Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

N,N-diethyl-m-toluamide; deet

EC Number: 205-149-7
CAS Number: 134-62-3

CLH-O-0000001412-86-161/F

Adopted

9 June 2017

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

9 June 2017

CLH-O-0000001412-86-161/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: **N,N-diethyl-m-toluamide; DEET**

EC Number: **205-149-7**

CAS Number: **134-62-3**

The proposal was submitted by **Sweden** and received by RAC on **17 June 2016.**

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

PROCESS FOR ADOPTION OF THE OPINION

Sweden 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 **29 June 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **15 August 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Betty Hakkert**

Co-Rapporteur, appointed by RAC: **Riitta Leinonen**

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 **9 June 2017** by **consensus**.

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	616-018-00-2	N,N-diethyl-m-toluamide; deet	205-149-7	134-62-3	Acute Tox. 4* Skin Irrit. 2 Eye Irrit. 2 Aquatic Chronic 3	H302 H315 H319 H412	GHS07 Wng	H302 H315 H319 H412			
Dossier submitters proposal	616-018-00-2	N,N-diethyl-m-toluamide; deet	205-149-7	134-62-3	**Modify** Acute Tox. 4 **Remove** Aquatic Chronic 3	**Modify** H302 **Remove** H412	**Retain** GHS07 Wng	**Modify** H302 **Remove** H412			
RAC opinion	616-018-00-2	N,N-diethyl-m-toluamide; deet	205-149-7	134-62-3	**Modify** Acute Tox. 4 **Remove** Aquatic Chronic 3	**Modify** H302 **Remove** H412	**Retain** GHS07 Wng	**Modify** H302 **Remove** H412			
Resulting Annex VI entry if agreed by COM	616-018-00-2	N,N-diethyl-m-toluamide; deet	205-149-7	134-62-3	Acute Tox. 4 Skin Irrit. 2 Eye Irrit. 2	H302 H315 H319	GHS07 Wng	H302 H315 H319			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The substance *N*,*N*-diethyl-m-toluamide (hereafter referred to as DEET) has an existing harmonised classification and labelling (CLH) in Annex VI of Regulation (EC) No 1272/2008 (hereafter referred to as CLP). This proposal for classification and labelling from the Dossier Submitter (DS) considers the the hazard classes carcinogenicity, mutagenicity and reproductive toxicity as well as acute toxicity, specific target organ toxicity following single exposure (STOT SE) and aquatic chronic toxicity. Existing classifications for skin irritation and eye irritation were not assessed in the CLH report, and as a consequence are not evaluated by RAC. The CLH proposal is based on information available in the Competent Authority (CA) report (CAR) prepared under directive 98/8/EC.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The dossier included one acute oral, two acute dermal, and one acute inhalation toxicity studies. All studies were performed according to US EPA Guidelines (equivalent to OECD test guidelines) and included 5 animals/sex/group.

In addition, several case reports of DEET poisoning in humans were presented. These cases mainly reported neurological effects. As the levels of exposure were unknown and the exposure duration varied, these studies were judged by the DS to be of limited relevance for classification.

The acute oral toxicity study in rats tested dose levels of 1000, 2000, and 4000 mg/kg bw. The mortality of males and females combined for each dose was respectively 1/10, 7/10, and 9/10 animals. The calculated LD_{50} (by Probit) was 1892 mg/kg bw for both sexes combined, thus classification in Category 4 was proposed by the DS for acute oral toxicity.

As the acute dermal limit tests provided LD_{50} values in rabbits and rats were, respectively, > 2000 mg/kg bw and > 5000 mg/kg bw, no classification for acute dermal toxicity was proposed by the DS.

For acute inhalation toxicity, only a limit test was available. Rats were exposed for four hours to 2 mg/L DEET aerosol, at which no mortality occurred. As this concentration lies below the upper boundaries for acute classification, the DS noted that it cannot be excluded that it would meet the criteria for classification. However, based on the available data, no classification was proposed by the DS.

Comments received during public consultation

Two comments were received that addressed acute toxicity, one from a MSCA and one from an industry organisation. Both agreed with the proposed classification in Category 4 for acute oral toxicity.

Assessment and comparison with the classification criteria

Oral

One acute oral toxicity study in rats was presented, in which the LD_{50} was calculated to be 1892 mg/kg bw for both sexes combined. As this is the only LD_{50} value available and the substance itself was tested, the ATE (Acute Toxicity Estimate) for DEET is also 1892 mg/kg bw.

In addition, the CLH report refers to a review by Schoenig and Osimiz (2001) in which several suicide attempts with DEET are described. In two cases that resulted in death, one patient had also consumed, ethanol and cannabinoids, and the other chlorpromazine-HCl and hydralazine-HCl. In another suicide attempt resulting in death reported by a poison control centre, a patient drank 8 oz (237 mL) of DEET, resulting in cardiorespiratory arrest and status epilepticus. Also a study by Osimitz (2006) reported a successful suicide by ingestion of DEET, although the dose was not reported.

The human evidence presented has several limitations that reduces its value for the determination of a hazard classification and category. All reported cases of ingestion of high levels of DEET were suicide attempts and the amount of DEET ingested was reported only in one case. Moreover, in two cases also other substances were taken that may have been (partly) responsible for the fatal outcome. RAC agrees with the DS that although the human data does not contradict classification for acute oral toxicity, these reports have too many limitations to be used for classification by themselves.

