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: -

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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 explodability 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

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 thermoter 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

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-^{3}H]-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.

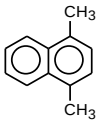
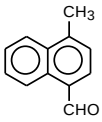
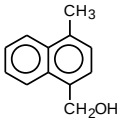
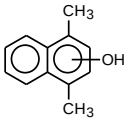
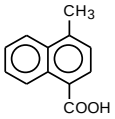
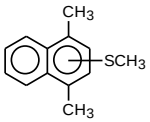
Table 2.1.1-1 Total balance of tritium following a single intraperitoneal administration of [ring-U-^{3}H]-1,4-dimethylnaphthalene.

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

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 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.

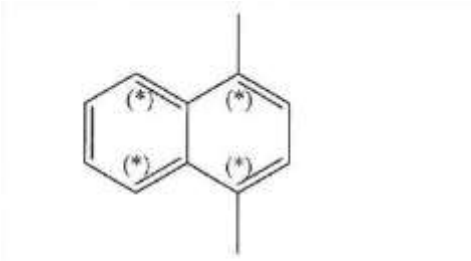
2.1.2 Study 2

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 ^{14}C label.
Each molecule has only one ^{14}C.

In this GLP study, 3 male rats received a single oral dose of 28 mg/kg bw of [^{14}C]-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 sacrifices 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)

The nature of the urinary ^{14}C 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 naphtols 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-dimethyl-methylthionaphthalene; 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 naphtols) 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 [^{14}C]-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%
subtotal	*69.7%*	*71.7%*	*73.4%*	*71.6%*
0-24 hr Feces	16.5%	10.6%	13.2%	13.4
24-48hr Feces	14.3%	20.5%	7.5%	14.1
subtotal	*30.8%*	*31.1%*	*20.7%*	*27.5%*
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 [^{14}C]-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**

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%
N-Acetylcysteine-4-methyl-1-naphthoic acid (isomer) Hydroxy-*N*-acetylcysteine-dihydro-1,4-dimethylnaphthalene (isomer)	2.5%
N-Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.2%
N-Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.5%
Hydroxy-*N*-acetylcysteine-dihydro-1,4-dimethylnaphthalene (isomer)	3.2%
Hydroxy-*N*-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%
N-Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.3%
N-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

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-^{14}C]-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**~~Table~~ 2.1.3-1). Urine, faeces and cage wash contained 76%, 14% and 1.5% of the total administered dose respectively.

Liver, 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 [^{14}C]-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
Hindquarter Muscle	0.017	NA

Sample	TRR (mg eq/kg)	% Administered Dose Recovered
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 [^{14}C]-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 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.

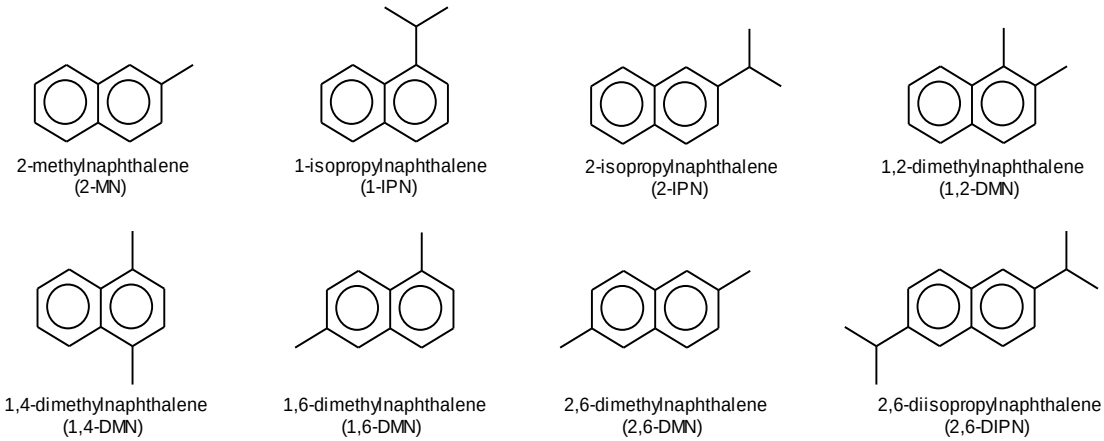
2.1.4 Study 4 – Kinetic information for other naphthalenes

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 ~~Figure~~ 2.1.4-1. Physical-chemical properties of these alkylnaphthalenes are listed in Table~~Table~~ 2.1.4-1.

