HET COLLEGE VOOR DE TOELATING VAN GEWASBESCHERMINGSMIDDELEN EN BIOCIDEN

BIJLAGE II bij het besluit d.d. 24 december 2014 tot toelating van het middel Care Plus Anti-Insect DEET Spray 50%, toelatingnummer NL-0009273-0000

Product Assessment Report

Care Plus Anti-insect DEET Spray 50%

24 December 2014

Internal registration/file no:	20120119 TNB
Authorisation/Registration no:	NL-0009273-0000
Granting date/entry into force of authorisation/ registration:	24 December 2014
Expiry date of authorisation/ registration:	24 December 2024
Active ingredient:	N,N-diethyl-meta-toluamide (DEET)
Product type:	PT19

Biocidal product assessment report related to product authorisation under Regulation (EU) 528/2012

Contents

1 General information about the product application

1.1 Applicant

Company Name:	Tropenzorg B.V.
Address:	De Huchtstraat 14
City:	Almere
Postal Code:	1327 EE
Country:	The Netherlands
Telephone:	+31 36 533 47 11
Fax:	+31 (0)36 534 49 75
E-mail address:	info@tropicare.eu

1.2 Current authorisation holder

Not applicable.

1.3 Proposed authorisation holder

Company Name:	Tropenzorg B.V.
Address:	De Huchtstraat 14
City:	Almere
Postal Code:	1327 EE
Country:	The Netherlands
Telephone:	+31 36 533 47 11
Fax:	+31 (0)36 534 49 75
E-mail address:	info@tropicare.eu

1.4 Information about the product application

Application received:	7 February 2012
Application reported complete:	21 February 2014
Type of application:	Application for authorisation of a biocidal product
Further information:	Not applicable

1.5 Information about the biocidal product

1.5.1 General information

Trade name:	Care Plus Anti-insect DEET Spray 50%
Manufacturer's development code number(s), if appropriate:	Not applicable

Product type:	PT19
Composition of the product (identity and content of active substance(s) and substances of concern; full composition see confidential annex):	48.5% pure active substance Substance of concern: ethanol
Formulation type:	AL
Ready to use product (yes/no):	Yes
Is the product the very same (identity and content) to another product already authorised under the regime of directive 98/8/EC (yes/no); **If yes: authorisation/registration no. and product name:** **or** **Has the product the same identity and composition like the product evaluated in connection with the approval for listing of active substance(s) on to Annex I to directive 98/8/EC (yes/no):**	No

1.5.2 Information on the intended use(s)

Overall use pattern (manner and area of use):	Repellent against mosquitoes and ticks on humans (PT19) for use outdoors and indoors in well ventilated areas.
Target organisms:	Mosquitoes (Culicidae): • House mosquitoes - *Culex spp.* • Malaria mosquitoes - *Anopheles spp.* • Yellow fever mosquitoes - *Aedes spp.* Ticks (Ixodoidea) (not tested against tropical tick species)
Category of users:	Non-professionals
Directions for use including minimum and maximum application rates, application rates per time unit (e.g. number of treatments per day), typical size of application area:	• Apply Care Plus DEET sparingly and carefully to parts of the body that are not covered by clothing, divide the product evenly over the skin • Wear long pants and/or long sleeved shirt. • Keep the spray bottle at least 15 cm from the skin, do not spray directly on the face. To protect the face from insect bites, first spray or spread a small quantity of Care Plus DEET onto the palm of the hand and then spread on the face. Frequency: use only once a day (max).
Potential for release into the	Yes

environment (yes/no):	
Potential for contamination of food/feedingstuff (yes/no)	No
Proposed Label:	See SPC
Use Restrictions:	• Do not use on children under 17 years of age. • Wear long pants and/or long sleeved shirt. • Apply Care Plus DEET sparingly and carefully to parts of the body that are not covered by clothing. Do not apply under clothing. • Spread the lotion evenly over the skin. • Do not apply to parts of the body that could easily come into contact with the eyes or mouth (such as hands and forearms). • Avoid contact with mucous membranes, eyes, nose (nasal passages) and lips. To protect the face from insect bites, first apply or spread a small quantity of Care Plus DEET onto the palm of the hand and then spread on the face. • Do not apply Care Plus DEET to damaged skin (such as cuts and scratches or sunburn). • Do not apply Care Plus DEET to areas around joints such as behind the knees or inside the elbows where skin folds normally occur. • When used in combination with sun block lotion, apply sun block first then wait 30 minutes before applying Care Plus DEET. • Wash your hands thoroughly with soap and water before eating or drinking. • Application of Care Plus DEET is not advised for sensitive groups such as pregnant and breast feeding women. • Wash the areas of skin that have been treated when protection is no longer needed or if side effects occur. • Do not reuse the container for any other purpose. It is preferable to dispose of any remaining product to a waste recycling centre as hazardous waste rather than to domestic waste.

1.5.3 Information on active substance

Active substance chemical name:	IUPAC name: *N,N*-diethyl-*m*-toluamide CA name; *N,N*-diethyl-3-methylbenzamide Common name (non-ISO): DEET

CAS No:	134-62-3
EC No:	205-149-7 (EINECS)
Purity (minimum, g/kg or g/l):	970g/kg
Inclusion directive:	2010/51/EU
Date of inclusion:	1 August 2012
Is the active substance equivalent to the active substance listed in Annex I to 98/8/EC (yes/no):	Yes: same source as evaluated for approval of the substance.
Manufacturer of active substance(s) used in the biocidal product:	Please refer to the SPC.

1.5.4 Information on the substance of concern

Substance chemical name	Ethanol
CAS No:	64-17-5
EC No :	200-578-6 (EINECS)
Purity (minimum, g/kg or g/l):	96%
Typical concentration (minimum and maximum, g/kg, or g/l):	Approximately 47% in the formulation
Original ingredient (trade name):	Ethylalcohol 96% with 5% IPA

1.6 Documentation

1.6.1 Data submitted in relation to product application

New studies concerning the product Care Plus Anti-Insect DEET 50% Spray have been submitted with respect to physical-chemical properties of the product, analytical methods, toxicity and efficacy. The studies are listed in Annex 1.

1.6.2 Access to documentation

The applicant has submitted a letter of access of the owner of the data on the active substance DEET submitted for the inclusion of DEET in the Union list of approved substances of EU Regulation 528/2012.

2 Summary of the product assessment

2.1 Identity related issues

General information

This assessment report contains the evaluation of a product based on the active substance DEET (*N,N*-diethyl-*m*-toluamide). DEET was evaluated and included in Annex I of Directive 98/8/EC for PT19 as part of the review programme for existing substances. The manufacturing site of DEET was evaluated as part of the EU review.

Product specific information

The product contains ethanol, which is considered a substance of concern.

2.2 Classification, labelling and packaging

2.2.1 Labelling of the biocidal product

Based on the profile of the substances the provided toxicology of the preparations, the characteristics of the co-formulants, the method of application and the risk assessment for the operator, the following labeling of the preparations is proposed:

Care Plus DEET Anti-Insect 50% Spray

The identity of all substances in the mixture that contribute to the classification of the mixture *:			
-			
Pictogram:	GHS02 GHS07	Signal word:	Danger
H-statements:	H225	Highly flammable liquid and vapour	
	H319	Causes serious eye irritation	
P-statements:	P101	If medical advice is needed, have product container or label at hand.	
	P102	Keep out of reach of children	
	P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.	
	P260	Do not breathe vapours	
	P271	Use only in well-ventilated areas	
	P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.	
Supplemental Hazard information:	-		
Child-resistant fastening obligatory?			No
Tactile warning of danger obligatory?			Yes

* according to Reg. (EC) 1272/2008, Title III, article 18, 3 (b)

2.2.2 Packaging of the biocidal product

non-professional use

Packaging applied for	Packaging considered acceptable by the RMS	Packaging authorised for the Dutch market
60mL HDPE bottle with spray attachment	60mL HDPE bottle with spray attachment	60mL HDPE bottle with spray attachment

2.3 Physico/chemical properties and analytical methods

The applicant has provided access to the Annex I dossier via a Letter of Access. The physical and chemical properties for the active substance DEET are detailed in the Annex I dossier, Doc IIIA, Section 3.

2.3.1 Physico-chemical properties

The product Care Plus DEET Anti-Insect Spray 50% is one of multiple similar ready to use liquid products with a similar composition:

- Care Plus DEET Anti-Insect lotion 50%

- Care Plus DEET Anti-Insect Spray 40%
- Care Plus DEET Anti-Insect Gel 30%
- Care Plus Anti-Insect DEET 50% Spray
- Care Plus Anti-Insect DEET Spray 20%
- Care Plus Anti-Insect DEET Spray 30%
- Care Plus Anti-Insect DEET Roll-on 30%

A comparison of the various compositions is included in the confidential section.

Table 1: Physico-chemical properties of the biocidal product:

	Method	Purity/Specification	Results	Reference
Physical state and nature	Visual	Not specified	Liquid	Product safety data sheet
Colour	Visual	Not specified	Colourless	Product safety data sheet
Odour	Olfactory	Not specified	Characteristic odour	Product safety data sheet
Explosive properties	Theoretical assessment	Not applicable	Not explosive based on the classification of the individual components of the formulation.	
Oxidizing properties	Theoretical assessment	Not applicable	Not oxidising based on the classification of the individual components of the formulation.	
Flash point	Theoretical assessment	Not applicable	Based on the ethanol/water content, a flash point of 23℃ is expected. Considering this is on the threshold between a cat 2 and cat 3 flammable liquid, the product will be labelled as a cat 2 flammable liquid as the influence of other components in the product is difficult to predict. The calculation method does not take into account other components. Therefore, this statement is considered not acceptable.	Hristova, M., 2010
	EC A9 GLP study	1) Care Plus Anti-insect Deet spray 40% 2) Care Plus Anti-	Closed-cup method at 101.3 kPa:	Brux A., 2014

	Method	Purity/Specification	Results	Reference
		insect Deet spray 50% 3) Care Plus Anti-insect Deet roll-on 30% 4) Care Plus Anti-insect Deet gel 30% 5) Cactuz 20% DEET	1) 16.0℃ 2) 16.0℃ 3) 15.0℃ 4) 15.0℃ 5) 16.0℃ Cactuz 20% DEET is an alternative trade name for the 20% DEET spray.	
Auto flammability	Theoretical assessment	Not applicable	Not self-igniting based on the classification of the individual components of the formulation.	
Other indications of flammability	Theoretical assessment	Not applicable	None based on the classification of the individual components of the formulation.	
Acidity / Alkalinity	Theoretical assessment	Not applicable	Not applicable – the product is not water based and is expected to have a pH in the neutral range when diluted with water, based on its composition.	Delezuch, E., 2013
Relative density / bulk density	Not stated.	50% DEET, batch 11NP038-4	For an acceptable study, please see below (Brux A., 2014a) Density: 0.888 g/mL at unstated temperature. The test method is inaccurate: a 25mL volumetric flask is used to determine the density by weighing the empty bottle and then weighing it again, filled to the mark with product. In addition, the temperature was not reported.	Vrijenhoek, T., 2011
	EC A3 GLP study	1) Care Plus Anti-insect Deet spray 40% 2) Care Plus Anti-insect Deet spray 50%	Anton Paar densitometer 1) D_4^{20} = 0.874 2) D_4^{20} = 0.893 3) D_4^{20} = 0.947	Brux A., 2014a

	Method	Purity/Specification	Results	Reference
		3) Care Plus Anti-insect Deet roll-on 30% 4) Care Plus Anti-insect Deet gel 30% 5) Cactuz 20% DEET	4) D_4^{20} = 0.960 5) D_4^{20} = 0.839	
Storage stability – stability and shelf life		DEET 30% gel, batches 11054, 10130, 08264	Stable for 33 months in HDPE. See table 2 for details.	Anonymous
		DEET 50%, batches 961231, 961206, 970826	Stable in HDPE at ambient temperatures (18 – 25 ℃). Properties investigated: density, DEET content, refraction index, sample weight. See table 3 for details.	Vreugdenhil, J., 2002
		40% DEET lotion, batches 970710, 970827 and 980422	Stable in HDPE at ambient temperatures (18 – 25 ℃). Properties investigated: density, DEET content, refraction index, sample weight. See table 4 for details.	Vreugdenhil, J., 2002a
Effects of temperature			In general, the components of the product are not heat sensitive. However, the product is flammable. Therefore, the product should not be exposed to heat and/or ignition sources.	
Effects of light			The product should preferably be stored in dark places and not exposed to direct sunlight.	
Reactivity towards container material			See above	
Technical characteristics in dependence of the			Not applicable. The product is a ready to use	

	Method	Purity/Specification	Results	Reference
formulation type			liquid.	
Compatibility with other products			Not applicable. The product is not to be mixed with other products.	
Surface tension			Not applicable	
Viscosity			Not applicable	
Particle size distribution			Not applicable	

Table 2: Shelf life data DEET 30% Gel

	Batch 11054		Batch 10130		Batch 08264	
	Initial	5 months	Initial	14 months	Initial	33 months
Refraction index	1.410	1.406	1.407	1.408	1.408	1.408
Density	n.d.	0.953	n.d.	0.953	n.d.	0.954
IR identity	Ok	Ok	Ok	Ok	Ok	Ok
DEET content (%w/w)	30.7	30.5	31.0	30.5	30.6	30.3
Appearance	n.d.	Conform	n.d.	Conform	n.d.	Conform

Table 3a: shelf life data DEET 50, batch 961206

	Initial	3 months	6 months	12 months	18 months	26 months	37 months	50 months	66 months
Mean sample weight	n.d.	62.03	61.9974	61.9078	61.8883	61.7038	61.6607	61.6607	61.4607
DEET content (%)*	103.4	103.4	100.0	102.4	96.6	103.0	102.8	103.4	104.8
Refraction index	1.4412	1.4482	1.4441	1.4408	1.448	1.4419	1.4446	1.4426	1.4401
Density	0.897	0.8962	0.8952	0.8986	0.8954	0.8991	0.9006	0.9001	0.8998
Appearance	Colourless	No change	No change	No change	No change	No change	No change	No change	No change

** reported relative to the specification of 50% DEET*

Table 3b: shelf life data DEET 50, batch 961230

	Initial	3 months	6 months	12 months	18 months	26 months	36 months	50 months	66 months
Mean sample weight	n.d.	61.8197	61.8200	61.7246	61.7013	61.6269	61.5493	61.4748	61.3752
DEET content (%)*	97.8	98.8	103.4	94.6	99.1	103.8	101.3	101.8	103.0
Refraction index	1.4522	1.4439	1.4439	1.442	1.4391	1.4424	1.4447	1.4427	1.4357
Density	0.8969	0.8965	0.8952	0.8989	0.8964	0.8983	0.8998	0.8994	0.8968
Appearance	Colourless	No change	No change	No change	No change	No change	No change	No change	No change

** reported relative to the specification of 50% DEET*

Table 3c: shelf life data DEET 50, batch 980422

	Initial	3 months	10 months	16 months	21 months	34 months	50 months
Mean sample weight	n.d.	58.5635	58.7094	58.6526	58.5876	58.4676	58.2813

DEET content (%)*	103.6	103.6	99.8	106.4	100.7	102.2	102.2
Refraction index	1.4347	1.4399	1.4448	1.4449	1.4382	1.4362	1.4357
Density	0.8880	0.8935	0.8736	0.8921	0.8975	0.8973	0.8968
Appearance	Colourless	No change	No change	No change	No change	No change	No change

** reported relative to the specification of 50% DEET*

Table 4a: shelf life data DEET 40, batch 970710

	Initial	6 months	12 months	19 months	25 months	30 months	43 months	59 months
Mean sample weight	68.6584	68.5821	68.5270	68.4533	68.3993	68.3431	38.1743	67.9734
DEET content (%)*	100.2	96.1	100.4	104.2	105.3	102.8	103.3	100.8
Refraction index	1.4210	1.4249	1.4297	1.4251	1.4244	1.4239	1.4259	1.4302
Density	0.8734	0.8784	0.875	0.8781	0.8741	0.8795	0.8788	0.8769
Appearance	Colourless	No change	No change	No change	No change	No change	No change	No change

** reported relative to the specification of 40% DEET*

Table 4b: shelf life data DEET 40, batch 970827

	Initial	6 months	12 months	18 months	24 months	30 months	42 months	58 months
Mean sample weight	n.d.	67.0181	66.9723	66.9290	66.8795	66.8310	66.6967	66.5225
DEET content (%)*	102.2	97.2	99.8	98.3	101.2	95.1	101.9	101.5
Refraction index	1.4190	1.4233	1.4235	1.424	1.4323	1.4251	1.4223	1.4208
Density	0.8750	0.8777	0.8736	0.8762	0.873	0.8782	0.8783	0.8786
Appearance	Colourless	No change	No change	No change	No change	No change	No change	No change

** reported relative to the specification of 40% DEET*

Table 4c: shelf life data DEET 40, batch 980422

	Initial	3 months	10 months	16 months	21 months	34 months	50 months
Mean sample weight	n.d.	67.6916	67.6296	67.5644	67.5002	67.3387	67.1975
DEET content (%)*	104.5	103.5	102.9	100.0	100.0	100.3	100.5
Refraction index	1.4250	1.4318	1.4299	1.4323	1.4258	1.4287	1.4302
Density	0.8690	0.874	0.8762	0.873	0.8785	0.8776	0.8769
Appearance	Colourless	No change	No change	No change	No change	No change	No change

** reported relative to the specification of 40% DEET*

A shelf-life of 5 years (60 months) in HDPE is considered sufficiently supported by the data provided.

