 Effects of 1,4-dimethylnaphthalene to *Strongylocentrotus droebachiensis*.

Substance	Test	Concentration range [mg/L]	Percentage of normal embryos after		
			6 hours	48 hours	96 hours
Control	1	0-0	96	94	95
1,4-dimethylnaphthalene	1	2.92-1.57	76	50	0
1,4-dimethylnaphthalene	1	0.95-0.54	95	92	94
Control	2	0-0	98	97	97
1,4-dimethylnaphthalene	2	3.09-1.08	86	50	0
1,4-dimethylnaphthalene	2	2.06-0.56	96	90	0*
1,4-dimethylnaphthalene	2	1.03-0.31	94	94	94

* slight inhibition

Remarks by RMS

The NOEC for effects of 1,4-dimethylnaphthalene on sea urchin embryo development as determined in this test was 0.57 mg/L.

4.4.4 Chronic toxicity to algae or aquatic plants

STUDY 1

Reference/notifier	: IIA, 8.6/01, DocID 535A-103	Exposure duration	: 7-day
Year of execution	: 2002	Nominal conc	: 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L
GLP statement	: Yes	Dosing method	: Semi-static
Guideline	: OPPTS 850.4400 (draft) = OECD 221 (proposed draft)	Acceptability	: Acceptable
Test substance	: 1,4-dimethylnaphthalene, batch 01C-01	IC ₅₀	: 1.1 mg/L
Purity	: 98.8%	NOEC	: 0.15 mg/L
Species	: <i>Lemna gibba G3</i>		
Water solubility	: 5 mg as/L		

Substance	Species	Method	T	pH	Duration	Criterion	Value
			[°C]		[d]		[mg as/L]

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

1,4-dimethylnaphthalene	<i>Lemna gibba</i> G3	Semi static	25.3 26.0	–	8.0 9.2	–	7	IC ₅₀	1.1
-------------------------	--------------------------	----------------	--------------	---	------------	---	---	------------------	-----

Description

Fronds of duckweed, *Lemna gibba* G3, were exposed to six concentrations of 1,4-dimethylnaphthalene, batch 01C-01, purity 98.8%, a negative control (culture medium) and a solvent control (DMF 0.1 mL/L) under static-renewal conditions for seven days. Acclimatisation for at least two weeks. Nominal test concentrations selected were 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L. Test medium was 20XAAp medium, pH 7.5. Test chambers contained 200 ml test solution. Three replicates for each test treatments and controls and one replicate without duckweed at the highest test concentration of 5.0 mg as/L. Renewal of test solutions on days 3 and 5 of the test. Four plants with in total 12 fronds were added to each replicate. Frond numbers were determined on days 3, 5 and 7. Observations of chlorosis, necrosis, break-up of duckweed colonies, death and any other abnormalities in plant or frond appearance were also performed on days 3, 5 and 7.

Measured test concentrations were determined at the beginning of the test, on day 3 and 5 of “new” and “old” test solutions and at test termination. IC₅₀ values were calculated based on replicate frond counts on day 7 of the test.

Conditions. Temperatures ranged from 25.3 to 26.0°C, pH 8.0 – 8.3 in the new prepared solutions and 8.3 - 9.2 in the old solutions. Continuous light (4460 – 5240 lux).

Chemical analysis. Samples were analysed by HPLC with fluorescence detection at 225 nm after diluting with acetonitrile and 50% (v/v) acetonitrile in a water solution. Recovery of the fortified samples ranged from 93.3 to 99.7% of nominal, LOQ 0.020 mg as/L.

Calculations and statistics. Mean frond numbers and percent inhibition were performed using TOXSTAT version 3.5. IC₅₀ was determined by linear interpolation. Correction of frond number for normality and homogeneity of variances was carried out using Shapiro-Wilks and Levene’s tests, respectively. Negative and solvent controls were compared using a t-test, which showed no statistically significant difference. Treatment groups were compared to pooled control groups using ANOVA and Bonferroni’s test.