Considering that the ATE in rats of 1892 mg/kg bw lies below the limit of 2000 mg/kg bw, RAC supports the classification of DEET in Category 4 for acute oral toxicity (**Acute Tox. 4; H302**).

Dermal

RAC agrees with the DS that as the LD_{50} values of two studies were above the limit of 2000 mg/kg bw, **no classification for acute dermal toxicity** is warranted.

Inhalation

No mortality was observed at a single dose of 2 mg/L DEET aerosol. The clinical signs of toxicity included ocular and nasal discharge, irregular respiration, dyspnoea, hunched posture, and hypoactivity. All effects were reversible within three days. Although 2 mg/L is below the highest classification limit for dusts and mists of 5 mg/L, the dossier submitter argued that it may be technically challenging to achieve a concentration of 5 mg/L and particles of respirable size.

This is in accordance with paragraph 3.1.2.3.2. from the CLP Regulation, which states that dusts and mist should ideally be tested at a maximum dose of 2 mg/L. At this dose, particles have a mean mass aerodynamic diameter (MMAD) between 1 and 4 microns and will deposit in all regions of the rat respiratory tract.

RAC agrees that it cannot be excluded that classification for acute inhalation toxicity is justified, considering only a limit study is available in which a single dose is tested below the limit of classification. However, as no mortality was observed in this study, **no classification for acute inhalation toxicity** is warranted.

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

Summary of the Dossier Submitter's proposal

Three oral dog studies are summarised in the CLH dossier by the DS for STOT SE, i.e. two 8-week studies with two dogs/sex/dose and one 52-week study with four dogs/sex/dose. Dogs showed clinical signs of neurotoxicity in all three studies, although only in one 8 week study it

was clear that the neurological symptoms occurred after a single dose. This study was terminated on day 5 of dosing due to the severity of the neurotoxic symtoms. The symptoms observed included abnormal head movements, ptyalism, emesis, abnormal biting and scratching, as well as convulsions.

The LOAEL for emesis and ptyalism was 125 mg/kg bw, while ataxia and abnormal head movements occurred after administration of 225 mg/kg bw on the day of dosing. One dog showed also prostration, convulsions, abnormal gait and dilated pupils after a single administration of 225 mg/kg bw.

No histopathological findings were found in neuronal tissues in the 52 week study. The results of *in vitro* assays indicated that DEET induces neuroexcitation via octopamine receptors and is capable of blocking Na^+ and K^+ channels.

In addition, two neurotoxicity studies in rats were presented, but these were limited in quality and reporting. One reported an increased response time to heat stimulus and decreased rearing activity after a single dose of 500 mg/kg bw. The second study showed a transient increase in locomotor activity after oral exposure to 218-683 mg/kg bw/day in animals from a 2-generation study. As the neurotoxic effects in rats were limited to dose levels within the limits of classification for acute oral toxicity and there were no signs of neurotoxicity in the chronic studies in rats and mice, the effects in rats were not further considered for classification by the DS.

There are reports describing neurological effects in humans following use of products containing DEET, which have been summarised in the CLH dossier under acute toxicity. Neurological symptoms were reported after both dermal application and oral ingestion. The most reported symptom was seizures, but also headaches, ataxia, disorientation, drooling, movement disorder, cephalalgia, drowsiness, trembling, and opistotonos were described. In some cases, secondary diagnosis was possible, including encephalitis, parainfectious encephalopathy, and underlying seizure disorder (not disproportionally represented).

As the relationship between the exposure to DEET and the reported symptoms was unclear, due to possible underlying diseases of the patients and limited information on the exposure, these reports had not been further included in the discussion on STOT SE in the CLH dossier.

The DS concluded that, although clinical signs of neurotoxicity were observed in dogs at doses below the guidance value for classification for STOT SE 1, it is not possible to assess whether these effects occurred near doses that are lethal in dogs. As there were also no clear neurotoxic effects observed in other mammalian species tested, the evidence is not sufficiently conclusive to fulfil the criteria for classification. Therefore, no classification was proposed by the DS for STOT SE.

Comments received during public consultation

Three comments were received that addressed STOT SE, two from MSCA's and one from an industry association. The industry association agreed with no classification. One MSCA noted that it is difficult to conclude on this endpoint due to the small amount of data with single exposure although there were clear acute neurological symptoms in dogs (see also the comments under reproductive toxicity).

The second MSCA agreed that dogs were more sensitive than rats, but pointed out that there are no data to support that findings in rats are more relevant to humans - especially since there are several human case reports of neurotoxicity, which cannot be disregarded. In addition, the MSCA asked to include several additional studies. The MSCA stressed that as no mortality was reported in the dog studies, it could not be concluded that the effects occur close to the lethal dose based on the rat LD_{50}. Based on these considerations, the MSCA requested discussion on classification for STOT SE 1 by RAC.

Assessment and comparison with the classification criteria

Substances are usually classified as STOT SE 1 when they have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, it can be presumed to have the potential to produce significant toxicity in humans following single exposure.