2.3.2 Analytical methods

Analytical method for determination of the active substance in the biocidal product
The methods for the active substance DEET and the impurities in the technical active substance are described in detail in the Annex I dossier. This also holds for the residue analytical methods for DEET.

For the product, a HPLC-UV method (ANA-00237) was provided (de Vries, E.S., 2012).

Specificity
Chromatograms of a blank, calibration standard and products (20% spray, 40% spray, 50% spray, 30% gel and 30% roll-on) were provided.

Linearity
A grand total of six linearity determinations were reported:
Set 1: 0 – 36.8 and 0 – 49 mg/L, in both cases r^2 = 0.99996, n=5 and n=6 respectively
Set 2: 0 – 36.8 and 0 – 49 mg/L, r^2 = 0.9996 and 0.99990, n=5 and n=6 respectively
Set 3: 0 – 36.8 and 0 – 49 mg/L, r^2 = 0.99972 and 0.99986, n=5 and n=6 respectively

The method is linear over a range of 0 – 49mg DEET/L.

Accuracy
The recovery values reported below are based on products with information on the factory mixing ratio's. This is considered a worst-case situation compared to spiking a blank formulation.

Care Plus Anti-Insect DEET	recovery (%)					
	individual samples					mean
Spray 20%	98	105	105	104	105	103
		105	105	104	105	105*
Gel 30%,	101	103	100	102	103	102**
Roll-on 30%	101	104	103	102	102	102**
Spray 40%	99	101	101	101	99	100
Spray 40%	99	99	99	100	100	99
Spray 50%	99	101	101	101	101	101
Lotion 50%	101	99	101	100	99	100
* not taken into further consideration. ** identified as statistically significant different from 100, p (two-tailed) = 0.0367 and 0.0093 for the gel and roll-on respectively (One sample t-test 2014, GraphPad Software, Inc.)						

Because, the accuracy levels found for the 20% Spray were not within the acceptable range, on lab scale, the 20% and 30% spray were formulated at lab-scale. This procedure is comparable to spiking a blank formulation. Data generated using these samples show that the recoveries of the active substance are within acceptable limits.

Care Plus Anti-Insect DEET	recovery (%)					
	individual samples					mean
Spray 20%*	105	103	103	103	98	102
Spray 20%*	103	103	103	102	101	102**
Spray 30%	100	100	100	100	100	100
* the 20% sample was analysed twice. The second set of measurements was performed on the same is the measurements in the 30% formulation. ** identified as statistically significant different from 100, p (two-tailed, One sample t-test 2014, GraphPad Software, Inc.)						

Precision

Care Plus Anti-Insect DEET	Sample no.	std	std rel. (%)	Horwitz RSD (repeat ability)
Spray 20%	1408149-01	0.594	2.88*	1.71
Gel 30%,	1408149-02	0.344	1.12	1.61
Roll-on 30%	1408149-03	0.365	1.19	1.61
Spray 40%	1408149-04	0.434	1.08	1.54
Spray 40%	1408149-05	0.324	0.82	1.54
Spray 50%	1408149-06	0.458	0.91	1.49
Lotion 50%	1408149-07	0.354	0.71	1.49

** 0.68% not taking into account an outlier*

Every sample was injected 5 times, resulting in the standard deviations reported in the table above. One measurement of the data set for the 20% Spray was significantly lower than the other four (individual values 19.6, 21, 20.9, 20.7, 21). Not taking into account the value 19.6, the RSD would be 0.68%. Based on a Grubbs test, the value was considered an outlier.

The applicant has provided additional precision data for the 20% Spray product because it was not possible to retrieve the cause of the outlier. Five injections of the product resulted in concentrations found in the range of 19.6 – 21.0 %w/w with a RSD of 2.53%.

The lab reasoned that due to the relatively low concentration of DEET in the product compared to the other products, part of the sample may evaporate. Analysis was again repeated, also including the 30% Spray, taking additional care (capped sample vials) to avoid evaporation of solvents. With the additional measures, the RSD was 0.80% for the 20% Spray and 0.18% for the 30% Spray (n = 5).

Based on the above, the method used is considered sufficiently precise.

Method description

The analytical method is based on dissolving about 0.2g of sample in ethanol in a 50mL volumetric flask. Any further dilution required is performed in ethanol prior to HPLC analysis.

HPLC conditions: RP-18 column (Merck Lichropshere 100 125-4), 0.8 mL/min flow, 10µL injection volume, 10% methanol eluent, detection at 205nm.

Analyte	Principle of method
Technical active substance as manufactured:	GC-FID

Impurities in technical active substance:	GC-FID with GC-MS for confirmation of the identity
Active substance in the formulations	HPLC-UV

Residue analytical method in air
The EU review of DEET concluded that a residue analytical method for air may be required at the product authorisation stage.

The Technical Notes of Guidance state that an analytical method is required if the vapour pressure exceeds 0.01 Pa and/or the product is sprayed or occurrence in air is otherwise probable (IIA4.2b). Therefore considering the vapour pressure of DEET and the intended use, a residue analytical method is required.

A new residue analytical method (Miller, C., 2013) was developed and validated according to SANCO/825/00 rev 8.1, which adequately addresses the requirements of the Regulation.

Method description
Air is drawn through a Tenax cartridge for 6 hours at 1L/min (360L air) at 35℃ and 80%RH and at 20℃ and 30%RH, followed by desorption with acetone and dilution in methanol, followed by analysis by HPLC-MS/MS with external standardisation.

Conditions

Instrument:	AB Sciex API 4000 (Analyst 1.4.2 software), Waters Acquity UPLC		
Mode:	Ion spray		
Column:	C18, 2.1mmx50mm, 1.7µm.		
Mobile phase A:	water:methanol:formic acid (90:10:0.1 v:v:v) + 0.01M ammonium formate		
Mobile phase B:	methanol:formic acid (100:0.1 v:v)		
Gradient	Time	%A	%B
	0	40	60
	1	40	60
	1.5	5	95
	2.5	5	95
	3	40	60
	4	40	60
Injection volume:	10 µL		
Flow rate:	0.5 mL/min		
Retention time:	approx. 0.6 minutes		

Validation data
The method validation is reported in table 2.3.2-1.

No matrix effect of the Tenax sorbent was observed.

Discussion and conclusion
The LC-MS/MS method submitted is acceptable and complies with SANCO/825/00 rev 8.1 and TNsG validation requirements. The required LOQ of 0.225mg/m^3, based on the lowest AEL_{acute} of 0.75 mg/kg bw/day, is met.

At 35℃ and 80%RH breakthrough was detected at 10% of the nominal fortification rate. At 20℃ and 30%RH, the breakthrough was 6%. The lab co nsiders this to be acceptable. Considering the accuracy (87 – 110% overall, mean 90 – 103% per fortification level) and the repeatability (RSD ≤6% per fortification level) of the method are acceptable, the breakthrough volume of up to 10% still allows sufficiently accurate measurements and is therefore considered a minor deficiency.

In August 2011, RMS Sweden evaluated additional data, including a residue analytical method for water (Sadgrove, L., 2010). This method was validated using two transitions (192->119m/z and 192->91m/z). Considering the method for water is highly specific, the method for air can be considered highly specific as well. No additional confirmatory method is required.

Table 2.3.2-1 Validation data for the residue analytical method for air

Target analyte	Method / equipment	Specificity	Linearity	Accuracy (min-max (mean)) (%)		Repeatability (% RSD)	Reference
DEET	LC-MS/MS 192->119 m/z 35℃, 80%RH	No interference	0.2 – 5ng/L, n=9 r^2 = 0.9994 y=799154x+ 54527.8	Control (n=2)	Not detected	-	Miller, C., 2013
				0.225mg/m^3	101 - 105 (103)	1.7 (n=5)	
				2.25mg/m^3	87 – 93 (90)	3.3 (n=5)	
	LC-MS/MS 192->119 m/z 20℃, 30%RH	No interference		Control (n=2)	Not detected	-	
				0.225mg/m^3	94 – 110 (101)	6.0 (n=5)	
				2.25mg/m^3	97 - 105 (101)	2.8 (n=5)	

2.4 Risk assessment for Physico-chemical properties

Care Plus DEET Anti-Insect 50% Spray is a colourless liquid with a characteristic odour. It is not explosive, not oxidising, but based on its flashpoint, is considered highly flammable (flammable liquid cat 2). A determination of the pH was not performed and is not considered necessary for this type of product. The product is ready to use, therefore no technical characteristics need to be investigated. In HDPE, the product is expected to remain stable for at least 5 years.

2.5 Effectiveness against target organisms

2.5.1 Function

This product is an insect repellent (PT19) based on 48.5% DEET (w/w).

2.5.2 Organisms to be controlled and products, organisms or objects to be protected

Care Plus Anti-insect DEET 50% Spray is used to repel ticks and mosquitoes, in particular house mosquitoes, malaria mosquitoes and yellow fever mosquitoes.

This product is an insect repellent for outdoor use, or indoor use in well ventilated areas, that should be applied to the skin of exposed body parts with the purpose to protect the uncovered skin of humans against biting ticks and mosquitoes.

2.5.3 Effects on target organisms

Care Plus Anti-insect DEET 50% Spray differs from the product described in the CAR of DEET since the concentration of the active substance is higher. Therefore new laboratory studies have been provided with the mosquitoes *Culex quinquefasciatus*, *Anopheles gambiae* and *Aedes aegypti* and the tick *Ixodes ricinus*. Note that these studies have been performed using Care Plus DEET Anti-insect Lotion 50%. Since the lotion and the spray

have a comparable composition, results of the lotion are assumed to be representative for Care Plus Anti-insect DEET 50% Spray. The results are described in B5.10-03 and B5.10-04 and are summarised in Table 2.5.3.0 below.

Table 2.5.3.0: *Efficacy of the active substance from its use in the biocidal product - Care Plus DEET Anti-insect Lotion 50%.*

Test substance	Test organism(s)	Test system/concentrations applied/exposure time	Test results: effects, mode of action, resistance*	Reference
Care Plus DEET Anti-insect Lotion 50%	*Culex quinquefasciatus*	Arm-in-cage test: 5 volunteers exposed both treated forearms (2 products were tested at the same time, approx 1.67µl repellent per cm^2) into the test cages with mosquitoes (approx. 1000 mosquitoes of a mixed population in terms of sex and age, meaning about 500 "blood thirsty" female mosquitoes). Exposition was started 1 hour after treatment of the volunteer forearms, and the test ended 11 hours after treatment. During that time the test was performed at intervals of one hour. The exposure time of volunteer forearms was 5 minutes per interval. The number of bites as well as number of landings was recorded. The tests ended when the relevant volunteer got two bites within one test interval or within two subsequent test intervals. Biting activity was checked with an additional volunteer (negative control, untreated forearm area of approx. 25 cm^2). No positive control. Test method: BPD BioG B591-01 (according to the guidelines of EPA)	One person received the first bite after 9 hrs, the four other persons received the last bite after 11+ hrs (could not be determined as test was stopped after 11 hrs). This results in an average PT of 10 hrs.	B5.10-04
Care Plus DEET Anti-insect Lotion 50%	*Anopheles gambiae*	Test description: see above. For one person the test was stopped after 10 hrs.	One person received the first bite after 9 hrs, for one person the test was stopped after 10 hrs (10+), the three other persons received the last bite after 11+ hrs (could not be determined as test stopped after 11 hrs). This results in an average PT of 10 hrs.	B5.10-04
Care Plus DEET Anti-insect Lotion 50%	*Aedes aegypti*	Test description: see above. The test was ended 15 hours after treatment.	Two persons received the first bites after 7 hrs, the last one after 14 hrs. This results in an average PT of 9 hrs.	B5.10-04
Care Plus DEET Anti-	*Ixodes ricinus*	The forearm of 10 volunteers (5 male, 5 female) was treated from the elbow to the wrist with approx 1.67µl repellent per cm^2,	None of the ticks crossed the second mark on the treated	B5.10-03

insect Lotion 50%		leaving the lowest 5 cm of the arm near the wrist untreated. The arm was held vertically and a tick was placed on the untreated area, 1 cm below the treated area (first mark). Ticks remaining motionless or walking down the arm were stimulated to walk in direction of the treated area by means of a clean hairbrush. Ticks falling off from the untreated zone were again placed on the starting line. Ticks not walking onto the treated zone within one minute were removed, scored as "repelled" and replaced by a new tick. Ticks walking onto the treated skin were given an additional 1 min to cross the second mark 3 cm in direction to the elbow. If the tick, within that time period, did not cross the second mark, it was removed, scored as "repelled", and replaced by a new tick. Ticks crossing the second circular mark were likewise removed, but scored as "not repelled". A total of five ticks were tested every 30 min on each volunteer up to 5 hours after application of the repellent. Ticks were screened for activity on the untreated control arm of the same volunteer (negative control); only ticks that walked up and crossed the second mark on the control arm were further used for testing. Test method: EPA OPPTS 810.3700	skin during the 5 hours of the test, resulting in a mean PT of at least 5 hours.	

* PT = protection time, defined for mosquitoes as the time from application until two bites within one test interval were received or when two bites within two subsequent test intervals were received and for ticks as the period between the time of application of the repellent to time of a tick crawling onto human skin and crossing the second mark on the treated skin.

These tests show the efficacy of Care Plus DEET Anti-insect Lotion 50% against mosquitoes in simulated-use tests (arm-in-cage) and against ticks in a laboratory test. The results of the arm-in-cage studies show that Care Plus DEET Anti-insect Lotion 50% repels *Culex* spp, and *Anopheles* spp for an average of 10 hrs; *Aedes* spp. are repelled for on average 9 hrs (minimum 7 hrs). The laboratory test with *Ixodes ricinus* shows that ticks are repelled for at least 5 hours. The product was not tested against tropical tick species, some of which are known to be more aggressive.

The Draft TNsG on PT18 and PT19* states that to show efficacy of products intended for use as repellent on skin or clothes against mosquitoes both simulated-use tests (arm-in-cage) **and** field studies showing repellence in the field need to be provided. However, this guidance was not available during the process of data collection by the applicant and in line with the draft note for guidance discussed at PA&MRFG** 'competent authorities should therefore accept data based on the latest available guidance published (or applicable) on the date when the applicant can reasonably be expected to start collecting data, and not require re-alignment to any subsequently published guidance for the purpose of granting authorisation or mutual recognition'. In the TNsG on product evaluation*** that was available during data collection, no details are given on the data requirements for repellents. The CA of the Netherlands is of the opinion that the simulated-use laboratory

tests (arm-in-cage studies) are worst case scenarios and that field studies can be waived under the prerequisite that comparable product, comparable dosage and a sufficient number of test persons are used in lab studies provided. In recent dossiers 10 test persons are required per test, in this case only 5 test persons were used. The tests were done well and the data from different Care Plus Anti-insect DEET products with different concentrations of DEET are consistent and thereby support each other. The tests against ticks was done with 10 test persons, conform the accepted standard test method.

The CA NL is therefore of the opinion that the tests are acceptable and support the claim for the product Care Plus Anti-insect DEET 50% Spray for repellence of mosquitoes, including more aggressive tropical species. The average protection time against Culex and Anopheles is 10 hours, against Aedes the average protection time is 9 hours. For the claim of repellency of ticks a good test was provided against the European sheep tick. The protection time against ticks is at least 5 hour. Some tropical tick species, however, are known to be more aggressive. No tests against these species was provided. Therefore a warning will be added to the SPC and to be put on the label that the product may be less effective against certain tropical tick species. This to warn the traveller to tropical countries using the product to stay alert.

*BPD 98/8/EC: Technical Notes for Guidance: TNsG on Product Evaluation, Insecticides, acaricides and products to control other arthropods (PT 18) and Repellents and attractants (only concerning arthropods) (PT 19). *European Commision, Directorate-General Environment,* CA-Sept10-Doc.6.2b

****Draft note for guidance. Relevance of new guidance becoming available during the process of authorisation and mutual recognition of authorisations of biocidal products. CA-July 12-Doc.6.2d. PA&MRFG-July 12-Doc.8.

***TNsG on Product Evaluation, ECB, February 2008.

2.5.3.1 Dose

The active substance is incorporated in a ready for use spray at a concentration of 48.5 % and is used by the general public (non-professional users). The product is used as a topical application of exposed body parts and should be used only once a day.

2.5.3.2 Mode of action

DEET repels biting and sucking insects without time delay. The mechanism of action of the active ingredients in insect repellents is not revealed yet; however, their effectiveness is determined experimentally.

2.5.3.3 Limitations

No limitations for efficacy are mentioned.

2.5.3.4 Resistance and resistance management strategy

There is no known instance of target insects developing resistance to DEET. It is unlikely that resistance will occur for DEET, since there is only low selection pressure because the insects that are repelled do not die, and there are many other food sources available for these insects. Therefore, it is considered unnecessary to take actions to prevent development of resistance by target organisms.

