Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

1,4-dimethylnaphthalene

EC Number: 209-335-9
CAS Number: 571-58-4

CLH-O-0000006734-69-01/F

Adopted

5 December 2019

5 December 2019

CLH-O-0000006734-69-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **1,4-dimethylnaphthalene**

EC Number: **209-335-9**

CAS Number: **571-58-4**

The proposal was submitted by **the Netherlands** and received by RAC on **8 February 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **1 April 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **31 May 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Nathalie Printemps**

Co-Rapporteur, appointed by RAC: **João Carvalho**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **5 December 2019** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	1,4-dimethylnaphthalene	209-335-9	571-58-4	Asp. Tox. 1 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 2	H304 H319 H400 H411	GHS07 GHS08 GHS09 Dgr	H304 H319 H410		M=1	
RAC opinion	TBD	1,4-dimethylnaphthalene	209-335-9	571-58-4	Asp. Tox. 1 Acute Tox. 4 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 3	H304 H302 H319 H400 H412	GHS07 GHS08 GHS09 Dgr	H304 H302 H319 H410		oral: ATE = 1300 mg/kg bw M=1	
Resulting Annex VI entry if agreed by COM	TBD	1,4-dimethylnaphthalene	209-335-9	571-58-4	Asp. Tox. 1 Acute Tox. 4 Eye Irrit. 2 Aquatic Acute 1 Aquatic Chronic 3	H304 H302 H319 H400 H412	GHS07 GHS08 GHS09 Dgr	H304 H302 H319 H410		oral: ATE = 1300 mg/kg bw M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

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

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

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

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

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

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

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

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

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

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

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity - Oral route

For acute oral toxicity, one oral toxicity study in rats was available using doses up to 2500 mg/kg. The study was similar to OECD TG 401 (GLP). A limit dose study conducted earlier than the main study with 5000 mg/kg was also included in LD_{50} calculation (10 dosed rats died). Death occurred in groups dosed with 1700 mg/kg and higher. The calculated acute oral LD_{50} was 2 730 mg/kg (95 % confidence interval: 2346-3178 mg/kg bw). On this basis, the DS proposed no classification for 1,4-DMN.

Acute toxicity - Dermal route

No deaths occurred in an acute rabbit dermal toxicity study performed according to OECD TG 402 (GLP). The acute dermal LD_{50} was greater than 2000 mg/kg in the study. On this basis, no classification was proposed by the DS.

Acute toxicity - Inhalation

In a study in line with OECD TG 403 (GLP), the LC_{50} observed in rats exposed to a single dose of 1,4-DMN (aerosol) was found to be greater than 4.16 mg/L. One death out of 10 animals occurred at this dose level. The DS reasoned that, if an actual dose of 5 mg/L was used, would result in no difference in the toxicity outcome and proposed no classification.

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

Acute toxicity: oral

The LD_{50} of 1,4-DMN was above 2000 mg/kg in the available rat acute toxicity study (vehicle was corn oil). Based on the results of the preliminary study performed in the *in vivo* micronucleus assay, mice seemed more sensitive than rats as the LD_{50} was below 2000 mg/kg. Although this range-finding study was not designed for acute toxicity classification, the study was GLP, well-performed to investigate acute toxicity of 1,4-DMN and used the same vehicle as in the rat study. There is no information in the dossier supporting that mice would not be a relevant species. Therefore, according to the CLP criteria, a **classification as Acute Tox. 4, H302 is warranted** via the oral route.

Regarding the ATE, the default converted value of 500 mg/kg for Acute Tox. 4 may be too conservative as no death occurred at this dose level in the mice study. As the LD_{50} is according to the study between 1300 and 1650 mg/kg bw, RAC proposed an **ATE of 1 300 mg/kg bw**.

Acute toxicity: dermal

The LD_{50} of 1,4-DMN in rabbits was greater than 2000 mg/kg in both sexes. **No classification via the dermal route is warranted** according to the CLP criteria.

