Diethyl-*m*-toluamide (DEET)

[134-62-3]

Insect Repellent

Review of the Toxicology Literature for the Topical Insect Repellent Diethyl-m-toluamide (DEET)

SCIENTIFIC EVALUATION AND ASSESSMENT

SUMMARY

Insect repellents are used to prevent nuisance bites from mosquitoes (as well as ticks, biting flies, and mites) and may aid in lowering disease transmission from these pests e.g. Malaria and West Nile virus. *N*, *N*-diethyl-*m*-toluamide (DEET) seems to be most effective and is the best studied insect repellent currently available to the general public. It is reported to effectively control 'mosquitoes, biting flies, biting midges, black flies, chiggers (mites), deerflies, fleas, gnats, horse flies, no see-ums, sand flies, small flying insects, stable flies and ticks' (USEPA, 1998).

DEET has been available for use world-wide for 40 years, however testing of more than 20,000 other compounds has not resulted in another marketed chemical product with the duration of protection and broad-spectrum effectiveness of DEET. It, as well as other insect repellents, is marketed in the United Kingdom in a variety of forms and concentrations. Aerosol and pump-spray products are intended for skin applications as well as for treating clothing. Liquid, cream, lotion and stick products enable direct skin application.

The following document reviews the relevant metabolism and toxicological data currently available. A number of toxicology studies were not available, and the EPA evaluation has been cited in these instances. A formal data call was not undertaken. A brief overview of the key data in the toxicology review is given below.

ADME studies

The dermal penetration and absorption of DEET has been extensively studied in laboratory animals using *in-vitro* and *in-vivo* methods. The percentage absorbed was dependent on the solvent used, concentration of DEET, method used to determine absorption (e.g. recovery in urine following dermal application or comparison of kinetics following intravenous or dermal application) and extent of occlusion used. A number of studies using human volunteers and *in-vitro* methods with human skin samples are available that confirm the influence of solvent and formulation on absorption of DEET. From these studies, it is suggested that 20% of DEET can be absorbed for a 15% ethanolic solution of DEET and 12% for 100% DEET.

In a study submitted to the EPA, the oral absorption of DEET in rats was estimated to be 96%. In this study absorbed DEET was rapidly distributed and excreted mainly in the urine as metabolites. The predominant pathways of metabolism involved oxidation of the methyl group on the aromatic ring and N-deethylation of the amide moiety. The available studies in animals that used dermal administration also showed that absorbed DEET was excreted predominantly via the urine.

Two studies have examined the metabolism of DEET following topical application to humans. The available information suggests a qualitatively and quantitatively similar to that documented in rats following oral administration. Overall, it would seem that a value of 20% for dermal absorption could be used for risk assessment. In addition the ADME of DEET in experimental animals and humans is similar.

Toxicology: Studies in Experimental Animals

Data on the acute toxicity, irritancy (skin/eye), and skin sensitisation of DEET are available in a number of studies. Limited data are available on individual formulations. DEET is of low acute oral (LD_{50} 2170-3664 mg/kg bw) and inhalation (LC_{50} 5.95 mg/l) toxicity in the rat and low acute dermal toxicity (LD_{50} 4280 mg/kg bw) in the rabbit. One particular study suggests that the toxicity of DEET may be decrease with age and additionally females may be more susceptible than males. DEET is a mild skin irritant in rabbits and is not a skin sensitiser in guinea pigs. DEET is an eye irritant, although evidence of reversal by 168 h suggests that DEET may not induce serious damage to eyes but should be regarded as an eye irritant.

There was no evidence of toxicologically significant effects on reproduction in rats, or on development in rats and rabbits. DEET was not carcinogenic to rats or mice. DEET was not mutagenic *in-vitro* in *Salmonella typhimurium* (in presence/absence of exogenous metabolic activation), did not induce clastogenicity in CHO cells *in-vitro*, and did not induce Unscheduled DNA Synthesis in rat hepatocytes, *in-vitro*. These data were provided for the EPA review.

NOAEL's for all repeat dose, reproduction, developmental and carcinogenicity studies were all above 100 mg/kg/day). At higher doses in these studies effects on body weight gain were reported which were due to reduce food intake. Thus no toxicologically significant effects were documented from an adequate range of studies.

Evidence of treatment-related effects was documented in the acute neurotoxicity and sub-chronic neurotoxicity study, however the effects were mild and only seen at the top dose levels. The EPA evaluation was that these effects should not be used in risk assessment.

Toxicology: Evidence from human case reports:

An overview of all the published case reports detailing adverse effects documented in individuals exposed to DEET is presented. Twenty-nine case reports were found. These predominantly came from the U.S.A. The effects reported have to be considered in the context of the large proportion of the US population regularly using DEET containing insect repellents. There are no formulation details available for these case reports and thus effects of co- formulants can't be excluded. Seven involved dermal effects (although several involve more than one individual), 14 reported CNS toxicity (a total of 18 cases), 3 predominantly psychosis, 1 reported adverse effects on reproduction and 4 reported a range of effects including CNS toxicity and dermal effects (4 cases).

With regard to the dermal exposure cases, there was evidence of severe skin reactions in one report. It is uncertain what role co-formulants played in these reactions and no information on formulation details are available. The findings from this case report have not been reproduced on such a scale in other reports and are not consistent with the large-scale use of DEET products by millions of people every year. The evidence is suggest that DEET products under normal conditions of use do not cause irritancy but may, in some circumstances where exposure is high and/or prolonged, give rise to severe irritation of the skin and in a very cases skin sensitisation.

Risk assessment

Possible approaches to risk assessment are reviewed. In particular, the risk assessment methodologies of the USA and Canada are considered, as well as an approach suggested by the DEET Joint Ventures group. Issues relating to endpoint selection, particularly relating to neurotoxicity are discussed.

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This report has been prepared by the Department of Health Toxicology Unit at Imperial College

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1 INTRODUCTION

This introduction aims to give information on the active ingredient (section 1.1) and to detail the use of the active ingredient in manufacturer products (section 1.2)

1.1 Identity of the Active Substance

Sections 1.1.1 to 1.1.13 present details of the active substance (i.e. DEET) that is found in insect repellent products.

1.1.1 Substance Name

N,N-Diethyl-3-methyl-benzamide
DEET
Diethyl toluamide
N,N-Diethyl-3-methylbenzamide
N,N-Diethyl-m-toluamide
M-Delphene

1.1.2 CAS Number

134-62-3

1.1.3 Other Common Names and Synonyms

3-Methyl-N,N-diethylbenzamide
4-09-00-01716 (Beilstein Handbook Reference)
AI 3-22542
AI3-20218
Amincene C-EM
Autan
BRN 2046711
Benzamide, N,N-diethyl-3-methyl-
Bepper DET
Caswell No. 346
Chemform
DEET
DET (insect repellent)
DETA
DETA-20
Delphene
Detamide
Dieltamid
Diethyl-m-toluamide
Diethyltoluamide
Diethyltoluamidum [INN-Latin]
Dietiltoluamida [INN-Spanish]
EINECS 205-149-7

ENT 20,218
ENT 22542
EPA Pesticide Chemical Code 080301
Flypel
M-Det
MGK
MGK diethyltoluamide
Metadelphene
Muscol
N,N-Diethyl-3-methylbenzamide
N,N-Diethyl-m-toluamide
N,N-Diethyl-meta-toluamide
NSC 33840
Naugatuck DET
Off
Repel
Repper DET
Repper-DET
Repudin-Special
m-DETA
m-Delphene
m-Toluamide, N,N-diethyl-
m-Toluic acid diethylamide

1.1.4 Molecular Formula and structural formula, molecular weight

Molecular Formula : $C_6H_4CH_3CON(C_2H_5)_2$
$C_{12}H_{17}NO$

Structural Formula:
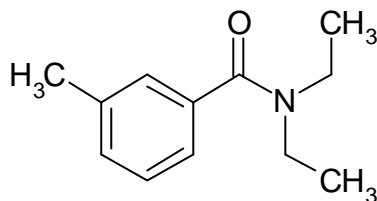
Molecular Weight: 191.26

1.1.5 Popular UK Manufacturers/Retailers

The following is an illustrative list of UK manufacturers and retailers selling insect repellents containing DEET in the UK.

Ardern Healthcare
Boots
Cameron Products UK
Jaico
Jungle Formula
J. Pickles & Sons
Mosi-guard
Nomad Travel

This list is not exhaustive and has been collected from the Internet until October 2002.

1.1.6 Chemical Class

Aromatic amide (N, N-dialkylarylamide)

1.1.7 Function

Insect Repellent

1.1.8 Specification of Purity of the Active Substance

Technical DEET is composed of more than 95% *m*-DEET isomer. Ortho (*o*-DEET) and para (*p*-DEET) isomers are slightly more and less toxic than *m*-DET, respectively (Ambrose and Yost, 1965).

1.1.9 Regulatory History

United Kingdom

DEET is not regulated as a pesticide under the Control of Pesticides Regulations 1986, or as a medicine under the Medicines Act 1968. Insect repellents are controlled by the Biocides Product Directive (98/8/EC), as enacted by regulations in the UK in May 2000.

A review process is under discussion by EU competent authorities, such as the Health and Safety Executive for the UK. It is likely that any review of used insect repellents will not take place for several years.

USA

DEET was first developed by the U.S. Department of Agriculture for military use in 1946 and was first registered in the United States in 1957.

DEET is an EPA-registered "insecticide". In 1991, notice was sent to manufacturers, formulators, producers, and registrants of DEET-containing products regarding new label requirements implemented under the Label Improvement Program (LIP) established in 1980 (USEPA, 1991). The new label requirements were a result of EPA concern for individuals who may be hypersensitive to DEET, based on reports of adverse reactions from topical applications of the chemical and from accidental exposures, such as swallowing or spraying in the eye. The EPA mandated that all labels should include the following statements:

- Read all directions before using this product
- Do not spray in enclosed areas. (For sprays and aerosols only)
- Do not apply over cuts, wounds, or irritated skin
- Do not apply to eyes and mouth and do not apply to the hands of young children. Do not spray directly on face
- Use just enough repellent to cover exposed skin and/or clothing. Do not use under clothing. Avoid overexposure. Frequent reapplication and saturation is unnecessary for effectiveness
- After returning indoors, wash treated skin with soap and water. Wash treated clothing
- Use of this product may cause skin reactions in rare cases. If you suspect that you or your child is reacting to this product, wash treated skin, then call your local poison control centre. If you go to a doctor, take this repellent with you

In April 1998, the EPA issued a Re-registration Eligibility Decision (RED) for the chemical DEET (US EPA, 1998). For EPA recommendations on the use of DEET following this decision, see section 1.2.3.

Canada

Personal insect repellents containing DEET have been registered in Canada since 1957. As of July 2001, there were 127 registered end-use products containing DEET, all personal insect repellents, from 41 registrants (Re-evaluation Decision Document RRD2002-01, Pest Management Regulatory Agency, Canada).

1.1.10 Organisms Controlled

DEET is generally considered to be an effective insect repellent for control of biting flies, biting midges, black flies, chiggers (mites), deerflies, fleas, gnats, horse flies, mosquitoes, no see-ums, sand flies, small flying insects, stable flies and ticks (USEPA, 1998). It is also used as a prophylactic agent against several insect-borne diseases, such as Lyme disease, human granulocyte ehrlichiosis, encephalitis, malaria, dengue fever, yellow fever

and Rocky Mountain spotted fever. It should be noted that the EPA carried out no assessment of efficacy.

1.1.11 Mode of Action

Mosquito attraction to a host involves a complex behavioural cascade of responses to long-range and short-range stimuli, both olfactory and visual. At close range, lactic acid is an important and possibly essential trigger for landing on the host. Indeed, it is thought that the presence of lactic acid is the particular stimulant that directs the mosquito to land. Therefore, the unique effectiveness of DEET is considered to be due to its ability to mask the sensory perception of lactic acid on the skin.

1.1.12 Effects on Harmful Organisms

DEET is primarily used to repel biting pests such as mosquitoes and ticks. Therefore, no direct effects on harmful organisms should occur.

1.1.13 Field of Use

Application of DEET can be direct to the human body or onto clothing while being worn. In this way, DEET may be used as an insect repellent either indoors (households and domestic dwellings) or outdoors.

1.2 Identity of the Manufacturers Product

Section 1.2.1 to section 1.2.11 present details of manufacturers insect repellent products available in the UK that contain the active substance (i.e. DEET). Some information on insect repellents from the USA is presented for comparison.