2.5.4 Evaluation of the label claim

Both a Dutch and an English SPC are added to the PAR (see 1.5.2).
A warning is added that the product may be less effective against certain tropical tick species.

2.6 Exposure assessment

2.6.1 Assessment of exposure to humans and the environment

General information toxicology

The applicant has submitted an effect and exposure assessment for the product Care Plus DEET Anti-Insect 50% Spray. The human health exposure and risk assessment of the Care Plus DEET Anti-Insect 50% Spray is examined by the eCA NL appropriately according to standard requirements. One new human toxicological study with the product Care Plus DEET Anti-Insect 50% Spray has been provided. No new studies have been provided concerning the active substance and human health exposure. The product was not a reference product in the EU-review program for inclusion of the active substance in Annex I of Directive 98/8/EC. The eCA NL has revised this risk assessment for the human health aspect. See for more detail section 2.7.

General information environment

The product was not a reference product in the EU-review program for inclusion of the active substance in the Union list of approved substances of EU Regulation 528/2012. The applicant has not submitted an effect and exposure assessment for Care Plus Anti-Insect DEET 50% Spray. Therefore the RMS NL has included a risk assessment for the environmental aspect. See for more detail section 2.8 below.

2.7 Risk assessment for human health

Care Plus DEET Anti-Insect 50% Spray is a ready-to-use spray for non-professional use containing N,N-diethyl-*m*-toluamide (DEET) at concentration of 48.5% w/w (pure active substance). During the Annex I active review stage a product with an DEET concentration of 15% has been evaluated.

For this authorisation application, no new studies were submitted with the active substance or concerning human exposure that were not already evaluated during the Annex I active review stage. Detailed data on the toxicity of the active substance can be consulted in Doc IIA of the final Assessment Report (March 2010) for DEET, PT19.

The product Care Plus DEET Anti-Insect 50% Spray was not a reference product in the EU-review program for inclusion of the active substance in Annex I of Directive 98/8/EC. A skin irritation study was submitted with a comparable product (see 2.7.1.3 for results). This study has not been evaluated in the CAR of DEET. The applicant has submitted statements to address acute oral, dermal, inhalation toxicity, eye irritation and sensitisation based on the calculation rules according to CLP-Regulation (EC) 1272/2008. For dermal absorption of DEET the applicant provided a statement that the value of 20% used in the CAR of DEET can be used in the risk assessment.

2.7.1 Hazard potential

2.7.1.1 Toxicology of the active substance

The toxicology of the active substance was examined extensively according to standard requirements. The results of this toxicological assessment can be found in the CAR. The threshold limits and labelling regarding human health risks listed in Annex 4 “Toxicology and metabolism” must be taken into consideration.

2.7.1.2 Toxicology of the substance(s) of concern

The product Care Plus DEET Anti-Insect 50% Spray contains ethanol as a potential substance of concern. The content of ethanol in the formulation is 47%.

Ethanol is notified according to the biocides review programme (for PT1-4). A draft CA-report is not yet available. For ethanol a Council's Dutch Expert Committee on Occupational Standards (DECOS) evaluation (2006) is available. Although according to the EU-draft guidance on substances of concern a quantitative evaluation for ethanol is not necessary in the EU, the eCA NL performed a risk characterisation for ethanol based on the following List of Endpoints.

List of Endpoints

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands sets health-based recommended occupational exposure limits for chemicals in air
at the workplace in 2006. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards (DECOS).
Epidemiological studies suggest that consumption levels below 10-12 grams of ethanol per day will probably not cause liver cirrhosis. However, the Committee on Alcohol consumption
and reproduction concluded that at these consumption levels effects on fertility and development have been reported. Even long term oral exposure to levels of 1-12 gram ethanol
per day might result in effects on the development (like increased incidence of spontaneous
abortion, foetal death, pre-term delivery and decreased length of gestation) and fertility, according to the Committee on Alcohol consumption and reproduction. Considering the fact that the maximal alcohol concentration in blood after one (oral) drink is approximately 10-100
times higher than the ethanol concentration in blood after inhalatory exposure to 1300 mg/m^3, the committee was of the opinion that a HBC-OCRV (Health based calculated occupational
cancer risk value) of 1300 mg/m^3 is low enough to protect against these effects. Other toxic effects manifest after exposure to higher exposure levels. DECOS calculates an HBC-OCRV of
1300 mg/m^3, resulting in a breast cancer risk of 4 additional death cases per 1000 ($4*10^{-3}$) deaths for 40 years.
In addition, DECOS recommends a short term exposure limit (STEL) of 1900 mg/m^3 TWA 15
minutes and a skin notation, as dermal exposure can substantially contribute to the body burden of ethanol.
Furthermore, in 2008 a worker exposure limit of 260 mg/m^3 8h-TWA value and the 1900 mg/m^3
15 min-TWA were set by the Dutch Health Council.
From the available meta-analysis and pooled studies, the committee concluded that drinking of one glass of alcoholic beverage per day the internal intake will be 10 gram ethanol.

In the report of DECOS it is stated that, as a worst case estimate, a penetration rate of 0.7 $mg/cm^2/h$ can be used to calculate the internal dose after dermal exposure. Although there are no exact values available for dermal absorption of ethanol, values of 1-2% dermal absorption are usually used for ethanol based on studies and the penetration rate recommended by DECOS in the Netherlands. The EFSA guidance on dermal absorption (2012)[1] recommends the value of 25% for formulations containing >5% substance.

[1] EFSA Guidance on dermal absorption. EFSA Journal 2012;10(4):2665

Therefore the eCA NL has performed the risk assessment by considering two values for dermal absorption of ethanol: 25% and 1-2%.

2.7.1.3 Toxicology of the biocidal product

The toxicology of the biocidal product was examined appropriately according to standard requirements. The product was not a reference product in the EU- review program for inclusion of the active substance in Annex I of Directive 98/8/EC.
The basis for the health assessment of the biocidal product is laid out in Annex 4 "Toxicology – biocidal product".

A GLP-compliant skin irritation study with the product has been submitted by the applicant to address skin irritation. The results of this study is presented below. A sample of a comparable product (50% DEET) was tested for acute dermal irritating properties in an experiment with three albino rabbits, according to EEC Directive 92/69/EEC, method B.4 and OECD Guideline no. 404.

At 1 h after the patch removal, very slight erythema and very slight oedema were observed in the rabbits. At 24 h and 48 h after the patch removal, well-defined erythema and slight oedema were observed in the three rabbits. In addition, slight scaliness was observed in all rabbits. The average scores for erythema and oedema at 24 h are 2 for erythema and 2 for oedema and at 48 hours 1.7 for erythema and 2 for oedema.

At 72 h after the patch removal, very slight or well-defined erythema, slight scaliness and very slight or slight oedema were observed in the three rabbits. The average scores for erythema and oedema at 72 h are 1.7 for erythema and 1.7 for oedema. At 7 days after removal, no signs of skin irritation were observed in any of the three rabbits.

2.7.2 Exposure

The product Care Plus DEET Anti-Insect lotion 50% contains the active substance DEET and the substance of concern ethanol.

The intended use of the product is exclusively by dermal application. The exposure assessment is based on a maximum application frequency of 1 time per day, as indicated in the instructions of use. Dermal route is the main path of exposure, but contributions to exposure via inhalation of the product during application of the repellent and via hand to mouth contact are possible. In the CAR of DEET it has been concluded that inhalation exposure cannot be fully ruled out and therefore a recommendation on ventilation is considered necessary. Moreover, based on the vapour pressure of DEET of 0.11 Pa at 20 ºC and 0.23 Pa at 25 ºC and considering that the formulation will be applied by spraying, respiratory exposure can potentially occur. The product can be applied indoors and outdoors. By using the safety phrases P260 according to Regulation 1272/2008/EC ("Do not breathe spray") and P271 ("Use only in well ventilated areas") according to Regulation 1272/2008/EC inhalational fraction can be excluded in the risk characterisation calculations.

Oral exposure by hand-to-mouth transfer is not considered to be a significant route of exposure because the smell and taste of DEET acts as a self deterrent against this type of activity. More importantly, the product contains an ingredient that acts as a strong deterrent for ingestion (Bitrex). However, the efficacy of Bitrex was discussed at a Technical Meeting where it was concluded that Bitrex may not be effective in preventing ingestion in all age groups, in particular children < 12 years old. Therefore the oral route is still considered to be possible and the calculations for hand to mouth transfer are included by the ECA NL in the worst case exposure calculations. The potential for exposure to DEET is summarized in the table below.

Potential for exposure to DEET:

Exposure path	Industrial use	Professional use	General public	Via the
Inhalation	-	-	X	-
Dermal	-	-	X	-
Oral	-	-	X	-

2.7.2.1 Exposure of professional users

The product is not intended for professional use.

2.7.2.2 Exposure of non-professional users and the general public

In Annex 6 “Safety for non-professional operators and the general public”, the results of the exposure calculations for the active substance for the non-professional user are laid out.

Active substance DEET:
A user survey study has been performed in the USA involving human use and exposure to insect repellents containing DEET (Boomsma and Parthasarathy, 1990 (III-A6.14)). This study is part of the data package for DEET and is presented in Doc III of the final CAR. The human health exposure scenario for adult consumers at the 75th percentile of use, applying the representative product containing DEET as an insect repellent was used for the risk characterizations. The use of the 75th percentile was considered acceptable since the user study had a large number of study subjects and the measured exposure was similar to the default exposure value of the TNsG. In this study, the average active ingredient content was estimated to be 26.1%. The 75th percentile of human dermal exposure per application of the formulation containing 26.1% DEET is estimated to be 1.5 g active substance for males, 1.0 g for females, 1.66 g for children aged 13-17 years and 1.42 g for children aged <12 years based on the results of the survey study. Daily exposure for different age groups was calculated by considering a body weight of 70, 60, 62.8 and 25.5 kg for males, females, children > 12 years of age and children <12 years of age, respectively. The same values for body weight were also used in the CAR of DEET.

Exposure due to hand to mouth transfer has also been included in the calculations as a worst-case approach. According to the TNsG on human exposure, part II, 2002 it is expected that adults will ingest the amount applied to fingers. The surface of the fingers is approximately 4% of the treated body surface. For the age groups 13-17 years and < 12 years the ingested amount is considered to be the amount on the whole hands, i.e. approximately 8% of the treated body surface (head, arms, hands, legs and feet according to US EPA Child-Specific Exposure Factors Handbook, 2002).

A dermal absorption value of 20% was used to calculate internal exposure in humans.

Substance of concern ethanol:
Based on the USA user survey study with DEET-containing repellants the 75th percentile of human dermal exposure per application is estimated to be 5.75 g product for males, 3.83 g product for females, 6.36 g product for children aged 13-17 years and 5.44 g product for children aged <12 years. As a consequence for Care Plus DEET Anti-Insect 50% Spray with an ethanol concentration of 45.5% the 75th percentile of external dermal exposure per application is estimated to be 2.62 g ethanol for males, 1.74 g ethanol for females, 2.89 g ethanol for children aged 13-17 years and 2.48 g ethanol for children aged <12 years.

Indirect exposure of general public
The degree of indirect exposure is considered negligible as the primary route of exposure is direct application to the skin.

2.7.2.3 Exposure to residues in food

The application of the formulation does not result in residues to which consumers might become exposed.

2.7.3 Risk Characterisation

2.7.3.1 Risk for Professional Users

The product is intended for non-professional use only.

2.7.3.2 Risk for non-professional users and the general public

Active substance DEET:
It was decided at TM I and II 2009 that risk characterisation for DEET products should be performed for two daily applications and by using the 75th percentile of human dermal exposure based on the USA survey study. However, the instructions of use provided by the applicant indicate a maximum exposure frequency of once per day. When considering the application rate of once per day and 75th percentile from the USA survey study, the estimated exposures to DEET after dermal application of Care Plus DEET Anti-Insect Spray 50% in percentages of the $AEL_{repeated\ dermal}$ for adult males, adult females, children >12 years and < 12 years are presented in Table 1.

Table 1. The ratio of the estimated dermal exposure to DEET to $AEL_{repeated\ dermal}$ for Care Plus DEET Anti-Insect 50% Spray. One application per day has been considered.

48.5% DEET	**Exposure/$AEL_{repeated\ dermal}$**
Dermal	
Male:	0.97
Female:	0.76
>12 yr:	1.20
<12 yr:	2.53

If only dermal exposure is considered, the use of the product Care Plus DEET Anti-Insect 50% Spray, once per day is considered acceptable only for adults. Furthermore, reverse reference calculations in Annex 6 show how many times per day the formulation can be applied dermally without exceeding the AELs. For example, if only dermal exposure is considered, to exceed the $AEL_{repeated\ dermal}$ of 8.2 mg/kg bw/day, Care Plus DEET Anti-Insect 50% Spray can be applied 1.03, 1.32, 0.82 and 0.4 times per day for adult male, adult female, child >12 years and <12 years respectively. Thus for children < 12 years old and for children > 12 years old the application once per day is not considered acceptable.

As a worst-case approach, the eCA has also performed the assessment of the oral exposure, considering potential ingestion of 4% of the total applied product by adults (amount on fingers). No separate oral exposure assessment was performed for children (< 17 years old), as no acceptable risks were identified for this age group when only dermal exposure was considered. The resulting oral exposure estimates were compared with $AEL_{acute\ oral}$ of 0.75 mg/kg bw/day. From the calculation given in Annex 6 it can be seen that higher risk characterization ratios are calculated for oral exposure in comparison with dermal exposure. The reverse dose calculations in Annex 7 further show that for Care Plus DEET Anti-Insect 50% Spray only 1.9% and 2.4% of the estimated external dose per application at the 75th percentile of use for adult males and adult females, respectively, can be ingested before an $AEL_{acute\ oral}$ of 0.75 mg/kg bw/day is exceeded. If as a worst-case an ingestion of 4% of the applied product is considered for adults, the exposure area in adults

would have to be reduced to avoid exceeding the AEL. However, in the PA-MRFG meeting it has been agreed that labelling instructions with the intent to reduce the treated skin area are not accepted as an adequate risk mitigation measure; thus this restriction cannot be considered by the eCA NL.

However, in the CAR of DEET it was concluded that the oral dose is likely to be largely overestimated given the short half life after oral exposure in dogs and rats and the rapid achievement of C_{max}. The hand to mouth behaviour is more frequent in small children, and considering the presence of Bitrex in the formulation, it was concluded in the CAR that the contribution of oral exposure for children > 2 years old and adults is considered negligible. Respectively, an age limit of 2 years is proposed in the CAR of DEET as a cut-off for considering oral exposure. As a consequence the contribution of oral exposure due to use of Care Plus DEET Anti-Insect 50% Spray is considered to be negligible for adults.

In summary, based on the risk assessment the application of Care Plus DEET Anti-Insect 50% Spray once per day is considered acceptable only for adults (above 17 years old). As a consequence the use of Care Plus DEET Anti-Insect 50% Spray on children (younger than 17) cannot be authorised.

Substance of concern ethanol:
Based on the survey study the 75th percentile of human dermal exposure per application of Care Plus DEET Anti-Insect 50% Spray is estimated to be 2.62 g ethanol for males, 1.74 g ethanol for females, 2.89 g ethanol for children aged 13-17 years and 2.48 g ethanol for children aged <12 years. Although the exact dermal absorption percentage is unknown, the values of 1-2% are usually used in the Netherlands based on studies and the penetration rate recommended by DECOS. The EFSA Guidance on dermal absorption recommends a value of 25% for formulations containing > 5% substance. As the use of Care Plus DEET Anti-Insect 50% Spray in children is not considered acceptable, only the exposure calculations for adults have been performed by the eCA NL. If as a worst-case 25% dermal absorption is considered, the expected internal dermal exposure to ethanol will be 6.6% (2.62 x 0.25/10)x 100%) of the expected ethanol intake by drinking one glass of alcoholic beverage (10 g ethanol per day) for adult males and 4.4% for adult females. The 1-2% dermal absorption percentages result in internal dermal exposure of 0.26-0.52% of the expected ethanol intake by drinking one glass of alcoholic beverage (10 g ethanol per day) for adult males and 0.17-0.34% for adult females. Based on these results the e-CA NL concludes that no unacceptable risk results from the presence of ethanol as a substance of concern in the formulation Care Plus DEET Anti-Insect 50% Spray.

Conclusions
Because the product Care Plus DEET Anti-Insect 50% Spray is intended for intentional exposure on skin and to be used by the general public, including elderly and unhealthy subjects, a conservative approach based on the risk characterisation should be taken when approving the product.

A recommendation on ventilation should apply since the inhalational fraction is excluded in the risk characterisation calculations. Therefore Care Plus DEET Anti-Insect 50% Spray needs to be carrying safety phrases P260 according to Regulation 1272/2008/EC (“Do not breathe spray”) and P271 (“Use only in well ventilated areas”) according to Regulation 1272/2008/EC.

The use of the product Care Plus DEET Anti-Insect 50% Spray once per day is considered acceptable only for adults. The product must not be used on children < 17 years old. Therefore, the restrictions “Do not use more than once a day” and “Do not use on children (<17 years old)” have to be written on a prominent position on the label.