Acute toxicity: inhalation

In an OECD TG 403 study, 10 rats (5/sex) were exposed, whole-body, to 4.16 mg/L/4h of 1,4-DMN (aerosol). Only one death occurred at 4.16 mg/L. Thus, the LC_{50} in rats is expected to be higher than the 5 mg/L cut-off for acute inhalation toxicity classification. Therefore, RAC agrees with the DS **not to classify 1,4-DMN for acute inhalation toxicity**.

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

Summary of the Dossier Submitter's proposal

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

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

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

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

No human data are available in the dossier.

In the acute inhalation toxicity study, at 4.16 mg/L, clinical signs suggestive of respiratory irritation (dyspnea, eye and nasal discharge) were observed in several rats. These clinical signs were reversible by day 2 and no abnormalities were observed at necropsy (including the rat that died). The substance is not a skin irritant. As no histopathological findings were observed, RAC agrees with the DS's proposal not to classify 1,4-DMN for STOT SE 3; H335 for respiratory tract irritation.

In the available acute oral toxicity study, hunched posture and hypoactivity were consistently observed among treated animals from 1300 mg/kg. Ataxia and coma were only noted in animals exposed to high dose levels ≥ 2500 mg/kg i.e. close to the LD_{50} (calculated to be 2730 mg/kg). As hunch posture and hypoactivity are not specific clinical signs, RAC agrees with the DS that STOT SE 3; H336 for narcotic effects is not warranted.

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

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

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

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

Comments received during public consultation

No specific comments were received.

Assessment and comparison with the classification criteria

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

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

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

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

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

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

- For conjunctival oedema, scores were ≥ 2 in 5 out of 6 animals.

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

There is no data available. No classification is proposed.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC agrees with the DS proposal for **no classification due to lack of data**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

1,4-DMN was tested in a Local Lymph node assay (LLNA) in mice, conducted according to OECD TG 429 (GLP-compliant). The vehicle used in the study was acetone/olive oil (4:1 v/v). In this study, 1,4-dimethylnaphthalene was not found to be a skin sensitiser since stimulation index were below 3 up to 100 % w/w concentration. On this basis, no classification was proposed by the DS.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

No human data were available in the dossier.

RAC considers the available LLNA study reliable. Although a dose-response was observed in the stimulation index values calculated for the substance concentrations (2.1, 2.4 and 2.8 at 25, 50 and 100 %, respectively), no classification is warranted as the values were below the cut-off criteria of 3 for a positive result in an LLNA study.

RAC agrees with the DS that **no classification is warranted for 1,4-DMN**.

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

Summary of the Dossier Submitter's proposal

As no adverse findings were observed in the repeated dose toxicity studies at dose triggering classification STOT RE, the DS proposed no classification.

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

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

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

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

Assessment and comparison with the classification criteria

In vitro data

Two bacterial gene mutation assays were negative.
The first test was equivalent to OECD TG 471 (GLP-compliant) except that:

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

Overall, RAC considered this negative study reliable with limitations.

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

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

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

In vivo data

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

mg/kg. The increase was within the historical control range and was due to a single female. Therefore, RAC agrees that the study was negative.

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

In silico data

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

Comparison with the classification criteria

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

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

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

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

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

The DS also provided information from ATSDR on the alkyl derivatives 1-methylnapththalene (1-MN) and 2-methylnaphthalene (2-MN). In two published studies, using the same protocols, 1-MN and 2-MN induced bronchioalveolar proteinosis and bronchiolar/alveolar tumours in mice following 81-week dietary exposure (ATSDR, 2005). The postulated mode of action (MoA) is that the tumour formation is mediated by the formation of a highly reactive epoxide. Although evidence of the formation of this reactive epoxide was observed following a single intraperitoneal administration of 1,4-DMN, no evidence was observed following oral administration of 1,4-DMN (single exposure, 28 mg/kg). The DS concluded that the induction of systemic pulmonary alveolar

proteinosis and pulmonary tumours was thus unlikely for 1,4-DMN. The DS also pointed out that mice may be more susceptible than rats to this type of lung tumours based on potential differences in metabolism between mice and other species as seen with naphthalene.