Results

Mean measured test concentrations were 0.15, 0.31, 0.61, 1.2, 2.3 and 4.4 mg/L representing 88-100% of nominal. Mean measured test concentration at the 5.0 mg as/L abiotic treatment was 88% of nominal. Doubling time of frond number in the controls is about 3 days. Mean number of fronds after 7 days was 73 in the negative control and 75 in the solvent control and decreased treatment-related. Frond numbers after 7 days were 79, 67, 53, 35, 18, and 14 at 0.15, 0.31, 0.61, 1.2, 2.3, and 4.4 mg as/L. Percent inhibition increased from 8.6% to 81% at 0.31 to 4.4 mg as/L, at 0.15 mg as/L a stimulation of growth was noted of 6.8%. Frond numbers and percent inhibition were significant reduced at 0.31 mg as/L and onwards. Chlorotic and necrotic fronds were observed at 0.31 mg as/L and onwards. 7-days IC₅₀ for frond number is reported to be 1.1 mg as/L (95% CI 1.1 – 1.2 mg as/L), NOEC of 0.15 mg as/L.

Remarks by RMS

Water quality parameters were within accepted limits. The results, a 7-day IC₅₀ for frond numbers of 1.1 mg as/L and the NOEC value of 0.15 mg as/L, based on mean measured test concentrations is accepted.

5 REFERENCE LIST

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Annex point / reference number	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
IIA, 2.1/02	1993	1,4-DMN Batch H5510 - Color, Physical state, Odor, Boiling point, Specific gravity, pH, Flammability, Impact explosability, Viscosity. Ricerca, Inc., Analytical Services Painesville, Ohio USA report no. 4373-93-0226-AS GLP, Unpublished
IIA, 2.3/01	1993b	1,4-DMN - Vapor pressure. Ricerca, Inc., Analytical Services Painesville, Ohio USA report no. 4373-93-0249-AS GLP, Unpublished
IIA, 2.3/02	2003a	Calculation of Henry's Law Constant for 1,4-dimethylnaphthalene. Weterings Consultancy BV, Rosmalen, The Netherlands, 18 November 2003
IIA, 2.6/01	1993	1,4-DMN - Solubility Ricerca, Inc., Analytical Services Painesville, Ohio USA report no. 4373-93-0250-AS-001 GLP, Unpublished
IIA, 2.8/01	1978	Fluorimetric determination of partition coefficients of naphthalene homologues in octanol-water mixtures J. Environ. Sci. Health Part A, 13 (8): 595-602 Not GLP, Published
IIA, 2.14/01	1991	633 Organic chemicals, surface tension data. Chemical Engineering 98 (3): 140-2, 144, 146, 148, 150 Not GLP, Published

Annex point / reference number	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
IIA, 5.1/01	2000	Disposition and metabolism of 1,4-dimethylnaphthalene in rat. Int. J. Occup. Medicine Environ. Health, 13(4): 325-334 Not GLP, Published
IIA, 5.1/02	1983	In vivo and in vitro metabolism of 2-methylnaphthalene in the Guinea pig. Drug Metabolism and Disposition 11(2): 152-157 Not GLP, Published
IIA, 5.1/03	1982	Effects of inducers and inhibitors of cytochrome P-450-linked monooxygenases on the toxicity, in vitro metabolism and in vivo irreversible binding of 2-methylnaphthalene in mice. The Journal of Pharmacology and Experimental Therapeutics 221(3): 517-524 Not GLP, Published
IIA, 5.1/04	1981	Distribution and excretion of monoisopropylnaphthalene in rats. Bulletin of Environmental Contamination and Toxicology 26: 626-633 Not GLP, Published

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Annex point / reference number	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
IIA, 5.1/05	1979	Alkylnaphthalenes. III. Absorption, distribution, accumulation, and excretion of 2-isopropyl-naphthalene in rats. Eisei Kagaku 25(6): 327-333 Not GLP, Published
IIA, 5.1/06	1998	Disposition and metabolism of 1,2-dimethylnaphthalene in rats. International Journal of Occupational Medicine and Environmental Health 11: 305-317 Not GLP, Published
IIA, 5.1/07	2002	Disposition and metabolism of 1,6-dimethylnaphthalene in rats. Toxicology Letters 134: 227-235 Not GLP, Published
IIA, 5.1/08	1982	Metabolism of 2-methylnaphthalene in the rat in vivo. I. Identification of 2-naphthoyleglycine. Drug Metabolism and Disposition 10, 128-133 (<i>cited in: BUA 1990</i>) Not GLP, Published
IIA, 5.1/09	1990	Methylnaphthalene, I. 1-Methylnaphthalin, 2-Methylnaphthalin, II. 2,6-Dimethylnaphthalin. Gesellschaft Deutscher Chemiker, Beratergremium für umweltrelevante Altstoffe, BUA-Stoffbericht 47, March 1990 Not GLP, Published
IIA, 5.1/10	1978	Alkylnaphthalenes. I. Absorption, tissue distribution and excretion of 2,6-diisopropyl-naphthalene in rats. Chemistry and Pharmacology Bulletin 26(10): 3007-3009 Not GLP, Published
IIA, 5.1/11	1982	Urinary metabolites of 2,6-diisopropyl-naphthalene in rats. Drug Metabolism and Disposition 10(4): 429-433 Not GLP, Published
IIA, 5.1/12	1985	Biliary metabolites of 2,6-diisopropyl-naphthalene in rats. Bulletin of Environmental Contamination and Toxicology 35: 745-749 Not GLP, Published
IIA, 5.1/13	1979	Alkylnaphthalenes. II. Tissue accumulation of 2,6-diisopropyl-naphthalene administered continuously to rats. Eisei Kagaku 25(4): 221-224 Not GLP, Published
IIA, 5.1/14	1998	Metabolism and toxicity of diisopropyl-naphthalene as compared to naphthalene and monoalkyl naphthalenes: a minireview. Toxicology 126: 1-7 Not GLP, Published
IIA, 5.1/15	2005	Toxicological profile for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. US DHHS, Agency for Toxic Substances and Disease, August 2005 Not GLP, Published