Substances are classified in Category 1 for STOT SE on the basis of:

(a) reliable and good quality evidence from human cases or epidemiological studies; or

(b) observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. The oral guidance value for STOT SE 1 is ≤ 300 mg/kg bw.

Unfortunately, no good quality epidemiological studies on the effects of DEET are available. There are several case reports of clinical neurological effects in humans after accidental or intentional dermal application or ingestion of DEET, as summarized in the CLH report and in the table "Overview of human case reports of neurotoxic symptoms" (under "Additional key elements" in the background document). RAC notes that it is very difficult to determine the relative incidence of these adverse effects, as DEET is used on a very large scale and all studies found were (old) case reports. It is also not reported whether these patients took other agents, such as for the prevention of malaria. As a result, the actual exposure is often unknown and other diagnoses could not always be ruled out. Thus, while these human case studies could be supportive evidence for classification for STOT SE, RAC considers that they are not conclusive on their own.

In animal experiments, neurological symptoms were observed in dogs, which included ptyalism, emesis, ataxia, convulsions, and abnormal head movements after oral exposure to DEET (see table "Overview of the symptoms reported in the three dog repeated dose studies" below). The number of animals used in these studies was small and in two studies it was unclear whether the effects occurred after single exposure or repeated exposure. On the other hand, very similar effects occurred in all three studies and in one study (A6.3.1(2)) the effects were so severe that the study was terminated after 5 days. It should be noted that in study A6.3.1(2), DEET was administered in a single bolus, while in studies A6.3.1(1) and A6.5(1), the dose was divided over two administrations per day. Thus, the doses of 400 mg/kg bw/d were administered 200 mg/kg bw twice a day. All three studies reported that the symptoms started within 15-60 minutes after dosing. The LOAEL for neurological effects in dogs was 125 mg/kg bw/d.

Table: Overview of the symptoms reported in the three dog repeated dose studies

Neurotoxic effect reported in dogs	Dose (mg/kg bw/d) and incidence in studies A, B or C
Ptyalism	125 (B: 1/4) 175 (B: 3/4) 200 (A) 225 (B: 2/4) 400 (A)
Abnormal head movements	125 (B: 1/4) 225 (B: 3/4) 400 (A) 400 (C: 1/8)
Emesis	0 (B: 1/4) 125 (B: 3/4) 175 (B: 1/2)* 225 (B: 3/4)
Ataxia	225 (B: 2/4) 400 (C: 1/8)
Convulsions	125 (B: 1/4) 225 (B: 1/4) 400 (C:1/8)
Prostration, abnormal gait, pupils dilated	225 (B: 1/4)

Study A = Reference (CAR and CLH report) A6.3.1(1): Dosing 8 weeks, 0, 50, 100, 200, 400 mg/kg bw/d (divided over 2 equal doses)
Study B = Reference (CAR and CLH report) A6.3.1(2): Dosing 5 days (intended as 8 weeks), 0, 75, 125, 175, 225 mg/kg bw/d
Study C = Reference (CAR and CLH report) A6.5(1): Dosing 1 year, 0, 30, 100, 400 mg/kg bw/d (divided over 2 equal doses)
* Reported as such in the CLH report.

There is no information to explain why dogs seemed to be more sensitive to the neurological effects of DEET than rats.

The CAR (Competent Authority Report, 2010) for the use of DEET in biocides also evaluated the same studies presented in the CLH dossier. In the CAR, it was stated that despite the limitations of the human case reports and dog studies: "these effects reported in humans were of a neurological origin and neurotoxic effects were observed in test animals at high oral doses and therefore neurotoxicity is considered an endpoint of significance for risk assessment especially with respect to children." An acute AEL of 0.75 mg/kg bw/d was set for oral exposure based on the NOAEL of 75 mg/kg bw/d from the 5 day study in dogs (study A6.3.1(2)).

The symptoms observed in the dog studies included seizures, ataxia, emesis, abnormal head movements, and ptyalism. However, only seizures and possibly ataxia are considered severe enough to warrant classification. Seizures were observed in three animals in total, in two of them at below the guidance value of 300 mg/kg bw for STOT SE 1 and after correction for caloric demand (generally not used in classification and labelling for acute effects), the doses are more in line with the guidance values for STOT SE 2. Non-lethal effects in other species, if any, were observed at dose levels that are already covered by the classification as Acute Tox. 4 (H302).

Although there are self-reported cases of neurological effects in humans, compared to the size of the exposed human population, the number of adverse effects reported is relatively small. Moreover, there are several confounding factors that hamper the interpretation of these findings. As a consequence, RAC considers that this information is not conclusive for classification and can only be used as supportive information.

Conclusion

There are clinical signs of neurotoxicity observed in studies in dogs and some self reported cases of neurotoxic symptoms in humans. Severe effects were only seen in a few dogs at doses which when corrected for caloric demand are in line with the values for STOT SE 2. The human case

reports do not allow conclusions on classification on their own. The effects seen in other species do not fulfil the criteria for classification. In deciding between classification for STOT SE 2 and no classification, RAC noted the overlap with the classification guidance values for STOT SE 2 and category 4 for acute oral toxicity. On the this basis therefore, RAC agrees with the proposal of the dossier submitter for **no classification for STOT SE**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The CLH report (as well as the CAR) for DEET included *in vitro* tests in bacteria (in the *Salmonella typhimurium*-reverse mutation assay), in mammalian cells (*in vitro* cytogenicity test (CHO cells), *in vitro* gene mutation assay in mammalian cells (CHO- HPGRT mutations) and in an unscheduled DNA synthesis test (rat primary hepatocytes). These tests did not indicate genotoxic potential. However, a publication reported a positive *in vitro* comet assay in primary human nasal mucosa cells with a concentration-dependent genotoxic response to DEET (Tisch *et al.*, 2002).