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

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

Comments received during public consultation

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

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

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

Assessment and comparison with the classification criteria

Data on 1,4-DMN

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

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

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

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

Data on 1-MN and 2-MN

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

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

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

*p< 0.05

RAC notes several limitations in these studies:

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

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

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

Data on naphthalene

Naphthalene induced in rats, following long-term inhalation, neuroblastoma of the nasal olfactory epithelium in both sexes and respiratory epithelium adenomas in males. No lung tumours were observed in rats. In mice, exposed 104-weeks by inhalation, an increase in alveolar/bronchiolar adenomas were observed in females in presence of inflammatory changes. Alveolar/bronchiolar

adenoma and carcinoma were also increased in males but within historical control range values. Pulmonary alveolar proteinosis was not reported in mice. Based on the rat nasal cavity, the substance was classified Carc. 2. The relevance of the mouse lung adenomas were considered unlikely due to species differences in pulmonary metabolism (summary from the Substance Evaluation conclusion of UK on naphthalene published in 2018).
Evidence of carcinogenicity of the structurally-related naphthalene has been hypothesised to be due to metabolism *via* CYP-mediated ring epoxidation to reactive metabolites such as the 1,2-epoxide or 1,2-quinone derivatives in the nasal epithelium. The metabolic formation of ring epoxides is a relatively minor pathway for alkyl naphthalene derivatives, whereas it is one of the major pathway for naphthalene (see background document). Moreover, quinone derivatives are not formed with alkylated naphthalene compounds. As major differences in the metabolism of naphthalene and alkylated naphthalene (1-MN, 2-MN or 1,-DMN) exist, the use of naphthalene carcinogenicity data as evidence for 1,4-DMN carcinogenicity is not supported by RAC.

Overall conclusion

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

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

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

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility/effects on or via lactation

The potential of 1,4-DMN to adversely affect sexual function and fertility was investigated in an extended one-generation reproduction toxicity (EOGRTS) study in rats (OECD TG 443, GLP). In this study, test material was administered via the diet. Male and female reproductive parameters and organs were not affected by treatment.

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

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

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

Developmental toxicity

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

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

Comments received during public consultation

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

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

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

Considering developmental toxicity, no comments were received.

Assessment and comparison with the classification criteria

Sexual function and fertility

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

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

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

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

* p≤0.05

As effects were observed in both males and females and as developmental delays were noted in both groups, RAC considers that the delays in the onset of puberty may be due to a generalised growth delay. Parental toxicity observed at the top dose consisted of decrease body weight gain, decreased food consumption, increased relative liver weight (38 % in males and 57.5 % in

females) and cholesterol. At the mid dose, a decrease in food consumption was seen at several time points in females and increased liver relative weight (16 %) and cholesterol were already noted (see tables below).

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

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

*Significantly different from control p≤0.05

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

Developmental toxicity

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

There are also three poorly reported published developmental toxicity studies performed in mice or rats with methylnaphthalene (exact substance unknown) or 2,6-diisopropylnaphthalene. As there are existing data on 1,4-DMN and as potential toxicity differences between 1,4-DMN and

2,6-diisopropylnaphthalene have not been investigated (no data on the toxicity profile of 2,6-diisopropylnaphthalene), these data are not further considered.

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

Adverse effects on or via lactation

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

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

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

No information is available on the quantity or quality of the milk produced by the dams, nor was the rat milk analysed for the presence of 1,4-DMN or its metabolites. Nevertheless, the substance was found in the milk of goat (metabolism study in livestock). Moreover, the substance is lipophilic and preferentially distributed to fat. Therefore, it is likely that the substance could be transferred to milk of rats.