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Annex point / reference number	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
IIA, 5.1/16	2001	Metabolic capabilities of CYP2F2 with various pulmonary toxicants and its relative abundance in mouse lung subcompartments. The Journal of Pharmacology and Experimental Therapeutics 296(2): 510-519 Not GLP, Published
IIA, 5.1/17	1980	Identification of urinary metabolites of 2-isopropyl-naphthalene in rats. Drug Metabolism and Disposition 8(6), 463-466 Not GLP, Published
IIA, 5.1/18	1984	Identification and determination of urinary and biliary metabolites of 2-isopropyl-naphthalene in rats. Eisei Kagaku 30(2), 91-95 Not GLP, Published
IIA, 5.1/19	2001	Early events in naphthalene-induced acute Clara cell toxicity. American Journal of Respiratory Cell Molecular Biology 24: 272-281 Not GLP, Published
IIA, 5.1/20	1990	Alkyl-naphthalene. XI. Pulmonary toxicity of naphthalene, 2-methylnaphthalene, and isopropyl-naphthalenes in mice. Chemistry and Pharmacology Bulletin 38(11): 3130-3135 Not GLP, Published
IIA, 5.1/21	2003	The role of glutathione in metabolism of selected dimethylnaphthalenes in rat. Int. J. Occup. Medicine Environ. Health, 16(3): 265-270 Not GLP, Published
IIA, 5.1/22	1974	Studies on metabolism of diisopropyl-naphthalene. National Defense Medical Journal 21(7): 273-290 Not GLP, Published
IIA, 5.1/23	2004	Metabolism and toxicity of 1,4 dimethylnaphthalene extrapolation from alkylated naphthalenes Not GLP, Unpublished
IIA, 5.1/23	2012a	Metabolism of 1,4-dimethylnaphthalene in rats. Report No. 2263W-1, 23 October 2012 GLP, Unpublished
IIA, 5.2/01	1993a	Acute oral toxicity of 1,4-dimethylnaphthalene (1,4-DMN) in rats. report no. L08456 - Study 6,7,8 GLP, Unpublished
IIA, 5.2/02	1993b	Acute dermal toxicity of 1,4-dimethylnaphthalene (1,4-DMN) in rabbit (limit test). report no. L08456 - Study 4 GLP, Unpublished
IIA, 5.2/03	1993	Acute inhalation toxicity of 1,4-dimethylnaphthalene (1,4-DMN) in rats. report no. L08456L001 GLP, Unpublished