No *in vivo* test was summarised in the dossier, as all *in vitro* tests were negative. However, the Californian EPA reported a dominant lethal test with Swiss mice in which the number of implantations was decreased if males were exposed to DEET (http://www.cdpr.ca.gov/docs/risk/rcd/deet.pdf). However, due to study deficiencies, the result was considered equivocal.

It was concluded that although the positive comet assay and equivocal dominant lethal test indicate that further genotoxicity testing may be needed, the existing data does not meet the criteria for classification for germ cell mutagenicity.

Comments received during public consultation

Two comments from MSCA's were received on mutagenicity, one agreed with no classification but both MSCAs requested further details on the positive studies. The DS provided more extensive study summaries in their response, which are presented below.

In vitro comet assay (Tisch *et al.*, 2002):

The authors noted that cells from the middle turbinate were more sensitive to DEET, possibly due to differences in the intracellular metabolism of this substance, DNA repair capacity or antioxidant defences.

	Solvent control	DEET 0.5 mM	DEET 0.75 mM	DEET 1.0 mM
Middle turbinate (% undamaged cells)	89.6 ± 5.7	51.4 ± 4.6	36.3 ± 3.4	20.4 ± 5.2
Inferior turbinate (% undamaged cells)	92.4 ± 4.6	65.4 ± 6.2	48.3 ± 5.5	28.3 ± 6.3
There were no significant cytotoxic effects according to the cell viability test (trypan blue exclusion test) shed.				

A brief description of the *in vivo* dominant lethal assay (Swentzel, 1978), as summarised by the Californian EPA is presented below:

"In a dominant lethal assay, 10 male ICR/Ha Swiss mice received a single dose of DEET (95% meta, remainder other isomers) at 600 mg/kg. Ten mice/group in the positive and concurrent control groups received 10 mg/kg of TEM and 5 mg/kg corn oil, respectively. The males were then cohoused sequentially with 3 untreated virgin female mice 5 days/week for 8 weeks.

Females were sacrificed 13 days after the midweek of their cohabitation with a male. Although the fertility index was not significantly different from the concurrent controls, the total percentage of dams with less than 8 implantations over 8 weeks was greater in the males exposed to DEET than in the control animals (11.6% vs. 3.1%). This study had several deficiencies including only one dose level, too few pregnant females per group, and no individual data."

Assessment and comparison with the classification criteria

There are four negative *in vitro* mutagenicity assays and one positive comet assay, as well as an equivocal result from a dominant lethal test in Swiss mice. No information was provided on structure activity relationships to known germ cell mutagens.

In the absence of human evidence, Category 1 is not applicable.

Both Category 1B and 2 require at least one positive *in vivo* mutagenicity assay, or for Category 2 positive evidence *in vitro* supported by structure activity relationships to known germ cell mutagens.

The only available *in vivo* study (Swentzel, 1978) has severe limitations, as only one dose was tested (instead of three), there are no individual data, no historical control data, no positive control data, and the fertility index and post-implantation loss were not significantly different from the controls. Only the percentage of dams with less than 8 implantations was decreased after 8 weeks, which is not a regular endpoint under the OECD guideline. As such, it is hardly possible to determine its relevance for the determination of mutagenicity.

Considering there is no reliable *in vivo* study available and the *in vitro* evidence is equivocal, RAC agrees with the DS that **the available data is too limited to determine whether classification for germ cell mutagenicity is warranted or not. For this reason, no classification is proposed for mutagenicity.**

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two oral carcinogenicity studies were presented in the CLH dossier: A 2 year study in rats (OECD TG 453) and an 18 month study in mice. No significant increase in tumours was observed in either study.

In the rat study, females given the highest dose (400 mg/kg bw/d) showed slightly increased cholesterol values, decreased body weight, and decreased food consumption. The incidence of renal cell adenomas was slightly increased in males at the lowest dose (10 mg/kg bw/d) to 3/60, compared to the two control group incidences of 1/60 and 0/60. However, as the difference with the control groups was small and no increase in adenomas was observed at higher doses, this finding was not considered to be related to the treatment. It should be noted that the survival rate in this study was <50%.

The only effects found in the mice study were a decrease in body weight and food consumption and an increase in liver weight. As there were no histopathological changes in liver tissues, the increase in liver weight was considered to be an adaptive response. The highest dose tested in this study was 1000 mg/kg bw/d.

An investigation by the Agency for Toxic Substances and Disease Registry (ATSDR) found an increased risk of developing testicular cancer in Swedish workers using insect repellents for ≥ 115 days. However, due to deficiencies in the study, the results were not considered conclusive.

As there is no robust information that raises a concern, no classification for carcinogenicity was proposed by the DS.

