Committee for Risk Assessment

RAC

Annex 1
Background document
to the 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

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

Adopted

9 June 2017

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: N,N-Diethyl-meta-Toluamide (DEET)

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

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

Index Number: **616-018-00-2**

Contact details for dossier submitter:

Swedish Chemicals Agency (KEMI), Po box 2, 172 13 Sundbyberg, Sweden

Version number: **4** **Date:** **2016-06-17**

CONTENTS

Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***N,N-diethyl-m-toluamide (IUPAC)*** ***DEET (synonym)***
EC number:	***205-149-7***
CAS number:	***134-62-3***
Annex VI Index number:	***616-018-00-2***
Degree of purity:	***≥ 97% (w/w)***
Impurities:	***No impurities present at ≥1%. None of the impurities present at lower levels are considered relevant for the classification of the substance.***

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Acute Tox. 4, * H302 Eye Irrit. 2, H319 Skin Irrit. 2, H315 Aquatic Chronic 3, H412
Current proposal for consideration by RAC	Acute Tox. 4, H302 Remove Aquatic Chronic 3, H412
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4, H302 Skin Irrit. 2, H315 Eye Irrit. 2, H319

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Hazard class not assessed in this dossier			
2.2.	Flammable gases	Hazard class not assessed in this dossier			
2.3.	Flammable aerosols	Hazard class not assessed in this dossier			
2.4.	Oxidising gases	Hazard class not assessed in this dossier			
2.5.	Gases under pressure	Hazard class not assessed in this dossier			
2.6.	Flammable liquids	Hazard class not assessed in this dossier			
2.7.	Flammable solids	Hazard class not assessed in this dossier			
2.8.	Self-reactive substances and mixtures	Hazard class not assessed in this dossier			
2.9.	Pyrophoric liquids	Hazard class not assessed in this dossier			
2.10.	Pyrophoric solids	Hazard class not assessed in this dossier			
2.11.	Self-heating substances and mixtures	Hazard class not assessed in this dossier			
2.12.	Substances and mixtures which in contact with water emit flammable gases	Hazard class not assessed in this dossier			
2.13.	Oxidising liquids	Hazard class not assessed in this dossier			
2.14.	Oxidising solids	Hazard class not assessed in this dossier			

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.15.	Organic peroxides	Hazard class not assessed in this dossier			
2.16.	Substance and mixtures corrosive to metals	Hazard class not assessed in this dossier			
3.1.	Acute toxicity - oral	Acute Tox. 4 H302		Acute Tox. 4* H302	
	Acute toxicity - dermal				conclusive but not sufficient for classification
	Acute toxicity - inhalation				conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Hazard class not assessed in this dossier			
3.3.	Serious eye damage / eye irritation	Hazard class not assessed in this dossier			
3.4.	Respiratory sensitisation	Hazard class not assessed in this dossier			
3.4.	Skin sensitisation	Hazard class not assessed in this dossier			
3.5.	Germ cell mutagenicity				conclusive but not sufficient for classification
3.6.	Carcinogenicity				conclusive but not sufficient for classification
3.7.	Reproductive toxicity				conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure				conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Hazard class not assessed in this dossier			
3.10.	Aspiration hazard	Hazard class not assessed in this dossier			
4.1.	Hazardous to the aquatic environment	Removal of existing harmonized classification		Aquatic Chronic 3 H412	conclusive but not sufficient for classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
		as Aquatic Chronic 3, H412			
5.1.	Hazardous to the ozone layer	Hazard class not assessed in this dossier			

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

Labelling: Signal word: Warning
Hazard statements: H302, H315, H319
Precautionary statements: Responsibility of the applicant
Pictograms: GHS07

Proposed notes assigned to an entry:

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The previous classification and labelling was adopted prior to the review of DEET under the biocide directive 98/8/EC. The previous classification was made according to Directive 67/548/EEC, pursuant to Commission Directive 93/72/EEC (1 September 1993) adapting to technical progress for 19th time and Commission Directive 2001/59/EC (6 August 2001) adapting to technical progress for 28th time. The current entry in Annex VI, Table 3.1 of the CLP regulation is a translation of the classification adopted at the 28th ATP into CLP.

2.2 Short summary of the scientific justification for the CLH proposal

This proposal for classification and labelling considers hazard classes' carcinogenicity, mutagenicity and reproductive toxicity and those hazard classes for which a change to the existing classification is proposed. The proposal made is based on information available in the Competent Authority (CA) report prepared under directive 98/8/EC. The study summaries (Doc IIIA) of the CA report are included in a confidential attachment to the IUCLID dossier.

Human health hazards

Acute Tox. 4; H302: Based on the results of an acute oral toxicity study in Sprague-Dawley rats, the oral LD_{50} value for DEET is 1892 mg/kg. This value is within the range given for classification in category 4 of the hazard class "acute toxicity" in the CLP regulation.

Environmental hazards

DEET has a current classification Aquatic Chronic 3, H412.

The most reliable test to show that degradation of DEET has occurred is the OECD guideline 301 B ready biodegradable test (see table 21) where you can see an ultimate degradation of the test material as it is mineralizes to carbon dioxide.

There is another biodegradation test carried out according to OECD guideline 301D (Closed Bottle Test) but this test only showed inherent biodegradability and not ready biodegradability and there were also difficulties to interpret the results.

According to the MITI database (CITI 1992) DEET cannot be considered ready biodegradable (0% biodegradation as determined by BOD). This record was based on a test that was performed in 1983 and according to another guideline (OECD 301C).

The OECD Guideline 301C is less reliable than 301 B because it was not sure if it was the toxicity of DEET to the microorganism that caused the lower degradation of DEET or not. This is more likely to happen in the OECD 301 C test than in other 301 tests because the test substance was introduced at higher concentration (100 mg/L) in the OECD 301C test. That DEET might have a toxicity to microbes is shown in an acute toxicity study of phospohorent bacteria (Kaiser and Palabrica 1991 in Weeks 2011).

The open literature was searched for additional data to clarify the issue. However, these data did not allow any firm conclusions, except that analysis of influent and effluent in a German STP (Knepper et al, 2004, Water Science and Technology 50: 301-308) indicated that the rate of elimination was highly variable, and that DEET was not completely degraded within the STP.

Monitoring studies show that DEET is found in groundwater and surface water. This is considered as somewhat contradictory given that the substance was found to be readily biodegradable. It is concluded that the wide-spread, more or less continuous use together with the hydrophilic nature of DEET results in a wide-spread contamination of waters, including groundwater, at low concentrations. Under limited circumstances, like heavy rainfall or dominated effluent flows with very little dilution where relatively high concentrations of DEET may occur in rivers or streams and aquifers (Weeks, 2011).

Bioaccumulation potential of DEET in aquatic organisms is low.

In the large dataset on both acute and long term toxicity of aquatic organisms, the most reliable studies show a low toxicity.

As a result of this, it is suggested that DEET should not be assigned any classification for environment. Thus, it is suggested that DEET is declassified in relation to the current environmental classification.

2.3 Current harmonised classification and labelling

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

Classification
Hazard Class and Category Code(s): Acute Tox. 4 *, Eye Irrit. 2, Skin Irrit. 2, Aquatic Chronic 3
Hazard Statement Code(s): H302, H319, H315, H412

Labelling
Pictogram, Signal Word Code(s): GHS07, Wng
Hazard Statement Code(s): H302, H319, H315, H412
Suppl. Hazard statement code(s): none

2.4 Current self-classification and labelling

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

Five groups of notifications are listed in the C & L inventory (December 2nd, 2014). Four of these groups propose the following classification:

Acute tox 4: H302, Skin irrit. 2:H315, Eye irrit. 2: H319 and Aquatic chronic 3: H412

The proposal made by the fifth group differs from the above with respect to the classification for environmental hazards. This group proposes Aquatic acute 3 instead of Aquatic chronic 3.

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) No1272/2008 (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 the RAC. The CLH proposal is based on information available in the Competent Authority (CA) report (CAR) prepared under directive 98/8/EC.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

A justification is not required for active substances in biocidal products and plant protection products (cf. Article 36(3) CLP Regulation).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

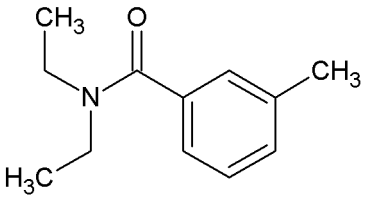
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	205-149-7
EC name:	N,N-diethyl-m-toluamide DEET
CAS number (EC inventory):	134-62-3
CAS number:	134-62-3
CAS name:	Benzamide, N,N-diethyl-3-methyl-
IUPAC name:	N,N-diethyl-m-toluamide
CLP Annex VI Index number:	616-018-00-2
Molecular formula:	$C_{12}H_{17}NO$
Molecular weight range:	191.27 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
DEET	99.0%w/w	97.8 – 99.7%w/w	Information not claimed to be confidential.

Current Annex VI entry:

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
No impurities present at ≥1%. None of the impurities present at lower levels are considered relevant for the classification of the substance.			All impurities are listed as confidential information in IUCLID section 1.2.

Current Annex VI entry:

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

The test material used in the experimental studies reported for the physico-chemical properties were of purified (99.3%w/w) or technical (97.8-98.6%w/w) quality. No further information (e.g. content and identity of impurities) is available on the composition of the batches used in testing of the physico-chemical properties.

The test material used in the experimental studies in section 4 (human health hazard) was mainly of technical quality. The purity of the test material used in the *in vitro* mammalian gene mutation study was unknown.

The purity of the test material used in the environment studies used for classification purposes (hydrolysis, ready biodegradability, adsorption/desorption, acute toxicity to fish, growth inhibition test on algae, inhibition of respiration in activated sludge) was 98.3% except for the study of acute toxicity to aquatic invertebrates in which the purity was not reported.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Liquid	A3.7(2)	measured
Melting/freezing point	Not determined (assumed to be < -20°C)	Justification for A.3.1.1	Not required if melting point is < -20°C
Boiling point	284.2ºC at standard pressure	A3.9	measured
Relative density	D_4^{20} = 0.998	A3.14(1)	measured
Vapour pressure	Measured: 0.64 Pa at 32.4°C 2.81Pa at 41.8°C 8.76 Pa at 52.0ºC Extrapolated: 0.11 Pa at 20.0ºC 0.23 Pa at 25.0ºC	A3.2	
Surface tension	58.0 mN/m at 1 g/L concentration and 20°C Material is surface active	A3.12	measured
Water solubility	11.2 g/L in distilled water (unstated pH) at 25°C	A3.9	measured
Partition coefficient n-octanol/water	Log P_{ow}= 2.4 at pH 6 and 22°C	A3.9	measured
Flash point	144°C	A3.12	measured by non-equilibrium closed cup method
Flammability	Not applicable to liquids	-	
Explosive properties	Not considered as explosive	Justification for A.3.15	Theoretical considerations based on structural properties in agreement with waiving criteria in CLP
Self-ignition temperature	Not determined	Justification for A3.11	
Oxidising properties	Not considered as an oxidizer	Justification for A.3.16	Theoretical considerations based on structural properties in agreement with waiving criteria in CLP
Granulometry	Not data available		

Property	Value	Reference	Comment (e.g. measured or estimated)
Stability in organic solvents and identity of relevant degradation products	Not generally tested. Stable at 15%w/w in denatured alcohol at 54°C for 2 weeks, at 40°C for 3 months and at 20°C for 3 months.	B3.7(1), B 3.7 (2)	DEET as manufactured does not contain organic solvents. Stability tested in commercial product
Dissociation constant	Not determined	Justification for A.3.16	DEET is not dissociable within the environmentally relevant pH range.
Viscosity	2.19×10^{-5} m^2/s at 20°C 8.25×10^{-6} m^2/s at 40°C	A3.14(1)	Measured (kinematic viscosity)

2 MANUFACTURE AND USES

2.1 Manufacture

This information is not necessary for classification purposes.