2.7.3.3 Risk for consumers via residues

The acute or chronic exposure to residues in food resulting from the intended uses is unlikely to cause a risk to consumers. Regarding consumer health protection, there are no objections against the intended uses. The restriction “Avoid contact with food” has to be written on a prominent position on the label.

2.8 Risk assessment for the environment

2.8.1 Effect Assessment

No studies were submitted with the product authorisation application for the active substance or for the product that were not already evaluated during the review stage for inclusion of DEET in the Union list of approved substances of EU Regulation 528/2012. Detailed data on the fate and distribution of DEET in the environment and the effect of the active substance on environmental organisms can be consulted in Doc IIA of the final Assessment Report (March 2010) for N,N-diethyl-*m*-toluamide (DEET, PT19). Fate and effects data are only provided in this Assessment Report for the parent structure, as DEET is ready biodegradable and no major (>10%) transformation products were formed in studies of hydrolysis and aquatic phototransformation.
The PNEC derivation is also described in detail in the Assessment Report for DEET, section 4.3.1 of Doc IIA and a summary is included in the table below.

Table 2.8.1-1 Summary of the PNECs derived for DEET in the different compartments.

Compartment	Organism	Endpoint	AF	PNEC
Freshwater	Green algae (*Selenastrum capricornutum*)	ErC_{50} = 43 mg/L	1000	0.043 mg/L
STP	Microorganisms from an activated sludge	EC_{50} > 1000 mg/L	100	10 mg/L
Sediment	Sediment-dwelling organisms	Equilibrium partitioning	-	0.0741 mg/kg ww
Soil	Green algae (*Selenastrum capricornutum*)	Equilibrium partitioning	-	0.0379 mg/kg ww

PNECs were not calculated for the air compartment, as there are no data on biotic effects in the atmosphere. Furthermore, DEET is not expected to be subject to long range air transport (half life is less than 2d), or contribute to global warming (although the substance has a vapour pressure (0.23 Pa) higher than 0.01 Pa, the Henry´s law constant is low (3.93E-3 Pa*m^3/mol and DT50 is less than 2d; cf the TNsG on Annex I inclusion), ozone depletion in the stratosphere (atmospheric lifetime is <<1 year, and it does not contain Cl, Br or F substituents) or acidification (the AP, Acidification Potential is low[2]).

The available avian acute lethality data are not appropriate for extrapolation to chronic dietary uptake conditions (cf TGD II3.8.3.5). PNECs were therefore not calculated for oral uptake from the food chain (to quantify the risk of secondary poisoning). No further avian data were required, because DEET has a low potential for bioconcentration and bioaccumulation (log Kow <3; cf TGD II3.8.2) and primary poisoning is considered not relevant for this type of product. In addition, DEET is extensively metabolized and excreted through the urine in all assessed mammals.

[2] De Leeuw F. 1993. Assessment of the atmospheric hazards and risks of new chemicals: Procedures to estimate ”hazarard potentials”. Chemosphere 27(8): 1313-1328. AP=(MWSO2/MWDEET)*(nN+ nCl + nF + 2*nS)/2= (64.06/191.28)*1/2 = 0.17).

2.8.2 Exposure Assessment

Major emissions from the application of mosquito and tick repellents result from indoor showering or bathing with emission via the STP to surface water and sediment (waste phase). Direct emission to surface water and sediment can result from outdoor showering or bathing after application of the product on the skin (waste phase).
Emission to fresh water is expected to be worst case. Therefore risk for the marine environment is considered covered by the freshwater risk assessment.
For the proposed applications emissions during the application phase and the service life of the products are also considered less relevant and these routes are therefore not assessed.

Indirect emission

The water compartment (both inland and marine) is expected to be indirectly exposed to DEET mainly from STP effluents, and because of the physiochemical character of the substance, the emissions will continue to primarily remain in this compartment (supported by level III fugacity modelling). The most relevant environmental compartment of concern for DEET is therefore the aquatic.
According to a usage study described in Boomsma & Parthasarathy (section III-B6.6(2) of the final CAR of DEET), on average 1.2 g of active ingredient of a repellent containing 20% DEET is consumed per application, of which 0.9 g (75%) is applied to the skin and 0.3 g (25%) to the clothes. One can also assume some of the product to be "spilled" during application (a direct release to the air compartment) and absorbed by the skin during the "leave on phase''.
In IC5, UC36 (cosmetic odour agents; p 226 in the TGD II), 5% of the applied amount (for substances having vapour pressure below 100 Pa) is assumed to be emitted to the air. This figure was therefore adopted. All absorbed DEET (6.4%) is assumed to be metabolized (and excreted primarily as urine metabolites). Therefore, the rest of the initially applied dose (88.7%) is assumed to be released to the STP (see Figure 1).

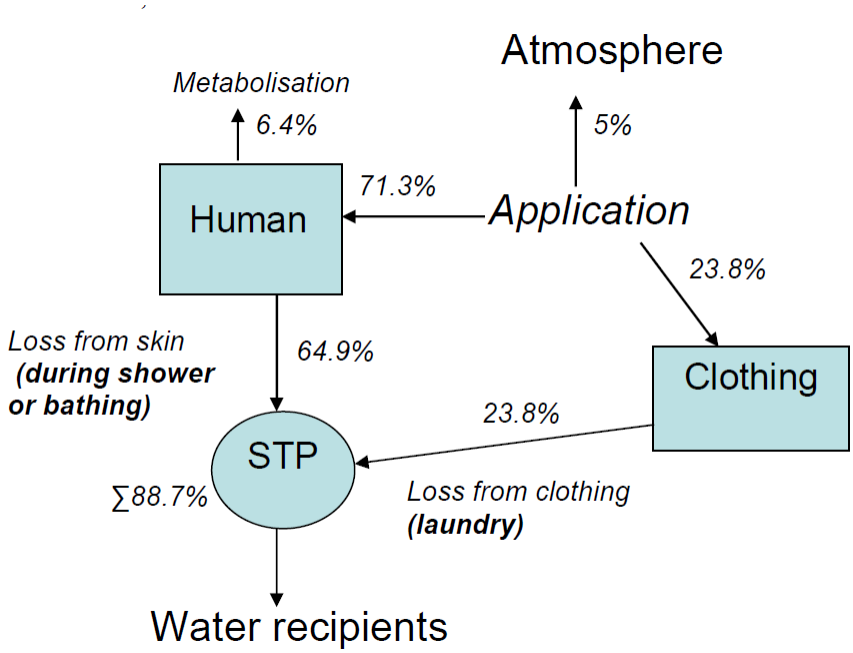


Figure 1 Assumed flows of DEET into the STP and environment. All percentages are referring to the initially applied dose.

Final environmental exposure will to a large extent depend on whether households are connected to STPs equipped with at least secondary (biological) treatment. Other efficient

treatment processes include ozonation and PAC (Powdered Activated Carbon) addition, although these are more common in drinking water treatment[3].
In the following sections, PECs are derived by using the draft Emission Scenario Document (ESD) for PT19 (repellents and attractants). These calculations are based on data on amount consumed by individuals. The TNsG on human exposure sets a default value for the amount of dermally applied repellent product to 6 g. Estimated PEC values are compared to monitoring data found in some recent publications in scientific peer reviewed journals.

Direct emission

At the Technical Meeting I (2009) several member states had questions about possible direct emissions due to swimming for these type of products. DE presented a swimming scenario at TM II 2011 (draft CAR for lauric acid) and proposed to include this scenario in the ESD for PT19 which DE has drafted. The draft ESD for PT19 which contains a modified swimming scenario when compared to the one applied in the draft CAR for lauric acid, has been distributed to Member States Competent Authorities at 18th September 2013 for e-consultation. DE requested other member states to submit data on natural swimming lakes in order to revise the swimming scenario for inclusion in the draft ESD for PT19. NL has developed a swimming scenario based on data from the more isolated freshwater swimming lakes to which officially the function 'swimming water' is assigned and has submitted these data to DE for inclusion in the ESD for PT19. Both the ESD and NL swimming scenarios are applied in the risk assessment for Care Plus Anti-Insect DEET 50% Spray.

2.8.2.1 PEC_{STP}, $PEC_{surface\ water}$ and $PEC_{sediment}$ – indirect emission

PEC_{STP} and local concentrations in surface water (Clocalwater, or $PEC_{surface\ water}$) were calculated using the draft ESD for PT19 and SimpleTreat 3.1. The input parameters used in SimpleTreat 3.1 are listed in Appendix I.
According to the calculation formula for emission rate to STP (cf table 3-7 in draft ESD for PT19), Elocalwater (Emission rate to wastewater (standard STP), kg/d), i.e. the inflow of DEET to an STP during an emission episode, can be calculated from the formula:
$E_{localwater} = N_{local}*N_{appl}*F_{inh}*F_{water}*Q_{formappl}*C_{formweight}*F_{penetr}*10^{-6}$

If using the input values in table 2.8.1.2-1, $Elocal_{water}$ is 2.67 kg/d for the Care Plus Anti-Insect DEET 50% Spray product. This value is used as input for the PT19 scenario in EUSES 2.1.2.

Table 2.8.1.2-1 Input values used to estimate Elocalwater (Emission rate to wastewater) in accordance with the draft ESD for PT19.

Input parameters (abbrev.)	Explanations	Input value	Remark
N_{local}	Number of inhabitants feeding one STP	10 000	Default according to draft ESD PT19 and TGD Part II
N_{appl}	Number of applications per day	1	According to the list of intended uses, the product is applied 1 time per day.

[3] In a study of simulated treatment processes on spiked raw water samples for drinking water use, the most efficient DEET removal process was ozonation, although high reduction also can be achieved by PAC addition (dose dependent). The simulated treatment processes compared were chemical (Alum coagulation, Ferric coagulation, Softening), PAC treatment and oxidation (chlorination and ozonation). Westerhoff et al. 2005. Fate of endocrine-disruptor, pharmaceutical and personal care product chemicals during simulated drinking water treatment processes. Environ Sci Technol 39: 6649-6663

Input parameters (abbrev.)	Explanations	Input value	Remark
F_{inh}	Fraction of inhabitants using product	0.37	According to the final CAR for DEET 37% (F_{inh} = 0.37) of the population is using any insect repellent.
F_{water}	Fraction released to wastewater	0.887	See figure 1
$Q_{formappl}$	Consumption of product per application	6 g	The TNsG on human exposure sets a default value for the amount of dermally applied repellent product to 6 g.
$C_{formweight}$	Amount of active substance in product	485 g/kg	i.e. 48.5% w/w (information submitted by the applicant)
F_{penetr}	Market share of products applied for this purpose	0.28	According to the final CAR for DEET (Default value in draft ESD for PT19 is 0.5)

Table 2.8.2.1-2 summarises the concentrations in STP effllluent as well as the PECs in surface water and sediment.

Table 2.8.2.1–2 PEC_{STP}, $PEC_{surface\ water}$ and $PEC_{sediment}$ for indirect emission to surface water and sediment via the STP due to body cleaning

PEC_{STP} (mg/L)	$PEC_{surface\ water}$ (mg/L)	$PEC_{sediment}$ (mg/kg ww)
1.68E-01	1.68E-02	2.90E-02

2.8.2.2 $PEC_{surface\ water}$ and $PEC_{sediment}$ – direct emission

The estimation of the local PECs for the aquatic compartment only includes surface water and sediment for the “swimming”-pathway because of direct entry of b.p. in the environment.

ESD swimming scenario

The development of the ‘swimming scenario’ is based on a proposal made by the Competent Authority Germany at TM II/2011, and the comments made thereafter by other Competent Authorities, and industry. A detailed description of the derivation of default parameter referring to the volume of the surface water body, the number of swimmers, and the swimming season is given in Appendix 6.4 of the draft ESD for PT19. The average number of swimmers per day is set at 1500 per default.

No information is available regarding the fraction of swimmers using an insect repellent. Surface water bodies are often located in forested areas where the occurrence of biting and sucking arthropods is likely. Furthermore, some surface water bodies have camping locations nearby and campers can be assumed to be equipped more often with an insect repellent than daily visitors of the lake. As a best guess it is assumed, that 2% of the swimmers use an insect repellent before entering the surface water body. Visits of swimmers at Dutch fresh- and seawater sites lasted 41-79 minutes per occasion in 2007 and 2009. It can be expected that during this time period treatment with a repellent will take place only once.

The fraction released to the surface water body is set to 1 per default. The volume of the water body is 1.2 million m^3 per default.

The rate constant for biodegradation in surface water for DEET which is readily biodegradable is set to 0.047 d^{-1} according to Table 7 (EU TGD, 2003). Time-weighted average concentrations of the repellent were calculated for emission periods of 1 day and 91 days.

The $PEC_{surface\ water}$ and $PEC_{sediment}$ for direct emission to the aquatic environment due to swimming based on the swimming scenario included in the draft ESD for PT19 are included in Table E.2.8.2.2-1. Biodegradation of DEET in the water compartment has been taken into account for the PEC calculations.

Table E.2.8.2.2-1 PEC/PNEC ratios for direct emission to the aquatic environment due to swimming based on the ESD swimming scenario including biodegradation.

	$PEC_{surface\ water}$ (mg/L)	**$PEC_{sediment}$ (mg/kg ww)**
Natural swimming areas	2.33E-09	4.02E-09

NL swimming scenario
There are 450 official swimming locations in the Netherlands owned by one of the 19 regional water boards and concern the more isolated lakes and 220 official swimming locations owned by Rijkswaterstaat (the executive arm of the Dutch Ministry of Infrastructure and the Environment), these locations concern swimming locations along side rivers etcetera.
The swimming lakes from water boards are included in the data analysis as these concern the more isolated swimming lakes. For each water board approx. 5-10 swimming locations have been selected, the total number of swimming lakes selected is 72. Parameters collected are the average and high number of swimmers per day during the period of access (swimming season from 1 May till 30 September) and the volume of the swimming area or of the entire lake. The water depth in the swimming area is estimated to be 1.5 m if not reported and in case a chain with balls borders the swimming area. According to the Dutch ''protocol zwemwaterlocaties in binnenwater'' (protocol swimming locations in inland waters) a swimming area should be delineated at a depth of 1.5 m in case the swimming area is defined.
Deep lakes can be stratified and thus only a certain part of the lake is susceptible to mixing. Information on which water volume of the lake gets mixed is mostly lacking and therefore mixing of the entire water volume of a lake is assumed in the data analysis.
Please be aware that mixing/dilution can have a big impact on the PECs for the water and sediment compartments.

It is assumed that 1% of the swimmers uses a repellent and that the entire amount of a single application of 6 g applied is washed off daily during swimming. Using these data the 10 percentile, 90 percentile and average PEClocal $_{water}$ with (TWA 30 days) and without degradation were calculated. For these PEC $local_{water}$ the PEC local $_{sediment}$ was calculated with the equilibrium partitioning method according to equation no. 50 of the TGD, see Table E.2.8.2.2-2.

Table E.2.8.2.2-2 90 percentile $PEC_{surface\ water}$ and $PEC_{sediment}$ for direct emission to the aquatic environment from swimming based on 30 days TWA concentrations. Calculations are based on the Dutch swimming scenario.

Scenario	**$PEC_{surface\ water}$ (mg/L)**	**$PEC_{sediment}$ (mg/kg ww)**
High density swimmers in lake	1.28E-02	2.21E-02
High density swimmers in swimming area	1.03E-01	1.78E-01
Average density swimmers in lake	4.30E-03	7.41E-03
Average density swimmers in swimming area	3.03E-02	5.22E-02

2.8.2.3 Exposure monitoring – data published in the open literature
Publications in scientific peer reviewed journals regarding DEET concentrations in the environment were used to compare the calculated values with measured data.
Before making comparisons between measured and modelled data one needs to be aware of the uncertainty associated with measured values, due to temporal and spatial variation.

Temporal fluctuations are of special concern when it comes to PEC estimations of DEET; the highest values expected during peak bug season. There may also be geographical variations. These monitoring data should therefore only be regarded as examples of DEET concentrations found in order to evaluate the calculated PEC values, not as substitutes. The highest surface freshwater concentration found in a study of 56 american streams was 1.1 µg/L, which is 9 times lower than the worst case $Clocal_{water}$ of 0.01 mg/L, see table 2.8.2.2-2.

A few data on DEET in American raw waste water influents (150 and 365 ng/L) have been found in the open literature (Snyder et al. 2006)[4]. These values are at least 3644 times lower than the lowest concentration in influent calculated (1.33 mg/L).
DEET concentrations in Norwegian and German STP effluents (10-60 ng/L and 130 ng/L respectively)[5], are at least 1307 times lower than what was estimated through model calculations (0.17 mg/L). The Norwegian data are from an STP without biological treatment whereas the German data are from an STP with biological treatment. The DEET concentrations found in the German influent was 0.21 µg/L, before the biological treatment step, which is more than 6333 times lower than estimated from the calculations.

Table 2.8.2.3-1 Environmental monitoring data for DEET from open peer reviewed scientific literature.