Comments received during public consultation

Two comments submitted by MSCAs on carcinogenicity asked for more details, including on the reliability of the studies, the reason for the low survival rate in the rat study, the numerical data for the effects (rather than just percentages), and the results and limitations of the study on Swedish workers, also with regard to testicular effects in other studies.

The DS clarified that the reliability of the mouse study was high, but the reliability of the rat study was lower due to the low survival rates. The survival per group in the rat study varied from 16-31 animals out of 60, without a correlation with the dose. The study report gave no explanation for the low survival rate.

Testicular effects in other studies included reduced testis weight, tubular degeneration of testis, and luminar debris in epididymides of hamsters at above 300 mg/kg bw/d. Increased relative testis weight was observed in a 90 day oral rat study at 1000 mg/kg bw/d and reduced testis weight in a 8 week dog study at 400 mg/kg bw/d. The weight reductions were not accompanied by histopathological findings and no testicular effects were found in the one year dog study.

The study on Swedish workers was a case-control study to the risk factors for testicular cancer. The odds ratio for insect repellants was 1.7 based on 39 cases and 54 controls. It was stated in the study that most insect repellents in Sweden contain DEET as active ingredient. However, it is not clear how many workers were truly exposed to DEET and what their level of exposure was.

Assessment and comparison with the classification criteria

To classify a substance for carcinogenicity in Category 1, there should be either human studies that show a causal relationship between exposure to the substance and the development of cancer, and/or sufficient evidence to demonstrate carcinogenicity in animals. Category 2 is warranted if there is limited evidence of carcinogenicity.

For DEET, one epidemiological and two animal carcinogenicity studies are available. The epidemiological study has severe limitations, as the exposure was to insect repellents and not specifically to DEET, there were no exposure data, and the group sizes of cases and controls were small. It is also unclear from the summaries provided whether the study controlled for confounding factors. Thus, a causal relationship between testicular cancer in humans and exposure to DEET cannot be adequately established based on this study.

The carcinogenicity study in rats showed a slight increase in the number of renal cell adenomas in males at 10 mg/kg bw/d (3/60 animals). However, also this study had limitations, in particular the high mortality, for which no explanation was provided. More importantly, no increase in tumour incidence was observed at 30 and 100 mg/kg bw/d in males or at any dose in females.

A carcinogenicity study in mice found no increase in the tumour incidence at any dose, up to 1000 mg/kg bw/d.

It is the opinion of RAC that **the available evidence is insufficient for classification of DEET for carcinogenicity according to the CLP Regulation**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The dossier includes a 2-generation study in rats and two prenatal developmental toxicity (PNDT) studies (OECD TG 414) in rats and rabbits.

Fertility

No effect was found on any of the reproductive parameters in the 2-generation study. However, as this study was performed prior to the current guidelines, sperm morphology and motility were not investigated (as well as other parameters).

Testicular effects in other studies included reduced testis weight, tubular degeneration of testis, and luminar debris in epididymides of hamsters at 611 and 3136 mg/kg bw/d. Increased relative testis weight was observed in a 90 day oral rat study at 1000 mg/kg bw/d and reduced testis weight in a 8 week dog study at 400 mg/kg bw/d. The weight changes were not accompanied by histopathological findings. No testicular effects were found in the one year dog study (400 mg/kg bw/d) or a 2 year rat study (100 mg/kg bw/d).

As the observed effects in repeated dose studies were inconsistent and there were no observations or effects on reproductive parameters in the 2-generation study, no classification for fertility was proposed by the DS.

Development

The only developmental effect reported in the 2-generation rat study was a significant decrease of pup body weight at the end of the lactation period in the F1 and F2 at a dose of 5000 ppm (218-713 mg/kg bw/d).

In the PNDT in rats, the % preimplantation loss was slightly but not significantly increased at the highest dose (750 mg/kg bw/d). The number of implantations/litter was unaffected. The foetal body weights/litter were also reduced at this dose. However, at this dose significant signs of maternal toxicity were observed, including reduced food consumption and reduction of body weight and body weight gain. Two dams were sacrificed in moribund state on GD 7.

No embryotoxic or teratogenic effects were observed in the PNDT in rabbits at doses up to 325 mg/kg bw/d. One high dose female showed neurological effects and died. In the other females, signs of toxicity were limited to decreased food consumption and body weight gain from GD 6-9.

In addition to the studies in the CLH dossier, a report by WHO INCHEM included older Russian studies in rats that reported gonadotoxic and embryotoxic effects of DEET. A short summary of a dermal study (100-1000 mg/kg bw/d) was cited that reported increased embryon loss, postnatal death, lagging development, and reduced pup weight. As there was no information on maternal toxicity and the study quality is unknown, these findings were not considered sufficient for classification.

As no developmental effects were found in the PNDT studies in rats and rabbits, no classification is proposed for development by the DS.

Comments received during public consultation

Two comments were submitted by MSCA's on reproductive toxicity. One agreed there is no evidence of reproductive toxicity. The second noted that the reliability score of 1 is not appropriate for the 2-generation study due to the deviations from the current OECD test guideline.

Assessment and comparison with the classification criteria

Fertility

The only reproductive toxicity study that evaluated effects on fertility was a 2-generation study in rats (218-713 mg/kg bw/d). No effects on fertility were observed in this study, although not all parameters were investigated.