1.2.1 Trade Names for DEET-Containing Products Currently Available for Application to Skin

Manufacturer	Preparation	Active ingredient
Ardern Healthcare	Ben's 100 Spray	95% DEET
	Ben's 30 lotion	30% DEET
Boots	Repel Plus Spray	20% DEET
	Repel Plus Pump	20% DEET
	Repel Plus Gel	20% DEET
	Repel Plus Roll-on	20% DEET
	Repel Plus Wasp Spray	unknown
Cameron Products	BFZEE Zone 3 tissue wipes	30% DEET
Jaico	Mosquito Milk Roll-on	24% DEET
Jungle Formula	Extra Strength Pump Spray	50% DEET
	Extra Strength Liquid	50% DEET
J. Pickles & Sons	Mijex Super Strength Roll-on	60% DEET
	Mijex High Strength Spray	50% DEET
	Mijex Gel	20% DEET
	Mijex Stick	10% DEET
Lifesystems	Expedition 50	50% DEET
	Expedition 2- Roll on	20% DEET
Mega-grip	Child Formulation	20% DEET (+ Citronella)
	Adult Formulation	50% DEET (+ Citronella)
Mosi-guard	DEET Pump Spray	50% DEET
Nomad Travel	DEET Spray	50% DEET
	DEET Lotion	50% DEET

Table 1.1: Popular Insect Repellents Containing DEET (UK Market)
This is not a complete listing and is only representative of the range sold in the UK. Data collected from Internet web sites in October 2002.

1.2.2 Trade names for DEET-containing products currently available for application to clothing/bedding only

Manufacturer	Preparation	Active ingredient
Lifesystems	Expedition 100 spray	100% DEET
Mega-Grip	Clothing Formulation	99% DEET + Citronella
Nomad Travel	Neet DEET	100% DEET

Table 1.2: Popular Insect Repellents Containing DEET (UK Market)
This is not a complete listing and is only representative of the range sold in the UK. Data collected from Internet web sites in October 2002.

1.2.3 Recommended Use

<u>United Kingdom</u>

A review of the use of DEET containing insect repellent products marketed in the UK has not been undertaken to date. Insect repellents are currently regulated under the Biocides Product Directive (98/8/EC), as enacted by regulations in the UK in May 2000. The following table (table 1.3) provides information on the manufacturers' recommended use of these products.

Manufacturer	Preparation	Active ingredient(s)	Manufacturers recommended use
Ardern Healthcare	Ben's 100 Spray	95% DEET	10 hours protection; not for use by children or by the pregnant (from www.ardernhealthcare.co.uk)
	Ben's 30 lotion	30% DEET	8 hours protection; family use (not infants) (from www.ardernhealthcare.co.uk)
Cameron Products	BFZEE tissue wipe (zone 3)	30% DEET	Apply to exposed skin. 4-5 hours protection. Not for use for children under 2 years. (from www.bfzee.com)
Boots	Repel Plus Spray	20% DEET	<u>Face</u>: Spray onto hands then wipe face <u>Body</u>: Spray 20cm from skin <u>Children</u>: Suitable for 6 years and over. Apply as above. Renew application every 3 hours or as necessary. Reapply after swimming. (from product label)
	Repel Plus Pump	20% DEET	<u>Face</u>: Spray onto hands then wipe face <u>Body</u>: Spray 15cm from skin <u>Children</u>: Suitable for 6 years and over. Apply as above. Renew application every 3 hours or as necessary. Reapply after swimming. (from product label)
	Repel Plus Gel	20% DEET	Apply lightly to exposed areas. Suitable for 6 years and over Renew application every 3 hours or as necessary. Reapply after swimming. (from product label)
	Repel Plus Roll-on	20% DEET	Apply lightly to exposed areas. Suitable for 6 years and over Renew application every 3 hours or as necessary. Reapply after swimming. (from product label)
	Repel Plus Wasp Spray	unknown	6 hours protection: suitable for 3 years and over (from www.boots.co.uk)
Jaico	Mosquito Milk Roll-on	24% DEET	Apply several stripes over exposed parts of body. Total coverage not necessary. Offers 8 hours protection. Suitable for 3 years and over. (from product label)

Table 1.3: Recommended Use of DEET Containing Insect Repellents
These recommendations are taken from the labelling of manufacturer products and reflect the manufacturer product assessment. This is not a complete listing. The products listed are representative of the range sold in the UK. Data collected from Internet web sites and product labelling until October 2002.

Manufacturer	Preparation	Active ingredient(s)	Manufacturers recommended use
Jungle Formula	Extra Strength Pump Spray	50% DEET	10 hours protection. Apply when necessary. Suitable for children aged 10 or over (from www.jungleformula.com)
	Extra Strength Liquid	50% DEET	
J. Pickles & Sons	Mijex Super Strength Roll-on	60% DEET	Not recommended for children or babies.
	Mijex High Strength Spray	50% DEET	Reapply every six hours or after swimming
	Mijex Gel	20% DEET	Reapply every six hours or after swimming
	Mijex Stick	10% DEET	Reapply every six hours or after swimming
			(www.jpickleshealthcare.com)
Lifesystems	Expedition 50	50% DEET	Can be used by children without sensitive skin. Application lasts 8 hours. Reapply after swimming
	Expedition 2- Roll on	20% DEET	Not Specified (www.gear-zone.co.uk)
Mega-grip	Child Formulation	20% DEET (+ Citronella)	Not Specified
	Adult Formulation	50% DEET (+ Citronella)	Not Specified
			(From www.megagrip.co.uk)
Mosi-guard	DEET Pump Spray	50% DEET	Not recommended for skin application. Not for use on children aged six or under. (www.masta.org)
Nomad Travel	DEET Spray	50% DEET	In hot and humid climates, re-application may need to be as much as once an hour (due to fast 'sweat-off' time)
	DEET Lotion	50% DEET	In hot and humid climates, re-application may need to be as much as once an hour (due to fast 'sweat-off' time)
	Neet DEET	100% DEET	Only for use on clothing (cotton) (from www.nomadtravel.co.uk)

Table 1.3 (continued): Recommended Use of DEET Containing Insect Repellents
These recommendations are taken from the labelling of manufacturer products and reflect the manufacturer product assessment. This is not a complete listing. The products listed are representative of the range sold in the UK. Data collected from Internet web sites and product labelling until October 2002

USA

In April 1998, the EPA issued a Re-registration Eligibility Decision for the chemical DEET (USEPA, 1998). After completing a comprehensive re-assessment of DEET, the EPA concluded that, as long as consumers follow label directions and take proper precautions, insect repellents containing DEET do not present a health concern. Human exposure is expected to be brief, and long-term exposure is not expected. Based on extensive toxicity testing, the EPA believed that the normal use of DEET does not present a health concern to the general population.

Most of the changes to DEET registrations required by the EPA concerned label directions and claims (see below). The EPA also encouraged companies to provide a company telephone number or toll-free number on all product labels for consumers to call for additional product information and to report incidents.

Statements required on ALL DEET product labels (USEPA, 1998)

- Read and follow all directions and precautions on this product label
- Do not apply over cuts, wounds, or irritated skin
- Do not apply near eyes and mouth. Apply sparingly around ears
- Do not apply to children's hands
- Do not allow children to handle this product
- When using on children, apply to your own hands and then put it on the child
- Use just enough repellent to cover exposed skin and/or clothing

- Do not use under clothing
- Avoid over-application of this product
- After returning indoors, wash treated skin with soap and water
- Wash treated clothing before wearing it again
- Use of this product may cause skin reactions in rare cases
- If you suspect a reaction to this product, discontinue use, wash treated skin, and call your local poison control centre
- If you go to a doctor, take this product with you

Additional statements required on aerosol and pump spray formulations containing DEET product labels (USEPA, 1998)

- Do not spray in enclosed areas
- If used on the face spray on hands first and then apply sparingly and avoid eyes. Do not spray directly onto face.

Canada

Personal insect repellents containing DEET have been re-evaluated by the Pest Management Regulatory Agency (Re-evaluation Decision Document RRD2002-01, Pest Management Regulatory Agency, Canada). The following decisions were noted.

- Children under six months of age were advised not to use personal insect repellents containing DEET
- Children between the ages of six months and two years may use one application of DEET (10% or less) in situations where there is a high risk of complications from insect bites. The product should be applied sparingly, not to face and hands and prolonged use should be avoided.
- Children between two and 12 years may use up to three applications per day of a low concentration product (below 10%). The product should be applied sparingly, not to face and hands and prolonged use should be avoided.
- Children older than 12 years and adults may use products containing DEET up to 30% concentration.

1.2.4   Type of Products

Preparations are available in the United Kingdom as Aerosol/Non-aerosol sprays, roll-on sticks, gels and lotions (see table 1.1).

1.2.5   Application Rate

There is no specific data available. Application rates vary depending on the concentration of DEET in the product (see table 1.3). As a general rule, higher concentrations of DEET are suggested to provide longer-lasting protection.

1.2.6   Concentration of Active Substance in Material Used

In the UK, concentrations of DEET in insect repellents range between 10% and 100% (see table 1.3).

1.2.7   Method of Application

Repellents may be applied directly to skin or clothing, window screens, bedding, mesh insect nets, tents, or sleeping bags. If DEET-treated garments are stored in a plastic bag between wearing, the repellent effect can last for many weeks.

1.2.8   Necessary Waiting Period or Other Precautions to Avoid Effects

Not specified

1.2.9   Procedures for Washing Off in Emergency

The following procedures are recommended for washing off DEET in cases of emergency (as reported on the National Toxicology Program (NTP) website).

Skin: Immediately flood affected area with water. Gently wash all affected skin areas thoroughly with soap and water.

Eye: Flush injured person's eyes with water or normal saline solution for 20 to 30 minutes

1.2.10   Emergency Measures in Case of Accident

The following clean up and first aid procedures are recommended in case of accident (as reported on the NTP website)

Skin Contact: Gently wash all affected skin areas thoroughly with soap and water while removing and isolating all contaminated clothing.
If symptoms persist such as redness or irritation develop, immediately call a physician and be prepared to transport the injured person to a hospital for treatment

Inhalation: Immediately leave the contaminated area; take deep breaths of fresh air. If symptoms (such as wheezing, coughing, shortness of breath, or burning in the mouth, throat or chest) develop, call a physician and be prepared to transport the injured person to a hospital for treatment.

Eye Contact: First check the injured person for contact lenses and remove if present. Flush injured person's eyes with water or normal saline solution for 20 to 30 minutes while simultaneously calling a hospital or poisons control centre.
Do not put any ointments, oils or medication in the injured person's eyes without specific instruction from a physician.
Immediately transport the injured person after flushing eyes, to hospital even if no symptoms (such as redness or irritation) develop.

Ingestion: Do not induce vomiting. If the injured person is conscious and not convulsing, give 2 or 3 glasses of water to dilute the chemical and immediately call a hospital or poisons control centre. Be prepared to transport to hospital if advised by physician.
If the injured person is convulsing or unconscious, do not give anything by mouth, ensure injured person's airway is open and lay on his/her side with the head lower than body. Do not induce vomiting. Immediately transport to hospital.

1.2.11 Availability

__United Kingdom__

At this time, there is no specific information concerning the availability of DEET-containing insect repellents to the UK population. However, there are Internet sites available to purchase these insect repellents and many are available as over-the-counter preparations in the UK.

__USA__

It is estimated that approximately 38% of the US population use DEET-containing repellents annually (Veltri *et al*., 1994; Selim *et al*., 1995). As of September 1998, 225 DEET products were registered with the EPA. The large number of products and high level of use in the USA is due somewhat to an increased level of problematic biting insects that may carry disease. Therefore, this is not analogous to the availability of repellents in the United Kingdom.

In the USA, insect repellents are prepared in many different application types (e.g. aerosol and non-aerosol sprays, lotions, sticks, foams, and towelettes) and have concentrations ranging from approximately 4 percent to 100 percent.

2 PHYSICAL AND CHEMICAL PROPERTIES

Property	Information
Molecular Weight	191.26
Appearance physical/colour	Colourless to light-yellow liquid
Odour	Nearly odourless
Boiling point	160°C (at 19mm Hg)
Melting point	-45°C
Relative density	0.996
Solubility in water	<0.1 g/100ml at 20°C
Solubility in other solvents	Soluble in ethanol, ether, benzene, isopropanol, chloroform, carbon disulphide, alcohol Sparingly soluble in petroleum ether and glycerin Miscible with 2-propanol, cottonseed oil and propylene glycol
Vapour Density	6.7
Vapour Pressure	1 mmHg at 111°C 2.54×10^{-3} mmHg at 25°C
Flash point	155°C
Evaporation rate (butyl acetate=1)	<1
Refractive index	1.5212 (at 20°C)
Reactivity	Incompatible with strong acids, strong bases and strong oxidising agents
Stability in water	Hydrolyses slowly in the presence of water
Stability over storage	Sensitive to prolonged exposure to moisture. Solutions of this chemical in water, DMSO, 95% ethanol or acetone should be stable for 24 hours under normal laboratory conditions
UVmax (in cyclohexane)	274nm (shoulder) (epsilon=600)

Table 2.1: Physical and Chemical Properties of DEET

2.1.1 Summary of Physical and Chemical Properties

DEET (N,N-diethyl-*m*-toluamide) is an all-purpose, insect repellent for the individual. It contains a minimum of 95% of the *meta* isomer, the most effective form of diethyl toluamide, as a technical active ingredient.