Met c

Met c

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.

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-[^{3}H]-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 [^{14}C]-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-isopropylnaphthalene 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 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-isopropylnaphthalene in the blood, liver, kidneys, brain, heart, spleen and muscle were observed at 2 h after the administration (first time point). Disappearance of 2-isopropylnaphthalene 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-isopropylnaphthalene from these tissues was slower than from the other examined tissues. No information is provided on the distribution and excretion of metabolites of 2-isopropylnaphthalene (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-isopropylnaphthalene in the diet for 14 or 28 days (reported to be equal to 13-18 mg/kg bw/day). Again, the highest 2-isopropylnaphthalene 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-isopropylnaphthalene in the diet during 28 days, the 2-isopropylnaphthalene content of blood and skin decreased to about one-third and the 2-isopropylnaphthalene content of the adipose tissue decreased to about 50% within the first 24 h after withdrawal, and then decreased more slowly. Disappearance of 2-isopropylnaphthalene 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-isopropylnaphthalene 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-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 and Maruyama, 1979 (IIA 5.1/05)).

In rats orally administered 2-isopropylnaphthalene 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-isopropylnaphthalene 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-isopropylnaphthalene 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-isopropylnaphthalene 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-isopropylnaphthalene-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[^{14}C]naphthalene (6.25 µCi/kg bw ~ 1 mg/kg bw) plus 100 mg/kg bw non-labelled monoisopropylnaphthalene, both consisting of 60% 2-isopropylnaphthalene and 40% 1-isopropylnaphthalene, in olive oil. Other groups of male rats (3 animals/time point) received 100 mg/kg bw unchanged 1-isopropylnaphthalene in olive oil by stomach tube. The tissue concentration patterns of unchanged 1-isopropylnaphthalene resembled those of 2-isopropylnaphthalene 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)

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)).

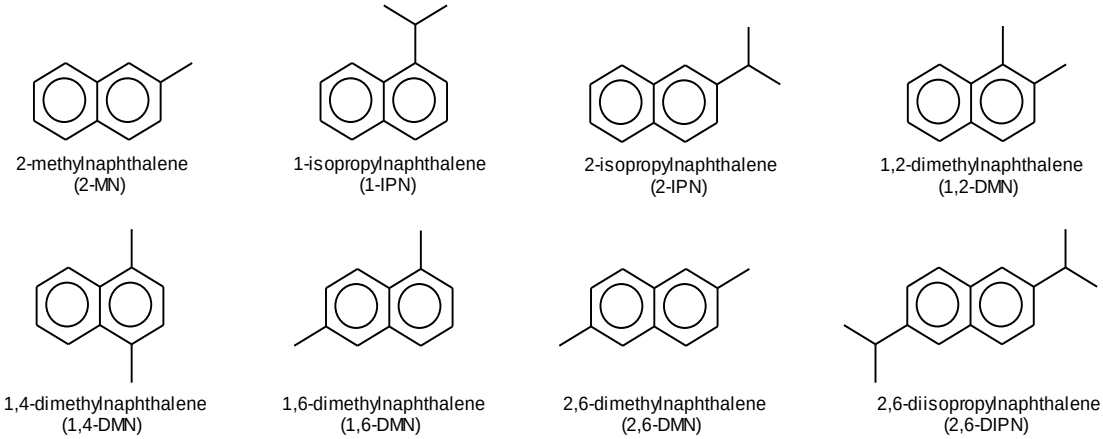
A study in mice with ^{3}H-labeled diisopropylnaphthalene was available in the Chinese language. Based on abstract, figure legends and tables (in English), radioactivity reached maximum levels in various organs at 2 to 4 h after oral administration (dose unknown). The only exception was the adipose tissue 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)).

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, alkylnaphthalenes 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 alkylnaphthalenes

	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]	3				4 h: adipose tissue > kidney > liver > lung 24 h: kidney > adipose tissue, liver, lung	Griffin et al.1982
1-IPN/2-IPN	Oral	2	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
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.