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

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

Based on the kinematic viscosity of 1,4-DMN of 5.9 mm^2/s at 25 °C, , classification as Asp. Tox. 1; H304 was proposed by the DS.

Comments received during public consultation

Two MS agreed with the DS's proposal.

Assessment and comparison with the classification criteria

According to the criteria, the substance shall be classified if it is a hydrocarbon and its kinematic viscosity is ≤ 20.5 mm^2/s at 40 °C.

As kinematic viscosity decreases with higher temperature, it is expected that kinematic viscosity of the substance at 40 °C would fulfil the criteria. Therefore, RAC agrees with the DS to **classify 1,4-DMN as Asp. Tox. 1, H304**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

1,4-dimethylnaphthalene (1,4-DMN) currently has no classification in Annex VI to CLP. The DS's proposal for consideration by RAC was to classify 1,4-DMN as Aquatic Acute 1 (M=1) and Aquatic Chronic 2. This was based on the lowest EC_{50} value of 0.54 mg/L for *Daphnia magna* and the lowest NOEC value of 0.030 mg/L for *Pseudokirchneriella subcapitata* and the substance being rapidly degradable for classification purposes.

Degradation

No experimental information on hydrolysis is available and it is indicated that HydroWin QSAR (EPIWeb 4.0) cannot be used to estimate the hydrolysis half-life for 1,4-DMN. A hydrolysis DT_{50} of 5.27×10^5 day (20°C) estimated by EUSES 2.1 is reported in the dossier but it is considered not reliable by the DS. Based on the structure of the substance, hydrolysis is expected to be very slow.

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

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

The dossier also includes a water-sediment simulation study with the substance, which, however, is not considered reliable by the DS because the test substance was quickly lost from the system due to volatilisation and adsorption to rubber stoppers used in the test vessels. No DT_{50} values could be calculated in the study.

No soil simulation studies are available for the substance. However, information from public literature studies on degradation of 1,4-DMN or other similar PAHs by soil or composter microorganisms is included in the dossier. In three of these studies, isolated bacteria were able to degrade 1,4-DMN or 2,6-DMN. In two of the studies, unspecified DMNs were exposed to composter microorganisms and biodegradation and/or volatilisation of DMNs were observed. According to the DS, no reliable DT_{50} values can be obtained from these studies, but the studies indicate that soil microorganisms can degrade 1,4-DMN.

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

Bioaccumulation

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

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

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

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

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

Aquatic toxicity

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

Acute toxicity

Two acute toxicity studies on fish, performed following OECD TG 203, are available for 1,4-DMN. In one of the studies, a 96-h LC_{50} value of 0.67 mg/L (95% CL of 0.57 to 0.80 mg/L) based on mean measured concentrations was determined for *Oncorhynchus mykiss* under flow-through conditions. In the other, study, a 96-h LC_{50} value of 1.4 mg/L (95% CL 0.86 – 1.7 mg/L) is reported for *Pimephelas promelas*, based on mean measured concentrations. It is noted that in the *Pimephelas promelas* study, DMF was used as a solvent but its concentration is not reported. Both studies met the validity criteria indicated in OECD TG 203 and are considered reliable by the DS.

The dossier includes an acute toxicity study with *Daphnia magna* performed under flow-through conditions and following OECD TG 202. The study consisted of two tests. In the first test, only 15% immobilisation was observed at the highest concentration, and hence, an EC_{50} value of > 0.48 mg/L was reported based on mean measured concentrations. In the second test, higher concentrations were used and a dose-related increase of immobilisation, up to 100%

immobilisation at the highest concentration, was observed. A 48-h EC_{50} value of 0.54 mg/L (95% CL 0.33 – 0.94 mg as/L) based on mean measured test concentrations was determined. The mean measured concentrations were 30-45% of the nominal concentrations but they were constant during the tests. The validity criteria of the OECD TG 202 were met.