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Annex point / reference number	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
IIA, 5.2/04	1993c	Acute dermal irritancy/corrosivity study of 1,4-dimethylnaphthalene (1,4-DMN) in rabbits. report no. L08456 - Study 2 GLP, Unpublished
IIA, 5.2/05	1993d	Primary eye irritancy study of 1,4-dimethylnaphthalene (1,4-DMN) in rabbits. report no. L08456 - Study 1 GLP, Unpublished
IIA, 5.2/07	2011	Assessment of contact hypersensitivity to 1,4-dimethylnaphthalene in the mouse (Local Lymph Node Assay) Project 495316, 4 March 2011 GLP, Unpublished
IIA, 5.3/01	2003	90-Day oral (diet) toxicity study of 1,4-dimethylnaphthalene in rats. report no. 02-154 GLP, Unpublished
IIA, 5.4/01	1993	Mutagenicity test on 1,4-dimethylnaphthalene in the Salmonella/mammalian-microsome reverse mutation assay (Ames test). report no. 15683-0-401 GLP, Unpublished
IIA, 5.4/02	2007	Reverse mutation in five histidine-requiring strains of <i>Salmonella typhimurium</i> report no. 2782/2-D6171 GLP, Unpublished
IIA, 5.4/03	2005	Evaluation of the mutagenic activity of 1,4-dimethylnaphthalene in an <i>in vitro</i> mammalian cell gene mutation test with L5178Y mouse lymphoma cells report no. 424711 GLP, Unpublished
IIA, 5.4/04	1993	Genotoxicity test on 1,4-dimethylnaphthalene in the assay for unscheduled DNA synthesis in rat liver primary cell cultures. report no. 15683-0-447 GLP, Unpublished
IIA, 5.4/05	1993	Mutagenicity test on 1,4-dimethylnaphthalene in an <i>in vivo</i> micronucleus assay. report no. 15683-0-455 GLP, Unpublished
IIA, 5.4/06	2007	1,4-Dimethylnaphthalene - <i>In vivo</i> mouse bone marrow slide scoring for the micronucleus assay report no. 7931-100 GLP, Unpublished
IIA, 5.4/07	2007	1,4-Dimethylnaphthalene - Measurement of unscheduled DNA synthesis in rat liver using an <i>in vivo/in vitro</i> procedure report no. 1000/31-D6172 GLP, Unpublished

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Annex point / reference number	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
IIA, 5.5/01	2000	IUCLID Dataset bis(isopropyl)naphthalene Substance ID: 38640-62-9 European Commission, European Chemicals Bureau, 18 February 2000 Not GLP, Published
IIA, 5.5/02	2002	TOX/2002/38. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, Submission of new data and evaluation of Di-isopropyl-naphthylene (DIPN) url: http://cot.food.gov.uk/pdfs/2002-38DIPN.PDF Published
IIA, 5.5/03	1977	Long-term chronic toxicity and carcinogenicity of DIPN in rats. (<i>cited in: ECB 2000</i>) Not GLP, Unpublished
IIA, 5.5/04	1993	Chronic toxicity and carcinogenicity studies of 1-methylnaphthalene in B6C3F1 mice. Fundamental and Applied Toxicology 21: 44-51 Not GLP, Published
IIA, 5.5/05	1997	Chronic toxicity and carcinogenicity studies of 2-methylnaphthalene in B6C3F1 mice. Fundamental and Applied Toxicology 36: 90-93 Not GLP, Published
IIA, 5.5/06	1985	Endogenous lipid pneumonia in B6C3F1 mice. In: Jones TC, Mohr U, Hunt RD, eds. Respiratory system: Monographs on pathology of laboratory animals. New York: Springer-Verlag, 166-168 (<i>cited in: ATSDR 2005</i>) Not GLP, Published
IIA, 5.5/07	1992	Ultrastructural analysis of pulmonary alveolar proteinosis induced by methylnaphthalene in mice. Experimental Toxicology and Pathology 44, 47-54 (<i>cited in: ATSDR 2005</i>) Not GLP, Published
IIA, 5.5/08	1988	The alveolar type II epithelial cell: a multifunctional pneumocyte. Toxicology and Applied Pharmacology 93(3): 472-483 (<i>cited in: ATSDR 2005</i>) Not GLP, Published
IIA, 5.5/09	1992	Relationship of cytochrome P450 activity to Clara cell cytotoxicity. II. Comparison of stereo-selectivity of naphthalene epoxidation in lung and nasal mucosa of mouse, hamster, rat and Rhesus monkey. Journal of Pharmacology and Experimental Therapy 261(1): 364-372 (<i>cited in: ATSDR 2005</i>) Not GLP, Published
IIA, 5.5/10	2001	Inhaled naphthalene causes dose dependent cell cytotoxicity in mice but not in rats. Toxicol Appl Pharmacol 173(2): 114-119 (<i>cited in: ATSDR 2005</i>) Not GLP, Published