Effects on reproductive organs were also investigated in repeated dose studies in rats, hamsters, and dogs.

In rats, an increased relative, but not absolute, testicular weight was observed in a 90 day study at 1000 mg/kg bw/d, but not in a 2 year study at 100 mg/kg bw/d. As the weight increase had no histophatological correlate, was not found in other rat studies (2 year and 2-gen) and only occurred at a very high dose where there were also other signs of toxicity (kidney effects), it is unlikely to be a specific effect on fertility.

A 90 day range finding study in hamsters showed reduced testis weight (only the absolute weight change was statistically significant) and tubular degeneration of testis at 611 and 3136 mg/kg bw/d (see table "Summary of the results of a 90-day range-finding study in hamsters" under "Additional key elements" in the background document). In addition, luminar debris were seen in the epididymides, but since the epididymis was only subjected to microscopic examination if there were macroscopic findings, the finding is only supportive of the other effects.

Considering that the mortality was 27%, the highest dose exceeded the maximum tolerable dose. However, the reduction in testis weight and tubular degeneration were also observed at the dose of 611 mg/kg bw/d. At this dose, general toxicity was limited to a decrease in body weight and increased relative brain and kidney weights. The latter were secondary to the lower body weight, as the absolute kidney and brain weights were unaffected.

The decrease in absolute testis weight was on average 41%. However, this decrease was not evenly spread over the animals, but the result of a higher incidence in small testis (see tables "Individual data of the control group of the hamster study" and "Individual data of the 611 mg/kg bw/d group of the hamster study", both in the background document) and small epididymis (data not shown). Small testes also occurred in the control group and in both groups coincided with tubular degeneration.

Unfortunately, there are no historical control data available to verify the usual variation of these endpoints. Additionally, the literature data on hamsters is very limited, and no test protocol could be found specifically for this species. However, as indicated in Hubrecht and Kirkwood (2010) hamsters should be kept at a minimum of 14 h light to eliminate hibernation. When hamsters are kept at shorter light periods and in particular, if the period is then changed to an even shorter light period, this may induce regression of the reproductive system, including a reduction in testis weight and tubular degeneration (Larkin *et al.*, 2001; Donham *et al.*, 1996; Breckon and Cawood, 1985). The hamsters in the 90 day study were kept at a 12/12 h cycle; unfortunately it was not stated how they were housed before the study started. There was no link between food consumption or body weight and testis weight. Thus, it may be that the photoperiod had an influence on the gonadal weight, but with the current information, this can neither be proven nor disproven.

Reduced testis weight was also observed in an 8 week dog study at 400 mg/kg bw/d. The weight changes were not accompanied by histopathological findings. Reduced testis weight in short term dog studies should be interpreted with caution, as this may result from delayed development due to toxicity. As no testicular effects were found in the one year dog study at the same dose level (400 mg/kg bw/d), the effect in the 8 week study is considered of limited relevance.

In addition, a reduction in the number of female mice with 8 or more implantations was found in the dominant lethal assay discussed in the section on mutagenicity after a single dose of 600

mg/kg bw. The reporting was very limited and it is very difficult to judge the significance of this finding with only one dose level administered and without any indication of the historical control range.

Considering the contradictions and limitations in the outcome of the studies, classification as Category 1B is not appropriate. A case could be made for both classification in Category 2 and for no classification for fertility. The main reason for classification would be the testis effects in the 90 day hamster study. However, this study has several limitations, in particular the limited experience with the test species, high inter-individual variations in the effects, and the lack of historical control data. It should also be noted that the guidance paragraph 3.7.2.3.1. states that "in case of contradictions between the standard repeat dose studies and reproductive studies, the result from the latter should be considered more relevant." The hamster study was not a standard repeated dose study, and there is also a 2-generation study in rats available, which did not show any testis effects. Although it cannot be excluded that this is a species specific effect, in this case there is all the more reason to doubt the relevance of hamsters as a result of their seasonal changes in reproductive parameters.

RAC concludes that, although there are some indications of testicular toxicity, **the weight of the evidence is not sufficient to warrant classification for fertility**.

Development

RAC agrees with the DS that the Russian studies are too poorly reported to draw any conclusions from the effects described. The developmental effects reported in the 2-generation and PNDT rat studies were too slight to support classification and no developmental effects were found in the rabbit PNDT.

As no developmental effects were found in either rats or rabbits, RAC concludes that **no classification for developmental toxicity is warranted** according to the CLP Regulation.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

N,N-Diethyl-meta-Toluamide (DEET) is a biocide used as an active ingredient in insect repellents and attractants. The current environmental classification in Annex VI, Table 3.1 of the CLP Regulation is Aquatic Chronic 3, H412. The DS proposed to declassify the substance based on low toxicity to aquatic organisms, on low bioaccumulation potential and on rapid degradability of the substance.

DEET has very high solubility in water (11.2 g/L, distilled water at 25°C). DEET is moderately volatile or volatile according to the vapour pressure of 0.23 Pa at 25°C but only very slightly volatile from water surface (Henry's law constant 3.93E-3 Pa m3/mol). DEET is also surface active (surface tension 58.0 mN/mat 1 g/L and 20°C). The purity of the test material used in the environmental studies was 98.3% except for one study in which the purity was not reported.