2.2 Identified uses

Active ingredient in insect repellent products.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not addressed in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Following oral administration of DEET to rats, 85-95% was absorbed and excreted in the urine. There was no major difference in the rate of urinary excretion between sexes but the rate differed between administration routes. The lowest excretion rate was observed following dermal administration. For the oral route, an increasing order of excretion was observed following a single oral low dose, a single oral high dose and a repeated oral low dose, respectively. The greatest difference between excretion rates was observed for the dermal and the oral routes. The rate of dermal absorption of DEET was slower than the rate of oral absorption.

Upon oral or dermal administration, 74-91% of the administered radioactivity was excreted via urine, approximately 3-7% was excreted via faeces and 0.21-0.67% remained in tissues. The highest tissue levels were observed in spleen, liver and kidneys. DEET was completely metabolized in all orally or dermally treated groups with little or no excretion of the parent compound in urine. The two major metabolites identified were metabolite A (or m-[(N,N-diethylamino) carbonyl] benzoic acid) and metabolite B (or m-[(ethylamino) carbonyl] benzoic acid (A6.2(1))). The pattern of excretion following oral administration indicates that the rate of metabolism is the determining factor for the elimination rate. No bioaccumulation was noted following oral administration.

Studies investigating plasma levels in rats and dogs were performed in order to compare maximum plasma levels and area under the curve (AUC) at the NOAELs set in animal studies with the maximum plasma levels and AUCs in humans. Peak plasma levels in rats and dogs were reached approximately one hour after oral administration of doses comparable to NOAELs (200 and 75 mg/kg, respectively). The peak level in humans dermally administered a dose representing the 95th percentile of the estimated exposure from use and the peak level in rats dermally exposed to a dose comparable to the NOAEL of 1000 mg/kg bw/day were reached after approximately 8 and 4 hours of exposure, respectively. These studies also show a more rapid absorption in rats following oral administration compared to dermal (A6.2 (1)) and a slower elimination from blood after dermal exposure.

4.1.2 Human information

Following dermal application on skin, low levels of DEET and/or metabolites of DEET appeared in plasma within two hours indicating absorption of the substance. When exposure was terminated, levels of radioactivity declined rapidly in both plasma and urine demonstrating a rapid excretion of the dermally absorbed fraction. Based on analyses of urine samples collected over 5 days following dermal application of DEET (either as a 15% (w/w) solution in ethanol or as the undiluted technical grade material), less than 9% (20% if corrected for total recovery) of the dose was absorbed through the skin during an 8-hour exposure period (A6.2.(3)). More than 80% of applied DEET was recovered in skin wipes and rinses. The absorption was slightly higher if DEET was applied diluted

in ethanol however the difference may also be due to inter-individual differences (i.e. only 6 individuals were included in the study). The mean excretion of the administered radioactivity in urine and faeces was 8.3% and 0.08%. A mean of 80% of the administered radioactivity was found in association with applicators, swabs, rinses and coverings whereas tape strips only contained 0.07%. When corrected for recovery, the dermal absorption value is 20%. There is no evidence of accumulation in the skin.

4.1.3 Summary and discussion on toxicokinetics

The rate of absorption is slower following dermal absorption compared to oral absorption.
Absorbed DEET is extensively metabolized to two major metabolites, metabolite A (m-[(N, N-diethylamino) carbonyl] benzoic acid) and metabolite B (or m[(ethylamino)carbonyl] benzoic acid) in both rats and humans.
DEET is rapidly excreted, mainly via urine, and did not show bioaccumulation in the tests performed.

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral U.S. EPA Health Effects Testing Guideline OPPTS 870.1100, Rat, Sprague-Dawley derived/ albino/ Male and female, 5 sex/group 1000, 2000 and 4000 mg/kg bw	LD50 (males + females) =1892 mg/kg bw (95% Confidence Limits: 1652-2204 mg/kg bw) Females:1000 mg/kg bw ≤ LD50 (females) < 2000 mg/kg bw <u>Mortality:</u> 1000: 0/5 m, 1/5f 2000: 2/5 m, 5/5f 4000: 4/5 m, 5/5f <u>Clinical signs prior to death:</u> hypoactivity, hunched and/or prone posture. <u>Clinical signs in surviving animals:</u> hunched or prone posture, hypoactivity, reduced faecal volume and/or piloerection.	Fulfils CLP criteria for classification Reliability 1	A6.1.1
Dermal U.S. EPA Health Effects Test Guidelines, OPPTS 870.1200 Limit test, Rat, Sprague-Dawley derived/ albino Male and female, 5 sex/group 5000 mg/kg	LD50 (males + females) > 5000 mg/kg bw No mortality	None Reliability 1	A6.1.2(1)
Dermal U.S. EPA Pesticide Assessments Guideline, 81-2 Limit test Rabbit, New Zealand/albino Male and female, 5/sex/group 2000 mg/kg	LD50(males + females) > 2000 mg/kg No mortality Erythema and edema at all test sites persisting to Days 6 or 7.	None Reliability 1	A6.1.2(2)
Inhalation U.S. EPA Health Effects Test Guidelines OPPTS 870.1300 Limit test Rat, Sprague-Dawley derived/ albino Male and female, 5 sex/group 2.02 mg/L, 4h, aerosol	LC50(males + females) > 2.02 mg/L No mortality	None Reliability 1	A6.1.3

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Single doses of 1000, 2000 or 4000 mg DEET/kg bw was administered to groups of male and female rats. Hypoactivity and hunched and/or prone posture were observed in animals prior to death but also in surviving animals. There were no gross abnormalities in animals surviving to termination but discoloration of the lungs, liver, intestines and/or gastrointestinal tract were noted in all decedents.

The LD_{50} in males calculated by Probit analysis is 1944 mg/kg bw (with 95% confidence limits of 1595-2474 mg/kg bw) and the LD_{50} in females is calculated to be between 1000 and 2000 mg/kg bw. The oral LD_{50} for sexes combined is 1892 mg/kg thus DEET meets criteria for classification in category 4.

4.2.1.2 Acute toxicity: inhalation

Rats were exposed in a chamber to a concentration of 2.02 mg DEET/L during four hours. All animals gained weight and no deaths occurred during the study. The signs of toxicity observed included ocular and nasal discharge, irregular respiration, dyspnea, hunched posture and hypoactivity.
With the exception of nasal discharge and dypsnea, clinical signs persisted in all animals after being removed from the exposure chamber. However, all animals recovered from the above symptoms by Day 3. No gross abnormalities were observed in tissues or organs at necropsy.
The test was performed in accordance with the US-EPA guideline for acute inhalation toxicity testing which accepts a maximum test concentration of approximately 2.0 mg/L as a limit dose. This concentration is below the limit dose of 5 mg/L recommended in the corresponding OECD/EU guidelines and the upper limit for classification in category 4. However, as discussed in the current OECD TG 403 and in the draft report of the expert consultation meeting on acute inhalation toxicity, it may be technically challenging to achieve both a concentration of 5 mg/L and particles of respirable size. The study is considered to fulfil the purpose to establish the acute toxicity by inhalation and the LC_{50} of DEET is thus considered to be greater than 2.02 mg/L.

4.2.1.3 Acute toxicity: dermal

The acute dermal toxicity of DEET was tested in rabbits and rats using doses of 2000 and 5000 mg/kg bw, respectively. All animals survived treatment. Rabbits were reported to be" active and healthy" during the course of the study. Erythema and oedema were observed at all the test sites on Day 1 and persisted until Days 6 or 7.

Similarly, rats were reported to be "active and healthy" during the course of the study with the exception of one female appearing hypoactive on Day 1 only. There were no gross abnormalities observed in any of the test animals. Based on the results from these two studies, the dermal LD_{50} is considered to be greater than 2000 and 5000 mg/kg in rabbits and rats, respectively.

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

The human data available include direct observations and published clinical case reports. There are no studies on manufacturing plant personnel available (medical surveillance data). A review of

published literature describing clinical case reports was recently performed addressing dermal exposure, oral ingestion, occupational exposure and data from poison control centres (A.6.12 (1)). Based on this data, the applicant for DEET in the biocides review concluded the following:

Dermal exposure: In 14 reported U.S. or Canadian cases, neurological symptoms are associated with exposure to DEET. However, an alternative diagnosis is possible in all but one case.

Oral ingestion: There are six reported cases but because of the intentional ingestions resulting in peak plasma levels >1000 times higher than seen following normal use of DEET, this information is not considered relevant for the assessment of adverse effects following normal consumer use of DEET (Schoenig and Osimitz, 2001).

Occupational exposure: None reported for DEET manufacturers or product formulators.

Data from poison control centres: Between the years 1985 and 1989, 9086 human exposures involving insect repellents containing DEET have been reported to U.S. poison control centres. In 98.9% of these cases there are either no effect or short-lived symptoms whereas in 66 cases (0.73%) symptoms were classified as moderate (all symptoms resolved)[1].

According to information from the applicant for DEET in the biocides review, *"beginning in 1995 until 2001, the DEET Joint Venture contracted Pegus Research, Inc. to operate the National Registry of Human Exposures to DEET. The purpose of the Registry was to collect detailed information from individuals who used DEET-containing insect repellents and reported serious adverse neurologic or systemic effects. The Registry allowed follow up on individual cases to determine exposure circumstances, medical data and whether causality between DEET exposure and symptoms could be established. Results of this study were presented in February 2006 (A.6.12.2 and published in Regul. Toxicol. Pharmacol. 2010 Feb;56(1):93-9).*

Of 242 total cases, 12 cases of major (temporary) severity were possibly related to DEET (seizure, other neurological, dermal, and other) and one case of major severity was probably related to DEET (non-neurological). Fifty-nine cases with seizures were reported with 90% of the seizure cases of major or moderate severity. People with underlying seizure disorder were not disproportionately represented (6.8%) in these 59 cases. The registry has limitations, for example being based on passively reported data, but at the same time relatively few reports of neurological adversity were found despite that several billions of applications of DEET are estimated under the timeframe of the register study."

According to the registry, seizures were more common in children than in adults as 42/59 subjects (71%) experiencing seizures were below 20 years age. The large majority of patients 49/60 (82%) showing other types of neurological symptoms were below 19 years age. Five cases (0.06%) were classified as major but it is noted that the case resulting in death was a deliberate suicide by ingestion of DEET (Osimitz, 2006).

The publication by Scoenig and Osimitz (2001) also reports observations of seizures in 7/11 clinical cases following dermal use of DEET (age of patients ranging from 3 to 8 years and one 28 year old). In six of these cases the outcome was recovery/full recovery, in one case the outcome was unknown and one case resulted in death. Additional symptoms such as headache, ataxia and

[1] DS: The "short-lived symptoms" include mild irritation to the skin or mucous membranes and "moderate symptoms" include eye irritation (treated at home), symptoms of ataxia and a possible seizure in one case after saturation of clothes with 17.9% DEET. Diminished sensation and hypertension occurred in one case one week after using a DEET product (Schoenig and Osimitz, 2001).

agitation was observed in one patient and rash and restlessness in another patient. In four patients (ages of 7.5, 8, 1.5 and 1.5 years respectively), signs of opisthotonos were observed in one patient, headaches, ataxia and disorientation in the second, acute encephalopathy in the third and ataxia, movement disorder, drooling, opisthotonos, opsoclonus and myoclonus in the fourth patient. Two of the patients died whereas two recovered from symptoms. Six of patients having seizures had a possible alternative diagnosis of idiopathic seizure and the last case had a possible alternative diagnosis of encephalitis, parainfectious encephalopathy. The patients having seizures had been dermally exposed to concentrations of 10 or 15% DEET with the exception of three patients for which the exposure concentration was unknown and two patients who also used products containing 100% DEET. Where known, the use pattern included daily applications during two weeks, nightly for 3 months and applications were described as both copious and brief. The two patients who died (patients without seizures) had used a 15% concentration on 10 occasions and they had frequently used a formulation with an unknown concentration. In both cases, an alternative diagnosis was possible. The remaining two patients had used products containing 10% (exposure via dermal application and ingestion) and 20% for 3 months. In both patients encephalitis, parainfectious encephalopathy (with addition of myoclonic encephalopathy in the second patient) were possible alternative diagnoses.