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Annex point / reference number	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
IIA, 5.5/11	2001	Toxicity and carcinogenicity study in F344 rats following 2 years of whole-body exposure to naphthalene vapors. Inhalation Toxicology 13(10): 931-950 (<i>cited in: ATSDR 2005</i>) Not GLP, Published
IIA, 5.5/12	1992	Toxicology and carcinogenesis studies of naphthalene (CAS No. 91-20-3) in B6C3F1 mice (inhalation studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. National Toxicology Program. NIH Publication No.92-3141. Technical report series no. 410 (<i>cited in: ATSDR 2005</i>) GLP, Published
IIA, 5.5/13	2000	Toxicology and carcinogenesis studies of naphthalene (CAS No. 91-20-3) in F344/N rats (inhalation studies). National Toxicology Program. NTP TR 500, NIH Publ. No. 01-4434 (<i>cited in: ATSDR 2005</i>) GLP, Published
IIA, 5.5/14	1995	Relationship of cytochrome P450 activity to Clara cell cytotoxicity. IV. Metabolism of naphthalene and naphthalene oxide in microdissected airways from mice, rats, and hamsters. Molecular Pharmacology 47(1): 74-81 (<i>cited in: ATSDR 2005</i>) Not GLP, Published
IIA, 5.5/15	1999	Specific dehydrogenation of 3-methylindole and epoxidation of naphthalene by recombinant human CYP2F1 expressed in lymphoblastoid cells. Drug Metabolism and Disposition 27(7): 798-803 (<i>cited in: ATSDR 2005</i>) Not GLP, Published
IIA, 5.5/16	2009	Interim one year report - Oral combined chronic toxicity/carcinogenicity study of 1,4-dimethylnaphthalene in rats, Experimur study no. 07-481, 28 May 2009 GLP, Unpublished
IIA, 5.5/17	2011	Oral (diet admixture) combined chronic toxicity/carcinogenicity study of 1,4-dimethylnaphthalene in rats Study No. 07-481, 31 March 2011 GLP, Unpublished
IIA, 5.6/01	1977	Two-generation reproduction studies on di-isopropyl naphthalene and diarylethane isomer (PCB substitutes) in mice. Japanese Journal of Hygiene 31(6): 637-643 Not GLP, Published
IIA, 5.6/02	2003	90-Day oral (diet) toxicity study of 1,4-dimethylnaphthalene in rats. report no. 02-154 GLP, Unpublished
IIA, 5.6/03	1993	Chronic toxicity and carcinogenicity studies of 1-methylnaphthalene in B6C3F1 mice. Fundamental and Applied Toxicology 21: 44-51 Not GLP, Published
IIA, 5.6/04	1997	Chronic toxicity and carcinogenicity studies of 2-methylnaphthalene in B6C3F1 mice. Fundamental and Applied Toxicology 36: 90-93 Not GLP, Published

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Annex point / reference number	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not
IIA, 5.6/05	2003	2,6-Diisopropyl-naphthalene (PC Code 055803), Biopesticide Registration Action Document. url: http://www.epa.gov/pesticides/biopesticides/ingredients/tech_docs/brad_055803.pdf Published
IIA, 5.6/06	1982	Safety evaluation of chemicals for use in household products (III). Teratological studies on 2-chloroethylbenzoate and methylnaphthalene in rats. Journal of the Osaka City Institute of Public Health and Environmental Sciences 1982: 83-90 Not GLP, Published
IIA, 5.6/07	2011a	Extended one-generation reproductive toxicity study of 1,4-dimethylnaphthalene administered orally (diet admixture) to rats Study No. 10-593, 31 March 2011 GLP, Unpublished
IIA, 5.6/08	2011b	Oral developmental toxicity (Segment II) study with 1,4-dimethylnaphthalene in rabbits Study No. 10-601, 31 March 2011 GLP, Unpublished
IIA, 5.7/01	2003	90-Day oral (diet) toxicity study of 1,4-dimethylnaphthalene in rats. report no. 02-154 GLP, Unpublished
IIA, 5.7/02	2005	Toxicological profile for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. US DHHS, Agency for Toxic Substances and Disease, August 2005 Not GLP, Published

Annex point/ reference no.	Year	Title Company, report no. Source (where different from company) GLP or GEP status (where relevant) Published or not
IIA, 7.1/01	1993	Microbial oxidation of dimethylnaphthalene isomers Applied and Environmental Microbiology 59(5), 1504-1506 Not GLP, Published
IIA, 7.1/02	1988	Metabolism of 2,6-dimethylnaphthalene by Flavobacteria Applied and Environmental Microbiology 54, 428-433 Not GLP, Published