Area information	**Analytical information**	**Concentrations found**	**Reference**
Seawater North Sea Sampling locations mostly coastal	Polymeric sorbent extraction + GC-MS LOQ: 26 pg/L Sampling period: June-July 1998 2x10L samples at 5m depth 15 sampling locations	Highest values 1.09 and 1.06 ng/L respectively [found in the German Bight; (53°40.00'N; 06°25.00'E) and (54°15.00'N; 07°48.00'E)] DEET was detected in all but two samples.	Weigel *et al.* 2002.
Seawater Tromsø Sound (Norway), (into which sewage is discharged)	Glass fibre filtration, sorbent extraction + GC/MS LOQ: 0.20 ng/L Sampling period: 2002 (most samples taken in April, the rest in October) 2.5L samples. 12 sampling locations	Range: 0.4-13 ng/L (STP data: 10 and 60 ng/L in April and October respectively)	Weigel *et al.* 2004. [Ref no. 8066] Chemosphere 56: 583-592

[4] Snyder et al, 2006. Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. Desalination. In press.

[5] Ref no 8066. Weigel et al. 2004. Determination of selected pharmaceuticals and caffeine in sewage and seawater from Tromsø/Norway with emphasis on ibuprofen and its metabolites. Chemosphere 56: 583-592

Area information	Analytical information	Concentrations found	Reference
Surface freshwater Las Vegas Wash, a waterway receiving tertiary treated municipal effluent from the city of Las Vegas, NV.	Whole water (incl dissolved and particulate phases) Solid Phase Extraction + LC/MS/MS 1L samples 3 replicates Reporting level: 1.0 ng/L	Average: 40 ng/L	Vanderford *et al.* 2003.
Surface freshwater 56 streams across the USA, some bias to streams downstreams intense urbanization and livestock	Whole water (incl dissolved and particulate phases) Continuous Liquid- Liquid Extraction + GC/MS Sampling period: 2000 Reporting level: 40 ng/L[a] Duplicate composite samples (from 4-6 vertical profiles)	Highest value: 1.1 µg/L (measured at urban site) Median concentration: 0.05 µg/L (all sites) Frequency of detection: 73.2%	Kolpin *et al.* 2002

[a] Reporting level: lowest concentration standard that could be quantitated reliably. Initially set to 0.04 µg/l, and then revised to 0.08 µg/l, but lower contrations reported if GC/MS criteria (retention time and abundance of three characteristic ions in the same ratio as that of standard) were met. Sandstrom et al, 2005.

Compared to monitoring data from STP influents/effluents all estimated values are conservative. Similarly, the estimated values were in the range of, or above the peak maximum measured concentration in fresh surface water.
DEET has been on the Dutch market for > 3 years (authorised since 1986). This period is sufficiently large to consider the market share to be established. DEET is included in the list of substances of concern relevant for surface water at drinking water abstraction points as established by VEWIN/CTGB. This list is based on monitoring data for eight Dutch drinking water abstraction periods and measured during period 2008-2012.
The active substance DEET was detected at several drinking water abstraction points in surface waters in the Netherlands. However exceeding of the drinking water limit occurred only occasionally. Based on the available data it can be concluded that the 90th percentile of the measurements over the period 2008-2012 is below the drinking water limit of 0.1 µg/L and for five out of eight drinking water abstraction points even below the detection limit of 0.02 µg/L, see Table 2.8.2.3-2.

Table 2.8.2.3-2 Monitoring data for DEET at Dutch drinking water abstraction points from surface water in the period 2008 – 2012

Abstraction point	Number of measurements above detection limit/ Number of measurements [n/N]	Number of measurements above drinking water limit/ Number of measurements [n/N]	Overall 90-percentile [µg/L]
Andijk	0/52	0/52	< d.l.*
Nieuwegein	8/65	0/65	< d.l.
Amsterdam-Rijn kanaal (Nieuwersluis)	21/52	0/52	< d.l.
Brakel	30/100	1/100	0.03

Abstraction point	Number of measurements above detection limit/ Number of measurements [n/N]	Number of measurements above drinking water limit/ Number of measurements [n/N]	Overall 90-percentile [µg/L]
Heel	17/59	1/59	0.05
Petrusplaat/Keizersveer	42/103	1/103	0.06
Scheelhoek/Stellendam	7/35	0/35	< d.l.
Drentsche Aa (De Punt)	0/125	0/125	< d.l.

*d.l: detection limit, in general the detection limit for DEET is 0.02 µg/L

Furthermore, the RIVM did not include this active substance on the recommended list of surface water to be monitored for drinking water from surface water[6] because all measured concentrations in the Rhine and Meuse were below the drinking water limit of 0.1 µg/L. From the general scientific knowledge collected by the CTGB about the products and their active substance, the CTGB concludes that there are no concrete indications for concern about the consequences of these products for surface water from which drinking water is produced when used in compliance with the directions for use. The standards for surface water destined for the production of drinking water are met.

2.8.2.4 PEC_{soil} and $PEC_{groundwater}$ – indirect emission

The estimation of the local PECs for the terrestrial compartment includes soil and groundwater:

- PEC_{soil} according to equation 66, chapter 2.3.8.5, EU TGD (2003);
- $PEC_{porewater}$ according to equation 68, chapter 2.3.8.6, EU TGD (2003) as a first worst-case estimation.

The estimation of releases to the soil compartment premises calculation of predicted concentrations of the a.s. in dry sewage sludge as part of a.s. load leaving a STP. Accumulation of the acute substance may occur when sludge is applied over consecutive years for persistent substances. Table 2.8.2.4-1 summarises the concentration in dry sewage sludge C_{sludge} as well as the PECs in soil and porewater.

Table 2.8.2.4–1 Csludge, PEC_{soil} and $PEC_{groundwater}$ for indirect emission to soil and groundwater due to body cleaning.

C_{sludge} (mg/kg)	PEC_{soil} (µg/kg ww)	$PEC_{porewater\ grassland}$ (µg/L)	$PEC_{porewater\ agricultural\ soil}$ (µg/L)
13.7	14.2	1.89	5.05

The calculated PEC for porewater was addressed further by the RMS as the drinking water limit for groundwater of 0.1 µg/L was exceeded. PECgw for the nine FOCUS groundwater scenarios, as developed for plant protection products, were calculated. Model used, input data and assumptions are shown in Table 2.8.2.4-2. The overall assumption being that the only exposure route to groundwater is via the application of sludge from STPs.

Table 2.8.2.4–2 Summary of data used and assumptions made to calculate PECgroundwater for DEET in FOCUS scenarios.

Parameter	Value
Model used:	FOCUS PEARL ver. 4.4.4.
Years of simulation:	26 (including 6 yrs “warming-up” period)

[6] Bakker, J. Biociden in oppervlaktewater voor drinkwaterproductie, National Institute of Public Health and the Environment, RIVM report 601712007, 2010, Bilthoven, The Netherlands.

Parameter	Value
Application rate:	0.069 kg/ha[a]
Application method:	To the soil surface
Date of application:	1 October annually for 20 years[b]
Molar mass:	191.3 g/mol
Vapour pressure:	0.23 Pa (25°C)
Water solubility:	11200 mg/L (25°C)
Kom:	25.1 L/kg[c]
Freundlich exponent 1/n:	0.9 (FOCUS default)
DT_{50} soil	30 days (12°C) [d]
Coefficient for uptake in plants:	0 (worst-case assumption)

a Calculated from SimpleTreat output concentration of DEET in dry sewage sludge of 13.7 mg/kg (see table 2.8.2.4-1), and application of 5000 kg dry sludge/ha and year to agricultural land (at a single event as suggested in the TGD, Part II 2.3.8.5).

b Autumn application assumed to represent a worst-case situation.

c Calculated from Koc as 43.3/1.724.

d In accordance with EUSES/TGD, Part II 2.3.6.5, for ready biodegradable substances.

The resulting PECgw (as FOCUS standard output; 80th percentile annual average PECgw at 1 m depth) are shown in Table 2.8.1.4-3. These results show that the predicted groundwater concentrations of DEET following the intended use of this substance are <0.1 µg/L for all FOCUS scenarios.

Table 2.8.2.4-3 80th precentile annual average PEC of DEET in groundwater (at 1 m depth) calculated for nine FOCUS scenarios, assuming application of sewage sludge from STP to land.

Scenario	PECgw, µg/L
Chateaudun	< 0.01
Hamburg	< 0.1
Jokioinen	< 0.01
Kremsmuenster	< 0.1
Okehampton	< 0.1
Piacenza	< 0.1
Porto	< 0.1
Sevilla	< 0.01
Thiva	< 0.01

As agreed at the Technical Meeting I in 2009, the Netherlands submitted available groundwater monitoring data on DEET to the RMS. In addition to a report[7] (in Dutch) presenting the results from screening the presence of 149 pesticides and some biocides in groundwater at 189 locations in the Netherlands in 2007, the results on DEET were also presented in an Excel file. Hence, details with regard to DEET from this monitoring program appear not to be available in the open literature. The monitoring data were collected by two provinces and two drinking water companies from the Southern part of the Netherlands. The majority of the samples were taken during July-December. DEET was the substance that was found above the detection limit (0.01 µg/L) at the highest number of occasions (30%). In 189 samples from 189 groundwater monitoring points 57 samples had a concentration >0.01 ug/L, and out of these three samples (1.6%) were above the drinking water limit, i.e. > 0.1 ug/L (range was 0.36-1.48 µg/L). The report also referred to monitoring data from 2003 during which DEET was found above the detection limit in 5% of the samples, and in no sample concentrations >0.1 µg/L were measured.

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

In the Netherlands, surplus sludge of public STPs is not applied for fertilization and soil improvement of agricultural soil. Therefore, leaching to groundwater is not expected and thus monitoring data for groundwater are not required for the Dutch authorisation of Care Plus Anti-Insect DEET 50% Spray.

2.8.2.5 PEC_{Soil} and $PEC_{groundwater}$ – direct emission

In the scenario for the swimming pathway the terrestrial compartment is not exposed and therefore not assessed.

2.8.2.6 PEC_{air}

The active substance DEET is moderately volatile. The vapour pressure is 0.11 Pa at 20℃. A Henry's law constant of 3.93×10^{-3} Pa m^3 mol^{-1} is reported, confirming its relatively low volatility.
AOPWIN model calculation estimates that DEET in the atmosphere reacts with photochemically produced hydroxyl radicals in air, with a half-life of 0.634 days (24 hr day; 0.5×10^6 OH/cm^3). This calculated half life is below the trigger of < 2 days that is used as cut-off value to identify chemicals that could be of potential concern for with the potential for long-range transport through the atmosphere. As the substance unlikely shows significant long-range transport, it is considered of no concern for ozone depletion.
Criteria for the examination of environmental risks to air are not specified in the form of a numerical standard. Therefore, effects on air quality only are taken into account when adverse effects are foreseen. The assessment of potential impacts on air quality, yet, is aimed to minimize the risk for stratospheric ozone depletion. There are no indications that this substance contributes to depletion of the ozone layer and the compounds are furthermore not listed as 'controlled substance' listed in Annex I of Regulation (EC) No 1005/2009 of the European Parliament, the environmental risk to air is considered acceptable.

2.8.2.7 Primary and secondary poisoning of birds and mammals

As the log K_{ow} is < 3 (2.4), a risk for bioconcentration and biomagnification is not expected (conform the biomagnification trigger value proposed for K_{ow} in the TGD).
As DEET is not bioaccumulative and the concentrations in surface water and soil are low, the risk for the primary and secondary poisoning is considered acceptable.

2.8.3 Risk Assessment

The risk characterisation for the environment is the comparison of the toxicity of the substance to the exposure estimates. Both aspects were already discussed in section 2.8.1 and 2.8.2, respectively, and only the relevant values are summarised below.

2.8.3.1 Aquatic compartment (incl. sediment and STP)

The PNEC values for the water compartment and STP microorganisms were calculated from toxicity data by using recommended assessment factors, see section 2.8.1. The PNEC for STP microorganisms is 10 mg/L which is based on and EC_{50} > 1000 mg/L and an assessment factor of 100.
Because only three acute aquatic tests were performed, all on freshwater species, the assessment factor for the freshwater compartment was 1000. For the sediment compartment, there are no toxicity data available. The low Koc value indicates that sorption to sediment is not likely. Nevertheless, a PNEC value of 0.0741 mg/kg ww for sediment has been calculated based on the equilibrium partitioning theory and PNECwater of 0.043 mg/L. As both the PEC and PNEC for sediment are based on equilibrium partioning with the PEC and PNEC for surface water, the risk assessment for the aquatic environment covers the surface water and sediment compartments.

<u>Indirect emission</u>

Even when making worst case assumptions for the local environment, none of the PEC/PNEC ratios exceed 1, see table 2.8.3.1-1.

Table 2.8.3.1–1 PEC/PNEC ratios for indirect emission to the aquatic environment via the STP due to body cleaning indoors.

	PEC (mg/L)	PNEC (mg/L)	PEC/PNEC
Microorganisms in STP	1.68E-01	10	1.68E-02
Aquatic environment	1.68E-02	0.043	3.91E-01

Direct emission

In Tables 2.8.3.1-2 and 2.8.3.1-3 the PEC and PEC/PNEC ratios for direct emission to surface water and sediment due to swimming are indicated, the PECs were calculated using both the swimming scenarios developed by Germany and The Netherlands for the PT19 ESD.

In the tables only the PEC/PNEC for freshwater is included as both the PEC and PNEC for sediment are based on equilibrium partitioning. Therefore the risk assessment for the aquatic environment covers the surface water and sediment compartment.

Table E.2.8.3.1-2 PEC/PNEC ratios for direct emission to the aquatic environment due to swimming in natural waters based on the ESD swimming scenario including biodegradation.

	PEC (mg/L)	PEC/PNEC
Natural swimming areas	2.33E-09	5.42E-08

The PEC/PNEC ratio for both surface water and sediment is < 1 for PECs calculated with the ESD scenario.

Table E.2.8.3.1-3 90 percentile PEC/PNEC ratios for direct emission to the aquatic environment from swimming in natural waters based on 30 days TWA concentrations. Calculations are based on the Dutch swimming scenario.

Scenario	PEC (mg/L)	PEC/PNEC	Number out of 72 lakes with PEC/PNEC > 1
High density swimmers in lake	1.28E-02	2.98E-01	2
High density swimmers in swimming area	1.03E-01	**2.40**	35
Average density swimmers in lake	4.30E-03	1.00E-01	0
Average density swimmers in swimming area	3.03E-02	7.05E-01	3

The presence of a high density number of swimmers in a swimming area will be occassional and the release of DEET into the swimming area can be considered intermittent. Furthermore, the DT50 of DEET is 15 days at 12℃ but degradation will be even more rapid at higher water temperatures, not unusual in shallow swimming areas warmed by the sun during the swimming season. During release the PEC/PNEC ratios are thus expected to be above 1 just for a short period of time and therefore the risk to aquatic and sediment organisms is considered acceptable.

2.8.3.2 Terrestrial compartment

For the soil compartment, there are no toxicity data available. The low Koc value indicates that sorption to soil is not likely. Nevertheless, PNEC values have been calculated based on equilibrium partitioning theory and PNECwater.

Even when making worst case assumptions for the local environment, the PEC/PNEC ratio does not exceed 1.

Table 2.8.3.2–1 PEC/PNEC ratios for indirect emission to soil due to body cleaning after product use.

PEC_{soil} (µg/kg ww)	PNEC (µg/kg ww)	PNEC/PNEC
14.2	37.9	3.75E-01

2.8.3.3 Groundwater compartment

In the EUSES modelling the porewater PEC in agricultural soil was above 1 µg/L. This result was further addressed by the RMS by calculating PECgw at 1 m soil depth for nine FOCUS groundwater scenarios in FOCUS PEARL v. 4.4.4 model, assuming that sludge from STP is applied to agricultural soil. The 80th percentile annual average PECgw were below the drinking water limit of 0.1 µg/L for all FOCUS scenarios.
Finally, monitoring data from The Netherlands indicate that DEET may have a potential to leach to groundwater. In 189 samples of groundwater in 2007, DEET was detected at >0.01 µg/L in 57 samples (30%) and in 3 of these samples (1.6%) concentrations were reported as >0.1 µg/L (range 0.36-1.48 µg/L).
It is not known to the RMS why the groundwater concentrations in three samples were exceeded, but considering that in the majority of the groundwater samples the 0.1 ug/L standard is not exceeded the RMS does not see sufficient ground for identifying an unacceptable risk.

2.8.3.4 Atmosphere

Although PEC/PNEC ratios could not be calculated, the physiochemical properties of DEET do not suggest that this substance will pose a significant threat to the atmospheric environment, see section 2.8.2.6.

2.8.3.5 Primary poisoning and secondary poisoning (non compartment specific effects relevant to the food chain)

Primary poisoning of birds and mammals due to intake of the product is not expected to be relevant. Considering the low acute toxicity of DEET to birds (LD50 1375 mg/kg bw) and the type of use intake by birds and mammals of the active substance via water is considered as negligible.
Although PEC/PNEC ratios could not be calculated, it can be concluded that no risk for secondary poisoning has been identified based on the low BCF value, see section 2.8.2.7.

2.9 Measures to protect man, animals and the environment

Toxicology

The instructions for use must contain the following indications:

- Do not use more than once a day.
- Do not use on children (< 17 years old)
- Avoid contact with eyes, mucous membranes and damaged skin.
- Avoid contact with food.
- Use only outdoors or in a well-ventilated area and do not inhale the product
- Keep this product away from children.