Technical DEET is a nearly colourless liquid with a faint characteristic odour. It is relatively stable, highly hygroscopic and sensitive to light. Technical DEET is practically insoluble in water and glycerine but miscible with several organic solvents.

3 TOXICOLOGY AND METABOLISM STUDIES

In this section, studies have been separated into absorption, distribution, metabolism and excretion (ADME) studies in animals (section 3.1), followed by ADME studies in humans (section 3.2). Following this, acute toxicity studies (including irritancy and sensitisation, (section 3.3), short-term toxicity studies (section 3.4), genotoxicity studies (section 3.5), long term and carcinogenicity studies (section 3.6), reproductive toxicity studies (section 3.7) and neurotoxicity studies (section 3.8), are described. Finally there is a section describing evidence of toxicity from human exposure, which reviews case reports of exposure to DEET in the literature (section 3.10).

3.1 Absorption, Distribution, Metabolism and Excretion in Animals

The studies described in this section have been separated into skin penetration studies (section 3.1.1), oral and dermal absorption (section 3.1.2), distribution (section 3.1.3), metabolism (section 3.1.4) and excretion (section 3.1.5) studies. These have been further divided into *in vivo* (i.e. whole body) and *ex vivo* (i.e. excised tissue) studies where relevant.

It should be noted that some authors distinguish between 'penetration' and 'absorption', while others use them interchangeably. Those who make the distinction suggest that 'penetration' occurs when a topically applied substance passes into the layers of skin below the epidermis, but no movement to the circulation is implied. On the other hand, 'absorption' occurs when a substance passes through the skin or mucosa or other lipid barriers and enters the bloodstream or lymphatic system of an organism with distribution to other tissues and subsequent metabolism (biotransformation) or elimination (clearance) (Robbins and Cherniack, 1986).

Due to the fact that DEET-containing insect repellents are available for topical use and that there is a large amount of data concerning this area of exposure, dermal exposure data has been summarised first.

3.1.1 Skin Penetration

3.1.1.1 *In Vivo*

The following studies of skin penetration were reported in the review of Robbins and Cherniack (1986).

i) Schmidt *et al.* (1959) reported penetrations in the guinea pig of between 19% and 48% of the applied dose, at 6 hours after dermal treatment (doses ranging from 0.99 - 1.14 mg DEET/cm^2).

ii) In comparison, Smith *et al.* (1963) reported from 7 to 30% penetration of DEET in the guinea pig, 2 hours after topical treatment of approximately 1mg/cm^2. In both this study and that of Schmidt et al. (1959), the quantity penetrating the skin was calculated by

subtracting the quantities collected in the evaporation and rinse traps from the quantity applied.

iii) Accumulation of DEET in the skin with persistence for long periods has been reported in mice. After topical application of ^{14}C DEET and washing the skin two hours later, an average of 21% ^{14}C remained on the skin 36 days after application (Blomquist & Thorsell, 1977).

3.1.1.2 *Ex vivo*

An *in vitro* method was used to determine the evaporation and percutaneous penetration of radiolabelled DEET applied to pigskin (Hawkins & Reifenrath, 1984). The dermal side of the skin was mounted on a penetration cell. Appearance of radioactivity in the fluid flowing past the dermal side of the skin indicated percutaneous penetration. An evaporation manifold, with replaceable vapour traps, was mounted on the stratum corneum side of the skin. Using the model, the influence of a number of factors (flow rate and composition of fluid in the penetration cell, and temperature and humidity of air flowing through the evaporation manifold) on the disposition of chemicals on fresh skin was determined. When the penetration cell flow was increased from 5 to 10 ml/hr of Tyrodes solution, or when porcine serum was substituted for Tyrodes solution as the fluid flowing through the penetration cell, no significant difference was found in total percutaneous penetration of several control compounds. However, total percutaneous penetration of DEET more than doubled when the air temperature was increased from 20 to 32°C, whereas total evaporation decreased.

3.1.1.3 Summary of Animal Skin Penetration Studies

The results from the *in vivo* skin penetration studies would suggest that between 7% and 48% of the applied dose of DEET could penetrate animal skin. However, it should be noted that the majority of these reports are relatively old and the limited information pertaining to experiment design and implementation have been from reviews. Papers containing experimental data using more up-to-date techniques have not been found. Therefore, the applicability of this data to human conditions cannot be evaluated. Although the *ex vivo* study described is more recent, it does not provide information on amounts of DEET found to penetrate skin. However, data from this paper does suggest that air temperature may be a factor for DEET penetration into the skin, although this would need further investigation and confirmation. Considering the skin penetration data from animal studies in total, it would appear that there has been no definitive skin penetration study carried out in animals.

3.1.2 Absorption

3.1.2.1 *In vivo*

Rat: Oral

i) A series of experiments consisting of one preliminary and six definitive experiments was conducted to determine the absorption, distribution, elimination and metabolism of DEET (Schoenig *et al*., 1996). The EPA used these data in a review of DEET (US EPA, 1998).

In the preliminary experiment, groups of CD® rats (4/sex) received a single dose (100mg/kg body weight/day) of radiolabelled DEET (ring-UL-^{14}C) by oral gavage. The blood radioactivity levels were measured at various intervals for 24 hours to determine the peak blood ^{14}C-Level.

In the definitive experiments, groups of rats (5/sex/dose regimen) received DEET by single oral low dose (100mg/kg), single oral high dose (500mg/kg) or repeated oral low dose (100mg/kg for 14 days). One group (5 rats/sex/group), the single oral low dose, was sacrificed at peak blood ^{14}C level to determine the radioactivity levels in various tissues. The results suggest that the peak blood level was reached 0.5 hours after oral dosing in males while in females it took about two hours. In addition, 85-91% of the administered radioactivity was found in the urine and 3-5% was recovered in the faeces. The overall quantitative pattern of excretion of radioactivity into the urine and faeces was similar for males and females in the three groups. However, the fastest rate of excretion was observed in the repeated oral dose group, followed by the single oral low-dose and then the single oral high-dose groups.

ii) A blood level study to define the plasma profile in rats administered DEET has been carried out (Goldenthal, 1999 (unpublished); Leveglia, 1998 (unpublished); both cited in Schoenig & Osimitz, 2001). An increase in time to respond in a thermal response test and decreased rearing activity detected by two measures of vertical movement (vertical activity and vertical time) in a motor activity test were observed in a rat acute neurotoxicity study (see section 3.8). These findings were observed at the 1-hour post-treatment time and at the 500mg/kg dose level. In order to compare the systemic exposure to DEET that rats received under this dosage regimen to that of humans under simulated conditions of human use, a blood level study was conducted at the highest level at which no effects were observed in the rat study (i.e. 200mg/kg). Eight groups, each consisting of three male and female rats, were used. One group of rats was humanely sacrificed for blood collection and subsequent DEET plasma analysis at each of eight time intervals over a 4-hour period following oral administration. Blood profiles and peak blood levels observed in these rats were much different than those observed in humans under normal use conditions. For example, peak plasma levels were observed within 15 to 45 minutes after dosing, after which time a plateau level or gradual decrease in plasma levels was observed during the 4 hour time period in which the plasma levels were measured. Average peak plasma levels were 9.58µg/ml in male rats and 13.61µg/ml in female rats. AUC values could not be determined because DEET plasma levels did not return to baseline over the 4-hour period in which they were measured in this study. A comparison of peak plasma levels between the rats and humans (Ohayon *et al*., 1997 (unpublished) in Schoenig & Osimitz, 2001) showed 21 to 30-fold differences.

In order to more fully characterise the time course of elimination of DEET from the plasma following an oral bolus dose, a second oral blood level study was conducted in rats. In the second study, the same dose level (200mg/kg) was administered, however the DEET plasma profile was evaluated over a 48-hour rather than a 4-hour period of time. Groups consisting of five male and five female rats were humanely sacrificed at each time interval. The data from this study showed that peal plasma levels occurred 30 minutes after dosing and there was a distinct difference between male and female rats in both maximum plasma levels and AUC. In this study, elimination of DEET from plasma after reaching peak levels was biphasic, indicating rapid absorption from the stomach followed by distribution in the body within 2 to 4 hours of administration, and subsequently eliminated from plasma within 12 hours. A comparison of peak plasma levels between rats and humans shows that the levels in male and female rats under these experimental conditions were 16 to 34 times higher than in humans, respectively.

Rat: Dermal

i) In the study of Schoenig *et al.* (1996), 5 rats/sex/ were given a single dermal dose of 100mg/kg. These rats were sacrificed at peak blood ^{14}C level to determine the radioactivity levels in various tissues. However, no peak blood level was found, instead a blood level plateau, which began approximately one and one-half hours after dosing and persisted until the termination of the study (24 hours after dosing), was found in both male and female rats. These data indicated that when DEET was dermally applied to rats a small amount of the test compound was continuously absorbed from the application site. In addition, 74-78% of the administered dose was found in the urine and 4-7% was found in the faeces.

ii) Dermal absorption of ^{14}C-ring-labelled DEET has been investigated in male Sprague Dawley rats to investigate the effects of site of application and multiple exposure (Moody *et al.*, 1989). Doses of 1 ìCi (44µg) ^{14}C DEET in 100µl acetone were applied to 4.2cm^2 shaved areas at mid-dorsal regions of rats. Non-occlusive gauze patches were used to protect the dosed area of the rats for 24 hours. The patches were then removed and the treated area was washed with 50% ($^v/_v$) soap solution. Urine samples were collected at 4 and 8 hours on the first day and at 24-hour intervals for 7 days post-treatment. Duplicate 4ml samples of urine or wash were counted in 10ml liquid scintillation counter (LSC) cocktail. The total percent radiolabel excreted (percent in urine + percent in wash) was corrected for incomplete excretion using percent recovery data on ^{14}C DEET injected intramuscularly. Absorption in rats dosed mid-dorsally was $36 \pm 8\%$, with a urinary excretion $t^1/_2$ of 20 hours.

In this study, the effect of multiple exposure was also investigated. Three 33µl doses (1µCi/100µl or 0.33µCi/dose) were applied at intervals approximating 10% of the calculated excretion half-life ($t^1/_2$). Rats ($t^1/_2$ = 20 hour) were dosed at 2-hour intervals. No significant difference ($p \geq 0.3$) was obtained between the total percentage absorbed dermally with single ($36 \pm 8\%$; $t^1/_2$ = 20 hours) as compared with three ($31 \pm 5\%$; $t^1/_2$ = 16 hours) applications at 2 hour intervals to rats.

iii) Absorption studies (Snodgrass *et al.*, 1982) were conducted in 6 male and 6 female Sprague Dawley rats (250-300g). Topical application of a 75% ($^w/_v$) solution of ^{14}C DEET was made to the mid-lumbar area of the back of each animal using a microlitre syringe. Rate of administration of DEET was 4µg/cm^2. Following topical application, the

area was covered with a non-occlusive patch. This was changed after 24 hours. Both 24-hour and 7-day patches were monitored at the end of the test for volatised repellent trapped in the appliance. Urine from each animal was collected daily for 7 days, the volumes measured and aliquots retained for radioactive assessment. Faeces were similarly collected and radioactivity measured. At the end of the 7 day test period animals were euthanised and representative tissue specimens collected from brain, muscle, fat, bone, skin, liver, lung, spleen, kidney, testes and blood to monitor retained activity.

Rapid penetration of the repellent occurred with at least 75% of the dose appearing in the urine within the first day. The percent of absorbed compound (i.e. that appearing in the urine and faeces) was measured as 43.6% for male rats and 32.8% for female rats.