Additional information in the form of clinical case reports from French poisoning centres was provided by the French CA. Of cases reported in the data base, 50.6% were related to DEET and in 61% of these cases, clinical symptoms were described. In a few cases (i.e. 14 cases), neurological symptoms sometimes in association with cephalalgia (8 cases), drowsiness (2 cases), ataxia (2 cases), trembling (4 cases), muscle spasm/convulsions (1 case), dizzy spells (2 cases), and eye trouble (1 case) were noted. Other clinical signs noted in a few cases include breathing problems such as cough (following exposure to repellent aerosol spray formulation), dyspnoea, vomiting and nausea. Convulsions were observed in a 39 year old man (with no underlying seizure disorder) during the third day using a cream containing 50% DEET applied two times daily. Furthermore, trembling was reported in four children (of 2, 2, 3 and 5 years age) after using lotions containing concentrations of 7, 13, 15 or 50% DEET respectively. There is no information with respect to outcome or possible alternative diagnoses.

In conclusion, reports available describe neurological effects in human following use of products containing DEET. Symptoms seem to be more severe in children. However, in many of these cases, effects may be due to an underlying disease or a possible alternative diagnosis cannot be excluded. Moreover, the exposure duration varies and the exact exposure levels are unknown thus it is unclear if effects occur as a result of excessive doses which would be an apparent misuse of the product. Therefore, the significance of this information on the assessment of acute toxicity is limited.

4.2.3 Summary and discussion of acute toxicity

4.2.4 Comparison with criteria

The oral LD_{50} is 1892 mg/kg and DEET thus meets criteria for classification in category 4, i.e. oral LD_{50} >300 but ≤ 2000 mg/kg bodyweight.

The acute dermal LD_{50} values of DEET in rabbits and rats are greater than 2000 mg/kg and 5000 mg/kg, respectively. Consequently, criteria for the least stringent category are not fulfilled (dermal LD_{50} >1000 but ≤ 2000 mg/kg bodyweight).

The acute inhalation LC_{50} of an aerosol of DEET is greater than 2.02 mg/L. This value is below the upper limit for classification in the least stringent category (i.e. inhalation (dust/mist) LC50 >1 but

≤ 5 mg/l) thus, strictly, it is not possible to exclude that the substance would meet criteria for classification in category 4. However, as discussed in the current OECD TG 403 and in the draft report of the expert consultation meeting on acute inhalation toxicity it may be technically challenging to both achieve a concentration of 5 mg/L and particles of respirable size. Taking into account that all animals gained weight and no deaths occurred during the study, no classification is proposed with respect to acute toxicity via inhalation.

4.2.5 Conclusions on classification and labelling

Based on the data available, the acute oral toxicity of DEET meets criteria for classification and labelling in Regulation (EC) 1272/2008). Classification Acute Tox. 4; H302 and labelling GHS07, Wng, H302 is thus confirmed.

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.

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

Table 11: Summary table of relevant studies

Method	Results	Remarks	Reference
Oral, 8 weeks U.S. EPA Pesticide Assessments Guideline 83-1 and OECD Guideline for the Testing of Chemicals, Health Effects No. 452 Beagle dog, 2/sex/group Capsule administration (divided into 2 doses/day) of 0, 50, 100, 200, 400 mg/kg bw/day, seven days a week	No effects on body weight at 200 mg/kg bw/day At 400mg/kg: Difference in group mean body weight compared to pre-test: males: ↑11% (↑18% in controls) females: ↓2.6% (↑19% in controls), one female lost weight during the study. Group mean food consumption at 400 mg/kg bw: males: ↓7% compared to controls) females: ↓47% compared to controls) The females also had decreased food consumption. Clinical signs observed at 200 mg/kg bw/day and 400 mg/kg bw/day may be indicative of neurological effects (effects generally occurred within 1hr of dosing) but no histopathology were performed for nerve tissue. NOAEL is set based on ptyalism observed at a dose of ≥200 mg/kg bw/day and abnormal head movements observed at 400 mg/kg bw/day. Emesis, relaxed nictating membrane were observed at 400 mg/kg bw/day but occurred also at other doses including controls and symptoms did not clearly increase with increasing dose levels. No mortalities. LOAEL= 200 mg/kg bw/day NOAEL=100 mg/kg bw/day	The study was performed as a range-finding study to a chronic toxicity study in dogs but has been compared to the OECD 407 guideline for Repeated dose 28 day oral toxicity study. Some deviations were found. Parameters that were not investigated included no neurotoxicity battery tests, blood clotting time not measured, thymus and spleen not weighed, no histopathology for spinal cord, small and large intestines, trachea, accessory sex organs, urinary bladder. Reliability 1	A6.3.1(1)
Oral, 8 weeks Beagle dog 2/sex/group, Capsule administration (single dose/day) of 0, 75, 125, 175 and 225 mg/kg bw/day The study was terminated early (day 5) due to the clinical signs observed. The test report does not clearly state the reason for	Clinical signs: ataxia and ptosis (effects generally occurred within 1hr of dosing) LOAEL= 125 mg/kg bw/day NOAEL= 75 mg/kg bw/day Clinical signs day 1 of study: 0 mg/kg bw/day:	The study did not follow a guideline but was conducted in accordance with generally accepted scientific principles. The study investigated mortality, clinical	A6.3.1(2)

termination but it is noted that one dog convulsed at 125 mg/kg bw and at 225 mg/kg bw and were removed from dosing.	emesis (food like) 1/4 75 mg/kg bw/day: Soft stool 1/4 125 mg/kg bw/day: emesis (frothy slight to moderate) 3/4, ptyalism (moderate) 1/4, abnormal head movements 1/4 175 mg/kg bw/day: soft stool, ptyalism (slight or moderate) 3/4, emesis (frothy) 1/2 225 mg/kg bw/day: abnormal head movements 3/4, emesis (frothy) 3/4, emesis (food like) 3/4, ptyalism, 2/4, ataxia (slight or marked) 2/4, prostration 1/4, abnormal gait 1/4, convulsions (marked) 1/4, pupils dilated 1/4 No mortalities. All animals gained weight during the 5 days on study apart from the 225 mg/kg bw/day group that lost weight compared to pretest body weight (1.4%). One female at 225 mg/kg bw convulsed 15 min post dose at day 1, had four episodes of a minutes duration throughout a 45 min period (the dog was removed from dosing).	investigations, body weight and food consumption. Reliability 2	
Oral (capsule), 1 year U.S. EPA Pesticide Assessments Guideline 83-1 (b) and OECD Guideline for the Testing of Chemicals, Health Effects No. 452 Dog, 4/sex/dose 0, 30, 100 and 400 mg/kg bw/day (two divided doses 7 days/week)	Decreased body weight (both sexes). Occasional ataxia, tremors, abnormal head movements and or convulsions in one dog (400 mg/kg bw without correlation in neuropathological examinations of peripheral or central nervous tissue. LOAEL = 400 mg/kg bw/day NOAEL = 100 mg/kg bw/day	Reliability 1	A6.5(1)
Oral (gavage) U.S. EPA TSCA Guidelines 798.6050, 798.6200, 798.6400 and 798.6500 Rat,Charles River Crl:CD®VAF/Plus® males and females 10/sex/group 0, 50, 200 and 500 mg/kg as single dose	Increased response time to heat stimulus and decreased rearing activity at one hour LOAEL = 500 mg/kg bw/day NOAEL = 200 mg/kg bw/day	Reliability 2 Limitations with respect to study design prevent a conclusion.	A6.9(1)
Based on the U.S. EPA TSCA Guidelines 798.6050, 798.6200,	Transient increase in locomotor activity	Reliability 3 Limited information	A6.9(2)

798.6400 and 798.6500 Rat, Crl: COBS®CD® males and females 20/sex/group, additional 10 animals/sex from the control group were also chosen for use as a 'sham' control in the passive avoidance test.	LOAEL (neurotox): 5000 ppm LOAEL (neuropath): > 5000 ppm, the highest dose tested NOAEL(neurotox): 2000 ppm NOAEL (neuropath): ≥ 5000 ppm For F1 males this corresponded to approximately: 500 ppm = 21.6 to 67.5 mg/kg/day 2000 ppm = 92.0 to 278 mg/kg/day 5000 ppm = 218 to 683 mg/kg/day, taken from the 2-generation study, dietary exposure	on historical control data, the type of equipment used in some of the measurements and the age of animals thus complicating an assessment of the relevance and sensitivity of the test used in the study.	A6.8.2

In a pre-test in dogs, oral administration of DEET for 8 weeks resulted in a NOAEL of 100 mg/kg/day based on clinical signs (abnormal head movements and ptyalism). The substance was administered in capsules two times per day at doses of, 50, 100, 200 and 400 mg/kg bw/day. The clinical observations made were considered to be related to treatment and were generally observed within 60 minutes of dosing. **Abnormal head movements were observed after a dose of 400 mg/kg/day and ptyalism was observed at a dose of 400 mg/kg/day and occasionally also at 200 mg/kg/day.** According to data in section 4.1.1, dogs (and rats) reach peak plasma levels approximately one hour after oral administration of doses comparable with NOAELs (200 and 75 mg/kg, respectively). The severity of the abnormal head movements increased with dose (however the use of few animals prevented an adequate statistical analysis). Also ptyalism (graded slight to moderate-marked) occurred in all treated animals with a dose-related increase of severity. Since the clinical signs noted in this study were reported on a weekly basis, it is not entirely clear if effects occurred already after the first dose.

Clinical signs on day 1 were also observed in another 8 week study in dogs following oral doses of 0, 75, 125, 175 and 225 mg/kg bw/day administered once daily in capsules. This study, which was performed as a dose finder for a chronic toxicity study, was terminated after five days exposure. The clinical signs observed in this study were the reason for administering doses on two separate occasions in the 8 week study described above. Clinical signs in this study occurred more frequently and with increasing severity at higher dose levels. Emesis, ptyalism, abnormal biting and scratching, abnormal head movements were observed at or above doses of 125 mg/kg bw/day. Convulsions occurred shortly after dosing in two dogs (in one female after a dose of 225 mg/kg bw/day on the first day of treatment and in one male after a dose of 125 mg/kg bw/day on the third day of treatment). **Ptyalism and frothy emesis were observed on the first day of treatment in all dose groups administered doses above 125 mg/kg bw/day. Other symptoms occurring on the first day of treatment included ataxia in 2/4 animals, abnormal head movements in 3/4 animals, and prostration, convulsions, abnormal gait and dilated pupils in one animal administered 225 mg/kg bw.** Based on these results, the LOAEL was set at 125 mg/kg bw/day and the NOAEL was 75 mg/kg bw/day.

In a 52-week study in dogs, the NOAEL was set at 100 mg/kg bw/day (dose divided into two administrations per day) based on body weight changes and clinical neurological signs (ptyalism and emesis) at 400 mg/kg bw/day. No mortality was observed and there were no histopathological effects on the brain, sciatic nerve or spinal cord. However, **in one of eight dogs administered 400 mg/kg/day, ataxia, abnormal head movements and convulsions occurred within 30 min of dosing on nine occasions over a five month period with onset during week 29**. Since the clinical

signs were reported on a weekly basis, it is not possible to conclude if any of the clinical signs appeared already after a single dose. Considering that there were no histopathological findings in neuronal tissues and since the effects observed resemble those observed in the acute toxicity studies, results are considered to support that neurological signs observed are acute effects. Moreover, there were no clear indications that effects increased over time.

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

Dogs orally administered DEET in diet for 8 weeks showed clinical signs of neurotoxicity shortly after receiving a single dose (A6.3.1(2)). Similar clinical signs of neurotoxicity were observed in two other studies in dogs but it could not be clarified whether or not effects occurred after a single dose or following repeated exposure to the substance (A6.3.1 (1) and A6.5(1)). The mode of action behind the neurotoxicity of DEET has been investigated in a published study performed in insects and rat neuronal cortex cells (Swale DR, Sun B, Tong F, Bloomquist JR (2014)). The results of a series of *in vitro* assays were considered to indicate that DEET induces neuroexcitation via octopamine receptors and it is capable of blocking Na^+ and K^+ channels. The latter was considered to contribute to numbness in lips or mouth of humans sensed following incautious application of DEET. Since the results indicated a low sensitivity of acetylcholinesterase to DEET, this was not considered a likely mode of action for the neurotoxicity observed in insects. The IC_{50} values concluded in patch clamp studies with rat cortical neurons were 0.7 µM for sodium channels and approximately 0.1 mM for potassium channels. However, it is difficult to compare these concentrations to expected in vivo exposure levels in humans and it is thus unclear how to incorporate this information in the hazard assessment in a meaningful way.