3 Decision

The Dutch CA considers that sufficient information has been provided to verify the outcome and conclusions, and permits the authorisation of Care Plus DEET Anti-Insect 50% Spray.

The formulation Care Plus DEET Anti-Insect 50% Spray has been applied for and evaluated as an insect repellent that should be applied to the skin of exposed body parts with the purpose to protect humans from mosquito and tick bites.

Based on the assessment, the Dutch CA concludes that these products can be safely used by non-professional user, taking into account the risk mitigation measures as indicated under 2.9.

4 Annexes

1. **List of studies reviewed**
2. **Analytical methods residues – active substance**
3. **Toxicology and metabolism –active substance**
4. **Toxicology – biocidal product**
5. **Safety for professional operators**
6. **Safety for non-professional operators and the general public**
7. **Residue behaviour**

Confidential Annex:

Product composition

Annex 1 List of studies reviewed

List of data submitted in support of the evaluation of the biocidal product

Section No / Reference No	Author	Year	Title Source (where different from company) Company, Report No. GLP (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
B3.4/01	Ewa Delezuch-Ntambala	2012	Flash Point Care Plus Anti Insect DEET “products” statement	Y	Toprenzorg B.V.
B3.4/02	Colipa	1994	Colipa Recommendation flammability labelling of cosmetics products	N	Colipa
B3.4/03	M. Hristova	2010	Estimation of water-alcohol mixture flash point	N	Public lit
B3.5/01	Ewa Delezuch-Ntambala	2013	Statement on pH Care Plus DEET products	Y	Tropenzorg B.V.
B3.7/02	Ewa Delezuch-Ntambala	2012	Stability statement Care Plus Anti Insect DEET ”products””	Y	Tropenzorg BV
B4.1-01	Masterlab B.V.	2011	Masterlab Analysis Prescription, Quantification of DEET with HPLC-UV and identification by IR, Doc nr ANA-00238	Y	Tropenzorg B.V.
B4.1/02	Masterlab B.V.	2011	Masterlab Validation report, Determination diethyl-m-toluamide DEET in DEET concentrates using HPLC-UV, version 1	Y	Tropenzorg B.V.
B4.1/03	Masterlab B.V.	2011	Specific gravity, Doc nr ANA-00236, version 1	Y	Tropenzorg B.V.
B4.1/04	Masterlab B.V.	2011	Refractive index, Doc nr ANA-00235, version 1	Y	Tropenzorg B.V.
B4.1/05	Masterlab B.V.	2011	Analyse Report	Y	Tropenzorg B.V.
B4.1/06	Ewa Delezuch-Ntambala	2013	Explanation analyse rapport Care Plus DEET products	Y	Tropenzorg B.V.

Section No / Reference No	Author	Year	Title Source (where different from company) Company, Report No. GLP (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
B4.1/07	PJM Janssen	2014	Addendum to: Validation report, Determination of diethyl-m-toluamide (DEET) in DEET-concentrates using HPLC-UV. Validation Report, determination DEET in DEET-concentrates, version 1.0, December 2012. GLP: no Unpublished	Y	Tropenzorg B.V.
B4.1/08	PJM Janssen	2014	Analytical method validation for DEET content in Care Plus mosquito repellents GLP: no Unpublished	Y	Tropenzorg B.V.
B5.10-01	K.-H. Lüpkes, BioGenius GmbH	2011	Repellent Efficacy of a Product on Human Arms against Mosquitoes: Mosquito repellent effect of a product against, House mosquito Culex quinquefasciatus, Malaria mosquito Anopheles gambiae and, Yellow fever mosquito Aedes aegypti. Product: CARE PLUS ANTI INSECT DEET lotion 50%, Report no: BIO 095-11, BioGenius GmbH,	Y	Tropenzorg B.V.
B5.10-02	H. Dautel, A. Gharbi, Insect Services	2011	Evaluation of Care Plus Anti Insect DEET lotion 50% against the European Sheep Tick, Ixodes ricinus, on human volunteers, Study report, BGN-IR-0311d, Insect Services, 31-10-2011	Y	Tropenzorg B.V.
B5.10-03	Ewa Delezuch-Ntambala	2011	Efficacy document summary : H. Dautel, A. Gharbi, Evaluation of Care Plus Anti Insect DEET lotion 50% against the European Sheep Tick, Ixodes ricinus, on human volunteers, Study report, BGN-IR-0311d, Insect Services, 31-10-2011	Y	Tropenzorg B.V.

Section No / Reference No	Author	Year	Title Source (where different from company) Company, Report No. GLP (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
B5.10-04	Ewa Delezuch-Ntambala	2011	Efficacy document summary : K.-H. Lüpkes, Repellent Efficacy of a Product on Human Arms against Mosquitoes: Mosquito repellent effect of a product against, House mosquito Culex quinquefasciatus, Malaria mosquito Anopheles gambiae and, Yellow fever mosquito Aedes aegypti. Product: CARE PLUS ANTI INSECT DEET lotion 50%, Report no: BIO 095-11, BioGenius GmbH,	Y	Tropenzorg B.V.
B6.1/02	Merck Publishing Group	1996	The Merck Index, Merck Publishing Group. Abstract for Ethanol	N	-
B6.1/05	European Food Safety Authority	2012	Conclusion on the peer review of the pesticide risk assessment of the active substance denatonium benzoate (approved as denathonium benzoate)	Y	European Food Safety Authority (EFSA)
B6.1/06	Cosmetic Ingredient Review Expert Panel	2005	Safety Assessment of Alcohol Denat. (…..) and the denaturants Denatonium Benzoate, Quassin, and Brucine Sulfate/Brucine		Cosmetic Ingredient Review
B6.1/07	Lubrizol Advanced Materials, Inc.	2011	Toxicology Studies and Regulatory Information, Pharmaceutical Bulletin 2;	Y	The Lubrizol Corporation
B6.2.1/01	TNO Chemistry	2003	TNO report V 5011/05 Acute dermal irritation/corrosion study with Care Plus DEET lotion (50%) in albino rabbits	Y	Tropenzorg BV
B 6.2.1/02	Ewa Delezuch-Ntambala	2013	Statement Cutaneous Corrosion/Irritation for Care Plus Anti Insect DEET “products” performed Acute Dermal Irritation/Corrosion with Care Plus lotion (50%) in albino rabbits - TNO report V 5011/05, 2003.	Y	Tropenzorg B.V.
B6.2.2/01	Ewa Delezuch-Ntambala	2013	Statement on Eye Irritation for Care Plus Anti Insect DEET “products	Y	Tropenzorg B.V.
B6.3/01	Ewa Delezuch-Ntambala	2013	Statement on Sensitization properties for Care Plus Anti Insect DEET “products”	Y	Tropenzorg B.V
B6.4/01	Ewa Delezuch-Ntambala		Statement on Dermal Absorption in Human Care Plus DEET products	Y	Tropenzorg B.V.
B 6.6-02	Ewa Delezuch-Ntambala	2013	Exposure to Care Plus Anti Insect DEET products	Y	public

Section No / Reference No	Author	Year	Title Source (where different from company) Company, Report No. GLP (where relevant) Published or Unpublished	Data protection claimed Y/N	Owner
B 6.6/03	RIVM	2007	How to assess childrens exposure RIVM 2007	Y	public
B 6.6/04	EPA	2011	Dermal Exposure Factors Chapter 7	Y	public
B 6.6/05	EPA	2011	Exposure Factors Handbook Chapter 8	Y	public
B 6.6/06	WHO	2009	WHO_HTM_NTD_WHOPES_2009.4	Y	Topenzorg BV
B 6.6/07	Nieverpack	2013	Manufacturing process description	Y	Tropenzorg BV
B 6.6/08	Niverpack	2005	Mileuvergunning Niverpack	Y	Tropenzorg BV
B 9/01	Borelis	2007	Packaging specification	Y	Tropenzorg BV
B 9/02	Borelis	2007	Packaging specification	Y	Tropenzorg BV
B 9/03	FRAPAK Packaging B.V.	2010	Packaging specification	Y	Tropenzorg BV
B 9/04	Calmar	2006	Packaging specification	Y	Tropenzorg BV

Annex 2 Analytical methods residues – active substance

N,N-diethyl-meta-toluamide

The analytical methods for residues are taken from the CA report to support the inclusion of DEET in annex I of Directive 98/8/EC. Where relevant, some additional remarks/information are given in italics.

Analytical methods for residues

Soil (principle of method and LOQ)	DEET: LC-MS/MS with 1 transition (LOQ: 0.01 mg/kg)
Air (principle of method and LOQ)	*DEET: LC-MS/MS (LOQ 0.225µg/m^3)**
Water (principle of method and LOQ)	DEET: LC-MS/MS (LOQ: 0.1 µg/L in surface water)
Body fluids and tissues (principle of method and LOQ)	DEET in blood plasma: HPLC-UV (LOQ 49.4µg/L) No confirmatory method is provided. No further data is required as DEET is not classified as toxic or highly toxic.
Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	Not required as the use pattern of DEET will not result in any contact with food or feeding stuffs.
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	Not required as the use pattern of DEET will not result in any contact with food or feeding stuffs.

** new data; see paragraph 2.3.2*

Annex 3 Toxicology and metabolism –active substance

N,N-diethyl-meta-toluamide

Threshold Limits and other Values for Human Health Risk Assessment

Summary			
	Value	Study	SF
AEL repeated dermal (general public)	8.2 mg/kg bw/day*	8-week study (dogs, oral capsule)	100
AEL acute oral (general public)	0.75 mg/kg bw/day	90 day study (rat dermal)	100
*Corrected for a dermal absorption of approximately 82 % in the rat			

Inhalative absorption	No data
Oral absorption	>80% based on urinary, faecal and tissue content (in the rat). In rats, 85-91% of administered radioactivity was found in urine.
Dermal absorption	Dermal rat approx. 82% (based on urinary excretion, faeces content, tissue content and skin). Humans: <20% based on urinary excretion, faecal and skin content, corrected for recovery). No information was provided on inhalational absorption.

Classification	
with regard to toxicological data (according to the criteria in Dir. 67/548/EEC)	Class of danger: Xn R phrases: 22 - 36/38
with regard to toxicological data (according to the criteria in Reg. 1272/2008)	Pictogram: GHS07 Signal word: Warning Acute Tox. 4, H302; Eye Irrit. 2, H319; Skin Irrit. 2, H315.

Annex 4 Toxicology – biocidal product

Care Plus Anti-insect DEET 50% Spray

General information

Formulation Type	spray
Active substance(s) (incl. content)	DEET (48.5%)
Category	PT19

Acute toxicity, irritancy and skin sensitisation of the preparation (Annex IIIB, point 6.1, 6.2, 6.3)	
Rat LD50 oral (OECD 420)	No study was submitted
Rat LD50 dermal (OECD 402)	No study was submitted
Rat LC50 inhalation (OECD 403)	No study was submitted
Skin irritation (OECD 404)	Not irritating
Eye irritation (OECD 405)	No study was submitted
Skin sensitisation (OECD 429; LLNA)	No study was submitted

Classification and labelling proposed for the preparation with regard to toxicological properties (Annex IIIB, point 9)	
Regulation 1272/2008/EC	GSH07 Warning H319 P101 P102 P260 P271 P305+P351+P338

Annex 5: Safety for professional operators

Care Plus Anti-insect DEET 50% Spray

The product is not intended for professonal use.

Annex 6: Safety for non-professional operators and the general public

Care Plus Anti-insect DEET 50% Spray

General information	
Formulation Type	spray
Active substance(s) (incl. content)	DEET (48.5%)
Category	PT19

<Active Substance>

Data base for exposure estimation	
according to	Appendix: Toxicology and metabolism – active substance/CAR

Exposure scenarios for intended uses (Annex IIIB, point 6.6)	
Primary exposure	Non-professional users (consumers; adults and children)
Secondary exposure, acute	Not relevant
Secondary exposure, chronic	Not relevant

The internal dermal exposure is calculated according to the following formula:

Internal dermal dose a.s. = (Number of applications) × (amount of product (75^{th} percentile based on survey data)) × (content a.s.) × (% dermal absorption) / body weight

The internal oral exposure is calculated based on the following formula:

Internal oral dose a.s. = (Number of applications) × (Amount of product (75^{th} percentile based on survey data)) × (content a.s.) × (% ingested amount) / body weight

The number of applications is considered to be one per day. For dermal absorption the value of 20% is used for DEET based on the CAR. Oral absorption is considered to be 100% as a worst-case approach. The % of the ingested amount is considered to be 4% for adults (product on fingers).

Primary exposure for one application for adults and children:

Internal exposure for one application	**48.5% DEET**
Dermal* (mg/kg bw/day)	
Male (0.164 mg/kg bw/day per 1% DEET)	7.95
Female (0.128 mg/kg bw/day per 1%	6.21
>12 yr (0.203 mg/kg bw/day per 1% DEET)	9.85
<12 yr: (0.427 mg/kg bw/day per 1% DEET)	20.71
Oral (mg/kg/bw/day)**	
Male (0.033 mg/kg bw/day per 1% DEET)	1.60
Female (0.026 mg/kg bw/day per 1%	1.26

**Based on the 75th percentile of human use rate (Boomsma and Parthasarathy, 1990) and considering one application per day corrected for a conservative dermal absorption of 20% in humans and body weights of 70 kg for male adult, 60 kg for female adult, 62.8 kg for children*

>12 years old and 25.5 kg for children < 12 years old. For clarity, in the first column the exposure values per 1% DEET based on the results of the user survey study are given.
*** Internal oral exposure is calculated by considering adults ingesting 4% of the external dermal dose (product on fingers). Oral exposure is considered to be 100% as a worst-case approach. For clarity, in the first column the exposure values per 1% DEET based on the results of the user survey study are given.*

Risk characterisation ratio per application for adults and children (internal exposure)

Risk Characterisation Ratio*	**48.5% DEET**
Dermal	
Male:	0.97
Female:	0.76
>12 yr:	1.20
<12 yr:	2.53
Oral	
Male:	2.13
Female:	1.68

* *Based on the 75th percentile of human use rate (Boomsma and Parthasarathy, 1990) and considering one application per day corrected for a conservative dermal absorption of 20% in humans and body weights of 70 kg for male adult, 60 kg for female adult, 62.8 kg for children >12 years old and 25.5 kg for children < 12 years old. The $AEL_{repeated\ dermal}$ of 8.2 mg/kg bw/da is used for the calculation of the RCR after dermal exposure. The $AEL_{acute\ oral}$ of 0.75 mg/kg bw/day is used for the calculation of the RCR after oral exposure.*

Reverse reference scenario for one application per day for adults and children*

	External dermal exposure per application (mg/kg bw/day)	**Internal dermal exposure per application (mg/kg bw/day)**	**$AEL_{acute\ oral}$/External dermal exposure**	**$AEL_{repeated\ dermal}$/ Internal dermal exposure**
48.5% DEET				
Male:	39.75	7.95	0.019	1.03
Female:	31.05	6.21	0.024	1.32
>12 yr:	49.25	9.85	Not calculated**	0.83
<12 yr:	103.55	20.71	Not calculated**	0.40

**Based on the 75th percentile of human use rate (Boomsma and Parthasarathy, 1990) and considering one application per day corrected for a conservative dermal absorption of 20% in humans and body weights of 70 kg for male adult, 60 kg for female adult, 62.8 kg for children >12 years old and 25.5 kg for children < 12 years old. The internal dermal exposure is compared with the $AEL_{repeated\ dermal}$ of 8.2 mg/kg bw/day. To estimate how much DEET applied to the skin can be ingested without exceeding the $AEL_{acute\ oral}$, the external dermal exposure is compared with the $AEL_{acute\ oral}$ of 0.75 mg/kg bw/day (considering 100% oral absorption this value also represents internal oral exposure).*
*** No separate assessment of potential oral exposure for children was performed, as no acceptable risks have been identified for this age group if only dermal exposure was considered.*

Conclusion:
Exposure of non-professionals and the general public to the biocidal product Care Plus DEET Anti-Insect 50% Spray containing 48.5% DEET as active substance is considered acceptable, if the biocidal product is used as intended and all safety advices are followed.

Annex 7: Residue behaviour

N,N-diethyl-meta-toluamide

The acute or chronic exposure to residues in food resulting from the intended uses is unlikely to cause a risk to consumers. Regarding consumer health protection, there are no objections against the intended uses.