Dog: Oral

A blood level study to define DEET plasma profile was carried out in dogs (Badalone, 1997 (unpublished) cited in Schoenig & Osimitz, 2001). Undiluted DEET was administered to dogs as a single oral bolus dose via gelatin capsules at levels where clinical signs, indicative of neurotoxicity (i.e. 75mg/kg/day), had been seen (see section 3.8). Four male and four female dogs were utilised in this four day study, and DEET plasma levels were profiled on the first and last days of this study. Both blood profiles and peak blood levels observed in these dogs were much different in those observed in humans. For example, peak plasma levels were observed within 30 minutes after dosing and plasma levels were back below the limit of quantitation within 3 to 4 hours after dose administration. Consistent with that found in humans was the fact that no differences were observed between male and female dogs and there was no evidence of accumulation of DEET in the blood following repeated doses. The overall mean peak plasma level was 14.7µg/ml. The overall AUC was 12.59µg hr/ml. A comparison of the overall effects between dogs and human (Ohayon *et al*., 1997 (unpublished) in Schoenig & Osimitz, 2001) showed a 33-fold difference (i.e. 14.7 versus 0.45µg.ml, respectively)

Dog: Dermal

i) An investigation of the influence of dose on the percentage percutaneous penetration of DEET was carried out in the hairless dog (Reifenrath *et al*. 1981). Six dogs (3/dose), whose weight varied from 11 to 23kg, were chosen. Topical doses of either $4µg/cm^2$ or $320µg/cm^2$ radiolabelled DEET were applied to the sides of dogs and covered with a protective patch. Radioactivity was determined in urine, protective patches and skin washes. The calculated penetrations were $11.6 \pm 4.2\%$ and $8.5 \pm 3.3\%$ of the applied dose ($4µg/cm^2$ or $320µg/cm^2$, respectively), indicating lower penetration with increased concentration, although this difference was not statistically significant.

ii) A study to evaluate DEET pharmokinetics was carried out in male beagle dogs, with respect to transdermal bioavailability (Qiu et al. (1997). Four animals were dermally treated with topical DEET formulations. Formulation A (OFF! Skintastic II Insect Repellent – 7.125% DEET, S.C. Johnson & Son, Racine, WI) was dosed to a rectangular 10cm×Mcm area (where M was the body weight of the animal in kg), in the anterior dorsal region along the backline at 15mg/DEET/kg. Three days prior to dermal application, the hair on the application site was closely clipped off. An appropriate amount of formulation A was applied by transferring onto the entire area and evenly spreading over within 1 minute without applying excessive pressure on the skin. At 15

and 30 minutes, and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16 and 18 hours post-dose, blood samples were collected and processed. The dosed area was not occluded in order to allow the evaporation of DEET to occur freely. Securing a plastic protection collar on the neck prevented oral ingestion of DEET by the animals. Surveillance was undertaken to deter the animals from abrading the dosed area against any object. At 10 hours post-dose, the remaining formulation A on the skin was removed by gently wiping the area with wet gauze and gauze wetted with mild soapy water in sequence. The animals were given formulation B (gel emulsion, 7.5% DEET, containing a polymeric gel base, a polymeric emulsifier, a non-ionic surfactant, and a polyethylene glycol cosolvent as the main ingredients) after a two-week washout. To ascertain whether the previous dosing of formulation A would influence the DEET bioavailability of formulation B, a blood sample was taken from each animal prior to dermal application. No DEET was found on the chromatograms of the samples.

Plasma samples were quantitated for DEET concentration using a newly established HPLC method after solid phase extraction. The limit of quantitation of the method was 15ng/ml. Pharmacokinetic values were determined by non-compartmental analysis. Peak plasma DEET concentration (Cmax) and the time at which Cmax was reached (tmax) were obtained by visual inspection of the plasma DEET concentration-time curve. Area under this curve and the first moment curve (AUMC) were determined by the trapezoidal rule using computer software.

DEET was rapidly absorbed into systemic circulation of the animals from formulation A with MAT (mean absorption time) of 2.05 ± 0.16 hours. At just 15 minutes post-dose, the plasma concentration of DEET reached 101.0 ± 28ng/ml, about 51% of Cmax (196.5 ± 12.0ng/ml), which was detected at 1.25 ± 0.29 hours post-dose. The transdermal absorption of DEET from formulation B was relatively slower, as greater MAT (2.66 ± 0.33 hours), lower Cmax (154.3 ± 8.7ng/ml), and the plasma concentration of DEET at 15min post-dose (17.7 ± 8.3ng/ml) were observed. The transdermal bioavailabilities of DEET were determined as $18.3\% \pm 0.7\%$ and $14.0 \pm 0.9\%$ respectively, for formulations A and B.

iii) Absorption studies (Snodgrass *et al*., 1982) were conducted in 3 male Beagle dogs (1 year old). Topical application of a 75% (w/v) solution of 14C DEET was made to the mid-lumbar area of the back of each animal using a microlitre syringe. Rate of administration of DEET was $4\mu g/cm^2$. Following topical application, the area was covered with a non-occlusive patch. This was changed after 24 hours. Both a 24-hour and 7-day patches were monitored at the end of the test for volatised repellent trapped in the appliance. Blood was drawn at timed intervals after injection to document tracer disappearance from the circulation. Urine from each animal was collected daily for 7 days, the volumes measured and aliquots retained for radioactive assessment. Faeces were similarly collected and radioactivity measured. At the end of the 7 day test period animals were euthanised and representative tissue specimens collected from brain, muscle, fat, bone, skin, liver, lung, spleen, kidney, testes and blood to monitor retained activity. Rapid penetration of the repellent through skin occurred with at least 75% of the dose appearing in the urine within the first day. The percent of absorbed compound (i.e. that appearing in the urine and faeces) was measured as 31.2%.

Rabbit: Dermal

i) Absorption studies (Snodgrass *et al*., 1982) were conducted in 6 female New Zealand White rabbits (3.5 – 4.0kg). Topical application of a 75% (w/v) solution of ^{14}C DEET was made to the mid-lumbar area of the back of each animal using a microlitre syringe. Rate of administration of DEET was 4µg/cm^2. Following topical application, the area was covered with a non-occlusive patch. This was changed after 24 hours. Both 24-hour and 7-day patches were monitored at the end of the test for volatised repellent trapped in the appliance. Urine from each animal was collected daily for 7 days, the volumes measured and aliquots retained for radioactive assessment. Faeces were similarly collected and radioactivity measured. At the end of the 7 day test period animals were euthanised and representative tissue specimens collected from brain, muscle, fat, bone, skin, liver, lung, spleen, kidney, testes and blood to monitor retained activity.

Penetration of the repellent occurred rapidly with at least 75% of the dose appearing in the urine within the first day. The percent of absorbed compound (i.e. that appearing in the urine and faeces) was measured as 38.3%.

ii) Various formulations of DEET were investigated for skin uptake in 16 male New Zealand White rabbits weighing 4kg (Domb *et al*., 1995). The animals were housed in metabolic cages for the 7 days of the study and allowed free access to food and water. Prior to application of the topical doses, the back and neck of the rabbits were shaved. Eight rabbits received a 1g/kg topical dose of 10% DEET in ethanol, and the other eight rabbits received a 1g/kg topical dose of 10% DEET in lipospheres. Topical administration was achieved by application of the material to a 64cm^2 area of rabbit skin, which approximates 10% of the body surface area in a 4kg rabbit. Rabbits received DEET-liposphere formulation dose of 1g/kg over a 8×8cm^2 skin area. A non-occlusive cloth was then secured to the top of the barrier wall in order to collect evaporated DEET. The residual dose was removed from the skin by washing and quantitated after 24hours.

A heparin lock was placed in the ear of the rabbit for collection of 1.0ml blood samples at 0, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180 and 210 minutes and then at 4, 5, 6, 8, 12, 16 and 24 hours and daily for 7 days. Urine was collected after 1, 2 and 3 days. The rabbits were sacrificed after 7 days and the application sites collected. The radioactivity of individual samples was quantitatively determined by liquid scintillation counting.

The absolute bioavailability of DEET from a 10% ethanol solution was reported to be 45%, whereas that from lipospheres was only 16%.

Monkey: Dermal

Dermal absorption of ^{14}C-ring-labelled DEET has been investigated in male rhesus monkeys to investigate the effects of site of application and multiple exposure (Moody *et al*., 1989). Doses of 1 ìCi (44µg) ^{14}C DEET in 100µl acetone were applied to mid-dorsal, forearm, forehead and ventral and dorsal regions in monkeys. Non-occlusive gauze patches were used to protect the dosed area of monkeys (with the exception of the forehead) for 24 hours. The patches were then removed and the treated area was washed with 50% ($^v/_v$) soap solution. Urine samples were collected at 4 and 8 hours the first day, and at 24-hour intervals for 7 days post-treatment. Duplicate 4ml samples of urine or wash were counted in 10ml liquid scintillation counter (LSC) cocktail. The total percent radiolabel excreted (percent in urine + percent in wash) was corrected for incomplete excretion using percent recovery data on ^{14}C DEET injected intramuscularly. The extent

and rate of absorption was highly dependent on site of application, with $14 \pm 5\%$ ($t^1/_2 = 4$ hours) penetrating the forearm, $33 \pm 11\%$ ($t^1/_2 = 6$ hours) the forehead, $27 \pm 3\%$ ($t^1/_2 = 7$ hours) the dorsal forepaw and $68 \pm 9\%$ ($t^1/_2 = 8$ hours) the ventral forepaw.

In this study, the effect of multiple exposure was also investigated. Three 33µl doses (1µCi/100µl or 0.33Ci/dose) were applied at intervals approximating 10% of the calculated excretion half-life ($t^1/_2$). Monkeys ($t^1/_2 = 4$ hour) were dosed at 0.5 hour intervals. No significant difference ($p \geq 0.3$) was obtained either between the total percentage absorbed dermally with single ($14 \pm 5\%$; $t^1/_2 = 4$h) as compared with three ($12 \pm 1\%$; $t^1/_2 = 4$ hour) applications at 0.5 hour intervals to monkey forearms.

3.1.2.2 *Ex vivo*

Ex vivo dermal absorption studies were conducted using different skin types (Moody & Nadeau, 1993). Total 48-hour percentage absorption values were; rhino mouse, $36 \pm 27.5\%$; human, $28 \pm 4.2\%$; rat, 21 ± 2.2 %; pig, $15 \pm 0.8\%$; tissue cultured Testskin, 13 ± 9.6 % and hairless guinea pig, $11 \pm 1.4\%$. Lag times for *in vitro* absorption ranged from 0.6 hours (human) to 1.9 hours in rats. Soapy water rinses 24 hours post-exposure ranged from 4% in rats to 18% in mice and total recoveries ranged from 70 % for Testskin to 93 % for human skin.

Comparative *in vivo* studies in rats and guinea pigs as part of the above investigation demonstrated 14-day urinary recoveries of 38 ± 10.3 % (rats) and 26 ± 5.4 % (guinea pigs). Total faecal recoveries were 1 ± 0.5 % and 3 ± 0.8 % and total tissue recoveries were 2 ± 0.4 % and 1 ± 0.3 %. Soapy skin washes at 24 hours recovered 8 ± 0.5 % in rats and 5 ± 2.8 % in guinea pigs. Skin removed from the site at 14 days contained 0.2 ± 0.11 % and 0.1 ± 0.06 % and total recoveries were 84 ± 9.2 % and 108 ± 2.9 %, respectively. The *in vitro* data therefore underestimated *in vivo* dermal absorption, possibly due to a depot effect.

3.1.2.3 Summary of Animal Absorption Studies

Oral and dermal absorption studies of DEET in animals are summarised below in Table 3.1. In rats, DEET was rapidly and extensively absorbed following oral dose administration. Absorption was much slower after dermal dosing. The percentage absorbed was dependent on the solvent used, concentration of DEET, method used to determine absorption (e.g. recovery in urine following dermal application or comparison of kinetics following intravenous or dermal application) and extent of occlusion used. These results are difficult to compare. More appropriate studies have been carried out in humans and these are discussed later (section 3.2).