One algal toxicity study following OECD TG 201 is available for 1,4-DMN. *Pseudokirchneriella subcapitata* algae were exposed to the test substance in closed test vessels with minimal headspace during 96 hours under static conditions. An E_rC_{50} value of 0.62 mg/L (95% CL 0.60 – 0.64 mg as/L) is reported after 72 hours of exposure and an E_rC_{50} of 0.60 mg/L after 96 hours. It is stated that constant exponential growth was not observed in the controls throughout the study; the growth peaked at 48 hours after which it slowed down. The cell number increased by a factor 16 within the first 48 hours, and hence, the first validity criterion of the current OECD TG 201 (2006) was met. This was the only validity criterion regarding control growth indicated in the OECD TG 201 (1984) that was in use at the time when the study was conducted (year 2002). However, the second validity criterion of the current OECD TG 201(i.e. the mean coefficient of variance for section-by-section specific growth rates in controls should be ≤ 35%) was not met. According to the annex of the CLH report, the mean variance of daily growth rate in the control was 41% at 48 hours, 58% at 72 hours and 79% at 96 hours.

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

The dossier also includes a semi-static study assessing the toxicity of 1,4-DMN to the aquatic plant *Lemna gibba*, performed in accordance with OECD TG 221 (Draft). A 7-d IC_{50} value for frond number is reported to be 1.1 mg/L (95% CI 1.1 – 1.2 mg/L), based on mean measured concentrations. According to the DS, the water quality parameters were within accepted limits and the results of the study are considered acceptable for classification.

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

Chronic toxicity

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

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

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

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

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

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

Comments received during public consultation

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

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

A company/manufacturer submitted a comment indicating that according to the CLP Guidance, in the chronic studies EC_{10} values (when available) should be preferred over NOEC values and provided an attachment with new calculations and statistical analyses for EC_{10} and NOEC values for the available OECD TG 201 algal study and OECD TG 215 fish study. This resulted in E_rC_{10} = 0.357 mg a.s./L for algae, and EC_{10} = 0.200 mg a.s./L for fish, which would lead to a classification as Aquatic Chronic 3. The DS agreed with the comment and modified their proposal for environmental classification accordingly.

Assessment and comparison with the classification criteria

Degradation

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

In the available ready biodegradation screening test (OECD TG 301C), 66% degradation of 1,4-DMN was observed after 28 days based on O_2 consumption. It is not clear whether the 10-d window was met but it does not apply to tests performed according to OECD TG 301C. Regarding the lack of abiotic control, RAC notes that if part of the test substance was lost from the test vessels by volatilisation, it would mean that the BOD, and consequently, the degradation of the test substance, were underestimated because the BOD was calculated considering the initial test substance concentration. Therefore, since the degradation of the test substance reached the pass level, the lack of abiotic control does not affect the conclusion of the study, and hence, RAC considers that the study can be considered acceptable for classification.

The available water-sediment simulation study is not relevant for classification as the test substance was lost from the test system through volatilisation and adsorption, and no DT_{50} values were determined. The five studies regarding degradation of the substance or similar substances by soil or composter microorganisms are not considered relevant for classification purposes. This is because based on the available information in the CLH report, in two of the studies the microorganisms were pre-exposed to 2,6-DMN and in other two studies composter microorganisms were used, and hence, pre-exposure to DMNs or other similar PAHs cannot be excluded. In the fifth study, it is not clear whether the used soil bacteria might have been pre-exposed to similar substances but no information on biodegradation rate is reported.