Section B5.10 Efficacy Data

Annex Point IIB5.10
TNsG: Pt. I-B5.10, Pt. III-Ch. 6

Specify where appropriate, e.g. wood-rotting fungi, laboratory study

			Official use only
		1 Reference	
1.1	**Reference**	H. Dautel , 2011-10-31, Study report BGN-IR-0311d, Evaluation of Care Plus Anti Insect DEET lotion 50% against the European Sheep Tick, Ixodes ricinus, on human volunteers, Insect Services.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Tropenzorg BV	
1.2.2			
1.2.3	Criteria for data protection	-	
1.3	**Guideline study**	-	
1.4	**Deviations**	-	
		2 Method	
2.1	**Test Substance (Biocidal Product)**	CARE PLUS ANTI INSECT DEET LOTION 50% Active: DEET Concentration: 50 % Batch: 11NP038-4 Deterrent: 10 mg Bitrex / litre)	
2.1.1	Trade name/ proposed trade name	Care Plus Anti Insect DEET lotion 50%	
2.1.2	Composition of Product tested	Product contains 50% w/w DEET denatureted ethanol and Bitrex (denatonium benzoate)as ingestion deterrent .	
2.1.3	Physical state and nature	Liquid, ethanol solution of DEET	
2.1.4	Monitoring of active substance concentration	Yes 50.3 g/100g concentration DEET in product were HPLC-UV	
2.1.5	Method of analysis	concentration DEET in product were HPLC-UV. Test were performed by Masterlab BV, 23-08-2011,	
2.2	**Reference substance**	No -	
2.2.1	Method of analysis for reference substance	-	
2.3	**Testing procedure**		
2.3.1	Test population / inoculum / test organism	The 10 volunteers comprised of 5 females and 5 males which were fully informed about the nature and the purpose of the test and of any health consequences involved. All persons voluntarily participated at the study	

		and signed a consent form. The age of the persons was between 20 and 59 years. Test persons were advised not to use any perfume or perfume-rich lotions at the test days nor to drink tee or consume caffeine during the test. Test organism: Ixodes ricinus ticks See Table 1.2
2.3.2	Test system	see Table 1.3
2.3.3	Application of TS	see Table 1.4
2.3.4	Test conditions	see Table 1.5
2.3.5	Duration of the test / Exposure time	A total of 5 ticks were tested every 30 min on each volunteer up to a time of 5 hours after application of product.
2.3.6	Number of replicates performed	The 10 volunteers and 5 ticks per 1 hour .
2.3.7	Controls	Yes, All ticks were screened for sufficient activity at the time of testing by performing a control run on the untreated arm. Only such ticks were used for testing that walked up at least 3 cm on the untreated arm of the same volunteer. Such screening was performed <60 min before a test run with each tick. Each tick was tested only once.
2.4	**Examination**	
2.4.1	Effect investigated	Generation of efficacy data on the repellency of the test Care Plus Anti Insect DEET lotion 50%) against Ixodes ricinus ticks.
2.4.2	Method for recording / scoring of the effect	Immediately after application (p. a.) of the repellent, a single prescreened nymph of I. ricinus was placed on the untreated skin about 1 cm below the repellent border and observed for a maximum of 1 min. It was protocolled whether • the tick walked onto the treated skin or not, • the tick walked back from treated skin to the untreated area or not, • the tick walked a distance of at least 3 cm on treated skin in direction to the elbow, thereby crossing the second mark, • the tick fell off from the skin. Most of the ticks immediately started walking when placed on the arm. Ticks remaining motionless or walking down the arm were stimulated to walk in direction of the treated area by means of a clean hairbrush. Ticks falling off from the untreated zone before walking onto the treated area were again placed on the starting line. Ticks not walking onto the treated zone within one minute were removed, scored as “repelled” and replaced by a new tick. Ticks walking onto the treated skin were given an additional 1 min to cross the second mark 3 cm in direction to the elbow. If the tick, within that time period, did not cross the second mark (either because it fell off, or remained on the treated area, or walked back to the untreated zone) it was removed, scored as “repelled”, and replaced by a new

tick. Ticks crossing the second circular mark were likewise removed, but scored as "not repelled".

2.4.3	Intervals of examination	Every 60 minutes, beginning 1 hour after treatment, lasting up to 5 hours maximum, Test Interval-30 minutes
2.4.4	Statistics	S.E. = Standard error of the mean
2.4.5	Post monitoring of the test organism	No

3 Results

3.1 Efficacy

1.) Protection time

In all tests performed, and with all volunteers, not a single tick walked more than 3 cm over treated skin in direction to the elbow throughout the whole test time of five hours.

Thus, the observed protection time was five hours.

2.) Repellency

As all of the ticks were repelled without any exception, the repellency was always 100 %, throughout the whole test period of five hours.

3.1.1 Dose/Efficacy curve

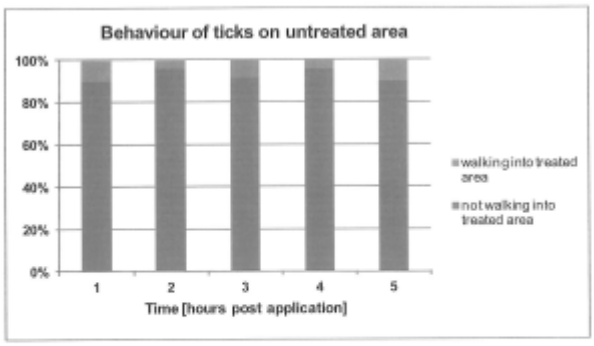


Fig. 1: Behaviour of the ticks on the untreated area of the arm. Ticks either walked onto the treated zone of the arm or remained within the untreated zone throughout the 1 min-observation period. Percentages are based on the total number of ticks used (n=100 per time period).

3.1.2	Begin and duration of effects	-
3.1.3	Observed effects in the post monitoring phase	-

Section B5.10 **Efficacy Data**
Annex Point IIB5.10 *Specify where appropriate, e.g. wood-rotting fungi, laboratory study*
TNsG: Pt. I-B5.10, Pt. III-Ch. 6

3.2 Effects against organisms or objects to be protected -

3.3 Other effects -

3.4 Efficay of the reference substance -

3.5 Tabular and/or graphical presentation of the summarised results

Table 2: Percentages of ticks displaying certain behaviours during the test. Mean values and standard errors of the mean (S.E.) are calculated from the means of the single volunteers.

Time [h] post application	Ticks not walking into treated area Mean ± S.E.	Ticks walking into treated area Mean ± S.E.	No. of ticks
1	90.0 ± 5.2	10.0 ± 5.2	100
2	96.0 ± 2.7	4.0 ± 2.7	100
3	92.0 ± 5.3	8.0 ± 5.3	100
4	96.0 ± 2.2	4.0 ± 2.2	100
5	90.0 ± 5.4	10.0 ± 5.4	100

3.6 Efficacy limiting factors Non-entry field

3.6.1 Occurrences of resistances -

3.6.2 Other limiting factors -

4 Relevance of the results compared to field conditions

4.1 Reasons for laboratory testing - Easier to use standard parameters in the laboratory,

4.2 Intended actual scale of biocide application - CarePlus Anti Insect DEET lotion 50%. gave slightly more than 5 hr protection against ticks.

4.3 Relevance compared to field conditions

Section B5.10 **Efficacy Data**
Annex Point IIB5.10 *Specify where appropriate, e.g. wood-rotting fungi, laboratory study*
TNsG: Pt. I-B5.10, Pt. III-Ch. 6

4.3.1	Application method	In the field, the application will be applied by hand.
4.3.2	Test organism	Test are also target organism. See 2.3.1
4.3.3	Observed effect	Yes, Care Plus Anti insect DEET Lotion 50% is effective as repellent against ticks for at least 5 hours.
4.4	**Relevance for read-across**	No

5 Applicant's Summary and conclusion

5.1	**Materials and methods**	The repellent efficacy of CARE PLUS ANTI INSECT DEET Lotion 50 % was tested against Ixodes ricinus ticks by a total of 10 human volunteers. Tests were performed according to the guidelines of the US Environmental Protection Agency (EPA). Briefly, the forearm of a person was treated from the elbow to the wrist, leaving the lowest 5 cm of the arm near the wrist untreated. The arm was held vertically and a tick was placed on the untreated area, 1 cm below the treated area. Ticks entering the treated skin and walking >3 cm in direction to the elbow were considered not repelled
5.2	**Reliability**	Reliability indicator: 1 Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant
5.3	**Assessment of efficacy, data analysis and interpretation**	The test lasted for four hours post application (p.a.). The test product repelled all of the ticks (n = 500) throughout the whole test period lasting up to five hours p.a. The resulting repellency therefore was 100 % and the protection time determined to be at least five hours. Most of the ticks (always >85 %) even did not crawl onto the treated skin area throughout the observation period. Most of the ticks that nevertheless did so either turned back or fell off before reaching the 3 cm-mark. The repellent thus proved to be highly effective as evaluated by the present assay.
5.4	**Conclusion**	Product Care Plus Anti insect DEET Lotion 50% is effective as repellent against ticks for 5 hours.
5.5	**Proposed efficacy specification**	Product Care Plus Anti insect DEET Lotion 50% is effective as repellent against ticks for 5 hours ticks.

Section B5.10 **Annex Point IIB5.10** **TNsG: Pt. I-B5.10,** **Pt. III-Ch. 6**	**Efficacy Data** *Specify where appropriate, e.g. wood-rotting fungi, laboratory study*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	*18-04-2014*
Comments	*It is a pity the tests were ended after 5 hours. Therefore the real protection time cannot be established.*
Summary and conclusion	*The tests were well done according to standard methods. The results and conclusions are valid. The protection time of 5 hours is probably an under-estimation of the real protection time because the tests were ended after 5 hours.*
	6 Comments from ... *(SPECIFY)*
Date	*Give date of comments submitted*
Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

1.2 Test organismEuropean Sheep Tick Ixodes ricinus

Criteria	Details
Species	Ixodes ricinus, European Sheep Tick,
Strain	The ticks used for the study were of the F4 laboratory generation originally collected in the area of Berlin, Germany.
Source	Insect Services
Laboratory culture	yes
Stage of life cycle and stage of stadia	Immature and adult ticks
Mixed age population	Yes,
Other specification	mixed sex
Number of organisms tested	500
Method of cultivation	The I. ricinus ticks were taken from a laboratory colony and were free of borreliae and FSME-virus. Immature and adult ticks had previously been fed on gerbils (Meriones unguiculatus) or rabbits (Oryctolagus cuniculi), respectively and were kept at constant 90 % r.h. and 20 °C/longday conditions (16L:8D) (standard conditions). The ticks used for the study were of the F4 laboratory generation originally collected in the area of Berlin, Germany. The age of the ticks was between 17 and 20 weeks (after their last moult). All ticks were randomized by transferring the ticks into a large tray. By walking around, the ticks mixed themselves. The ticks were then randomly taken from the tray and stored inside glass vials at standard conditions until experiment started. Throughout the tests only such ticks were used that readily moved or climbed out of their vial. Ticks remaining motionless on the bottom of their vial were not used. Furthermore, all ticks were screened for sufficient activity at the time of testing by performing a control run on the untreated arm (see below). Only such ticks were used for testing that walked up at least 3 cm on the untreated arm of the same volunteer. Such screening was performed <60 min before a test run with each tick. Each tick was tested only once.
Pretreatment of test organisms befor exposure	See above
Initial density/number of test organisms in the test system	-

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	a) General procedure The test managers applied the products on the forearm of the volunteers, the lowest 5 cm near the wrist remaining untreated. The border of the treated area was marked by a pencil. A second mark was applied at 3 cm above the border, i.e. in direction to the elbow. During tests, the person was sitting, keeping the forearm vertically with the fingertips or palm placed on a horizontal surface.
Number of vessels / concentration	-
Test culture media and/or carrier material	-
Nutrient supply	-
Measuring equipment	Immediately after application (p. a.) of the repellent, a single prescreened nymph of I. ricinus was placed on the untreated skin about 1 cm below the repellent border and observed for a maximum of 1 min. It was protocolled whether • the tick walked onto the treated skin or not, • the tick walked back from treated skin to the untreated area or not, • the tick walked a distance of at least 3 cm on treated skin in direction to the elbow, thereby crossing the second mark, • the tick fell off from the skin. Most of the ticks immediately started walking when placed on the arm. Ticks remaining motionless or walking down the arm were stimulated to walk in direction of the treated area by means of a clean hairbrush. Ticks falling off from the untreated zone before walking onto the treated area were again placed on the starting line. Ticks not walking onto the treated zone within one minute were removed, scored as **"repelled"** and replaced by a new tick. Ticks walking onto the treated skin were given an additional 1 min to cross the second mark 3 cm in direction to the elbow. If the tick, within that time period, did not cross the second mark (either because it fell off, or remained on the treated area, or walked back to the untreated zone) it was removed, scored as "repelled", and replaced by a new tick. Ticks crossing the second circular mark were likewise removed, but scored as **"not repelled".** By this way, a total of 5 ticks were tested every 30 min on each volunteer up to a time of four hours after application of product. A clean hairbrush was always used to transfer the ticks to the arms, and another hairbrush

	was always used to remove the ticks.

1.4 Application of test substance

Criteria	Details
Application procedure	The aim was to apply 1.67 µl repellent per cm² of skin. To do so, the area of the arm was calculated by measuring the length of the lower arm from the border of the treated area near the wrist to the elbow at its respective front and back as well as its circumference at 5 equidistant points across the arm area to be treated. The area such measured was then always completely and evenly covered by repellent. The repellent was applied using a disposable pipette. The repellent was then evenly distributed on the skin by the test manager by hand, wearing nitril gloves. The amount of repellent applied was calculated by weighing the repellent container before and after application as well as the nitril glove used for dispersal (EW420 balance (Kern, Germany) read to the nearest 0.1 mg). Remnant amounts on the glove not applied were subtracted from the above repellent mass applied.
Delivery method	-
Dosage rate	1.67 µl / cm² product on human skin
Carrier	-
Concentration of liquid carrier	-
Liquid carrier control	-
Other procedures	-

1.5 Test conditions

Criteria	Details
Substrate	Care plus Anti Insect DEET lotion 50%, Batch: 11NP038-4
Incubation temperature	Product tests were performed inside a room of 55.5 m² and a height of 3.1 m. Temperature 24.1 ± 0.5 °C
Moisture	Rel. humidity: 51.6 ± 1.5 %
Aeration	-
Method of exposure	-
Aging of samples	-
Other conditions	The 10 volunteers comprised of 5 females and 5 males which were fully informed about the nature and the purpose of the test and of any health consequences involved. All persons voluntarily participated at the study and signed a consent form. The age of the persons was between 20 and 59 years. Test persons were advised not to use any perfume or perfume-rich lotions at the test days nor to drink tee or consume

	caffeine during the test.

Section B5.10 Efficacy Data

Annex Point IIB5.10
TNsG: Pt. I-B5.10, Pt. III-Ch. 6

Specify where appropriate, e.g. wood-rotting fungi, laboratory study

			Official use only
		1 Reference	
1.1	**Reference**	K.-H. Lüpkes, 2011-09-27, Study no: Mo4251, Report no: BIO 095-11Biological, Test Report, Repellent Efficacy of a Product on Human Arms against Mosquitoes: Mosquito repellent effect of a product against, House mosquito Culex quinquefasciatus, Malaria mosquito Anopheles gambiae and, Yellow fever mosquito Aedes aegypti. Product: CARE PLUS ANTI INSECT DEET 50% LOTION, BioGenius GmbH,	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Tropenzorg BV	
1.2.2			
1.2.3	Criteria for data protection	-	
1.3	**Guideline study**	-	
1.4	**Deviations**	-	
		2 Method	
2.1	**Test Substance (Biocidal Product)**	CARE PLUS ANTI INSECT DEET LOTION 50% Active: DEET Concentration: 50 % Batch: 11NP038-4 Deterrent: 10 mg Bitrex / litre)	
2.1.1	Trade name/ proposed trade name	Care Plus Anti Insect DEET lotion 50%	
2.1.2	Composition of Product tested	Product contains 50% w/w DEET denatureted ethanol and Bitrex (denatonium benzoate)as ingestion deterrent .	
2.1.3	Physical state and nature	Liquid, ethanol solution of DEET	
2.1.4	Monitoring of active substance concentration	Yes 50.3 g/100g concentration DEET in product were HPLC-UV	
2.1.5	Method of analysis	concentration DEET in product were HPLC-UV. Test were performed by Masterlab BV, 23-08-2011,	
2.2	**Reference substance**	No -	
2.2.1	Method of analysis for reference substance	-	

Section B5.10 Efficacy Data

Annex Point IIB5.10
TNsG: Pt. I-B5.10, Pt. III-Ch. 6

Specify where appropriate, e.g. wood-rotting fungi, laboratory study

2.3	**Testing procedure**	
2.3.1	Test population / inoculum / test organism	Volunteers The volunteers were representative of potential users of the products in terms of attractiveness for mosquitoes. 5 volunteers were used (additionally 1 volunteer for untreated controls). Test organism: - Malaria mosquito Anopheles gambiae (see table 1.2.1) - Yellow fever mosquito Aedes aegypti, (see table 1.2.2) - House mosquito Culex quinquefasciatus, (see table 1.2.3)
2.3.2	Test system	see Table 1.3
2.3.3	Application of TS	see Table 1.4
2.3.4	Test conditions	see Table 1.5
2.3.5	Duration of the test / Exposure time	Test days: 2 (- one test day for A. aegypti and C. quinquefasciatus, - one test day for A. gambiae) Test Time per Day : Up to 12 hours maximum Test Intervals: Every 60 minutes, beginning 1 hour after treatment, lasting up to 12 hours maximum Test Period per: Test Interval 5 minutes
2.3.6	Number of replicates performed	5 volunteers were used (additionally 1 volunteer for untreated controls). The volunteers were representative of potential users of the products in terms of attractiveness for mosquitoes.
2.3.7	Controls	Yes, (untreated arm of volunteer)
2.4	**Examination**	
2.4.1	Effect investigated	Repellent Efficacy of a Product Care Plus Anti Insect DEET lotion 50% on human arms against mosquitoes
2.4.2	Method for recording / scoring of the effect	The tests were performed as Arm-in-cage tests. For this reason the forearms of volunteers were treated with product. 5 volunteers were used. For the tests the volunteers exposed both treated forearms (2 products were tested at the same time) into the test cages with mosquitoes (approx. 1000 mosquitoes of a mixed population in terms of sex and age, meaning about 500 “blood thirsty” female mosquitoes). Exposition was started 1 hour after treatment of the volunteer forearms, and the test ended maximally 14 hours after treatment. During that time the test was performed at intervals of one hour. The exposure time of volunteer forearms was 5 minutes per interval. The number of bites as well as number of landings was recorded. The tests ended when the relevant volunteer got two bites within one test interval or within two subsequent test intervals.
2.4.3	Intervals of examination	Every 60 minutes, beginning 1 hour after treatment, lasting up to 12 hours maximum, Test Period per Test Interval-5 minutes
2.4.4	Statistics	No

Section B5.10 **Efficacy Data**
Annex Point IIB5.10 *Specify where appropriate, e.g. wood-rotting fungi, laboratory study*
TNsG: Pt. I-B5.10, Pt. III-Ch. 6

2.4.5	Post monitoring of the test organism	No

3 Results

3.1 Efficacy

Repelency effect of Care Plus Anti Insect DEET lotion 50%

.