Species	DEET formulation	Dose	Dose Site	Quantitation	Absorption[1] (% of applied)	Reference
Oral						
CD® rats (10/group)	^{14}C-labelled DEET in Corn Oil	100mg/kg 500mg/kg	Gastric gavage (2ml/kgb.w.)	Radioactivity	<91 <93	Schoenig *et al.* (1996)
		100mg/kg for 14days			<94	
Dermal						
CD® rats (n=10)	^{14}C-labelled DEET	100mg/kg	Back	Radioactivity	<82	Schoenig *et al.* (1996)
Rat (n=8)	Acetone solution	10.5µg/cm^2	Mid-dorsal	Radioactivity	36[5]	Moody *et al.*
Monkey (n=8)	(0.44mg/ml, ^{14}C-labelled)		Forehead Forearm Ventral forepaw Dorsal forepaw		33[5] 14[5] 68[5] 27[5]	(1989)
Beagle dog (n=4)	Commercial cream (7.125%) Gel Emulsion (7.5%)	15mg/kg	Anterior dorsal	HPLC	17.5[2] 13.4[2]	Qiu *et al.* (1997)
Rabbit (n=8)	Liposphere (10%, ^{14}C-labelled) Ethanol solution (10%, ^{14}C-labelled)	100mg/kg	Back/neck	Radioactivity Radioactivity	16[3] 45[3]	Domb *et al.* (1995)
Rabbit	Ethanol solution (75%, ^{14}C-labelled)	4µg/cm^2	Mid-lumbar	Radioactivity	38.3[7]	Snodgrass *et al.* (1982)
Beagle Dog		4µg/cm^2	Mid-lumbar	Radioactivity	31.2[7]	
Rat (male)		4µg/cm^2	Mid-lumbar	Radioactivity	43.6[7]	
Rat female) (n=6)		4µg/cm^2	Mid-lumbar	Radioactivity	32.8[7]	
Hairless dog (n=3)	Ethanol solution (40mg/ml, ^{14}C-labelled)	4µg/cm^2 320µg/cm^2	Dorsal Dorsal	Radioactivity Radioactivity	11.6[7] 8.5[7]	Reifenrath *et al.* (1981)

Table 3.1: Urinary Recovery and Absorption of Topically Applied DEET in *In Vivo* Animal Studies

[1]Mean Value; [2]Assessed by non-compartmental method: blood samples collected for 18 and 16h respectively, after intravenous and dermal dosing; [3]Assessed by non-compartmental method: blood samples collected for 10 and 24h respectively, after intravenous and dermal dosing; [4] Assessed by non-compartmental method: blood samples collected for 24h, after intravenous and dermal dosing; [5]Assessed as percent ratio of ^{14}C radioactivity recovered in urine to total ^{14}C radioactivity dermally applied; urine collected for 7 days after dermal dosing; [6]Assessed as percent ratio of ^{14}C radioactivity recovered in urine and faeces to total ^{14}C radioactivity dermally applied; urine and faeces collected for 5 days after dermal dosing; [7]Assessed as percent ratio of the ^{14}C radioactivity recovered in urine to total ^{14}C radioactivity dermally applied; urine collected for 7 days after dermal dosing

3.1.3   Distribution

Rat: Oral

The distribution of DEET has been investigated in rats (Schoenig *et al*., 1996). This oral study was previously described (section 3.1.2.1). The liver, kidney, lung, spleen, whole blood, and the carcass contained higher radioactivity than any other tissues. However, total tissue ^{14}C residues only ranged from 0.15 to 0.67% of the administered dose, indicating that very little DEET was sequestered in the body. Whole blood levels were higher than plasma levels for all treatment groups except for dermal dosing, suggesting binding or uptake of DEET and/or metabolites to red blood cells.

Mouse: Dermal

i) Distribution studies in mice using autoradiography with ^{14}C DEET administered dermally at 15 mg/kg found high concentrations in the skin, lacrimal glands, liver, kidney and nasal mucosa with high levels in the bile, intestinal contents and urine (Blomquist and Thorsell, 1977; Blomquist *et al*., 1975). Enterohepatic elimination appears to be very small with only fractional amounts of the doses in the above dog and rabbit studies detected in the faeces. Intravenous exposures confirmed the distribution of the dermal studies with tissue uptake occurring earlier. Other investigators using high doses (2000mg/kg and more) and chemical determination of DEET (isomer not stated) produced different results, with high concentrations in the brain, lungs and adrenals (Glejberman & Veronkina, 1972 (russian text), cited by Robbins & Cherniack, 1996). Methodological differences may account for these divergent results. Residual amounts of ^{14}C DEET were detected in skin, fatty tissue and muscle of mice for one to three months after dermal application of 100mg/kg.

Dog: Intravenous

i) In the study of Qiu *et al*. (1997), described in a previous section, male beagle dogs were also administered a bolus dose 2.5mg/ml DEET solution prepared in 40% (v/v) ethanol into the cephalic vein. At 5, 15 and 45 minutes and 1, 1.5, 2, 3, 4, 5, 7, 9, 12, 15 and 18 hours post-dose, a 7ml blood sample was taken from the jugular vein by venopuncture. The sample was transferred into a heparinised tube and centrifuged at 500g for 15 minutes. The plasma was collected, stored at -20°C, and analysed within 1 week. After a 2-week washout, the animals were given a higher dose of DEET at 6.0mg/kg. Plasma DEET concentrations declined bi-exponentially with a rapid distribution phase. There was no statistically significant difference (P<0.05) in CL, Vss, MRT and $t^{1}/_{2}$ between the two doses, indicating that the pharmacokinetics of DEET in the beagle dogs was linear over the dose range. The Vss value (6.21 ± 0.32 l/kg) was approximately 9-fold higher than the total body water of a lean dog (0.7l/kg), indicating that DEET underwent extensive extravascular distribution.

Placental Transfer

Several investigators have reported placental transfer of DEET. Residues were reported up to three months after birth in the tissues of offspring of female rats exposed to 1000mg/kg. While other studies suggest the placental transfer of DEET, bioaccumulation was not demonstrated in mice or rabbits, indicating rapid foetal excretion. Dermal

application of ^{14}C DEET to pregnant rabbits from day 1-29 of gestation resulted in no evidence of radioactivity above background levels in full-term foetuses.

^{14}C DEET was administered (i.v.) to pregnant rabbits on day 15 of gestation to maximise placental transfer. Maternal blood, kidney, liver, spleen, lungs and foetuses were monitored for 24 hours. The foetuses had the lowest radioactivity of all specimens, one-sixth that of maternal blood. Pregnant mice were given radiolabelled DEET (i.v.). Little radioactivity was found in the foetuses, but the concentration of DEET in the uterine cavity close to the yolk-sac villi was reported to be greater than that in the maternal blood (Blomquist *et al*., 1975; Snodgrass *et al*. 1982)

3.1.3.1   Summary of Animal Distribution Studies

The study of Schoenig *et al*., 1996 suggests that only a small percentage of the applied dose of DEET be retained in body tissues. The majority of DEET is eliminated via excretion. Residual amounts of [^{14}C] DEET were detected in skin, fatty tissue and muscle of mice for one to three months after dermal application.

3.1.4   Metabolism

3.1.4.1   *In vivo*

Rat: Oral

A series of experiments consisting of one preliminary and six definitive experiments was conducted (Schoenig *et al*., 1996) to determine the absorption, distribution, elimination and metabolism of DEET. The EPA used this study in a review of DEET (US EPA, 1998).

In the preliminary experiment, groups of CD® rats (4/sex) received a single dose (100mg/kg body weight/day) of radiolabelled DEET by either oral (gavage) or dermal administration. The blood radioactivity levels were measured at various intervals for 24 hours to determine the peak blood ^{14}C-Level.

In the definitive experiments, groups of rats (5/sex/dose regimen) received DEET by single oral low dose (100mg/kg), single oral high dose (500mg/kg), repeated oral low dose (100mg/kg), or single dermal low dose (100mg/kg). Two groups (5 rats/sex/group), a single oral low dose and a single dermal low dose group, were sacrificed at peak blood ^{14}C level to determine the radioactivity levels in various tissues. The metabolism data indicated that the absorbed DEET was quantitatively metabolised, and the intact DEET was below the detection limit. Two major metabolites were found. One was formed by oxidation of the methyl group on the aromatic ring, and it represented 50% of the administered DEET. The other one was derived from oxidation of the methyl group of the aromatic ring and N-dealkylation of an ethyl substituent on the amide moiety. The second metabolite represents 18% of the administered DEET.

Rat: Inhalational

Hippuric, benzoic and toluric acid DEET metabolites were reported in the urine of rats and rabbits exposed to aerosol mists (Christensen *et al*., 1969 cited by Robbins &

Cherniack, 1986). However, quantities of these metabolites were not determined and DEET could not be measured in the urine.

Guinea Pigs: Route unknown

Recovery of ^{14}C in urine of guinea pigs after 7 days was equivalent to 95 % of the penetrating dose, but no unmetabolised DEET was detected in the urine (Schmidt et al, 1959, cited by Robbins & Cherniack, 1986).

3.1.4.2 *Ex vivo*

Rat

i) A study was carried out to establish the identity of metabolites arising from the phenobarbital-treated rat liver microsomal oxidation of N,N-diethyl-m-toluamide (DEET) (Constantino & Iley, 1999). The products formed (see Figure 3.1) from DEET and all its subsequent metabolites were identified by HPLC retention times, UV spectroscopy, mass spectrometry and by comparison with authentic standards.

It was shown that DEET (1a) produced three major metabolites, *N*-ethyl-*m*-toluamide (1b), *N*,*N*-diethyl-*m*-(hydroxymethyl)benzamide (2a) and *N*-ethyl-*m*-(hydroxymethyl)benzamide (2b). At lower concentrations, or extended reaction times, two minor metabolites, toluamide (1c) and *N*,*N*-diethyl-*m*-formylbenzamide (3a) were formed. 1b and 2a are primary metabolites and their formation followed Michaelis-Menten-type kinetics. At low DEET concentrations, ring methyl group oxidation was favoured. At saturation concentrations, methyl group oxidation and *N*-deethylation proceeded at similar rates. The rate of formation of 2b decreased with increasing DEET concentration, therefore it was concluded that 2b was a secondary metabolite of DEET and DEET acted as a competitive inhibitor of the metabolism of 1b and 2a.

Except for the primary amides, where *N*-dealkylation is impossible, metabolism of all subsequent compounds, 1b, 1c, 2a-c, 3a-c and 4a, b involved an *N*-deethylation (*N*Et$_2$ → *N*HEt or *N*HEt → NH$_2$) competitive with a ring substituent oxidation (CH$_3$ → CH$_2$OH, CH$_2$OH → CHO or CHO → CO$_2$H). Amide hydrolysis was not observed for any substrate and no evidence was obtained for N-(1-hydroxyethyl)amide intermediates.
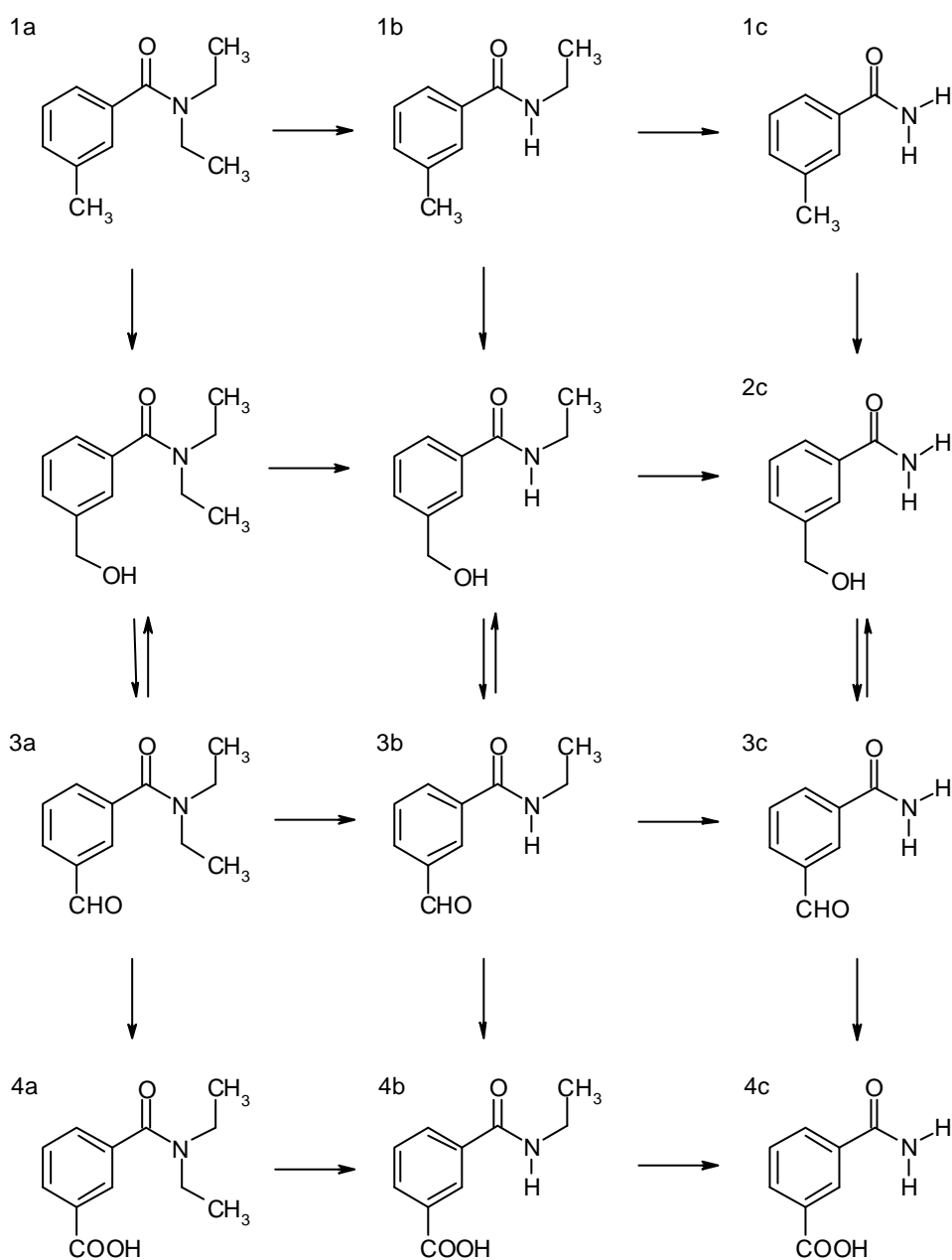
Figure 3.1: Pathways of DEET Metabolism
Horizontal lines represent N-dealkylation; the vertical direction represents ring-methyl oxidation (redrawn from Constantino and Iley, 1999)

ii) The metabolism of DEET by liver microsomes from male and female rats was investigated by Yeung & Taylor (1988). Mature (approximately 12 weeks) male and female Wistar rats were kept at 20°C with a 12 hour light/dark cycle. At the time of sacrifice, male rats weighed 275-300g and females 210-225g. Liver microsomes were prepared by differential centrifugation and the rate of metabolic oxidation of DEET was measured by taking aliquots from the reaction mixture at regular intervals. Aliquots were analysed for DEET and metabolites, particularly for *N,N*-diethyl-*m*-(hydroxymethyl)benzamide (BALC) and *N*-ethyl-*m*-toluamide (ET) by HPLC.