4.3.2 Comparison with criteria

According to section 8.2.1.7 (of CLP) *"Evidence from appropriate studies in experimental animals can furnish much more detail, in the form of clinical observations, and macroscopic and microscopic pathological examination, and this can often reveal hazards that may not be life-threatening but could indicate functional impairment. Consequently all available evidence, and relevance to human health, must be taken into consideration in the classification process, including but not limited to the following effects in humans and/or animals:*

(a) morbidity resulting from single exposure;

(b) significant functional changes, more than transient in nature, in the respiratory system, central or peripheral nervous systems, other organs or other organ systems, including signs of central nervous system depression and effects on special senses (such as sight, hearing and sense of smell);" etc.

In dogs, clinical signs of possible neurotoxicity, i.e. emesis and ptyalism, were observed already during the first day after administration of doses ≥125 mg/kg bw. In addition, ataxia and abnormal head movements occurred during day 1 following administration of 225 mg/kg. In one of the dogs also prostration, convulsions, abnormal gait and dilated pupils were observed during the first day of treatment. Similar signs were observed also in two other dog studies but at higher dose levels and, in both cases, it was not possible to clarify if effects occurred after a single administration. Based on these findings, classification STOT SE category 1 could be considered since neurological effects occurred already after a first dose of 225 mg/kg which is below the guidance value (≤ 300 mg/kg).

On the other hand, if the neurotoxic effects of DEET in insects would occur also in mammals, similar effects could be anticipated also for the other test species. There were some effects observed in two neurotoxicity studies in rats however the quality and thus the reliability of these studies was

considered low. Moreover, effects occurred at a dose level that was less than 5 times below the LD_{50} value. According to the CLP guidance (section 3.8.2.1.2), *"Care must be taken not to classify for STOT-SE for effects which are not yet lethal at a certain dose, but would lead to lethality within the numeric classification criteria. In other words, if lethality would occur at relevant doses then a classification for acute toxicity would take precedence and STOT-SE would not be assigned"*. The effects occurred at a dose close to the lower limit for classification in acute toxicity category 4 (i.e. 300 mg/kg bw >ATE ≤ 2000 mg/kg bw/d. Moreover, there were no clinical signs of neurotoxicity noted in the chronic studies in rats and mice or in the two-generation study. Clinical signs of neurotoxicity observed in the second developmental study were restricted to a dam who died.

Overall, the indications of neurotoxicity seem more or less restricted to dogs. The CLP guidance is not entirely clear if these types of effects represent unspecific toxicity rather than specific target organ toxicity and thus if the effects should be considered covered by the classification for acute toxicity. In the absence of information on the acute toxicity in dogs it is not known if the clinical signs of neurotoxicity observed occur within a range considered to be covered by the classification for acute toxicity (tentatively 5 times the LD_{50} value). No mortalities occurred in the dog study but considering that the study was terminated after 5 days based on the clinical signs observed, it seems reasonable to assume that the lethal dose is near 225 mg/kg bw.
Although there are reports describing neurological effects in humans following use of products containing DEET, it is not possible to elucidate if effects result from an underlying disease or if there may be an alternative diagnosis. Moreover, the exposure duration is rarely known and since the exact exposure levels are unclear it cannot be clarified if effects occur as a result of excessive doses and thus an apparent misuse of the product.

4.3.3 Conclusions on classification and labelling

Clinical signs of neurotoxicity were observed in dogs treated with DEET at doses below the guidance value for classification and labelling STOT SE in category 1. However, it is not possible to assess from the data available if these effects occur near doses that are lethal in dogs. Considering also that there were no clear neurotoxic effects observed in the other mammalian species tested, the overall conclusion made for this borderline case is that effects do not form conclusive evidence that criteria for classification are fulfilled. Therefore, no classification is proposed for STOT SE.

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.

Additional key elements

Additional human case reports from the National Institute of Environmental Health Sciences (NIEHS) report (1999) are presented in the table below.

Table: Overview of human case reports of neurotoxic symptoms

Route and exposure	**Sex and age of individuals**	**Symptoms**	**Reference**
Dermal, sprayed bedding and cloths with 15% DEET (180 ml), 2 w	F, 3.5 y	Encephalopathy, which included symptoms of tremors, crying spells, confusion, slurred speech, stiffening of extremities, and staggering gait.	Gryboski *et al.*, 1961*
Dermal, single application of two repellants, 1: 95%, 1: ?%	M, 5 y	Seizures. A urine sample collected 9-30 h after exposure revealed a DEET concentration of 0.003 µg/mL	Lipscomb *et al.*, 1992*
Dermal, < 3 applications	5M, 3-7 y 29 y	Seizures 8 to 48 h after application. Physical exams and laboratory tests were normal. One patient developed urticaria before his seizure.	Oransky *et al.*, 1989*
Oral, ~25 ml, 47.5%	F, 1 y	Unresponsiveness, seizure, and hypertonia. Patient showed a positive response to treatment with activated charcoal and a saline cathartic and was normal 20 h later.	Tenenbein, 1987*
Oral, 50 ml, 95%	F, 14 y	Unconsciousness, hypertonia, dilated pupils, and tremors.	Tenenbein, 1987*
	F, 16 y	Comatose state: no corneal, blink, gag, or deep tendon reflexes. Patient showed a positive response to treatment and was normal 30 h later.	
	F, 33 y	Unconsciousness, irregular breathing, comatose, pulseless, seizure during first 24 h after exposure, bowel infarction, and death on second day.	
	M, 26 y	Death. Urine level was 0.52 mmol/L. Drug screening was positive for cannabinoids.	
Oral, 15-25 ml, 95%	F, 19 y	Cardiac abnormalities, including right and left atrial enlargement 2 h post-ingestion. Patient returned to normal 24 h later.	Fraser *et al.,* 1995*
Dermal, spraying of insect repellent	F, 16 y	Light-headedness, nausea, vomiting, diarrhoea, tremors, chills, and hypotension. Patient returned to normal 24 h later.	Clem *et al.*, 1993*
Dermal, daily application for 3 w	M, 30 y	Pigmented skin, macular lesions, manic psychosis, including aggressiveness, hyperactivity, rapid speech, and grandiose delusions.	Snyder *et al.*, 1986*
Dermal, 15%, spraying on at least	F, 6 y	Reye-like syndrome that included lethargy, mood changes, nightmares,	Heick *et al.*, 1980*

10 locations on body		vomiting, colicky abdominal pain, headaches, ataxia, disorientation, convulsions, coma, and death 8 d after exposure.	

*From Tice and Brevard, 1999. DEET: Review of toxicological literature. National Institute of Environmental Health Sciences

Two more recent case reports have been found in addition to the previous cases (Wiles *et al.*, 2014; Hampers *et al.*, 1999). One concerns a 37 year old male who experienced seizure and cardiac arrest following ingestion of 6 ounces of a 40% DEET-containing solution (equal to 68 g , or 748 mg/kg bw). The second case was a 27 year old male who was presented with auditory hallucinations, confusion, disorientation, and agitation after spraying 25% DEET on his limbs and neck. In both cases, DEET was found in serum samples of the patients.

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, 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**.

4.4 Irritation

Hazard class not assessed in this dossier

4.4.1 Skin irritation

Hazard class not assessed in this dossier

4.4.2 Eye irritation

Hazard class not assessed in this dossier

4.4.3 Respiratory tract irritation

Hazard class not assessed in this dossier

4.5 Corrosivity

Hazard class not assessed in this dossier

4.6 Sensitisation

Hazard class not assessed in this dossier

4.7 Repeated dose toxicity

Hazard class not assessed in this dossier

4.8 Germ cell mutagenicity (Mutagenicity)

Table 18: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
U.S. EPA Pesticide Assessments Guideline, 84-2 In vitro gene mutation study in bacteria TA 1535, TA 1537, TA 98, TA 100, TA1538 Main test 1 +/- S9 mix, all strains 278, 555, 833, 2778, 5555, 8333 mg/plate Main test 2 (repeated due to excessive toxicity in 1st test) and confirmatory assay S9 mix all strains: 28, 83/84, 278, 833, 2778, 8333 µg/plate +S9 mix all strains: 2.8, 8.3, 28, 83/84, 278, 833, 2778, 8333 µg/plate Exposure time +/- S9 mix: 48h	Negative all strains	Reliability 1	A6.6.1
U.S. EPA Pesticide Assessments Guideline, 84-2 In vitro cytogenicity study in mammalian cells Chinese hamster ovary (CHO) cells Range finding: 0.0005 to 5.0 µl/mL Main tests: - S9 0.063, 0.125, 0.25, 0.50 and 1.00 µl/mL +S9 0.032, 0.063, 0.125, 0.25, and 0.50 µl/mL Exposure time: -S9 16h and + S9 2h	Negative Cytotoxicity by microscopic examination of the cell monolayer and mitotic index (MI) ≥1 µl/ml	Reliability 1	A6.6.2
OECD Guideline for the Testing of Chemicals No. 476 In vitro gene mutation assay in mammalian cells CHO/HPGRT forward mutation assay (CHO- K1 cells) Range finding, main and confirmatory tests: 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1.00, 5.00 µl/mL Exposure time: -S9 16.5h, +S9 4.75h	Negative Cytotoxicity by parallel cloning efficiency (PCE) 5.0 µl/mL (+S) Cytotoxicity by plating efficiency 1.00 and 5.0 µl/mL (-S9)	The purity of the test substance was not reported Reliability 2	A6.6.3(2)

Method	Results	Remarks	Reference
EPA Pesticide Assessments Guideline, 84-2 Unscheduled DNA synthesis assay (UDS) Rat primary hepatocytes Range finding: 10,3, 1, 0.30, 0.10, 0.03, 0.01, 0.003, 0.001 and 0.0003 µl/mL Main studies: 0.30, 0.20, 0.10, 0.03, 0.01, 0.003 and 0.001 µL/mL Exposure time: 18-20 h	Negative Cytotoxicity by LDH assay: Range: 10 (85%), 3 (95%), 1.0 (103%), and 0.3 (64%)µL/mL Main, parallel cytotoxicity test: 0.3 (66%), 0.2 (14%) and 0.1 (13%) µL/mL Repeat parallel cytotoxicity test: 0.3 (35%) and 0.2 (21%) µL/mL	Reliability 1	A6.6.3(1)

4.8.1 Non-human information

4.8.1.1 In vitro data

The *in vitro* genotoxicity of DEET was investigated 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). The results of these studies did not indicate a genotoxic potential of the substance. However, possible genotoxic effects have been described in a published study using alkaline microgel electrophoresis assay "comet assay" (Tisch, Schmezer et al., 2002[2]). In this study, primary nasal mucosa cells from biopsy samples were exposed during one hour to concentrations of 0.5-1.0 mM DEET or two other pesticides, i.e. permethrin and diazinon. A concentration-dependent genotoxic dose-response was observed for all three pesticides in the absence of cytotoxicity.

4.8.1.2 In vivo data

No *in vivo* tests were submitted for the review under 98/8/EC and this was considered acceptable since all *in vitro* tests were negative. According to a risk characterisation document prepared by The California Environmental Protection Agency (2000)[3], the results of a dominant lethal assay in Swiss mice showed a higher percentage of dams with less implantations if males were exposed to DEET. However, the study was claimed to have several deficiencies (including too few pregnant dams/group and lack of individual data) and the result was thus considered equivocal.

4.8.2 Human information

No data

4.8.3 Other relevant information

No data

---

[2] Tisch, M., P. Schmezer, M. Faulde, A. Groh and H. Maier (2002). "Genotoxicity studies on permethrin, DEET and diazinon in primary human nasal mucosal cells." Eur Arch Otorhinolaryngol 259(3): 150-153.