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

Bioaccumulation

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

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

No experimental BCF value is available for 1,4-DMN. The DS calculated a mean BCF of 5751 L/kg for the similar substance 1,3-DMN based on BCFs reported for the substance in a study (Jonsson *et al.* 2004) assessing the bioconcentration of a mixture of PAHs in sheepshead minnows (*Cyprinodon variegatus*). RAC notes that in the CLH dossier it is mentioned that both BCFs were

determined based on uptake and depuration rate constants (kinetic BCF) and based on the concentration of the substance in fish and water (steady-state BCF) but which type of BCFs values are reported is not stated. Furthermore, the BCFs are not lipid normalised. Based on the information found in Jonsson *et al.* (2004), the whole fish kinetic BCFs for 1,3-DMN were 3,979 and 8,313 L/kg and the steady-state BCFs were 3,426 and 4,761 L/kg at the low and high exposure concentrations, respectively. The kinetic BCF values are preferred as it seems that steady-state may not have been reached in the study, at least in the high exposure group. Based on the lipid weight based BCF_k values (41,020 and 85,696 L/kg for the low and high exposure groups) reported, the mean lipid content of the fish was 9.7%. RAC normalised the whole fish BCFs to 5% lipid content, which lead to BCFk values of 2,051, and 4,285 L/kg, for the low and high exposure concentrations, respectively.

RAC agrees with the DS that the BCF values determined for 1,3-DMN can be considered reliable. Structural similarity and relevant physico-chemical properties that influence bioavailability and uptake potential (e.g. water solubility, adsorption, lipophilicity) have been considered (AE 2.2 Assessment elements for Bioaccumulation potential of the ECHA's Read-Across Assessment Framework) when assessing whether read-across from 1,3-DMN to 1,4-DMN can be accepted in the bioaccumulation assessment. Based on their similar structures (only differing in the position of one of the methyl groups) and log Kow values, these two substances can be expected to have similar bioaccumulation potential, although it is noted that the different positions of the methyl groups in naphthalene could potentially cause some differences in the metabolism, and consequently in the BCF values, of the two substances. However, since the BCF values of 1,3-DMN are well above the cut-off value of 500 L/kg indicated in CLP, RAC agrees with the DS's proposal that 1,4-DMN has a bioaccumulation potential for classification purposes.

Acute toxicity

The available acute toxicity studies on 1,4-DMN with fish and aquatic invertebrates are valid and resulted in 96-h LC_{50} values of 0.67 mg/L for *Oncorhynchus mykiss* and 1.4 mg/L for *Pimephelas promelas* and in a 48-h EC_{50} value of 0.54 mg/L for *Daphnia magna*.

In the available algal toxicity study 72-h and 96-h E_rC_{50} values of 0.62 and 0.60 mg/L were determined for *Pseudokirchneriella subcapitata*. As stated in the CLH report, constant exponential growth was not observed in the control groups, and hence, the study does not fulfil the validity criteria of OECD TG 201. Furthermore, it is noted that the pH of the test mediums had increased by more than 1.5 units after 4 days in the controls and all treatment groups except in the highest test concentrations. According to ECHA Guidance R.7b, if in an algal growth inhibition test the exponential growth ceased in the control before the end of the test period, only data from the part of the test where exponential growth occurs and the validity criteria for the controls are fulfilled, should be used.

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

for both controls. Hence, based on these values, at 72 hours the validity criterion of the guideline is not met, whereas at 48 hours it was met.

In conclusion, after up to 48 hours of exposure, the growth in the control groups met the validity criteria, as the cell number had already increased by a factor of 16 and the coefficient of variance for section-by-section specific growth rates was ≤ 35%. Furthermore, based on the available information in the full study report, the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was ≤ 7%. Therefore, RAC considers that only the results up to 48 hours of exposure should be considered valid for classification. In the full study report, a 48-h E_rC_{50} of 0.58 mg/L (95% CL 0.55-0.61 mg/L) is reported.