Cytochrome P450 was also measured. Microsomes from males metabolised DEET much faster than did those of females (see table below), and it was also apparent that the rate of metabolism levelled off at 90 minutes in both cases.

	Male liver microsomes	Female liver microsomes
Cytochrome P450 (nmol/mg Protein)	0.94 ± 0.06	0.83 ± 0.07
% metabolism at 2 hours	58 ± 4.8	17 ± 1.6
$t^1/_2$ (min) for DEET	10 ± 1.5	15 ± 1.1
k_r (min-1) for DEET disappearance	0.0667 ± 0.007	0.0467 ± 0.002
k_r for BALC appearance	0.0777 ± 0.009	0.0273 ± 0.001
k_r for ET appearance	0.0970 ± 0.011	0.0346 ± 0.002
% metabolism at 2hours after 2 hour preincubation	9 ± 3	7 ± 0.5

Table 3.2: Metabolism of DEET by rat liver microsomes
From Yeung & Taylor (1988)

3.1.4.3 Summary of Animal Metabolism Studies

In a study submitted to the EPA and subsequently published (Schoenig *et al*., 1996), absorbed DEET was rapidly distributed and excreted mainly in the urine as metabolites. The predominant pathways of metabolism involved oxidation of the methyl group on the aromatic ring and N-deethylation of the amide moiety. The major metabolites seen were *N*,*N*-diethyl-*m*-(hydroxymethyl)benzamide (BALC) and *N*-ethyl-*m*-toluamide (ET). The available studies in animals that used dermal administration also showed that absorbed DEET was excreted predominantly via the urine.

3.1.5 Excretion

Rat: Oral

The study carried out by Schoenig *et al*. (1996) for the EPA (for details see section 3.1.2.1) indicated that the major route of DEET elimination in both male and female rats was via the urine. No marked difference was found in the total urinary or faecal radioactivity among the different dosing regimens. However, there was a difference in the rate of urinary elimination of DEET among the different dose groups. For example, repeated oral dose groups or pre-treatment groups showed the fastest rate of urinary elimination during the first four hours after dosing that any other dose groups. In contrast, the single dermal low-dose groups showed the slowest rate of urinary excretion, which may reflect the slow rate of dermal absorption.

Rat: Intravenous

Radiolabelled DEET (28.12µg; 2µCi) was given into the femoral vein of rats (Snodgrass *et al*., 1982). Urine from each animal was collected for 7 days, the volumes measured and aliquots retained for radioactivity assessment. Faeces was similarly collected and measured. Male and Female rats showed similar, rapid bioelimination of DEET with 97% of the recovered radiocarbon appearing in the first day. Total percentage of DEET recovered was 90% and 92% after 7 days for male and female, respectively. Little DEET was recovered in the faeces.

Rabbit: Intravenous

Radiolabelled DEET (70.3µg; 5µCi) was given into the marginal ear vein of rabbits (Snodgrass *et al*., 1982). Urine from each animal was collected for 7 days, the volumes measured and aliquots retained for radioactivity assessment. Faeces was similarly collected and measured. Rabbits showed a similar excretion pattern to that seen in the rat (reported above), however metabolic elimination was slower. Peak activities were reported 15 minutes after i.v. administration of ^{14}C DEET and half-life of 30 minutes. Total excretion of DEET was measured as 75% after 24 hours and 93% after 7 days. Little DEET was recovered in the faeces.

Guinea Pig: Route unknown

Schmidt *et al*., 1959 reported that urinary radioactivity peaked within 12 hours of administration of 1000µg/cm^2 DEET (cited by Robbins & Cherniack). After 24 hours the amount of DEET excreted was 82% of the penetrated dose, rising to 92% after 8 days. Little DEET was recovered in faeces.

Dog: Intravenous

i) The study of Qiu *et al*. (1997), described in a previous section contained some data on excretion. With MRT (mean residence time) measured as 2.34 ± 0.12 hours and $t_{1/2}$ (terminal elimination half-life) as 2.56 ± 0.29 hours, the elimination of DEET appeared to be fast. The mean CL (systemic clearance) was 2.66 ± 0.17 l/h/kg, approximately 2-fold higher than the renal blood flow which has been estimated as 1.38 l/h/kg, suggesting that DEET was partially cleared intrahepatically.

ii) Radiolabelled DEET (140.6µg; 10µCi) was given into the cephalic vein of dogs (Snodgrass *et al*., 1982). Urine from each animal was collected for 7 days, the volumes measured and aliquots retained for radioactivity assessment. Faeces was similarly collected and measured. Dogs showed a similar excretion pattern to that seen in the rat (reported above), however metabolic elimination was slower. Peak activities were reported 15 minutes after i.v. administration and a half-life of 35 minutes was calculated Total excretion of DEET was measured as 45% after 24 hours and 52% after 7 days. The reason for this low percentage of DEET eliminated was proposed as being due to less DEET absorbed, possible due to differences in skin anatomy. Little DEET was recovered in the faeces.

Mouse: Dermal

After application of 100 mg ^{14}C DEET/kg dermally to mice, peak blood activity occurred at one hour with complete elimination in 2-3 days (Lure *et al*., 1978, cited by Robbins & Cherniack, 1986)

3.1.5.1 Summary of Animal Excretion Studies

A summary of animal excretion studies is given below in table 3.2. It would seem that excretion via the urine is the predominant elimination route of DEET. Schoenig *et al*. (1996) data indicate that the rate of elimination may be increased in rats that received

multiple oral doses of DEET, suggesting that DEET can induce the liver microsomal enzyme system.

Species (n)[a]	Dose	Cumulative urinary excretion		Cumulative faecal excretion	
		% of applied	Time	% of applied	Time
Oral administration					
CD® rats (Schoenig *et al.* 1996)	100mg/kg 500mg/kg 100mg/kg for 14days	~86.8 ~88.9 ~90.6	7 days	~3.6 ~5.9	7days
Topical administration (ìg/cm2)					
CD® rat (Schoenig *et al.* 1996)	100mg/kg	<78	7 days	~7.1	7 days
Rat (Lure *et al.*, 1978, cited by Robbins & Cherniack, 1986)	330-400	42 68	6 hr 24 hr		
Guinea Pig (Schmidt *et al.*, 1959, cited by) Robbins & Cherniack, 1986)	1000	82[b] 89[b] 92[b]	24 hr 48 hr 8 days	1[b]	8 days
Mouse (Blomquist & Thorsell, 1977 cited by Robbins & Cherniack, 1986)	33	13 34	2.5 hr 2 days	< 3	2 days
Intravenous administration (mg)					
Mouse (Blomquist *et al.* 1975)	30	95.6 97.3	8 hr 40 hr	0.6	48 hr
Rat male (Snodgrass *et al.*, 1982)	28	87 90	24 hr 7 days	< 3	7 days
Rat female (Snodgrass *et al.*, 1982)	28	90 92	24 hr 7 days	< 3	7 days
Rabbit, (Snodgrass *et al.*, 1982)	70	75 93	24 hr 7 days	< 3	7 days
Dog, beagle, male (Snodgrass *et al.*, 1982)	141	45 52	24 hr 7 days	< 3	7 days

Table 3.3: Excretion of ^{14}C DEET in Animal Studies
(From Robbins & Cherniack,1986)
[a] Number of animals; [b] % of dose that penetrated (applied dose - that in rinse & traps); [c] l.d., limit of detection

3.2 Absorption, Distribution, Metabolism and Excretion in Humans

The studies described in this section have been separated into skin penetration studies (section 3.2.1), oral and dermal absorption (section 3.2.2), distribution (section 3.2.3), metabolism (section 3.2.4) and excretion (section 3.2.5) studies. These studies have been further divided into *in vivo* and *ex vivo* where relevant.

As previously explained, the term 'penetration' is used to describe when a topically applied substance passes into the layers of skin below the epidermis, but no movement to the circulation is implied. In contrast, 'absorption' is used to describe when a substance passes through the skin or mucosa or other lipid barriers and enters the bloodstream or lymphatic system of an organism with distribution to other tissues and subsequent metabolism or elimination.

In subsequent sections, dermal exposure data has been summarised first.

3.2.1 Skin Penetration

3.2.1.1 In vivo

i) *In vivo* experiments were performed using the top half of the evaporation/penetration cell maintained at 32°C with a circulating waterjacket (Spencer *et al.*, 1972). [14]C DEET and an ethanol control were applied to a $1.8cm^2$ area on the forearm at a dose of $25\mu g/cm^2$ (0.01μCi total dose). Following application, participants remained at rest in a room kept at between 25 and 30°C. The site was allowed to dry in air for one minute and the evaporation cell was affixed to the forearm. Dry air was passed over a $0.6cm^2$ exposed area of treated skin at 30ml/min and through a 6cm-collection tube (syringe) containing dry cotton. No DEET could be detected in the effluent from the cotton collection tube using a toluene wash capable of recovering $0.1\mu g/cm^2/h$ of DEET. The collection tube was changed at 15-minute intervals for 1 hour after application. The cotton from the collection tube and 2 rinse aliquots from the tube were placed in a scintillator cocktail and counted.

After the third collection period the cell was removed from the forearm. The treated area was rubbed vigorously with 2 toluene-soaked cotton swabs. Then 4 to 6 skin strippings were made with ¾ inch wide cellophane tape. The cotton balls and the skin-strip tape were placed in scintillation vials with scintillator solution. Neither cotton nor cellophane tape was found to affect the counting efficiency. The total recovery of ^{14}C DEET by this evaporation, wiping and stripping the skin was 48.3% (± 6.3%) of the applied dose. This would suggest that the remaining ^{14}C DEET penetrated the skin to layers below the strippable stratum corneum.

ii) Studies of skin penetration were reported in a review of Robbins and Cherniack (1986). Smith *et al.* (1963) reported a 7-13% penetration of DEET in 3 male and 2 female volunteers two hours after topical application of approximately $1mg/cm^2$. In a different experiment, these authors obtained penetration from approximately 9% at a dose of $1.86mg/cm^2$ to 56% at a dose of $77\mu g/cm^2$ in two male volunteers. In both these studies,

the quantity penetrating the skin was calculated by subtracting the quantities collected in the evaporation and rinse traps from the quantity applied. Hence, these values are subject to error.