[3] http://www.cdpr.ca.gov/docs/risk/rcd/deet.pdf

4.8.4 Summary and discussion of mutagenicity

There were no indications of mutagenicity in the four *in vitro* tests that were performed in accordance with recognized guidelines. However, a genotoxic response was observed in a published in vitro Comet Assay when primary nasal mucosa cells from biopsy samples were exposed to DEET.

4.8.5 Comparison with criteria

Criteria for classification in the least stringent category for germ cell mutagenicity reads:

"Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans.

The classification in Category 2 is based on:

– Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:

– Somatic cell mutagenicity tests in vivo, in mammals; or

– Other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

The results from the series of in vitro studies performed in accordance with recognized guidelines were negative. The genotoxic response observed in primary nasal mucosa cells in a published *in vitro* Comet Assay and the equivocal results found in a dominant lethal test in Swiss mice indicate that further genotoxicity testing may be needed. However, the existing data is not considered to provide convincing evidence that criteria for classification in category 2 are fulfilled.

4.8.6 Conclusions on classification and labelling

The data available is not considered to meet criteria for classification with respect to germ cell mutagenicity.

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.**

4.9 Carcinogenicity

Table 19: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
U.S. EPA Pesticide Assessments Guideline 83-5 OECD Guideline for the Testing of Chemicals, Health Effects No. 453 2-year combined toxicity & carcinogenicity, Dietary exposure, Charles River CD rats, males and females, 60/sex/group Males: 0 (Control 1 & 2), 10, 30 and 100 mg/kg/day Females: 0 (Control 1 & 2), 30, 100 and 400 mg/kg/day	Not considered carcinogenic NOAEL: 100 mg/kg/day (females) LOAEL: > 100 mg/kg/day (males), the highest dose tested in males, 400 mg/kg/day (females), based on decreased body weights and food consumption	In this study the survival was less than 50%. No satellite group with scheduled sacrifice at 12 months, was used.	A6.5(2) A6.7(1)
U.S. EPA Pesticide Assessments Guideline 83-2 18 month carcinogenicity study, dietary exposure, CD-1 mice, males and females 60/sex/group 0, 250, 500 and 1000 mg/kg/day	Not considered carcinogenic NOAEL≥1000 mg/kg LOAEL>1000 mg/kg Small changes in body weight increased liver weights without histopathologic correlation.		A6.7(2)

4.9.1 Non-human information

4.9.1.1 Carcinogenicity: oral

In a chronic toxicity/carcinogenicity study performed in rat, reduced body weights and food consumption were observed in female rats administered the highest dose (400 mg/kg/day). At week 104, the body weight was reduced by -17.1% and -19.4% compared to control groups 1 and 2. The food consumption was decreased by -7.5% and –8.8% compared to control group 1 and 2. There were no adverse effects observed in male rats at 100 mg/kg/day which was the highest dose tested. There were no treatment related clinical signs or differences in survival between the control and treatment groups. There were no treatment related urological or haematological changes. Cholesterol values were slightly increased in females administered 400 mg/kg/day, a statistically significant effect considered to be related to treatment. The high dose in males was chosen based on findings in previous studies in rats showing a susceptibility of males to renal toxicity. This was considered to be due to α2u-globulin accumulation. In the chronic toxicity/carcinogenicity study, a slight numerical increase in the incidence of renal cell adenomas was observed in males administered 10 mg/kg/day (3/60) compared to the control groups (1/60 and 0/60). However, renal cell adenomas were not observed in males administered 30 or 100 mg/kg/day or in any of the treated females. Considering the lack of a dose-response and that renal cell adenoma was present also in one male in the control group, the slight increase observed is not considered to be related to treatment.

In a carcinogenicity study performed in mice, decreased body weights were observed in both males and females administered 1000 mg/kg/day (-7.5 and -5.1% compared to male control groups C1 and C2 and by -5.7% compared to both female control groups C1 and C2). The food consumption was reduced in the 1000 mg/kg/day group and the change was statistically significant. A statistically significant difference in mean body weight was noted after week 16 also in males administered 250 mg/kg/day and after week 2 in both sexes administered ≥ 500 mg/kg/day that remained until study termination. The differences were slight (1-3g) and average food consumption was not affected. There were no treatment-related changes in haematological parameters in the 1000 mg/kg/day group and no treatment related clinical signs of toxicity. The absolute and relative (to body and/or brain weight) liver weights were increased in a dose-related manner in both males and females administered 500 and 1000 mg/kg/day. However, the increases were considered to represent adaptive rather than toxic changes since there were no histopathological findings in liver tissues noted. Based on the results, the NOAEL in this study was considered to be ≥ 1000 mg/kg bw/day for carcinogenicity and other long term effects.

The NOELs were 100 mg/kg/day for rats and 500 mg/kg/day for mice. Treatment with DEET did not increase the frequency of tumours and was thus not considered carcinogenic in any of the studies.

4.9.1.2 Carcinogenicity: inhalation

No data

4.9.1.3 Carcinogenicity: dermal

No data

4.9.2 Human information

4.9.3 Other relevant information

According to a public health statement from the Agency for toxic Substances and Disease Registry[4], a study investigating Swedish workers showed an increased risk of developing testicular cancer in those using insect repellents for 115 days or longer. However, due to deficiencies in the study, the results were not considered conclusive.

4.9.4 Summary and discussion of carcinogenicity

Treatment with DEET did not increase the frequency of tumours in rats and mice. No other robust information raising a concern for carcinogenicity has been found.

4.9.5 Comparison with criteria

The results of the chronic/carcinogenicity studies in rats and mice performed in accordance with recognised guidelines are not considered to meet criteria for carcinogenicity.

4.9.6 Conclusions on classification and labelling

DEET does not fulfil the classification criteria for carcinogenicity.

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

[4] http://www.atsdr.cdc.gov/consultations/deet/health-effects.html

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.

4.10 Toxicity for reproduction

Table 20: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Oral (in food), U.S. EPA guideline 83-4, corresponding to OECD 416Crl:COBS®CD® rats, male and female, 28/sex/group, 0, 500, 2000 and 5000 ppm, Before mating F0 >80 days, F1 >93 days, Duration of exposure in general F0, F1, F2 135/137 days for males and females respectively	No effects on reproduction Mottled kidneys with hyaline droplets, inflammation, regeneration, granular casts at all doses F1 males. Decreased body weight at 5000 ppm in males and females. Decreased body weights during lactation F1/F2 offspring at 5000 ppm. NOAEL (ppm) parental, F0: 2000 ppm (males)/ F1: ≤500 ppm (males), F0/F1: 2000 ppm (females), Offspring F1/F2: 2000 ppm	The study was performed prior to current guideline Sperm morphology and motility were not investigated, oestrus cycle length, vaginal smears were not investigated, vaginal opening, preputial separation and anogenital distance were not investigated, individual offspring body weights were only recorded at day 21 of lactation, brain, spleen and thymus weights were not recorded for the offspring, the only organ weights recorded for the parents were the testis weights of males that did not produce any offspring Reliability 1	A6.8.2
OECD 414, Oral (gavage), Sprague- Dawley (CD®) rats [Crl: CD® (SD)BR], 25 timed pregnant females/ dose group , Gestation day 6-15 0, 125, 250 and 750 mg/kg bw	NOAEL, maternal, 250 mg/kg bw. NOAEL, embryotox/teratogenicity, 250 mg/kg bw Decreased maternal body weights, clinical signs, and mortality (2 high dose dams) The substance was not teratogenic, embryotoxicity expressed as decreased foetal bw	Reliability 1	A6.8.1(1)

Method	Results	Remarks	Reference
OECD 414, Oral (gavage),New Zealand White rabbit, 13-16 timed pregnant females/dose group, Gestation day 6-18 0, 30, 100 and 325 mg/kg bw/day	NOAEL, maternal, 100 mg/kg bw. NOAEL, embryotox/teratogenicity, > 325 mg/kg bw Decreased maternal body weights, clinical signs of CNS effects in one female that died The substance was not teratogenic or embryotoxic at the highest dose tested	Reliability 1	A6.8.1(2)

4.10.1 Effects on fertility

4.10.1.1 Non-human information

There were no effects on reproduction in a two-generation study performed in rats using doses up to 5000 ppm (218 to 713 mg/kg bw/day). Parental F1 males were most sensitive with a NOAEL set at ≤500 ppm based on kidney effects. According to the study author, the kidney effect (only observed in males) was attributed to a chemically induced condition that is unique to male rats, i.e. alpha2µglobulin nephropathy. The body weights of parental females were slightly decreased compared to controls, in the highest dose group, this decrease was occasionally more than 10% compared to control females. The NOAEL for parental females is therefore considered to be 2000 ppm.

The body weights of pups were statistically significantly decreased (> 10%) compared to controls. The decrease was observed during the latter part of the lactation period in both F1 and F2 pups. Based on this finding, the NOAEL is set at 2000 ppm for the pups. The study was performed in 1989 and deviates slightly from the current OECD 416 guideline. Sperm morphology and motility were not investigated but spermatogenesis was investigated in animals that failed to produce offspring.

The results from the repeated dose toxicity studies do not raise a concern for testis and epididymides being targets for DEET toxicity. The relative testis weights were decreased at a dose of 400 mg/kg bw/day in a 56 week study in dogs but there were no histopathological evidence of damage. No testicular effects were reported at this dose level in the 2 year study in dogs. An increased testicular weight was observed in rats at doses above 1000 mg/kg bw in the 90 day study but there were no histopathological findings. No testis effects were observed in the chronic (2 year) study when the substance was tested up to a dose of 100 mg/kg bw/day, i.e. a non-toxic dose selected for high dose based on previous studies indicating that higher doses would not be tolerated by males due to kidney effects. Testis effects (testicular tubular degeneration) were observed at a higher frequency at doses of 611 and 3136 mg/kg bw/d in a 90 day study in hamsters. However, the relevance of this result may be questioned considering that there were no clear evidence of similar effects or of reproductive effects in the other species investigated. Overall, there are no observations or effects on reproductive parameters in the two-generation study indicating that sperm parameters need to be further evaluated.

The normality and the length of the oestrus cycle was not investigated by vaginal smears, neither were vaginal opening, preputial separation and anogenital distance investigated. There were no indications of ovary effects in the results from the repeated dose studies. Therefore, despite

deviations from the current OECD 416 guideline, the study was considered suitable for risk assessment and DEET is not considered to be a reproductive toxicant on the basis of these results.

4.10.1.2 Human information

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

The teratogenicity of DEET was investigated in two species, the rat and the rabbit. The studies were performed according to the OECD 414 guideline and both were preceded by dose finding studies. Since the studies were performed prior to the latest revision of the OECD guideline in 2001 there are some deviations from the current guideline; dams were treated only during the period of organogenesis and not until the scheduled sacrifice. This may have an impact on the assessment of potential effects during later stages of embryonal development however considering that the two-generation study in rats gave no further indications of embryotoxic or teratogenic effects at comparable dose levels, these studies were considered acceptable for the risk assessment under the BPD.

Significant treatment related signs of clinical toxicity were observed at 750 mg/kg/day in the rat teratogenicity study and two dams were sacrificed moribund on GD 7. There were no treatment related clinical signs observed in dams treated with 250 mg/kg/day or less. Maternal body weights were significantly reduced on gestation day 18 in animals administered 750 mg/kg/day (-5.2 % less than controls). Maternal body weight gains were significantly reduced at 750 mg/kg/day for gd 6-9 (a loss of -0.47g) and gd 6-15 (34.8 % less body weight gain than controls). The food consumption in dams administered 750 mg/kg/day were statistically significantly reduced throughout the gestation period i.e. from gd 6 to 15 (12% less than controls gd 6-15). The food consumption was significantly reduced during gd 6-9 (11% less than controls) also in animals administered 250 mg/kg/day, maternal but relatively unaffected for this dose for the rest of the period. Statistically significant increases in liver weights, both absolute and relative (as percent of final corrected body weight), were noted in rats treated with 750 mg/kg/day of DEET. Percent preimplantation loss was slightly, but not significantly, increased at 750 mg/kg/day. Gestational parameters, including the number of ovarian corpora lutea and implantations per litter were unaffected. The fetal body weights/litter was statistically significantly reduced at 750 mg/kg/day. A statistically significant increase in fetal sex ratio (% male fetuses) was noted at 250 mg/kg/day; however since this was not noted in the 750 mg/kg/day group, it was not considered to be related to treatment. There were no significant treatment-related fetal external, visceral or skeletal malformations or variations.