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

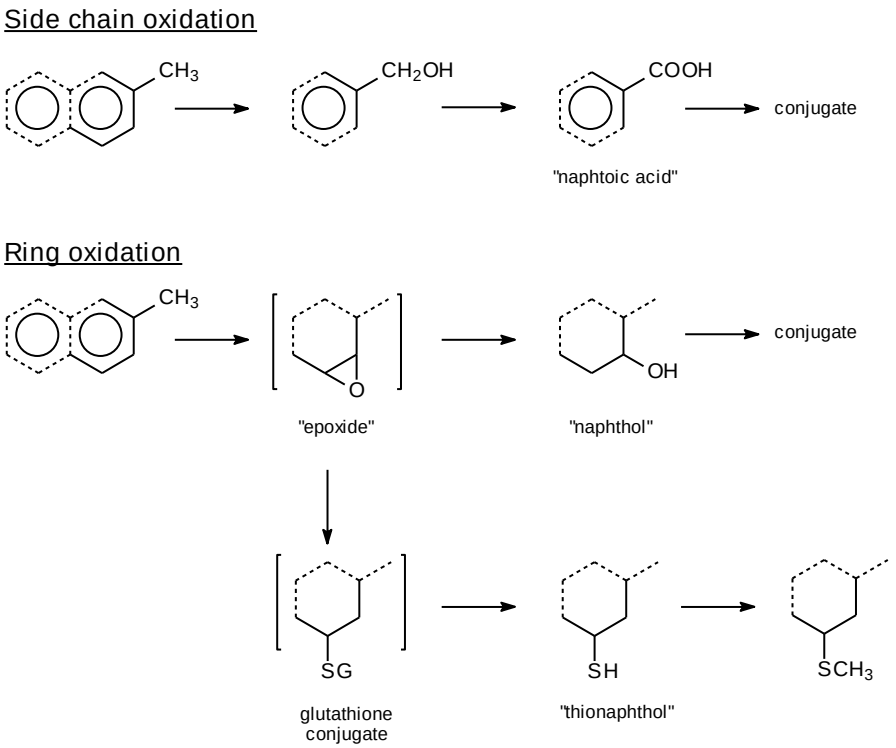
Day after halting 2,6-DIPN administration	**2,6-DIPN (average for 3 or 4 animals)**				
	Blood (µg/ml)	**Liver (µg/g)**	**Kidneys (µg/g)**	**Skin (µg/g)**	**Adipose tissue (µg/g)**
0	0.25	2.46	1.77	23.41	198.48
7	0	0	0	-	23.97
14	-	-	-	-	4.09
21	-	-	-	-	0.35
28	-	-	-	2.73	0.23
35	-	-	-	-	0.14

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



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 alkylnaph-thalene 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 alkylnaphthalenes 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

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 com-pounds 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 LD_{50}). 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

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%
N-Acetylcysteine-4-methyl-1-naphthoic acid (isomer) Hydroxy-*N*-acetylcysteine-dihydro-1,4-dimethylnaphthalene (isomer)	2.5%
N-Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.2%
N-Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.5%
Hydroxy-*N*-acetylcysteine-dihydro-1,4-dimethylnaphthalene (isomer)	3.2%
Hydroxy-*N*-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%
N-Acetylcysteine-4-methyl-1-naphthoic acid (isomer)	1.3%
N-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.