3.2.1.2 Ex vivo

i) Stinecipher & Shah (1997) evaluated the permeation characteristics of DEET from four commercial products, Everglades (95%), Repel Deerhunters (52.25%), Off! Skintastic (6.65%), and Skedaddle (6.2%), as compared to pure DEET (similar to 100%). Also studied were the effects of ethanol (the solvent for DEET) on the permeation of DEET and ethanol's potential for enhancing the dermal absorption of DEET. Permeation studies of DEET from commercial mosquito repellents and from solutions containing various percentages of ethanol were conducted across human skin using an infinite dose technique with a Franz diffusion cell. Permeation parameters such as steady-state flux (J-ss), lag time (t(l)), diffusion coefficient (D), permeability (P), and skin/vehicle partition coefficient (K) were obtained from the permeation profiles in each case. The cumulative amount of DEET permeated can be ranked according to the following order: neat DEET (100%) = Everglades (95%) > Repel (52.25%) > Skedaddle (6.2%) = Off Skintastic (6.65%). Pure DEET exhibited the highest flux value of 63.2 +/- 24.5μg/cm^2.h, while Off! Skintastic had the lowest value of 21.1 +/- 14.8 μg/cm^2.h. The t(l) and D values for each of the products were similar to that of pure DEET. The total amount of DEET permeated from 30-45% ethanolic solutions at the end of 36 h was significantly higher than that from pure DEET and from the 60-90% ethanolic solutions. The J(ss) P, and K values of DEET from the 30-45% ethanolic solutions were significantly higher than those from the 75-90% ethanolic solutions, while the t(L) and D values were similar for each solution. Therefore, there is potential for significant absorption of DEET after the dermal application of commercial mosquito repellents, and if ethanol is used as a solvent, it may enhance the permeation of DEET

ii) More recently, Stinecipher & Shah (1998) carried out percutaneous permeation studies on *meta*, *ortho* and *para* isomers of *N*,*N*-diethyltoluamide. Human skin samples (fat and other visceral debris removed) were obtained from elective plastic surgery patients. The skin was then washed with 0.1M, pH 7.4 phosphate buffer solution before freezing at –20°C. The full thickness skin samples were cut into 2cm^2 pieces and allowed to thaw overnight at room temperature in phosphate buffer solution prior to the *in vitro* percutaneous permeation experiments. Permeation studies were carried out with vertical Franz diffusion cells through full thickness human skin. The receptor compartment was filled with 0.1M phosphate buffer (pH 7.4) solution and was constantly stirred to ensure uniform distribution of DEET and maintain skin conditions. The temperature of the entire diffusion assembly was maintained at 37°C using a recirculating water jacket. Permeation studies with the isomers were conducted using the infinite dose technique (i.e. large excess of permeant is maintained in the donor compartment during the entire course of the experiment). The studies were carried out for 36 hours. Each isomer was applied to the skin in the donor compartment as either the neat isomer (78.13mg/cm^2), a 5mg/ml solution in water (7.81mg/cm^2), 10% isomer in 90% ethyl alcohol (46.88mg/cm^2), or 10% isomer in 45% alcohol (46.88mg/cm^2). For application of the neat isomer, 50 mg of each in the pure form was applied to the skin in the donor compartment. *m*-DEET, which is liquid

was applied as a 50µl aliquot. *o*-DEET and *p*-DEET, which are solids at room temperature were melted prior to application. These then solidified onto the skin when applied and cooled to room temperature. Aliquots of 1ml and 300µl of the isomers in the aqueous solution and in the ethanolic solutions, respectively, were applied to the surface of the skin. In each case the donor compartment was covered with a glass slip to prevent evaporation.

Aliquots of 300µl of the receptor fluid were withdrawn and replaced periodically with fresh phosphate buffer for 36 hours. Results are presented below.

[DEET isomer] (mg/cm^2)	Preparation	Percentage penetrated after 36 hours at 37°C		
		meta	*ortho*	*para*
7.81	5mg/ml in H_2O	13.27 ± 1.86	5.64 ± 0.40	13.73 ± 1.95
46.88	10% in 45% ethyl alcohol	7.37 ± 0.83	14.27 ± 2.02	12.50 ± 0.56
46.88	10% in 90% ethyl alcohol	1.33 ± 0.46	2.30 ± 1.57	0.81
78.13	Neat	0.57 ± 0.28	0.71 ± 0.16	0.43 ± 0.085

Table 3.4: Summary of the Human Permeation Study
As reported by Stinecipher & Shah, 1998

The results suggest that *meta*, *ortho* and *para* isomers do permeate through human skin. However, it would seem that the *meta* and *para* isomers favour penetration into the skin from an aqueous solution. It was suggested that the water may hydrate the skin and decrease its barrier function. In contrast, the higher content of ethanol corresponded with a lower percentage of skin penetration by each isomer. Steady-state fluxes were not affected by the composition of the vehicle, except for the ortho and para isomers in 45% ethanolic solutions, which had significantly higher penetration rates than with other vehicles. Despite the higher flux rates observed in some instances, the authors concluded that the toxic effects observed after topical use of DEET could not be directly attributed to the small amounts of the *ortho* or *para* isomers in the formulations.

iii) Evaporation rates were measured in a glass evaporation/penetration cell using abdominal skin samples obtained at autopsy, that had been stored frozen (Spencer *et al.*, 1979). Prior to experiment the full thickness skin sample was thawed and placed in the lower section of a permeability cell containing Ringers solution for 15 minutes. An ethanol solution of the labelled repellent was applied to a 1.2cm^2 area in the centre of the sample, at a dose of 25µg/cm^2. The ethanol was then allowed to evaporate for 1 minute. The upper half of the chamber was placed over the skin, and the chamber halves clamped together, forming an airtight seal. The chamber was maintained at 32°C (approximately skin temperature). Dry air was passed over a 0.6cm^2 exposed area of treated skin at 30ml/min and through a 6cm-collection tube (syringe) containing dry cotton. No DEET could be detected in the effluent from the cotton collection tube using a toluene wash capable of recovering 0.1µg/cm^2/h of DEET. The collection tube was changed at 15-minute intervals for 1 hour after application. The cotton from the collection tube and 2 rinse aliquots from the tube were placed in a scintillator cocktail and counted. After 1

hour the skin sample was removed from the cell; the skin surface was rinsed with the scintillator solution, which was collected in a vial and counted. The rinsed skin sample was then pyrolysed and the ^{14}C DEET absorbed by the skin was measured. The results show that $50.8 \pm 15.0\%$ of ^{14}C DEET was recovered in skin tissue (i.e. had penetrated the skin). Total recovery of ^{14}C DEET was estimated to be $79.5 \pm 10.3\%$.

iv) Using similar experimental conditions to those of Spencer *et al.* (1979), Reifenrath and Robinson (1982) obtained DEET skin penetration (absorption and oxidation) of approximately 30% penetration at 1 hour after application and of 36% after 12 hours, at a dose of $300\mu g/cm^2$. This study used abdominal human skin obtained at autopsy, which had had subcutaneous fat removed.

3.2.1.3   Summary of human skin penetration data

Human skin penetration data is summarised below in table 3.4.
Uncertainty about the degree of percutaneous absorption of DEET in humans may complicate an objective assessment of effects. Generally, the amount of DEET that permeates the skin is closely related to the repellent formulation. Using commercially available products, Stinecipher and Shah (1997) found that the cumulative amount of DEET that permeated human skin *in vitro* ranged from approximately 6% to 100%, depending upon the repellent tested. Earlier research suggested that approximately 9% to 56% of applied DEET permeated the skin, although only approximately 15% is systemically absorbed (Robbins and Cherniack, 1986). However, in vitro studies involving infinite-dose applications of DEET to human skin have agreed closely. Stinecipher and Shah (1997) calculated the steady-state flux of DEET, from epidermis to dermis at from 21 to $63\mu g/cm^2/hr$, while Moody et al. (1995) calculated it to be from 20 to $60\mu g/cm^2/hr$.

Sex & Age of individual	Chemical Form and Concentration	Exposure/ Observation Period	Results/ Comments	Reference
In vivo				
Age & sex n.p.	^{14}C DEET ($25\mu g/cm^2$)	1 hour after topical application	48.3% of applied dose penetrated	Spencer *et al* (1972)
3M, 2F (age n.p) 2 M (age n.p.)	DEET ($1mg/cm2$) DEET ($1.86mg/cm^2$- $77\mu g/cm^2$)	2 hours after topical application	7-13% of applied dose penetrated 9-56% of applied dose penetrated	Smith *et al.* (1963)
Ex vivo				
Excised human skin Age & sex n.p.	^{14}C DEET ($25\mu g/cm^2$)	1 hour after topical application	50.8% of applied dose penetrated	Spencer *et al* (1979)
Excised human skin (details n.p.)	^{14}C DEET ($300\mu g/cm^2$)	1 hour 12 hours	30% of applied dose penetrated 36% of applied dose penetrated	Reifenrath & Robinson, 1982)
Excised human skin (details n.p.)	m-DEET ($7.8mg/cm^2$) ($15mg/ml$ in H_2O) m-DEET ($46.88mg/cm^2$) 10% in 45% ethyl alcohol m-DEET ($46.88mg/cm^2$) 10% in 90% ethyl alcohol	36 hours	13.27% of applied dose penetrated 7.37% of applied dose penetrated 1.33% of applied dose penetrated	Stinecipher & Shah, 1998)

m-DEET (78.13mg/cm^2) Neat		0.57% of applied dose penetrated	

Table 3.5: Summary of skin penetration data

3.2.2   Absorption

3.2.2.1   In Vivo

<u>Dermal</u>

i) In a human dermal absorption study ((Selim *et al*., 1995), twelve healthy male volunteers, weighing between 54 and 87kg, were divided equally into two treatment groups. Volunteers in one treatment group received dermal applications of undiluted technical grade DEET (98.8% purity) fortified with a small amount of [^{14}C] DEET (uniformly labelled in the aromatic ring with a radiochemical purity of 98.9% (purity of 97.9% measured prior to use, and a specific activity of 22 mCi/mmol (14ìCi/mg)). Volunteers in the other group received dermal applications of technical grade DEET fortified with [^{14}C] DEET as a 15% solution in ethanol. Each volunteer in both groups received approximately the same total amount (mg) of DEET over the same 4x6cm application area located on the volar surface of the forearm. The quantity of DEET applied was defined in preliminary experiments by determining the volume of undiluted DEET that could be applied accurately and evenly over the 4x6cm application area. This was determined to be 15µl. Larger volumes were found to have a propensity to migrate outside of the prescribed area. The dose was applied evenly over the entire application site using calibrated glass micropipettes. The application site then was covered with an aluminium dome, which was secured in place by adhesive bandages. The dome contained air holes that allowed air to circulate over the application site but prevented loss of radioactivity due to accidental physical contact. The dosing solutions were left on the skin for 8 hr during which time the volunteers were allowed to move about under close supervision.

The dosing solutions were prepared so that each volunteer received approximately the same total amount of DEET and the same total amount of radioactivity. One dosing solution was neat technical grade DEET to which a small amount of [^{14}C] DEET was added. The ratio of unlabeled DEET to [^{14}C] DEET was approximately 50:1. A l5µl portion of this dosing solution containing 15mg (approximately) of DEET and 37.0 ìCi of radioactivity was applied to six volunteers in one treatment group. The other dosing solution was a 15% (^{w}/$_w$) mixture in ethanol of technical grade DEET spiked as described above with [^{14}C] DEET. A 100µl portion of this dosing solution containing 12mg (approximately) DEET and 36 ìCi of radioactivity was applied to the six volunteers in the other treatment group.

Blood samples were collected in heparinised tubes from an indwelling catheter placed in the median cubital vein of the ipsilateral arm (arm on which the dose was applied) and the contralateral arm (non application arm) at 0 (pre-dose), 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, and 120 hours after application of the dose. Plasma radioactivity in the ipsilateral arm was considered to be indicative of dermally absorbed radioactivity plus systemically circulating radioactivity. Plasma radioactivity in the contralateral arm was considered to

be indicative only of systemically circulating radioactivity. The whole blood samples were centrifuged to separate plasma from cells and the plasma samples were stored frozen in polypropylene tubes. A pre-dose urine sample was collected snit total urine was collected after application of the dose during the following time intervals: 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-60, 60-72, 72-84, 84-96, 96-108, 108-120, and 120-128 hr. The urine samples were weighed and stored frozen. All faecal samples were collected individually throughout the entire study period and stored frozen prior to analysis.

Eight hours after dose application, the protective coverings were removed and carefully stored in containers. In order to remove residual DEET, the application sites were wiped with cotton swabs soaked in isopropyl alcohol followed by isopropyl alcohol rinses. This removal procedure was conducted rapidly in order to minimise the potential effect on DEET absorption and to prevent extraction of DEET that may have penetrated the superficial layers of skin. The volume of the rinse was determined. The swabs and a portion of the rinses were stored separately in liquid scintillation vials for analysis. The application site then was covered with a dry gauze pad.

In order to assess the degree of penetration and accumulation of DEET into the superficial layers of the skin, the application sites were stripped with adhesive tape at approximately 1, 23, and 45 hr after removal of residual dosing material. The application sites were stripped with a total of 16 strips of tape at each time interval. The tape strips were pooled in groups of four and placed in liquid scintillation vials for analysis.

Data from the plasma samples obtained from the ipsilateral arm vein showed DEET absorption from the skin within 2 hr after dermal application and that during the subsequent 6 hr a fairly constant concentration of radioactivity in plasma was maintained. Concentrations of radioactivity in plasma from the ipsilateral arm vein rapidly declined to less than the limits of quantitation within 4 hr after removal of residual dosing material from the application sites. Measurements of radioactivity in the plasma samples collected from the contralateral (non-application) arm vein were below the limits of quantitation (twice background) at all intervals for both treatment groups.