In the teratogenicity study in rabbits, no treatment-related clinical signs were observed except for clinical signs and moribundity in 1/16 females administered 325 mg/kg/day. None of the females aborted or delivered early. Statistically significant decreases in maternal body weight gains were observed at 325 mg/kg/day from gd 6 to 9 (a body weight loss of -44.64 g in these animals) which were consistent with a reduced and statistically significant food consumption in this group throughout most of the treatment period (gd 6-18, 29.9% less than controls). There were no effects on gestational parameters at any dose level. There were no treatment-related external, visceral or skeletal malformations or variations observed in any fetuses in this study. Fetal body weights were unaffected by treatment. Based on these results, there was no evidence of teratogenicity at any dose.

4.10.2.2 Human information

4.10.3 Other relevant information

According to information in a report prepared by WHO INCHEM, gonadotoxic and embryotoxic effects of DEET have been observed in older studies in the rat (Gleiberman et al (1975, 1976)). The publications are only available in Russian but according to the first study focusing on embryotoxicity (Gleiberman et al (1975)), *"With application to the skin of an albino female-rat over the whole period of its pregnancy of the repellent diethyltolueneamide (DETA) in doses of 100 and 1000 mg/kg the overall embryonal fatality was found to go up, the size and weight of the rattlings to be down; there was recorded a lagging development of the newborns and a high postnatal death rate. The preparation could easily overcome the placental barrier, it was demonstrable in the placenta, fetuses, as well as in the bodies of newborn rattlings, even 3 months after their birth."*

The report also states that no embryotoxic effects were demonstrated in animals by Robins and Cherniak (1986).

4.10.4 Summary and discussion of reproductive toxicity

In summary, there were no teratogenic effects observed in the studies up to maternally toxic doses, embryotoxicity was only expressed as decreased foetal body weights (rats). According to secondary information available in a report prepared by WHO INCHEM, embryotoxic effects have been observed following dermal application of 100 or 1000 mg/kg bw/d. Considering that there is no information with respect to maternal toxicity and study quality and considering that oral doses up to 750 mg/kg bw/d did not cause developmental toxicity in rats when investigated in a robust guideline study, this information is not considered to overrule the conclusion made based on the results from studies in rats and rabbits (i.e. not teratogenic).

4.10.5 Comparison with criteria

The results of the studies on reproductive toxicity in rats and rabbits, which were performed in accordance with recognised guidelines, do not indicate that the substance causes reproductive or developmental toxicity. Consequently, criteria for classification are not considered fulfilled.

4.10.6 Conclusions on classification and labelling

DEET does not fulfil the criteria for classification and labelling for reproductive toxicity.

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.

Additional key elements

The table below summarises the results of the 90 day range finding study in hamsters (Study report, 1989), which was provided by the DS after the public consultation. Details of the individual data of the control group and the group with the dose of 611 mg/kg

bw/d, respectively, are given in the remaining tables in this section.

Table : Summary of the results of a 90 day range-finding study in hamsters

	0 mg/kg bw/d	**61 mg/kg bw/d**	**304 mg/kg bw/d**	**611 mg/kg bw/d**	**3100 mg/kg bw/d**	**Comments**
Mortalities	0/15	0/15	1/15	0/15	4/15	
Body weight w 13	174	171	156 (-10.3%)	152 (-12.3%)*	143 (-17.8%)**	Occasional decreases and stat. sign. from time to time at 611 mg/kg bw/d.
T. Body weight	174	172	156 (-10.3%)	157 (-9.7%)	145 (-17%)**	
Food Consumption	9.2	9.0 (-2.2%)	8.6 (-6.5%)	8.5 (-7.6%)	8.0 (-13%)	Occasionally stat. sign. lower during study but not on w13
Test. w/bw (%)	1.21 (± 0.58)	1.27 (± 0.60)	1.45 (± 0.71)	0.80 (± 0.53)	0.83 (± 0.67)	
Abs Test w	2.08 (± 0.97)	2.12 (± 0.91)	2.22 (± 0.98)	1.23* (± 0.78)	1.17* (± 0.86)	
Testes deg. tubular	4/15	5/15	5/14	12/15	11/15	
trace	1/15	2/15	1/14	3/15	0/15	
mild	2/15	3/15	2/14	5/15	9/15	
moderate	1/15	0/15	2/14	4/15	2/15	

*p<0.05; **p<0.01

Table: Individual data of the control group of the hamster study

Bw	**Bw gain**	**Food cons***	**Testis weight (Tw)**	**Tw/bw**
163	46	8.43	1.6	0.99
148	27	8.76	3.31	2.25
190	68	10.00	0.69	0.36
170	57	8.90	3.13	1.83
175	55	9.48	2.98	1.7
184	58	9.14	2.16	1.19
165	52	8.62	0.78	0.48
191	60	9.86	1.49	0.79
170	41	9.24	2.68	1.55
144	34	8.00	0.9	0.61
167	60	9.71	2.23	1.33
175	63	8.33	2.89	1.67
195	74	9.33	2.78	1.4
186	56	10.29	2.89	1.58

180	69	9.14	0.72	0.39

*Average of weeks 3, 7, and 13

Table: Individual data of the 611 mg/kg bw/d group of the hamster study

Bw	Bw gain	Food consumption*	Testis weight (Tw)	Tw/bw
166	47	8.86	0.86	0.51
164	43	8.95	2.21	1.33
132	15	7.86	2.48	1.86
145	37	8.76	2.41	1.66
145	22	7.29	0.41	0.28
153	41	8.67	0.91	0.59
157	46	8.43	0.83	0.53
172	50	10.05	0.55	0.32
155	42	9.52	1.2	0.76
175	49	9.76	2.66	1.55
156	26	8.67	0.89	0.56
135	26	7.86	0.61	0.31
173	44	9.19	0.73	0.41
136	23	8.10	0.77	0.56
118	15	7.62	0.89	0.74

*Average of weeks 3, 7, and 13

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; Brockon and Cwood, 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.

4.11 Other effects

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

The summaries and evaluations of the degradation studies with DEET is taken from the Final CAR March 2010, Swedish Chemical Agency and Weeks et. al (2011).

Table 21.Summary of relevant information on degradation (Final Car 2010, Swedish Chemical Agency 2010 and Weeks et al. 2011)

Method	Results	Remarks	Reference
Hydrolysis, OECD 111	Stable at pH 4, 7, 9 (< 10% decrease during 5 days at 50°C) DT50>1 year	experimentally determined Supportive study	A.7.1.1.1.1.
Ready biodegradability, OECD 301 B	Rapidly biodegradable (83.8% was degraded within the 10d window in the test at 28 days)	experimentally determined **Key study**	A.7.1.1.2.1
Inherent biodegradability, OECD 301 D(Closed bottle)	48% degradation at day 28 based on COD	Rapid degradation initial first 7 d and a halting degradation to 28 d. No explanation as to why the degradation halted	Kumar (2003) in Weeks et. al 2011
Japan MITI method, OECD 301 C	0% at day 28	Reliable with restriction, accepted data compilation	CITI(1992) in CAR 2010 Swedish National Chemical Agency

5.1.1 Stability

DEET was hydrolytically stable in sterile buffer solutions at pH 4, 7 CO_2 and 9 when incubated for 5 days at 50°C under dark conditions according to OECD guideline 111; the concentrations

decreased by less than 10% from the initial concentration within 5 days (A.7.1.1.1.1. in Final CAR March 2010, Swedish Chemical Agency.).

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

The biodegradability of DEET at 22±2°C was investigated in a ready biodegradability study based on CO_2 evolution according to OECD guideline 301B.

It was found that DEET is ready biodegradable, exhibiting 83.8% ultimate degradation (reached within 28d and the 10d window) (A.7.1.1.2.1 in Final CAR March 2010, Swedish Chemical Agency.). The study was conducted by Wildlife International, LTD for DEET European Union Joint Venture of the Wildlife International, Ltd. Biodegradation facility in Easton, Maryland and will only be summarized below.

In the CO_2 test, inoculated mineral medium is dosed with a known amount of test substance as the nominal sole source of organic carbon and aerated with CO_2 –free air. The CO_2 produced from the mineralization of organic carbon within the test chambers is displaced by the flow of CO_2 –free air and trapped as K_2CO_3 in KOH trapping solution. The amount of CO_2 produced by the test substance (corrected for that evolved by the blank inoculum) is expressed as a percentage of the theoretical amount of CO_2 (TCO_2) that could have been produced if complete biodegradation of the substance occurred. The test contained a blank control group, reference group and treatment group. Each group contained three replicate chambers. The blank control was used to measure the background CO_2 production of the inoculum and was not dosed with carbon source. The reference chambers were dosed with sodium benzoate, a substance known to be biodegradable, at a nominal concentration of 10 mg/C/L.

The inoculum (activated sludge) was collected from an STP that receives primarily domestic sludge. Initial mean cell concentration of the inoculum was 3.2E05 CFU/ml. The inoculum was used without adaptation. Test concentration was 10 mg C/l. No deviations from guideline were noted for this test, and all validity criteria in the OECD guideline were fulfilled.

A second study was conducted based on the OECD Guideline D (Closed Bottle test) which measured the consumption of oxygen as compared to the theoretical oxygen demand (ThOD) and chemical oxygen demand (COD): Kumar (2003a) conducted the test using mineral medium inoculated with river water and garden soil extract. The authors reported a rapid initial biodegradation reaching 30% ThOD and 37% COD by day 7. After day 7 biodegradation had essentially halted, reaching 40% ThOD and 48% COD on day 28. The biodegradation of the reference compound potassium hydrogen phtalate reached 87% based on ThOD and 95% based on COD, indicating that the microbial consortium was suitable active. No indication was made by the authors to explain the halted biodegradation. The level of biodegradation observed in this latter test can be categorized as "inherently biodegradable" but falling short of the rate necessary for the OECD definition of "readily biodegradable".(Weeks et al, 2011).

A third study was conducted based on OECD guideline 301 C (modified MITI test), which measures the loss of test material over time using a microbial consortium that originates as municipal wastewater sludge that is then preconditioned to a glucose-peptone media over the course of 30d (CITI 1992). No biodegradation was observed of DEET in this test (Weeks et al 2011).

The OECD Guideline 301C is less reliable than 301 B because it was not sure if it was the toxicity of DEET to the microorganisms that caused the lower degradation or not. This is more likely to happen in the OECD 301 C test than in other 301 tests because the test substance was introduced at higher concentration (100 mg/L) in the OECD 301 test. That DEET might have a toxicity to microbes is shown in an acute toxicity study of phospohorent bacteria (Kaiser and Palabrica in Weeks et al 2011)

Data on measured concentrations in surface water (marine and freshwater) and groundwater are summarised in section 5.2.3.

5.1.2.3 Simulation tests

No simulation tests for the compartments soil, water/sediment, or sewage treatment plants (STPs) were available. Such tests have not been considered necessary mainly because DEET was found to be ready biodegradable.

5.1.3 Summary and discussion of degradation

The most reliable test to show that degradation of DEET has occurred is the OECD guideline 301 B ready biodegradable test (see table 21) where you can see an ultimate degradation of the test material as it is mineralizes to carbon dioxide.

There is another biodegradation test carried out according to OECD guideline 301D (see table 21) but this test did only showed inherent biodegradability and not ready biodegradability and there were also difficulties to interpret the results.

The OECD Guideline 301C (an older modified MITI test 1983) is also less reliable than OECD 301 B 2002 because it was not sure if it was the toxicity of DEET to the microorganism that caused the lower degradation of DEET or not. This is more likely to happen in the OECD 301 C test than in other 301 tests because the test substance was introduced at higher concentration (100 mg/L) in the OECD 301C test. That DEET might have a toxicity to microbes is shown in an acute toxicity study of phospohorent bacteria (Kaiser and Palabrica 1991 in Weeks et al 2011).