-

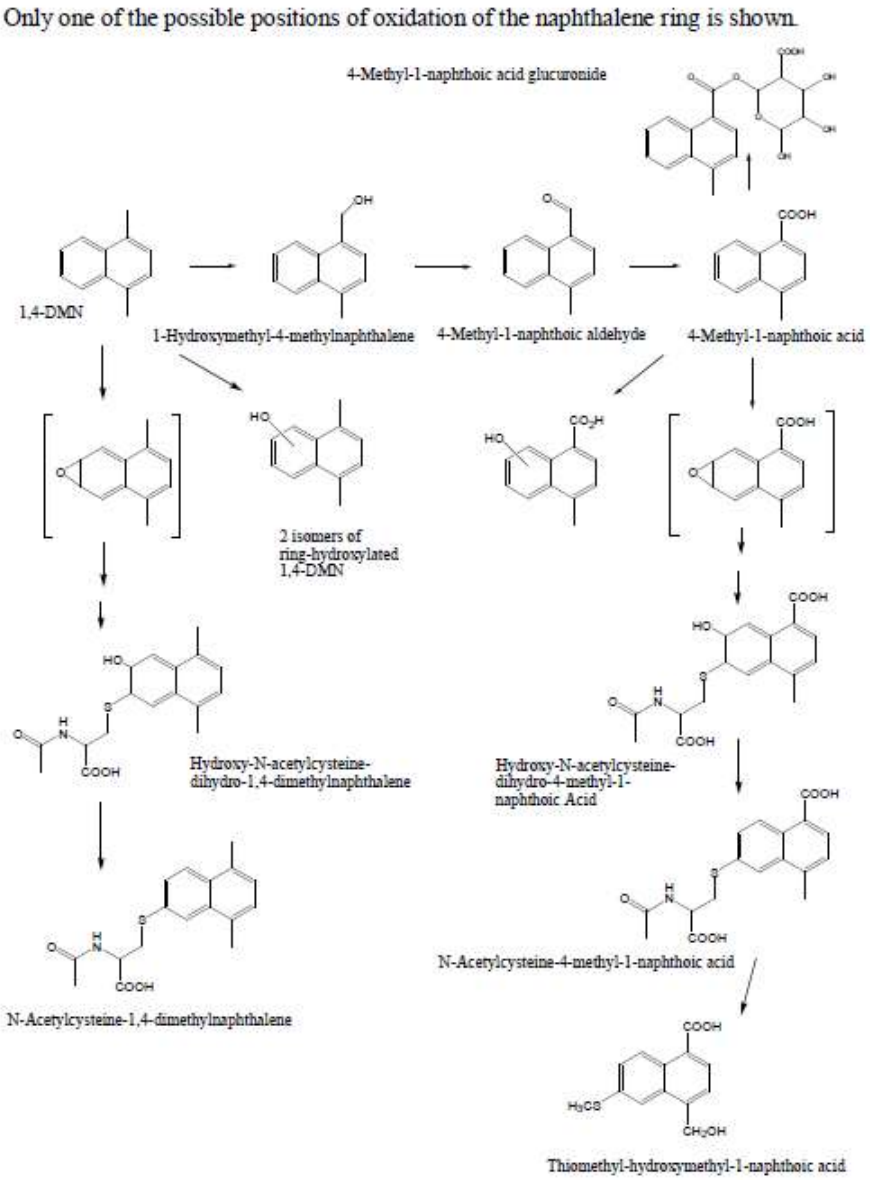
List of identified compounds following intraperitoneal administration of 1,4-dimethylnaphthalene in rats.

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:



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:

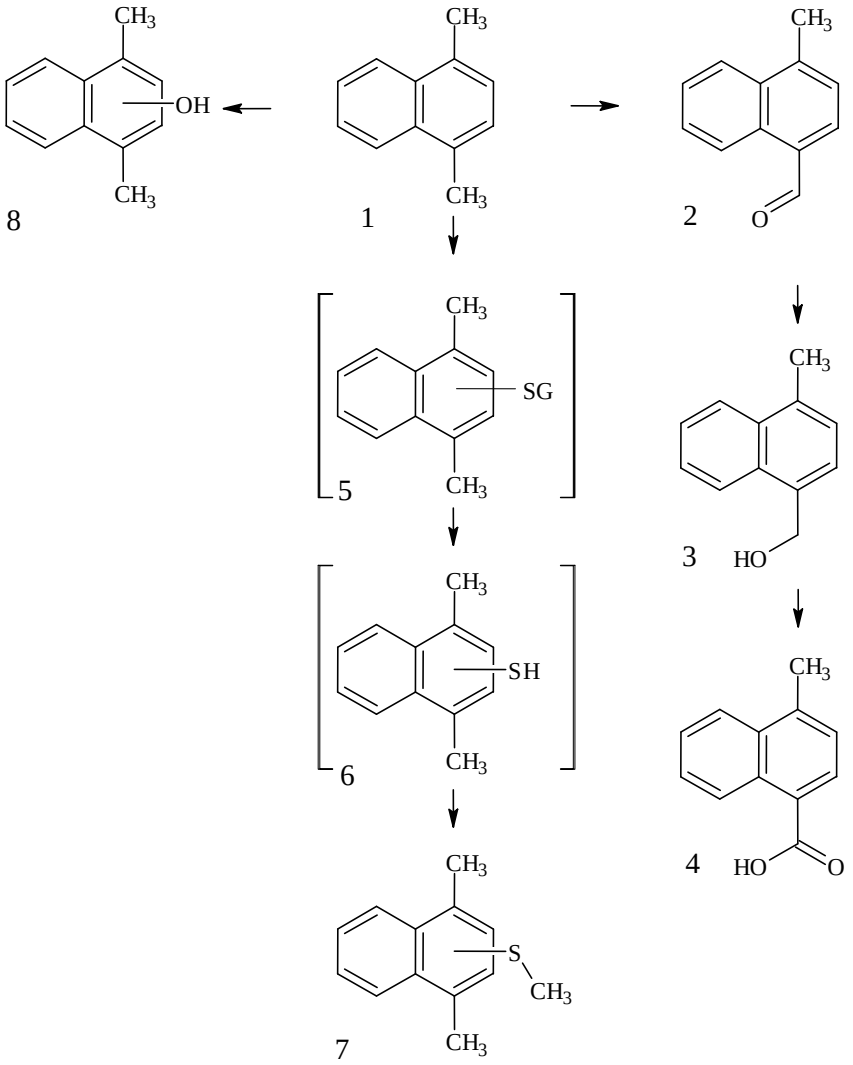
1) oxygenation of the alkyl side chains through an initial hydroxylation to the corresponding carboxylic acids: for 1,4-DMN this was initiated by hydroxylation on a methyl group, to form 1-hydroxymethyl-4-methylnaphtalene, that was further oxidised to 4-methyl-1-naphtoic acid (metabolite 4) (6.0% in urine). Another portion (equivalent to 6.1% of the dose) of the 4-methyl-1-naphtoic 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-dimethylnaphtalene derivates (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:



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:

a) side chain oxidation resulting ultimately in the carboxylic acid 4-methyl-1-naphthoic acid (4),

b) ring hydroxylation resulting in 1,4-dimethylnaphthols (2 isomers, (8)) via reactive epoxide with

c) 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.

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 (Crl: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.

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

	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

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.

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.

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 L08456L001	Exposure	:	single dose, whole-body, 4h
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 (Crl: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 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 mounth	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

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

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

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-dimethylnaphtalene, 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 ^{3}H-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.

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 ≥ 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:

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
Postive 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
Postive control	+	1123	905

Acceptability

The study is considered acceptable.

Conclusions

Under the test conditions, 1,4-dimethylnaphthalene did not induce revertants in *S. typhimurium.*

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 µg/plate in the presence of S9 and doses ≥ 200 µg/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 µg/plate, plus negative and positive controls. In experiment 2 the maximum concentration in the presence of S9 was reduced to 400 µg/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), mytomycin 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 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.

Table 3.8.1.2-1: Summary of the bacterial reverse mutation assay

Dose level (µg/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										
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

[1]Toxic, 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 µg/mL in the presence of S9 and doses ≥ 33 µg/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 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 first experiment the incubation time was 3 hours. A second experiment was carried out the 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 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.

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 ^{3}HTdR (46 Ci/mMole). The test material was soluble at and below 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
-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.

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

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 ≥ 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 ≥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 ≥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

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; Crl: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

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	ds wk 1-21	ds	

Dose (ppm)	**0**		**150**		**500**		**3750**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
						-tally)			
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/µL)	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/µL)	1203	850	1099	927	1073	858	954*	821	
Haematology (week 104/100)									
-white blood cell (10^3 cells/µL)	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/µL)	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*	
Pathology									
Macroscopy (week 52, 100/104)	Not toxicologically relevant effects								
Microscopy (week 52)									
-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	

Dose (ppm)	**0**		**150**		**500**		**3750**		**dr**
Sex	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
-erythrocytic accumulation	0	0	-	-	-	-	4	0	
Microscopy (week 100/104) Neoplastic lesions	Not toxicologically relevant effects								
Microscopy (week 100/104) 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.