In addition, a 7-d IC_{50} of 1.1 mg/L for *Lemna gibba* is reported in a study following OECD TG 221 (draft). RAC notes that according to the current OECD TG 221, for the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d^{-1}. Based on the information in the CLH report and its annex, the doubling time of frond number in the controls was about 3 days, and after 7 days the mean frond numbers were 73 and 75 in blank and solvent controls, respectively. RAC calculated mean growth rates based on these values and considering that the initial frond number was indicated to be 12. (Growth rates could not be calculated for each replicate as indicated in the guideline due to lack of data.) This resulted in mean growth rates of 0.258 d^{-1} and 0.262 d^{-1} for the blank and solvent controls, respectively. Hence, the growth in the control groups was slightly below the validity criteria of the current OECD TG 221. Furthermore, it seems that the reported IC50 is for frond number instead of for growth rate, which is the preferred endpoint. Therefore, RAC considers that the study and its reported results are not fully acceptable but they can be used as supporting information for classification, as lower acute values are available for other organisms.

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

Chronic toxicity

The available chronic toxicity tests with fish and aquatic invertebrates following OECD TGs 215 and 211, respectively, are valid and resulted in a 28-d NOEC of 0.09 mg/L for *Oncorhynchus mykiss* based on growth rate and a 21-d NOEC of 0.16 mg/L *Daphnia magna* based on reproduction, length and dry weight. During the public consultation, a company/manufacturer provided a document containing new statistical analyses performed for the raw data of the OECD TG 215 study with *Oncorhynchus mykiss,* and a 28-d EC_{10} of 0.200 mg/L (95% CL 0.159-0.251 mg/L) for growth rate (based on mean measured concentrations) is reported.

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

a juvenile life-stage. Therefore, RAC considers that, due to the limited test duration and lack of detailed data, these studies can only be used as supporting information for classification.

The chronic toxicity value reported for algae in the CLH dossier is the 72-h and 96-h NOE_rC of 0.030 mg/L for *Pseudokirchneriella subcapitata*. However, as stated above, in RAC's opinion, in the *Pseudokirchneriella subcapitata* study only results up to 48 hours of exposure are acceptable for classification. 48-h NOEC values were not determined in the original study. However, as stated above, during the public consultation, a company/manufacturer provided a document with new statistical analyses on the raw data of the study including E_rC_{10} and NOE_rC values for different study periods. RAC considers the new analysis reliable. The 48-h E_rC_{10} reported in the new document is 0.232 mg/L (95% CL 0.208-0.259 mg/L). Regarding the 48-h NOE_rC, a statistically significant difference in growth rates when compared to pooled controls was determined at concentrations of 0.11 mg/L and above. Furthermore, at the concentration of 0.053 mg/L the growth rate did not differ statistically significantly from the pooled control while at the lowest test concentration of 0.030 mg/L it did. RAC notes that the inhibition in growth rate at the lowest concentration was only 2.7% after 48 hours. Furthermore, based on the provided new information, the NOE_rC after 24 hours of exposure would be 0.053 mg/L. Therefore, since in the second lowest concentration (0.053 mg/L) no statistically significant inhibition was observed at 48 hours, it is not clear whether the observed low inhibition at 0.030 mg/L was caused by the test substance. In conclusion, RAC is of the opinion that a 48-h NOE_rC of 0.053 mg/L (based on mean measured concentration) can be considered reliable.

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

RAC agrees with the company/manufacturer's comment submitted during the public consultation and the DS's response to that comment: according to the current CLP Guidance, when EC_{10} values are available these are preferred over NOEC values in chronic toxicity studies. RAC notes that this applies in cases where EC_{10} and NOEC values are available for the same endpoint and he same study. RAC considers it more appropriate to use EC_{10} values for aquatic chronic classification because NOEC values strongly depend on the experimental design (number of doses, width of the inter-dose interval, etc.), whereas EC_{10} values are derived from the whole concentration-response curve. Therefore, in RAC's opinion, the relevant chronic values available for fish and algae are the 28d-EC_{10} of 0.200 mg/L for *Oncorhynchus mykiss* and the 48h-E_rC_{10} of 0.232 mg/L for *Pseudokirchneriella subcapitata*, respectively, even though these are significantly higher than the respective NOEC values. For the available *Daphnia magna* study only NOEC values are reported, and hence, they are used for classification.

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

Conclusion on Classification

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

Additional references

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

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

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).