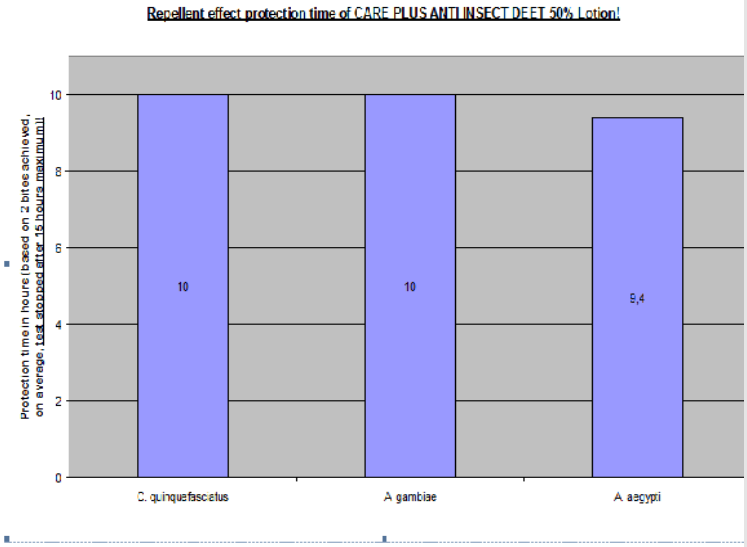
With the tested product the following protection times against bites (based on an average of 5 volunteers and two bites within one test interval or within two subsequent test intervals achieved were recorded:

- Culex quinquefasciatus: more than 10 hours of protection (at a mean of all volunteers),

- Anopheles gambiae: more than 10 hours of protection (at a mean of all volunteers) and

- Aedes aegypti: 9.4 hours of protection (at a mean of all volunteers).

3.1.1 Dose/Efficacy curve



3.1.2	Begin and duration of effects	-
3.1.3	Observed effects in the post monitoring phase	-
3.2	**Effects against organisms or objects to be protected**	-
3.3	**Other effects**	-
3.4	**Efficay of the reference**	-

Section B5.10 **Efficacy Data**
Annex Point IIB5.10 *Specify where appropriate, e.g. wood-rotting fungi, laboratory study*
TNsG: Pt. I-B5.10, Pt. III-Ch. 6

substance

3.5 Tabular and/or graphical presentation of the summarised results

Mosquito repellent effect / Protection time of product
CARE PLUS ANTI INSECT DEET 50% Lotion
against House mosquito *Culex quinquefasciatus*, Malaria mosquito *Anopheles gambiae* and Yellow fever
on the forearms of volunteers (summarized results).

Study: Mo4251
Method: BPD Bio(;B 951-01 (modified) → results based on 2 bites achieved →

test insects	protection time in hours (h), 5 volunteers: A	B	C	D	E
House mosquito *Culex quinquefasciatus*	>11h	>11h	>11h	>9h	>11h
	Ø >10h				
Malaria mosquito *Anopheles gambiae*	>11h	>11h	>11h	>9h	>10h
	Ø >10h				
Yellow fever mosquito *Aedes aegypti*	8h	7h	14h	7h	11h
	Ø 9.4 h				

Protection was given as long as 2 bites (within 1 test interval or within 2 subsequent test intervals) didn't occur, test interval: hourly. → →

3.6 Efficacy limiting factors Non-entry field

3.6.1 Occurrences of resistances -

3.6.2 Other limiting factors -

4 Relevance of the results compared to field conditions

4.1 Reasons for laboratory testing - Easier to use standard parameters in the laboratory,

4.2 Intended actual scale of biocide application - CarePlus Anti Insect DEET lotion 50%. gave slightly more than 10 hr protection against mosquitoes.

4.3 Relevance compared to field conditions

4.3.1 Application method In the field, the application will be applied by hand.

4.3.2 Test organism Test are also target organism. See 2.3.1

4.3.3 Observed effect Yes, CarePlus Anti Insect DEET lotion 50%. protection for more 10 hours against mosquitoes.

Se efficacy for spasis.

- Culex quinquefasciatus: more than 10 hours of protection (at a mean of all volunteers),
- Anopheles gambiae: more than 10 hours of protection (at a mean of all volunteers) and
- Aedes aegypti: 9.4 hours of protection (at a mean of all volunteers).

4.4 Relevance for No

Section B5.10 **Efficacy Data**
Annex Point IIB5.10 *Specify where appropriate, e.g. wood-rotting fungi, laboratory study*
TNsG: Pt. I-B5.10, Pt. III-Ch. 6

read-across

5 Applicant's Summary and conclusion

5.1 Materials and methods

The tests were performed as Arm-in-cage tests. For this reason the forearms of volunteers were treated with product. 5 volunteers were used.For the tests the volunteers exposed both treated forearms (2 products were tested at the same time) into the test cages with mosquitoes (approx. 1000 mosquitoes of a mixed population in terms of sex and age, meaning about 500 “blood thirsty” female mosquitoes). Exposition was started 1 hour after treatment of the volunteer forearms, and the test ended maximally 14 hours after treatment. During that time the test was performed at intervals of one hour. The exposure time of volunteer forearms was 5 minutes per interval.

The number of bites as well as number of landings was recorded.

The tests ended when the relevant volunteer got two bites within one test interval or within two subsequent test intervals.

5.2 Reliability

Reliability indicator: 1 Study conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of relevant

5.3 Assessment of efficacy, data analysis and interpretation

Checking of the biting activity with an exquisite volunteer (untreated forearm area of approx. 25 cm²) showed sufficient biting activity for the mosquitoes in all cases. With:

- Culex quinquefasciatus always 10 bites within 110 seconds (maximum) were found,

- Anopheles gambiae always 10 bites within 55 seconds (maximum) were found, and

- Aedes aegypti always 10 bites within 30 seconds (maximum) were found.

With the tested product the following protection times against bites (based on an average of 5 volunteers and two bites within one test interval or within two subsequent test intervals achieved) were recorded:

- Culex quinquefasciatus: more than 10 hours of protection (at a mean of all volunteers),
- Anopheles gambiae: more than 10 hours of protection (at a mean of all volunteers) and
- Aedes aegypti: 9.4 hours of protection (at a mean of all volunteers).

Concerning landings:

With Culex quinquefasciatus nearly no landings were found (with 2 of 5 volunteers starting with 1 landing after 10 hours). With Anopheles gambiae nearly no landings were found (with 1 of 5 volunteers after 10

Section B5.10 **Annex Point IIB5.10** **TNsG: Pt. I-B5.10,** **Pt. III-Ch. 6**	**Efficacy Data** *Specify where appropriate, e.g. wood-rotting fungi, laboratory study*

		hours 4 landings). With Aedes aegypti with the first volunteer 1 landing was found after 4 hours, partly up to 7 landings within a 5 minutes test interval were found
5.4	**Conclusion**	Product Care Plus Anti insect DEET Lotion 50% is effective as repellent against mosquitoes for 10 hours. - Culex quinquefasciatus: more than 10 hours of protection - Anopheles gambiae: more than 10 hours of protection - Aedes aegypti: 9.4 hours of protection
5.5	**Proposed efficacy specification**	Product Care Plus Anti insect DEET Lotion 50% is effective as repellent for 10 hours against mosquitoes (house mosquito ,malaria mosquito and yellow fever mosquito).

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	*18-04-2014*
Comments	*The studies were done with only 5 test persons and not with ten as is now standard in these type of studies. The studies, however, fit in a whole series of tests with this product in different formulations and DEET concentrations and this series of studies shows consistent results. The data and tests therefore support each other and the claims are sufficiently solid and valid.* *It was decided in e-consultation between MS to give Protection Time(PT) in whole hours. The average PT of 9.4 hours for Aedes will therefore be given as 9 hours Protection Time on the SPC and the label.*
Summary and conclusion	*The studies were well done. The conclusions are valid, with a remark that the protection time against Aedes mosquitoes will be put at 9 hours and not at 10 hours.*
	6 Comments from ... *(SPECIFY)*
Date	*Give date of comments submitted*
Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Tables for Method

1.2.1 Test organism Malaria mosquito Anopheles gambiae)

Criteria	**Details**
Species	Malaria mosquito Anopheles gambiae
Strain	strain BioGenius 07
Source	BioGenius GmbH
Laboratory culture	yes
Stage of life cycle and stage of stadia	7 days old minimum
Mixed age population	-
Other specification	mixed sex
Number of organisms tested	Approximately 1000
Method of cultivation	The adults are kept in cages (48 x 48 x 39 cm) with netting on the sides and upper part under day light conditions, additionally artificial light is supplied at 25°C ±1°C and 60% ±10% relative humidity. Cotton wool saturated with 10 % sucrose solution (Dextropur®) is placed in the cage as a drinking source. An artificial blood feeding system is used twice a week. Cattle blood with anticoagulant (CPD) is placed in a dish on a heating plate with a magnetic stirrer. After heating the blood to 40°C 50 ml filled into a pigs bowel and placed in the cages and the mosquitoes are able to suck the blood. A plastic soup tureen is used as an oviposition station which is filled with tap water. The eggs are placed in a plastic container (25 x 37 cm) with five liters of tap water and kept on a heater mat. Food for tropical fish (Vita®) is added once a day, except Sundays. After seven days the population (pupae) is transferred into buckets (10 liters) in an adequate amount of water. The hatched adults are used for testing or for breeding purposes again.
Pretreatment of test organisms before exposure	See above
Initial density/number of test organisms in the test system	1 female mosquito per approx. 216 cm^3 Approximately 1000 mix sex mosquitoes, where 500 female,

1.2.2 Test organism Yellow fever mosquito Aedes aegypti,

Criteria	**Details**
Species	Yellow fever mosquito Aedes aegypti,
Strain	Strain BioGenius 04
Source	BioGenius GmbH
Laboratory culture	yes
Stage of life cycle and stage of stadia	susceptible, 7 days old minimum
Mixed age population	-

Other specification	mixed sex
Number of organisms tested	Approximately 1000
Method of cultivation	The adults are kept in cages (48 x 48 x 39 cm) with netting on the sides and upper part under day light conditions, additionally artificial light is given during work hours at 25°C ±1°C and 60% ±10% relative humidity. Cotton wool saturated with 10 % sucrose solution (Dextropur®) is placed in the cage as a drinking source. An artificial blood feeding system is used once a week. Cattle blood with anticoagulant (CPD) is placed in a dish on a heating plate with a magnetic stirrer. After heating the blood to 40°C 50 ml filled into a pigs bowel and placed in the cages and the mosquitoes are able to suck the blood. The oviposition stations consist of beakers, half filled with tap water, with filter papers at the edges. Afterwards oviposition the filter papers are kept in a bucket (10 litres), the floor of which is covered with a 3 cm layer of humidified cellulose. A filter paper with eggs (two up to four weeks old) is placed in a plastic container (25 x 37 cm) with five litres of tap water and kept on a heater mat. Food for tropical fish (Vita®) is added once a day, except Sundays. After four to five days the population (pupae) is transferred into buckets (10 litres) in an adequate amount of water. The hatched adults are used for testing or for breeding purposes again.
Pretreatment of test organisms befor exposure	See above
Initial density/number of test organisms in the test system	1 female mosquito per approx. 216 cm^3 Approximately 1000 mix sex mosquitoes, where 500 female,

1.2.3 Test organism House mosquito Culex quinquefasciatus,

Criteria	**Details**
Species	House mosquito Culex quinquefasciatus,
Strain	strain BioGenius 05,
Source	BioGenius GmbH
Laboratory culture	yes
Stage of life cycle and stage of stadia	7 days old minimum
Mixed age population	-
Other specification	mixed sex
Number of organisms tested	Approximately 1000
Method of cultivation	The adults are kept in cages (48 x 48 x 39 cm) with netting on the sides and upper part under day light conditions, additionally artificial light is supplied during work hours at 25°C ± 1°C and 60% ±10% relative humidity. Cotton wool saturated with 10 % sucrose solution (Dextropur®) is placed in the cage as a drinking source. An artificial blood feeding system is used twice a week. Cattle blood with anticoagulant

	(CPD) is placed in a dish on a heating plate with a magnetic stirrer. After heating the blood to 40°C 50 ml filled into a pigs bowel and placed in the cages and the mosquitoes are able to suck the blood. A plastic dish with a diameter of 10 cm is used as an oviposition station which is filled up to a depth of two cm with tap water. The eggs are placed in a plastic container (25 x 37 cm) with five litres of tap water and kept on a heater mat. Food for tropical fish (Vita®) is added once a day, except Sundays. After six days the population (pupae) is transferred into buckets (10 litres) in an adequate amount of water. The hatched adults are used for testing or for breeding purposes again
Pretreatment of test organisms befor exposure	See above
Initial density/number of test organisms in the test system	1 female mosquito per approx. 216 cm^3 Approximately 1000 mix sex mosquitoes, where 500 female,

1.3 Test system

Criteria	**Details**
Culturing apparatus / test chamber	Test cages: macrolon, 90 cm long, 30 cm wide, 40 cm high (= 108.000 cm³) side walls made of gauze, two light cloth sluices at front side Others: micro-syringe
Number of vessels / concentration	Various test cages were used, each test cage was used for 2 test intervals
Test culture media and/or carrier material	-
Nutrient supply	-
Measuring equipment	**Test Criteria** - number of bites - additionally number of landings **Definition of Test Criteria** - Bite: A bite is the act of penetrating human skin by the mouthparts of a mosquito with ingestion of blood - Landing: A landing is the act of a mosquito alighting on human skin without biting End of Test / Criteria to Stop Test The tests were stopped for each volunteer after receiving two bites within one test interval or within two subsequent test intervals. Definition of Protection Time Protection time was given as long as no bites or maximum one bite was given. By receiving two bites within one test interval or within two subsequent test intervals the protection time ended.

1.4 Application of test substance

Criteria	Details
Application procedure	Amount of sample according to EPA requirements and test method BPD BioG B 591-01 Amount per 90 cm² forearm area: 150 µl (= 1.67 µl / cm²) Amount per edge of sleeve opening (approx. 1 cm width): 200 µl All amounts of all products were applied by µl
Delivery method	-
Dosage rate	1.67 µl / cm² product on human skin
Carrier	-
Concentration of liquid carrier	-
Liquid carrier control	-
Other procedures	-

1.5 Test conditions

Criteria	Details
Substrate	Care plus Anti Insect DEET lotion 50%, Batch: 11NP038-4
Incubation temperature	Room Conditions Temperature: 24 - 25 °C Light regime: weak day light
Moisture	Rel. humidity: 55 - 70 %
Aeration	-
Method of exposure	-
Aging of samples	-
Other conditions	The volunteers shall avoid alcohol, caffeine and fragrance products (e.g. perfume, cologne, hair spray, lotion etc.) for 12 hours before and during the test. Volunteers (test subjects) forearms are washed with unscented (fragrance free) soap, rinsed with water, then with a solution consisting of 70 % ethanol and 30 % water and dried with a towel. 90 cm² of each forearm of a volunteer is treated evenly with 150 µl of the test product by a syringe (liquid formulations taken directly from the products container). Beginning one hour after treatment a sleeve with an opening of 3.1 x 8 cm (approx. 25 cm²) is fastened around the arm in such a way that the opening is positioned completely over the treated area. The edges of the opening of the sleeve have been also treated with the test material (200 mg or µl) in a width of approx. 1 cm to prevent bites from the edge. The areas above the sleeves are protected by cloth a

	proboscis cannot penetrate; hands are protected by latex gloves. Volunteers shall avoid exertion which might increase perspiration, or abrasion, rubbing, touching or wetting the treated area. Both arms (two products were tested at one test day) are introduced into the cage through the cloth sluices and the number of bites and, additionally, landings per arm in a five minutes test period is registered. The test is repeated every hour up to a maximum of 12 hours, or finished before, if efficacy has ended (two or more bites within five minutes or within 2 subsequent testing's). Each test consists of five volunteers