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 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 (Murate, 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).*

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).*

TABLE 1
Incidences of Tumors in B6C3F$_1$ Mice Given 2-Methylnaphthalene in the Diet for 81 Weeks

	Female			Male		
Dose (%):	0	0.075	0.15	0	0.075	0.15
No. of mice:	50	49	48	49	49	49
Lesions	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 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	**NOAEL**par	:	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

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**
P animals								
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								
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								
Macroscopy	No treatment-related findings							
Microscopy	No treatment-related findings							

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)		22.1		22.0		21.9		22.1
- gestation index		100		100		100		95.8
Number of implantation sites		11.7		13.3		12.7		10.0
F_1 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							-22%*	-21%*
-PND 14				-8%*	-14%*	-16*	-45%*	-56%*
Pup body weight								
-at preputial separation (g)	161		163		168		144*	
-at vaginal patency (g)		118		122		118		105*
Sexual maturation								
- anogenital distance/litter (cm)	6.6/3.2		6.4/3.1		6.3/3.2		6.1/3.1	
- preputial separation/litter (days)	37		38		40*		46*	
- vaginal patency (days)	34		36		35		43*	
- onset of 1st oestrus (days)	2		2		2		1	
F1 Food consumption					-17%*	-10%	-50%*	-38%*
F1 body weights					-11%*	-11%*	-53%*	-46%*
Haematology								
PT (sec)	10.6	10.3	10.3*	10.3	10.3*	10.2	10.1*	10.1
aPTT (sec)	17.6	17.0	17.3	16.7	16.8	16.9	16.9	15.5*
Clinical chemistry								
Cholesterol (mg/dL)	58	50	66	51	73*	73*	80*	123*
gamma-GT	0.0	0.3	0.0	0.4	0.0	0.7	1.9*	9.2*
creatinin	0.34	0.35	0.30	0.32	0.29	0.29*	0.31	0.29*
triglycerides	58	30	50	33	44*	40	35*	43*
Urinalysis	No treatment related findings							

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} 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 ($p \leq 0.05$)
** 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 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 PDN 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.

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

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:	GLP statement	:	yes

		98.4%*			
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
			NOAELdev	:	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**
Maternal effects					
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				
Litter response					
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	
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 female	 36.5 35.4	 39.5 37.3	 38.6 37.2	 36.3 33.0	
Sex ratio	No treatment-related findings				
Examination of the foetuses					
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
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 P_1 consisted of 20 dayms. Of these 10 were killed on day 18 of gestuation 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 laction 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 oas 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 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 intak 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

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**	

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)	323	126	303	132	289	121	214*	86*	m
(% of control)			(94)	(105)	(89)	(96)	(66)	(68)	
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/µL)	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/
-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/
-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	
-basophilc 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 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**	
Sex	**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/µL)	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*
-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*

Dose (mg/kg food)	**0**		**10,000**	
Sex	**m**	**f**	**m**	**f**
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 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 humans[1].

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 mPa.s / 1.014 = 5.9 mm^2/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 [°C]	pH	Duration [d]	Transformation at end [%]	Classification
1,4-DMN	test medium	25	n.r.	28	66	readily biodegradable

[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

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 O_2/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.

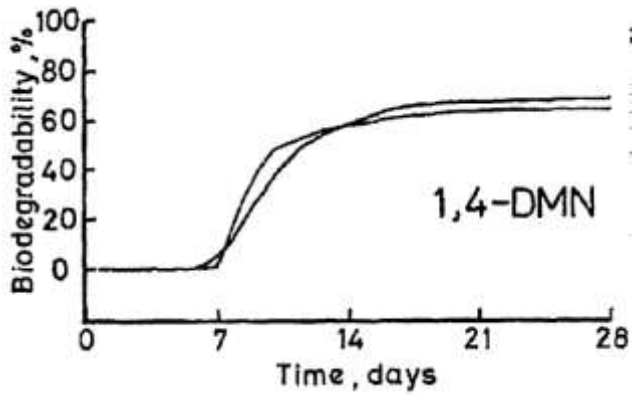
Remarks

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 Sub-stances	Acclimatization Period (days)	Degradation Level (%)			
		7 days	14 days	21 days	28 days
1,4-dimethyl-naphthalene	6 7	6 0	58 58	67 63	68 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 volatialization 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.