Degradation

DEET was hydrolytically stable in sterile buffer solutions at pH 4, 7 and 9 in a 5 day test at 50°C under dark conditions (OECD TG 111). According to the final CAR DEET is photolytically stable in sterile distilled water.

There are three ready biodegradability studies available on DEET. In a 28 day GLP OECD TG 301B study, 83.8%, degradation was recorded fulfilling the 10 day window, showing the substance is readily biodegradable. In a study based on the OECD TG 301D (Closed bottle test), mineral medium inoculated with river water and garden soil extract was used. According to the DS the level of biodegradation observed in the test shows inherent biodegradability more than ready biodegradability. The third study is based on OECD TG 301C (Modified MITI test). No biodegradation was observed in this test. The test substance concentration is, however, higher (100 mg) in this test compared to the two other tests, 10 mg/L for OECD TG 301B and 2-10 mg/L for OECD TG 301C, respectively. The DS is of the opinion that DEET might have been toxic to microorganisms at 100 mg/l because in an acute toxicity study of luminescent bacteria toxicity to microbes was shown (EC_{50} of 68 mg/L) which would explain the lack of biodegradation. EC_{50} (3 h) was greater than 1000 mg/L in an inhibition of respiration in activated sludge test (OECD TG 209). There are no study summaries available on OECD TG 301D and 301C. There are no degradation simulation tests available for any environmental compartment.

The DS provided measured concentrations of DEET in environmental compartments. The high frequency of positive samples both in ground and surface water indicates that DEET is wide-spread in aqueous media which seems to contradict the observatio that DEET is readily biodegradable.

Bioaccumulation

In a test equivalent to the OECD TG 117 (HPLC Method), the measured partition n-octanol/water coefficient (log K_{OW}) was 2.4 at pH 6 and 22°C for DEET. Since the method is not applicable to surface-active agents, there might still be some uncertainties with this value with DEET being mildly surface active. However, an estimation with ECOSAR Version 1.11 gives a log Kow of 2.258 (estimation EPISUITE Kowwin v.1.68) and supports the measured value. The QSAR estimated bioconcentration factor (BCF) is 22. Based on the estimated BCF the DS considered DEET having very little or no potential to bioaccumulate in the aquatic environment.

Aquatic toxicity

Table. Aquatic acute toxicity of DEET to aquatic organisms

Test species	Test method	Results	Remarks
Oncorhynchus mykiss	OECD TG 203; EEC Method C.1; OPPTS 850.1075, GLP, static	LC_{50}, 96 h: 97 mg/L (mm)	nominal concentrations maintained
Daphnia magna	U.S.EPA Ecol. Res.Series 660/3-75009; Standard Methods for the Examination of Water and Wastewater, GLP, static	LC_{50} (mortality), 51 h: 75 mg/L (mm) EC_{50} (abnormal behaviour), 51 h, 42 mg/L	nominal concentrations maintained, purity not reported
Selenastrum capricornutum	OECD TG 201; EEC Method C.3; OPPTS 850.5400, GLP, static	E_rC_{50}, 72 h: **41 mg/L** (mm) NOE_rC, 72 h: 7.6 mg/L (mm)	nominal concentrations maintained
Selenastrum capricornutum	OECD TG 201, GLP	E_rC_{50}, 72 h: 50-100 mg/L NOEC, 72 h, 24 mg/L	No study summary available
Pseudokirchneriella subcapitata NIES-35 (formerly *Selenastrum capricornutum*)	No standardized guideline, 96-hole microplate growth inhibition test	EC_{50}, 96 h: 41 mg/L NOEC, 96 h: 0.521mg/L	Published article, experimental details missing

There is aquatic acute toxicity data available on one fish test, one *Daphnia* test and three algae tests. The lowest reliable acute value is a 72 h E_rC_{50} of 41 mg/L for algae *Selenastrum capricornutum*.

Table. Chronic aquatic toxicity of DEET to aquatic organisms

Test species	Test method	Results	Remarks
Daphnia magna	EPA 850.1300	NOEC, 21 d: 14 mg/L (reproduction) **3.7 mg/L** (length)	Reliable, published article
Selenastrum capricornutum	OECD TG 201; EEC Method C.3; OPPTS 850.5400, GLP, static	E_rC_{50}, 72 h: 41 mg/L (mm) NOE_rC, 72 h: 7.6 mg/L (mm)	nominal concentrations maintained
Selenastrum capricornutum	OECD TG 201, GLP	E_rC_{50}, 72 h: 50-100 mg/L NOEC, 72 h, 24 mg/L	No study summary available
Pseudokirchneriella subcapitata NIES-35 (formerly Selenastrum capricornutum)	No standardized guideline, 96-hole microplate growth inhibition test	EC_{50}, 96 h: 41 mg/L NOEC, 96 h: 0.521 mg/L	Published article, experimental details missing

There is no aquatic chronic toxicity data available on fish. There is chronic acute data available from one *Daphnia* test and three algae tests. The lowest chronic values are a 21 day NOEC of 3.7 mg/L for *Daphnia magna* and a 72 h NOE_rC of 7.6 mg/L for algae *Selenastrum capricornutum*.