The current classification (“N - Dangerous for the environment, R52/53”, or “Aquatic Chronic 3 H412”) is presumably based on the result of the ready biodegradability test (MITI, 1983 see 2.2 Environmental hazard for more information of study and references) which is considered as less reliable than the OECD 301B test from 2002.

Hence, it can be concluded that DEET is not degraded in abiotic processes, but is readily biodegradable based on the above data. Since DEET is ready biodegradable and no metabolites were detected in the studies on hydrolysis, no data on potential metabolites formed in the environment is considered necessary.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

An adsorption/desorption screening test using HPLC determination (OECD 121) suggests that DEET probably has a very high mobility in soil and also would not adsorb significantly to sediment. In the screening test Koc was determined to 43.3 (A7.1.3 in the Final CAR March 2010, Swedish Chemical Agency).

5.2.2 Volatilisation

DEET can be considered as being moderately volatile. The vapour pressure is 0.23 Pa at 25°C (A3.2 in Final CAR March 2010, Swedish Chemical Agency.) and the calculated Henry´s law constant 3.93E-3 Pa*m^3/mol.

5.2.3 Distribution modelling

According to level III fugacity modelling (EPI Suite™ v.3.11), DEET would predominantly be distributed to the water compartment. For the modelling it was assumed that all emissions reach the water compartment first, since the primary route of DEET to the environment is expected to be via STP, following washing of skin and clothes after use of the repellent. In this fugacity modelling DEET would be distributed between different environmental compartments as follows: Water: 99.4%; Sediment 0.606%; Air 0.000402%; Soil 0.0274%.

In the context of risk assessment under the biocides directive 98/8/EC, further modelling was done to obtain Predicted Environmental Concentrations, but those results are not further presented here.

For the review under the biocides directive the open literature was searched for measured concentrations of DEET in environmental compartments. Monitoring data not published in the open literature were also made available from the Dutch authority CTGB. Finally, the Rapporteur Member State (SE) identified one European survey of groundwater after the review under the biocides directive was finalised (Loos et al, 2010, Water Research 44: 4115-4126). Results from groundwater, and fresh and marine surface waters are summarised in the table below. The reported *concentrations* should only be considered as examples, or as indications of the potential magnitude of residues in the environment since the measurements reflect a situation at a particular location at a particular point in time. By contrast, the *frequency* of positive detections may indicate whether a chemical has a wide distribution in the environment or not. In the case of DEET, the high frequency of positive samples indicates that the chemical indeed is wide-spread in aqueous media. This is considered as somewhat contradictory given that the substance was found to be readily biodegradable. The Rapporteur Member State (SE) therefore considered potential artefacts for the positive findings. The specificity of the analytical methods employed for the environmental samples could in most cases not be evaluated (with Loos et al, 2010, as an exception) but this is not considered to invalidate the observations. The main use area of DEET is as a repellent, however, additional areas of use have been reported (embalming and taxidermist fluids; drug excipient in matrix-type transdermal delivery systems).

DEET was also studied in Africa, Zambia groundwater and was found within groundwater at concentration up to 1.8 µg/L (wet season) and 0.4 µg/L (dry season). The five-fold increase in median DEET concentration following the onset of the seasonal rain highlights that more mobile compounds like DEET, rapidly migrate from surface to aquifer suggesting the aquifer is more vulnerable than previously considered (Sorensen et al, 2015).

Another study measuring pesticides in surface waters from four sub-basins in Argentina showed in basin samples from south east of Buenos Aires that DEET a maximum level of 0.701 µg/L. The results obtained are in agreement with studies performed in streams of United States, Australia and Netherlands, which showed the presence of DEET at concentrations up to 1.1 µg/L (Sandstrom et al.,Constanzo et al 2005 in Sorensen et al,2015).

In a study looking at the environmental release, environmental concentrations and ecological risk using chronic values that are available for Daphnia and algae that are available of DEET also confirms that under ordinary circumstances, DEET is not expected to occur at high enough to cause

any toxic effects to aquatic organisms. Under a limited number of circumstances, relatively high concentrations may occur in rivers and streams that are dominated by effluent flows, with very little dilution, but the expected DEET concentrations are still below levels that have been showing to be toxic (Aronson et al. 2011) .

Table 22: Summary of monitoring data

Location	Analytical information	Concentrations reported	Reference
20 groundwater sources in Africa, Kabwe, Zambia ,sampled at September 2013(dry season) and January 2014 (wet season)	Conducted by the UK Environmental Agency National Laboratory Service(NLS) using a multi-residue GC-MS method with a double-liquid extraction method with dichloromethane	Found in groundwater at concentration up to 1.8 µg/L (wet season) and 0.4 µg/L (dry season)	Sorensen et al,2015, Water Research 72: 51-63
Four surface water stations in Argentina sampled: Water samples collected : San Vicenta Sept 2011 rainfall 75 mm, Mistra sream May 2012 rainfall 5.2 mm Buenos Aires (including Azul) Sept 2012 and rainfall 61.2 mm	Solid-phaze extraction (SPE) using OASIS HLB 60 mg cartridges and ultra-high- pressure liquid chromatography coupled to tandem mass spectrometry (UHPLC/MSMS)	South East Buenos Aires maximum level of 0.701 µg/L	Geronimo et al., 2014, Chemosphere 107:423-431
164 groundwater monitoring stations across Europe, sampled autumn 2008	reversed-phase liquid chromatography (RP-LC) followed by electrospray ionization (ESI) mass spectrometry (MS); LOD 0.4 ng/l	frequency of detection: 83.5% median concentration: 1 ng/l 90th percentile conc: 9 ng/l max. concentration: 454 ng/l	Loos et al, 2010, Water Research 44: 4115-4126
189 groundwater locations in The Netherlands, sampled 2007 (mainly July-Dec)	method not known LOD 0.01 µg/l	frequency of detection: 30% 57/189 samples: >0.01 µg/l 3/189 samples: >0.1 µg/l max. concentration: 1.48 µg/l	Verhagen, de Coninck, and Vervest, 2008, Royal Haskoning, pp 71 (in Dutch), Also Excel-sheets provided by Dutch authority ctgb
seawater, North Sea; sampling locations mostly coastal; sampling period: June-July 1998 2x10 l samples at 5m depth, 15 sampling locations	GC-MS LOQ(limit of quantification) 26 pg/l (0.026 ng/l)	DEET was detected in all but two samples. max. concentrations: 1.09 and 1.06 ng/l respectively, found in the German Bight	Weigel et al., 2002, The Science of the Total Environment 295: 131-141
seawater Tromsø Sound (Norway), (into which sewage is discharged); sampling period 2002 (most samples taken in April, the rest in October) 2.5 l samples, 12 sampling locations	GC-MS LOQ (limit of quantification) 0.20 ng/l	DEET concentration range: 0.4-13 ng/l	Weigel et al., 2004, Chemosphere 56: 583-592
surface freshwater,	GC-MS	frequency of detection: 73.2%	Kolpin et al.,

56 streams across the USA, some bias to streams downstream intense urbanization and livestock production; sampling period:2000	Reporting level: 40 ng/l	median conc.: 0.05 µg/l max. concentration: 1.1 µg/l (measured at urban site)	2002, Environmental Science and Technology 36: 1202-1211

There is no known natural source of DEET. It is concluded that the wide-spread, more or less continuous use together with the hydrophilic nature of DEET results in a wide-spread contamination of waters, including groundwater, at low concentrations. Under limited circumstances, like heavy rainfall or dominated effluent flows with very little dilution where relatively high concentrations of DEET may occur in rivers or streams and aquifers (Weeks, 2011).

5.3 Aquatic Bioaccumulation

The summaries and evaluations of the accumulation studies with DEET is taken from the Final CAR March 2010, Swedish Chemical Agency. There is no information on reliability of the studies.

Table 23: Summary of relevant information on aquatic bioaccumulation (Final Car 2010, Swedish Chemical Agency 2010)

Method	Results	Remarks	Reference
Bioconcentration factor, QSAR (TGD III, 4.5.2.1)	22	Estimated Data sufficient for classification purposes under DSD or CLP **(key study)**	A 7.4.2.

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

DEET has a log P_{OW} of 2.4 (measured). The bioconcentration factor (BCF) in aquatic organisms was estimated to approximately 22, using a linear Quantitative Structure Activity Relationship (QSAR) model (eq. from TGD III, 4.5.2.1: Log BCF = 0.85 log Pow – 0.70). Based upon the estimated BCF, DEET is considered to have very little or no potential to bioaccumulate in the aquatic environment.

5.3.1.2 Measured bioaccumulation data

No data available.

5.3.2 Summary and discussion of aquatic bioaccumulation

Due to its hydrophilic properties DEET is not expected to bioaccumulate.

5.4 Aquatic toxicity

The summaries and evaluations of the aquatic studies with DEET is taken from the Final CAR March 2010, Swedish Chemical Agency. ECHA contributed with more information on the aquatic toxicity of DEET after they carried out a data search with SCOPUS and revealed new publications. The publication of Weeks et al (2011) contributed to the main new investigations in this area.

There is a large dataset on the acute and long term toxicity of aquatic organisms and the most relevant studies have been selected for the evaluation and environmental CHL proposal of DEET.

Table 24: Summary of relevant information on aquatic toxicity (Final Car 2010, Swedish Chemical Agency 2010 and Weeks 2011)

Method	Results	Remarks	Reference
Acute toxicity to fish *Oncorhynchus mykiss* OECD 203; EEC Method C.1; OPPTS 850.1075	LC_{50} (96 h) =97 mg/l	Reliable experimentally determined Supportive study	A7.4.1.1(1)
Acute toxicity to *Daphnia*, U.S. EPA Ecol. Res. Series 660/3-75009; Standard Methods for the Examination of Water and Wastewater (1980)	LC_{50} (51 h)= 75 mg/l (mortality) EC_{50} (51 h)= 42 mg/l (abnormal behavior)	Reliable experimentally determined Supportive study	A7.4.1.2(1)
Long term toxicity *Daphnia magna*. EPA 850.1300	21 d Reproduction NOEC (LOEC)=14(29)mg/l	Reliable Supportive study	Minderhout et al.(2008) in Weeks et al 2011
Growth inhibition algae, *Selenastrum capricornutum* OECD 201; EEC Method C.3; OPPTS 850.5400	E_rC_{50} (96 h)= 43 mg/l E_rC_{50} (72 h)=41 mg/l 96 h NOEC (LOEC)=15 mg/l (growth rate)	Reliable experimentally determined) **Key study**	A7.4.1.3(1) Desjardin et al (2002)
Growth inhibition algae, *Selenastrum capricornutum* OECD 201	E_rC_{50} (72 h)=100 mg/l NOEC (72 h) =24 mg/l	Reliable Supportive study	Rao (2003) in Weeks (2011)
Green algae *Pseudokirchenerielle subspicata* NIES-35, No standardized guideline, 96 –hole microplate growth inhibitiontest	96-h EC50 =41 mg/l 96-h NOEC=0.521 mg/l	Reliability with restriction: full experimental details not given	Harada et al(2008)
Inhibition of respiration in activated sludge, OECD 209; EEC Method C.11	EC_{50} (3 h) >1000 mg/l	Reliable experimentally determined Supportive study	A7.4.1.4

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

In a static test (OECD 203; EEC Method C.1; OPPTS) in the Final Car 2010) the 96-hour LC_{50} was determined to 97 mg/l in rainbow trout (*Oncorhynchus mykiss*) (A7.4.1.1 (1) Final CAR March 2010, Swedish Chemical Agency.). Measurements demonstrated that nominal concentrations were maintained over the study, and the endpoint was based on mean measured concentrations.

Summary

Overall it can be summarized that DEET has a low toxicity to fish EC50>1 mg/l

5.4.1.2 Long-term toxicity to fish

Long-term study in fish is not available.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

In a static test (U.S. EPA Ecol. Res. Series 660/3-75009, 1980) the 51-hour LC_{50} was determined to 75 mg/l in *Daphnia magna* (A7.4.1.2 (1) in Final CAR March 2010, Swedish Chemical Agency).