4.1.2 BOD_5/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 10^5 day (20°C).

4.1.4.2 Water-sediment degredation

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 water**	**T**	**pH**	**OM**	**Duration**	**Degradation at end**	**DT_{50} water**	**DT_{50} sediment**	**DT_{50} system**

		[g dwt/mL]	[°C]		[%]	[d]	[%]	[d]	[d]	[d]
[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-^{14}C]-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 O_2/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 O_2/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 CO_2 traps were sampled on days 14, 25 and 42. The 1st CO_2 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 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 µg/g and 518 µg/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 14C-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 Iow recoveries. After 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 14CO2 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% (± 3.5%) and 19.1% (± 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 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 degredation

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.

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-dimethyl-naphthalene. 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-live 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-methylnaphththalene 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-methylnaphththalene 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-live 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 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-isopropylnaphthalene 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 trimethylsilylization 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-Isopropylnaphthalene	22.3
2,6-Dimethylnaphthalene	15.5
2,6-Diethylnaphthalene	13.1
2,6-Diisopropylnaphthalene	16.0
2,7-Diisopropylnaphthalene	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 photodegration half-lives for 1,4-DMN could be derived. Nevertheless table 7.6.1. shows that the degradation rates of of alkylnaphthalenes from monomethylnaphthalene to diisopropylnaphthalene 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

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 (*Cyprinodon variegatus*)			
Water solubility	:	5.1 mg/L			

Substance	**Species**	**Duration [d]**	**Method**	**Exposure conc [µg/L]**	**BCF [L/kg wwt]**	**Based on**
1,3-dimethylnaphthalene	*Cyprinodon variegatus*	36 + 8	Continuous flow	2.74	3979	k_1/k_2
1,3-dimethylnaphthalene	*Cyprinodon variegatus*	36 + 8	Continuous flow	26.67	8313	k_1/k_2

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 (Kow), whereas k_1 values for three- and four-ring PAHs were lower despite their high log Kow values. Elimination rate constants (k_2) for the homologue series of naphthalenes and phenanthrenes generally increased with decreasing degree of alkylation and log Kow 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 Kow 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.

The time needed to eliminate 95% of the 1,3-dimethylnaphthalene in the fish ($T95 = 3.0/k_2$) 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 (*Oncorhynchus mykiss*)			
Water solubility	:	5.1 mg as/L			

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg/L]
1,4-dimethylnaphthalene	*Oncorhynchus mykiss*	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 LC_{50} 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 LC_{50} was 1.0 mg as/L, the 72-h LC_{50} was 0.89 mg as/L. The LC_{50} 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. LC_{50} 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 LC_{50} 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 method	:	Semi-static
Guideline	:	OPPTS 850.1075 = OECD 203 = EEC C.1	Acceptability	:	Acceptable
Test substance	:	1,4-dimethylnaphthalene, batch 01C-01	LC_{50}	:	1.4 mg as/L
Purity	:	98.8%			
Species	:	Fathead minnow (*Pimephales promelas*)			

Water solubility	:	5.1 mg/L

Substance	Species	Method	T [°C]	pH	Duration [h]	Criterion	Value [mg as/L]
1,4-dimethylnaphthalene	*Pimephales promelas*	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 LC_{50} of 2.3 mg as/L, 48-hours LC_{50} of 1.6 mg as/L, 72-hours LC_{50} of 1.4 mg as/L and 96-hours LC_{50} of 1.4 mg as/L (95% CL 0.86 – 1.7 mg as/L), based on mean measured test concentrations. All LC_{50} values were calculated using binomial probability method.

Remarks by RMS
Water quality parameters were within accepted limits. Concentrations of the solvent are not reported. The result 96-hours LC_{50} 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	:	*Daphnia magna*			
Water solubility	:	5.1 mg as/L			

Substance	**Species**	**Method**	**T [°C]**	**pH**	**Duration [h]**	**Criterion**	**Value [mg as/L]**
1,4-dimethylnaphthalene	*Daphnia magna*	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 $CaCO_3$, 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.