The algae 96-hole microplate growth inhibition test measured growth rate with light absorbance by photosynthetic pigments, using absorbance at 450 nm. This study gives the lowest chronic value 96 h EC_{50} of 0.521 mg/L. However, this study did not follow any guidelines,experimental details were missing and is not reliable according to the DS.

Comments received during public consultation

One industry organisation and one MSCA fully supported the DS proposal. One MSCA brought up the fact that although the DS noted that there is a large dataset available for DEET, this is not the case with many environmental studies. RAC agrees to this statement. In depth description of degradation studies is also missing although this property is crucial when considering declassification. The MSCA also wanted more information on aquatic toxicity and ready biodegradation studies e.g. GLP status, whether they meet validity criteria and deviations from the guideline. More information on those studies left out of the CLH Report was requested. They also saw it unlikely that halted degradation in the OECD TG 301D Ready biodegradation test would be due to toxicity to microorganisms.

Another MS supported the removal of classification in principle but wanted more information especially on ready biodegradation tests. They also proposed to consider Aquatic Chronic Category 4 classification for the substance. The DS informed that more information was available in the ECHA dissemination website and in a confidential IUCLID attachment. They also informed that they had chosen the OECD TG 301B to be the key study and therefore chose not to describe the other studies in as much detail. Another MSCA wanted more information on a chronic toxicity study to *Daphnia magna* showing 21 d NOEC of 3.7 mg/L since this study had not been evaluated in the biocide assessment and showed the lowest NOEC. The DS replied that as the study was published in an article, was reliable and was used as a supportive study they decided not to give more detailed information.

Assessment and comparison with the classification criteria

Degradation

DEET is hydrolytically and photolytically stable. There are three ready biodegradation studies available. OECD TG 301B test showing ready degradability (83.8% in 28 days), OECD TG 301D study showing more inherent degradability according to the DS and OECD TG 301C test where the substance dose of 100 mg/L is possibly caused inhibition to the bacteria. Unfortunately there are no study summaries available for RAC to evaluate the OECD TG 301D and OECD TG 301C studies.

Aquatic Bioaccumulation

The measured log Kow of 2.4 and an estimated log K_{OW} of 2.258 indicate that DEET has very little or no potential to bioaccumulate in the aquatic environment.

Aquatic toxicity

There are acute aquatic toxicity data available for fish, *Daphnia* and algae (table above). The lowest acute aquatic toxicity value is a 72 h E_rC_{50} of 41 mg/L for algae *Selenastrum capricornutum*.

There are chronic aquatic toxicity data available for *Daphnia* and algae (table above). No chronic toxicity data is available for fish. The lowest chronic toxicity value is 21 day NOEC (length) of 3.7 mg/L for *Daphnia magna*. The reliability of the *Daphnia magna* study is based on the DS statement. The study was not included in the CAR and reviewed under Directive 98/EEC and no details were given in the CLH Report or the responses to the Public Consultation comments. The 72 h NOE_rC for algae *Selenastrum capricornutum* is also between 1 and 10 mg/L.

In addition, there is a 96 hour algae NOEC for *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) of 0.521 mg/L but according to the DS this cannot be used as a basis for classification due to missing details of experimental conditions in a study not following any guidelines.

The QSAR calculations presented in the table above for acute toxicity follow the trend, algae being the most sensitive species (44.060 mg/L). For chronic toxicity the QSAR calculation show an estimate of 5.586 mg/L for *Daphnia*. The chronic fish toxicity, where measured test result is missing, is estimated to be 9.267 mg/L.

Comparison with the criteria

There are three ready biodegradation studies available with conflicting results. The OECD TG 301B test showing ready degradability (83.8% in 28 days). This study is considered the most reliable and best documented of all three. The OECD TG 301C test showed no biodegradation. This study was considered less reliable because it was unsure if the test concentration of 100 mg/L was toxic to microbes. RAC considers this explanation of toxicity in the MITI test to be plausible. The OECD 301D did not reach the pass-level for readily biodegradable, but there is at least a substantial degradation, which also contributes to the conclusion that the substance will not be persistent.

In addition, according to the CLP Guidance (Annex II.3.5) "*positive results in ready biodegradability tests could be considered valid, irrespective of negative results, when the scientific quality is good and the test conditions are well documented, i.e. guideline criteria are fulfilled, including the use of non-pre-exposed (non-adapted) inoculum*". Consequently,

RAC is of the opinion that DEET is rapidly degradable according to the classification criteria.

There is no measured BCF value available but based on the log K_{OW} DEET has very little or no potential to bioaccumulate.

The acute toxicity values for fish, Daphnia and algae are in the range of 10 to 100 mg/l and acute classification criteria ($LC/EC_{50} \leq 1$ mg/L) are not met.

The chronic toxicity values for Daphnia and algae are greater than 1 mg/L. There is no chronic data for fish. Using the surrogate method based on acute toxicity for fish, rapid degradability and no bioaccumulation potential the classification criteria for long term classification are not met. The same conclusion is reached using the chronic QSARs for toxicity. Thus, as DEET is considered a rapidly degradable substance, the classification criteria for long term classification are not met (EC_{10}/NOEC ≤ 1 mg/L for Aquatic Chronic 3 classification).

Overall, RAC agrees with the DS proposal to **remove the current classification for environmental hazards (Aquatic Chronic 3; H412) of DEET**.

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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).