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**

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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.

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

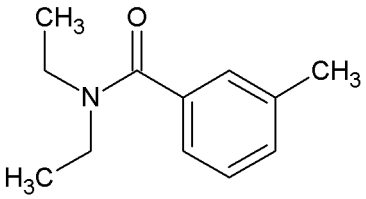
1 IDENTITY OF THE SUBSTANCE

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.

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.

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.

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

4.9.6 Conclusions on classification and labelling

DEET does not fulfil the classification criteria for carcinogenicity.

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.

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.

6 OTHER INFORMATION

7 REFERENCES

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8 ANNEXES

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