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.
EC_{50} of the first test is reported to be > 0.48 mg as/L, based on mean measured test concentrations.
The EC_{50} 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 EC_{50} 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_bC_{50}	:	0.32
			72-h E_rC_{50}	:	0.62
Purity	:	98.8%			
Species	:	*Pseudokirchneriella subcapitata*	72-h NOEC	:	0.030
Water solubility	:	5.1 mg as/L			(all values mm)

Substance	**Species**	**Method**	**T [°C]**	**pH**	**Duration [h]**	**Criterion**	**Value [mg as/L]**
1,4-dimethylnaphthalene	*Pseudokirchneriella subcapitata* [1]	Static	22.8 – 24.1	7.9 – 10.4	72	E_bC_{50}	0.32
1,4-dimethylnaphthalene	*Pseudokirchneriella subcapitata* [1]	Static	22.8 – 24.1	7.9 – 10.4	72	E_rC_{50}	0.62
1,4-dimethylnaphthalene	*Pseudokirchneriella subcapitata* [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.

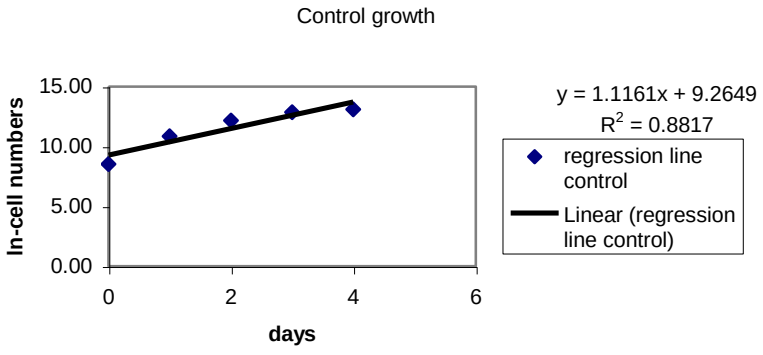
*Conditions.*23 ± 2 °C, continuous light (4300 lux) and continuous shaking at 100 rpm.

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_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. 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%).



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 method	:	Flow-through
Guideline	:	OECD 215	Acceptability	:	Acceptable
Test substance	:	1,4-dimethylnaphthalene (lot number 14D03M01-02)	NOEC growth rate	:	90 µg as/L
Purity	:	98.8%			
Species	:	Rainbow trout (*Oncorhynchus mykiss*)			
Water solubility	:	5.1 mg/L			

Substance	species	method	T [°C]	pH	Duration [d]	Criterion	Value [µg/L]
1,4-dimethylnaphthalene	*Oncorhynchus mykiss*	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.

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	:	No	Dosing	:	static

statement			method		
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 (*Gadus morhua*)			
Water solubility	:	5.1 mg/L			

Substance	**Species**	**Method**	**T [°C]**	**Duration [d]**	**Criterion**	**Value [mg/L]**
1,4-dimethylnaphthalene	*Gadus morhua*	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.

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 and1.03 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-dimethylnaphtalene	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	:	*Daphnia magna*			
Water solubility	:	5.1 mg as/L			

Substance	**Species**	**Method**	**T [°C]**	**pH**	**Duration [d]**	**Criterion**	**Value [µg as/L]**
1,4-dimethylnaphthalene	*Daphnia magna*	Flow-through	19.2 – 19.9	8.0 – 8.2	21	NOEC reproduction	160

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 $CaCO_3$, 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.

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 EC_{50} for mortality is reported to be > 339 µg as/L and the 21-day EC_{50} 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 (*Strongylocentrotus droebachiensis)*			
Water solubility	:	5.1 mg/L			

Substance	**Species**	**Method**	**T [°C]**	**Duration [d]**	**Criterion**	**Value [mg/L]**
1,4-dimethylnaphthalene	*Strongylocentrotus droebachiensis*	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. -

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-dimethylnaphtalene	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	:	*Lemna gibba G3*			
Water solubility	:	5 mg as/L			

Substance	**Species**	**Method**	**T [°C]**	**pH**	**Duration [d]**	**Criterion**	**Value [mg as/L]**
1,4-dimethylnaphthalene	*Lemna gibba 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. IC_{50} 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. IC_{50} 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 IC_{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.

Remarks by RMS
Water quality parameters were within accepted limits. The results, a 7-day IC_{50} 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

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