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Annex point/ reference no.	Year	Title Company, report no. Source (where different from company) GLP or GEP status (where relevant) Published or not
IIA, 7.1/03	1998	Composting of hazardous wastes and hazardous substances. In: Brown S, Angle JS, Jacobs L (Ed.), Beneficial co-utilization of agricultural, municipal and industrial by-products; Proceedings of the Beltsville Symposium XXII, Beltsville, Maryland, USA, May 4-8, 1997. Kluwer Academic Publishers, Dordrecht, The Netherlands, Norwell, Massachusetts, USA. ISBN 0-7923-5189-4., pp. 327-340. Not GLP, Published
IIA, 7.1/04	1996	Laboratory scale in-vessel composter for volatile emission analysis J. Environ. Qual. 25, 371-373 Not GLP, Published
IIA, 7.6/01	2004	Direct photolysis of 1,4-dimethyl-naphthalene using Quantitative Structure-Property Relationship (QSPR) models. Weterings Consultancy, 7 October 2004 Not GLP, Unpublished
IIA, 7.6/02	2000	Quantitative structure-property relationships for direct photolysis quantum yields of selected polycyclic aromatic hydrocarbons. The Science of the Total Environment 246: 11-20 Not GLP, Published
IIA, 7.6/04	1988	On the photolysis of alkylated naphthalenes in aquatic systems Chemosphere 17(4), 651-659 Not GLP, Published
IIA, 7.7/01	1983	Biodegradation of mono- and dimethyl substituted naphthalenes. Aromatikkusu 35(11/12), 287-292 Not GLP, Published
IIA 7.8/01	2003	Aerobic and anaerobic transformation of radiolabelled 14C-1,4dimethylnaphthalene in freshwater aquatic sediment systems. Wildlife Internation Ltd, Maryland USA report No 535E-102 Not GLP, Unpublished

Annex point / reference number	Year	Title Company Source (where GLP Published or not	Report different GEP	No. from company) status
IIA, 8.2/01	1993	1,4-Dimethylnaphthalene - Acute toxicity to Rainbow trout (Oncorhynchus mykiss) under flow-through conditions report no. 93-10-4955 GLP, Unpublished		
IIA, 8.2/02	2002	1,4-Dimethylnaphthalene - A 96-hour static renewal acute toxicity test with the Fathead minnow (Pimephales promelas) report no. 535A-101A GLP, Unpublished		

ANNEX TO ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 1,4-DIMETHYLNAPHTHALENE

Annex point / reference number	Year	Title Company Source (where GLP or Published or not	Report different from GEP	No. company) status
IIA, 8.2/03	2006a	1,4-Dimethylnaphthalene: A 28-day flow-through juvenile growth test with the Rainbow trout (<i>Oncorhynchus mykiss</i>) report GLP, Unpublished	no.	535A-105
IIA, 8.2/04	1984	Toxicity and chemical reactivity of naphthalene and methylnaphthalenes Aquatic Toxicology Not GLP, Published	5,	291-306
IIA, 8.2/07	2004	Bioconcentration, biotransformation, and elimination of polycyclic aromatic hydrocarbons in sheepshead minnows (<i>Cyprinodon variegatus</i>) exposed to contaminated seawater Environmental Toxicology and Chemistry Not GLP, Published	23(6),	1538-1548
IIA, 8.2/08	2003	Estimation of selected physicochemical properties for methylated naphthalene compounds Chemosphere 52, 869-881 Not GLP, Published		
IIA, 8.3/01	1993	1,4-Dimethylnaphthalene - Acute toxicity to Daphnids (<i>Daphnia magna</i>) under flow-through Springborn Laboratories, Inc., Wareham, Massachusetts USA report no. 93-104951 GLP, Unpublished		
IIA, 8.3/02	2006b	1,4-Dimethylnaphthalene: A flow-through life-cycle toxicity test with the cladoceran (<i>Daphnia magna</i>) Wildlife International Ltd., Easton, Maryland USA report no. 535A-104 GLP, Unpublished		
IIA, 8.4/01&1a	2002a	1,4-Dimethylnaphthalene - A 96-hour toxicity test with the freshwater alga (<i>Selenastrum capricornutum</i>) Wildlife International Ltd., Easton, Maryland USA report no. 535A-102 Analytical method verification for the determination of 1,4-dimethylnaphthalene in algal medium Wildlife International Ltd., Easton, Maryland USA report no. 535C-101 GLP, Unpublished		
IIA, 8.6/01	2002b	1,4-Dimethylnaphthalene - A 7-day static-renewal toxicity test with Duckweed (<i>Lemna gibba</i> G3) Wildlife International Ltd., Easton, Maryland USA report no. 535A-103 GLP, Unpublished		