The most important deviation from the OECD 202 guideline was that the study reported LC_{50} (based on lethality) rather than EC_{50} (based on immobility). Based on abnormal behaviour (surfacing, erratic movement, loss of equilibrium etc) reported in the study an EC_{50} of approximately 42 mg/l was determined. Measurements demonstrated that nominal concentrations were maintained over the study and the endpoints were based on mean measured concentrations.

Summary

Overall it can be summarized that DEET has a low toxicity to *Daphnia magna* EC50>1 mg/l

5.4.2.2 Long-term toxicity to aquatic invertebrates

A 21-d chronic bioassay study according to EPA 850.1300 was performed by measuring length and reproduction with *Daphnia magna* using GLP (Minderhout et al. 2008 in Weeks 2011). The study was reliable and the authors reported 21-d NOEC of 3.7 mg/l based on length and 14 mg/L based on reproduction.

5.4.3 Algae and aquatic plants

Inhibition of growth rate of algae (OECD 201; EEC Method C.3; OPPTS 850.5400) was studied in a static system with the freshwater green alga species *Selenastrum capricornutum* Printz (A7.4.1.3, in Final CAR March 2010, Swedish Chemical Agency and in Weeks et al (2011)

Final Car 2010, Swedish Chemical Agency 2010.). The duration of the study was 96 hours. Measurements demonstrated that nominal concentrations were maintained over the study and the endpoints were based on mean measured concentrations. Reported endpoints include: E_bC_{50} (72 h) 17 mg/l, E_bC_{50} (96 h) 18 mg/l, E_rC_{50} (72 h) 41 mg/l, E_rC_{50} (96 h) 43 mg/l. Growth rate is the preferred response variable in OECD 201 (2006), hence E_rC_{50} is considered more appropriate than E_bC_{50}. The control growth rate slowed down during the last 24 hours of the study (it was still above the validity criterion in the OECD test guideline 201, i.e., above 0.92/day). However, since the growth rate of the control was not constant over the duration of the study the results as reported per 96 hours were not considered as strictly valid. Therefore, the 72 hour E_rC_{50} is considered as the most appropriate endpoint of the test.

Another inhibition of growth rate study with the algae *Selenastrum capricornutum* (Rao 2003 in Weeks et al, 2011) was performed according to the guideline OECD 201. This study supports the study performed by Desjardin (2002) in Final CAR March 2010, Swedish Chemical Agency and has a good reliability (Klimisch score 1, according to Klimisch et.al. 1997) and follows GLP. The inhibition of growth rate was 72 h EC50=50-100 mg/l and 72 h NOEC =24 mg/l.

There was a study performed with the green algae *Pseudokirchneriella subcapitata* NIES-35 (Harada et al 2008 in Weeks 2011) using a 96 hole microplate growth inhibition test , a procedure as modified by Yasimita et al (2006) measuring growth rate with light absorbance by photosynthetic pigments, using absorbance at 450nm. The 96 h EC50=4.1 mg/l and the 96 h NOEC=0.521 mg/l. However, this study was not following any guidelines and the reliability was restricted meaning that full experimental details were not given (reliability category 2, evaluation system according to Weeks 2011 which would correspond to Klimisch score 2, according to Klimisch et.al. (1997)). The Algal Growth Inhibion (AGI) test they used in the experiment is not a standard test and was poorly described. A Dunnett´s method and Ecotox Statics version 2.6 d software package was used to determine NOEC but the methods were not explained and there was no reference to this article.

Costanzo et al. (2007) reported 24-h EC50 of 388 mg/L with *Chlorella protothecoides*. With the ECOSAR model (USEPA 2011, reliable with restricions, accepted calculation procedures), a 96 h EC50 of 25.4 mg/L and a chronic value (ChV) of 9.65 mg/L were calculated, both which compare well with the range of measured EC50 and NOEC values.

In the CLP guideline 2015 under section 4.3.2 Evaluation of available information and 4.1.3.2 general consideration informs you: ”Regarding the use of test data, in general, only reliable information (i.e. with a Klimisch reliability score of 1 (reliable without restrictions) or 2 (reliable with restrictions)) should be used for classification purposes. For larger data sets, preference should be given to information with Klimisch score 1, while information with Klimisch score 2 can be used as supporting information.”

Since we do have a large dataset on aquatic organisms and especially algae, the inhibition on growth rate study with the algae *Selenatrum capricornutum* ,with Klimish Score 1 and the endpoint 72 h EC50=41 mg/l and 96 h NOEC = 15 mg/l ,will be used classification purpose.

This is supported by the study of Rao (2003) with the same species and guideline and duration of the test a (72h E_r C50=50-100 mg/L and NOEC=24 mg/l and with the same Klimisch score 1.

Summary

Overall it can be summarized that DEET has a low toxicity to algae *Selenastrum capricornutum* EC50>1 mg/l

5.4.4 Other aquatic organisms (including sediment)

The effect of DEET on microbial activity in water (OECD 209; EEC Method C.11) was assessed by determining the level of inhibition of respiration of microorganisms present in activated sludge (A7.4.1.4 in Final Car 2010, Swedish Chemical Agency 2010). Exposure duration was three hours. DEET had only a minor inhibitory effect on aquatic microbial activity (26.8% inhibition at the highest tested concentration, 1000 mg/l), therefore EC_{50} (3 h) was set to >1000 mg/l.

Studies on toxicity to sediment-living organisms is not considered necessary since DEET is not expected to partition to and persist in aquatic sediments.

Summary

Overall it can be summarized that DEET has a low toxicity to microorganisms EC_{50}>1000 mg/l.

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

The criterion for acute toxicity is not fulfilled for any of the aquatic organisms (EC_{50} above 1 mg/L) for fish *Oncorhynchus mykiss*, invertebrate *Daphnia magna* and algae *Selenastrum capricornutum*).

DEET is considered as being rapidly biodegradable, since the most reliable rapidly biodegradation test according to OECD 301 B guideline showed a 83.8% ultimate degradation (reached within 28 d and the 10 d window) and the substance does not fulfil the criterion for bioaccumulation based on its log Kow < 4 and its BCF < 500.

For the chronic toxicity NOEC value is available invertebrates, representing one trophic level. There are no chronic toxicity studies carried out with fish and algae.

The criterion for chronic toxicity (NOEC<1 mg/L) is not fulfilled based on the long term reproduction study with *Daphnia* 21 d NOEC= 14 mg/L and the most reliable acute toxicity studies for both fish and algae with NOECs >1 mg/l.

It is suggested that DEET should not be assigned any classification for environment. Thus, it is suggested that DEET is declassified in relation to the current environmental classification.

The physiochemical properties of DEET do not suggest that this substance will be hazardous to the ozone layer.

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.

Additional key elements

Because there is no chronic toxicity data available on fish and because the reliability of the Daphnia test could not be evaluated by RAC due to missing data, RAC decided to use QSAR calculations in order to further elaborate on these data gaps. The calculations were made with ECOSAR version 1.11 with no user given data. The ECOSAR used log Kow of 2.258 and water solubility of 1911 mg/L for calculation. At first the substance was considered part of the Class Amides ECOSAR category, however acute algae data and chronic data for all three trophic levels was missing in the training set for this class. Hence, the Amide class predictions are not considered reliable. On the other hand, the test results in the training set and in the CLH Report were closer to the Neutral Organic SAR (baseline toxicity).

Table: ECOSAR v1.11. Baseline Toxicity QSAR for DEET

Species	End point	Predicted mg/L (ppm)
Fish	LC_{50} 96 h	92.180
Daphnids	LC_{50} 48 h	53.602
Green Algae	EC_{50} 96 h	44.060
Fish	ChV(1	9.267
Daphnids	ChV(1	5.586
Green algae	ChV(1	12.167

(1 ChV (Chronic Value) is the geometric mean between LOEC and NOEC. If LOEC not available NOEC can be used alone.

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**.

6 OTHER INFORMATION

7 REFERENCES

Aronson et al(2011). Environmental Release, Environmental Concentrations and Ecological Risk of N,N-Diethyl-m-Toluamide (DEET). Integrated Environmetal Assessment and Management.8(1)135-166.

Geronimo et al (2014). Presence of pesticides in surface water from four sub-basins in Argentina. Chemospere 107:423-431

Harada et al (2008). Green algae *Pseudokirchneriella subspicata* NIES-35, No standardized guideline, 96-hole microplate growth inhibitiontest.

Knepper TP. (2004). Analysis and fate of insect repellents. Water Sci Technol. 50(5):301-8.

Kolpin et al. (2002). Pharmaceuticals, hormones and other organic wastewater contaminants in U.S streams, 1999-2000: A national reconnaissance. Environmental Science and Technology 36: 1202-1211

Loos R, Locoro G, Comero S, Contini S, Schwesig D, Werres F, Balsaa P, Gans O, Weiss S, Blaha L, Bolchi M, Gawlik BM. (2010). Pan-European survey on the occurrence of selected polar organic persistent pollutants in ground water. Water Res. 44(14):4115-26.

MITI-CITI database (1992). Japanese Excisting New Chemical Inventory.

Sorensen et al (2015) Emerging contaminants in urban groundwater sources in Africa. Water Research.72:51-63.

Swale, D. R., B. Sun, F. Tong and J. R. Bloomquist (2014). "Neurotoxicity and mode of action of N, N-diethyl-meta-toluamide (DEET)." PLoS One 9(8): e103713.

Tisch, M., P. Schmezer, M. Faulde, A. Groh and H. Maier (2002). "Genotoxicity studies on permethrin, DEET and diazinon in primary human nasal mucosal cells." Eur Arch Otorhinolaryngol 259(3): 150-153.

Verhagen, de Coninck, Vervest (2008) Brede screening Bestrijdingsmiddelen Maasstroomgebied 2007. Royal Haskoning, pp 71

Weeks et al (2011). Environmental Assessment and Management. Assessment of the Environmental Fate and Ecotoxicity of n,N-diethyl-m- Toluamide (DEET). Integrated Environmental Assessment and Management 8:(1)120-134.

Weigel et al. (2002).Drugs and personal care products as ubiquitous pollutants: occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea. The Science of the Total Environment 295: 131-141

Weigel et al. (2004). Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromsø/Norway with emphasis on ibuprofen and its metabolites. Chemosphere 56: 583-592

Additional references

Additional references not included in the CLH report or RCOM Annex

Breckon G and Cawood AH, 1985. Photoperiodic control of meiosis in the male Syrian hamster (Mesocricetus auratus). J Reprod Fert 75, 177-181

Devin Wiles, Justin Yee, Uvidelio Castillo, Jason Russell, Henry Spiller, Marcel Casavant; A Lethal Case of DEET Toxicity Due to Intentional Ingestion. J Anal Toxicol 2014; 38 (9): 696-698. doi: 10.1093/jat/bku082

Donham RS, Goodear G, Stetson MH, 1996. Dissociation of spontaneous testicular recrudescence and photorefractoriness in the Syrian hamster. Journal of reproduction and fertility 108, 205-209

Hampers, L. C., Oker, E. and Leikin, J. B. (1999), Topical Use of DEET Insect Repellent as a Cause of Severe Encephalopathy in a Healthy Adult Male. Academic Emergency Medicine, 6: 1295–1297. doi:10.1111/j.1553-2712.1999.tb00147.x

Hubrecht R and Kirkwood J, 2010. The UFAW Handbook on the care and management of laboratory and other research animals. Eighth edition. Wiley-Blackwell

Larkin JE, Jones J, Zucker I, 2002. Temperature dependence of gonadal regression in Syrian hamsters exposed to short day lengths. Am J Physiol Regulatory Integrative Comp Physiol 282, 744-752. 10.1152/ajpregu.000299.2001

Study report (1989). Evaluation of DEET in a 90-day dose range finding study in hamsters.

8 ANNEXES

CAR doc IIIA including reference list of doc IIIA studies are provided as a confidential annex to the IUCLID dossier.