Means of 5.61 and 8.33% of the applied radioactivity were excreted in urine of volunteers from the undiluted DEET and 15% DEET in ethanol treatment groups, respectively. Most of the absorbed radioactivity was excreted within 12 hr after the start of dose application and by 24 hour nearly the entire absorbed radioactivity was excreted in the urine. Faecal excretion accounted for only 0.02 and 0.08% of the dose from the undiluted DEET and 15% DEET in ethanol treatment groups, respectively. Total radioactivity excreted in the urine and faeces ranged from 3 to 10% with a mean of 5.63% in the six volunteers applied undiluted technical grade DEET. The corresponding values for the six volunteers applied technical grade DEET as a 15% solution in ethanol were 4 to 14% and 8.41%, respectively.

The mean recoveries of radioactivity in the tape strips collected 1 hr after removal of the residual DEET were 0.08 and 0.07% of the administered dose in the groups of volunteers administered undiluted [^{14}C] DEET and [^{14}C] DEET as a 15% solution in ethanol, respectively. The mean recovery of radioactivity in the tape strips collected 23 and 45 hr after removal of the residual DEET was <0.01% of the administered dose in both treatment groups.

Recoveries of radioactivity from the applicators, swabs, skin rinses, gauze, and protective covers of the undiluted DEET and 15% DEET in ethanol treatment groups totalled 88.62

and 80.27% of the dose, respectively. Total mass balance as a percentage of applied radioactivity was 94.3% for the undiluted DEET group and 88.7% for the 15% DEET in ethanol group. HPLC analyses of the swab and skin rinse composites revealed that 98% of the recovered radioactivity in these components was unchanged DEET, although this data was not presented. A total of six metabolites were found; two of them were major metabolites that were found to be similar to those seen in a rat metabolism study. One metabolite resulted from oxidation of the methyl moiety on the aromatic ring of DEET to carboxylic acid while the other one was formed through N-dealkylation of an ethyl group from the amide moiety and the oxidation of the methyl group on the ring.

ii) The absorption of DEET was characterised in 4 humans (Feldmann & Maibach, 1970). $4\mu g/cm^2$ DEET in acetone was applied to the ventral surface of the forearm ($13cm^2$) using a microlitre pipette. The skin sites were not protected. The subjects were asked not to wash the area for 24 hours. All urine was collected for 5 days. The ^{14}C measured using liquid scintillation counting. The percentage of DEET applied that was absorbed after topical administration was 16.71%.

iii) Blood levels studies were carried out to define DEET plasma profile in humans following single and repeated dermal applications of DEET (Ohayon *et al*., 1997 (unpublished) in Schoenig & Osimitz, 2001). Three male and female human volunteers were administered undiluted DEET by the dermal route of administration at the 95th percentile of human use (3g/day for females and 4g/day for males). Both single and repeated (8hr per day for four consecutive days) applications were evaluated. DEET plasma levels were profiled on the first and fourth days of the study. The findings from this study show that DEET does not appear in the blood of humans until 1 to 2 hours after dermal application, after which time the DEET plasma levels gradually increase until the material is washed off 8 hours after application. It should be noted that DEET plasma levels had not reached a plateau prior to showering and may have increased further after 8 hours if showering had not occurred. The DEET plasma profiles and peak plasma levels were similar in males and females and did not increase after repeated dosing. The overalls mean peal plasma level was 0.45µg/ml. The overall mean area under the DEET plasma concentration versus time curve (AUC) was 3.51µg hr/ml.

3.2.2.2 Ex Vivo

A study was carried out to investigate the absorption of DEET *ex vivo* in human skin (Ross *et al*., 2000), in order to develop formulations of DEET with significantly reduced permeation using the basic principles and model of skin permeation based on Fick's laws of diffusion at steady state.
Human skin samples were obtained from elective plastic surgery, and the fat and other visceral debris were removed from the underside of the freshly excised skin. The skin was washed with 0.1M, pH 7.4 phosphate buffer solution before freezing at –20°C. The full thickness skin samples were cut into $2cm^2$ pieces and allowed to thaw overnight at room temperature in phosphate buffer prior to experiments. The permeation experiments were conducted with vertical Franz diffusion cells each having a receptor volume of 4.9ml and a diameter of 0.9cm. Pieces of full thickness human skin ($2cm^2$) that had been previously thawed were mounted on the receptor compartment of the diffusion cells. The

receptor compartment was filled with 0.1M, pH 7.4 PBS which was stirred continuously to ensure uniform distribution and maintain sink conditions, and the temperature of the entire diffusion cell assembly maintained at 37°C. Permeation studies were carried out using the infinite dose technique, using a large excess of permeant (46.88mg/cm^2). Homogeneous solutions consisting of 10% (100mg/ml) DEET and 45, 60, 75 and 90% Propylene glycol (PG) or 60, 75 and 90% polyethylene glycol 400(PEG 400) were evaluated for DEET permeation to study the effect of PG or PEG 400 on the permeation of DEET. An aliquot of 300μl (100mg/ml DEET) of the above homogenous solutions was applied to 0.64cm^2 of skin and then covered with a glass slip to prevent evaporation. Aliquots of 300μl of the receptor fluid were withdrawn and replaced periodically with fresh PBS for 36hours. Analysis of DEET was by HPLC.

Ternary phase diagrams of DEET with water and semipolar solvents, ethanol, PG and PEG 400, showed an increase in the aqueous solubility of DEET. This resulted in a linear decline in octanol/water PG with an increase in the concentration of the solvent. Permeation studies of DEET across human skin indicated that DEET's flux reduced with increasing PG concentration and the flux from 90% PG was 9.9+/-2.1 µg/cm^2 h, 6-fold lower than flux of pure DEET control, 63.2+/-24.5 µg/cm^2 h. Flux was reduced 6-fold from 60% PEG 400 solution, and permeation of DEET was totally prevented from 90% PEG 400 which was very viscous. However, a combination of 60% PEG 400 with 30% PG not only reduced permeation 9-fold but was suitable as a vehicle for formulation. The decrease in flux and permeability of DEET with increasing concentration of solvent appeared to be a direct result of decrease in skin/vehicle PC and octanol/water PC.

In vitro dermal absorption tests were conducted with the ^{14}C-ring-labelled DEET, dissolved in acetone and applied to skin sections (0.5 mm) from a dermatome at a dose rate of about 30mg/cm^2 (Moody & Nadeau, 1993). Skin permeation was determined using an automated in vitro dermal absorption procedure, and was calculated from the percentage recovery of ^{14}C-activity in the receiver solution. The total percentage of *in vitro* dermal absorption obtained by 48 hr post-exposure for human skin (n = 4) was 28 ± 4.2%. Lag times for DEET in vitro dermal absorption for human skin was 0.6hr.

3.2.2.3   Summary of Human Absorption data

A number of studies using human volunteers and *in-vitro* methods with human skin samples are available (see Table 3.5). These studies confirm the influence of solvent and formulation on absorption of DEET. The most appropriate study to use for risk assessment is the investigation undertaken for the EPA re-registration review (Selim et al 1995).

Sex & age of individual(s)	Chemical Form and Concentration	Exposure/ Observation Period	Results/ Comments	Reference
In vivo				
6 Males/group 20-29 years	15% [14]C DEET in ethanol (12mg; 36μCi)	4 x 6cm^2 on forearm for 8 hours	8.41% of applied dose recovered in urine 20% penetrated into skin	Selim *et al.*, 1995
	Undiluted ^{14}C DEET (15mg; 37μCi)		5.63% of applied dose recovered in	

			urine 12% penetrated into skin	
4, age & sex n.p.	^{14}C DEET in acetone ($4\mu g/cm^2$;$1\mu Ci$)	$13cm^2$ on ventral surface of forearm. Urine collected for 5 days	16.71% of applied dose absorbed	Feldmann & Maibach, 1970
Ex vivo				
Human skin sections ($2cm^2$)	10 % DEET in Propylene Glycol or Polyethylene Glycol	36 hours	Amount absorbed depended on formulation	Ross *et al.*, 2000
Human skin sections (0.5 mm)	^{4}C-ring-labelled DEET in acetone ($30mg/cm^2$)	48 hours	28% of applied dose absorbed	Moody & Nadeau, 1993

Table 3.6: *In Vivo* and *In Vitro* Dermal Absorption of DEET
Stinecipher and Shah (1997)

The study of Selim *et al*. reports that the radioactivity found in the urine, expressed as the percentage of the applied radioactivity, was 8.41% and 5.63% for a 15% solution of DEET and undiluted DEET, respectively. Very little radioactivity was found in the faeces (mean <0.1%). With dermal application, the majority of the applied radioactivity remained unabsorbed on the application site (78% of the applied dose for a 15% solution of DEET and 83% of the applied dose for undiluted DEET) and was recovered in skin rinsates, swabs, and protective coverings. Based on the amount administered, eliminated, found in tape-stripping, and unabsorbed (recovered in skin rinsates, swabs, applicator, and protective coverings), the amount of DEET penetrating into the skin was conservatively calculated to be 20% of the administered dose for 15% DEET and 12% for undiluted DEET. It should be noted that there was a difference in the total recovery between the two DEET treatment groups: in the 15% group, the total recovery was 89% of the administered dose while in the undiluted group, the total recovery was 94% of the administered dose. This difference is reflected in the variations seen in the calculated dermal absorption values (i.e., 20% vs. 12%).

3.2.3 Distribution

3.2.3.1 *In Vivo*

Oral

Limited information on DEET tissue distribution in humans is available. According to Tenenbein (1987), a 33 year old woman who died after ingesting 50ml of insect repellent containing 95% DEET had the following tissue levels of DEET: gastric lavage returns, 10.4mg/dl; blood, 16.8mg/dl: post-mortem blood, 11.2mg/dl; and liver, 17.7mg/dl. In a 26-year-old man who committed suicide by ingesting 50ml of 95% DEET, the following tissue DEET concentrations were obtained: blood, 24mg/dl; vitreous, 15mg/dl; and urine, 10mg/dl.

3.2.4 Metabolism

3.2.4.1 *In Vivo*

<u>Dermal</u>

i) Metabolism of DEET was studied in a report by Selim *et al*. (1995)(for details see section 3.2.2.1). The authors reported that at least six metabolites were present in the urine of human volunteers who were dermally treated with 12 or 15mg of DEET on the forearms. No intact ^{14}C DEET was found in any of the urine samples. One metabolite was identified as being formed from oxidation of the aromatic methyl group to a carboxylic acid group plus dealkylation of the amide group (N-ethyl-3-carboxylbenzamide). This metabolite represented 7.6% to a maximum of 25.5% of the radioactivity in the human urine samples that could be quantitated. The second metabolite identified was shown to be formed by the oxidation of the aromatic methyl group to a carboxylic acid group (N,N-diethyl-3-carboxylbenzamide). This metabolite accounted for 24% to 42.4% of the radioactivity obtained in human urine samples.

ii) Wu *et al*. (1979) characterised the urinary metabolites of N,N-diethyl-m-toluamide in a 30 year old male subject weighing 78kg. The subject applied 10.4g of DEET contained in a commercial insect repellent (not specified) to about 75% of his skin surface. Urine samples were collected individually over a 36-hour period and blood samples was drawn eight hours post-exposure. DEET and its metabolites were characterised using gas chromatography/mass spectrometry. The authors indicate that oxidation of the benzylic moiety of DEET to produce N,N-diethyl-3-carboxylbenzamide and hydroxylation of the side-chain to produce the glucuronide of N-hydroxyethyl-N-ethyl-m-toluamide were the predominant metabolic routes in humans. Between 10% and 14 % of the dose was excreted in the urine in the first hour as unmetabolised DEET.

3.2.4.2 *Ex Vivo*

A study was carried out to identify and quantify the oxidative metabolism of DEET by human liver microsomes and to compare the metabolism with that of rat and mouse liver microsomes (Usmani *et al*., 2002). Pooled human liver microsomes (HLM) (pooled from 10 donors) and human P450 isoforms expressed in baculovirus-infected insect cells (Sf9) (BTI-TN-5B1-4), CYP1A1, 1A2, 2A6, 1B1, 2B6, 2C8, 2C9*1 (Arg114), 2C18, 2C19, 2D6*1 (Val374), 2E1, 3A4, 3A5, 3A7 and 4A11 were used. In addition, gender specific, pooled and individual human liver microsomes were used. Rat liver microsomes (RLM) and mouse liver microsomes (MLM) were prepared from adult male Long Evans rats and adult male CD-1 mice, respectively. Total cytochrome P450 content was determined by a CO-difference spectrum method and protein concentration determined using a Bio-Rad protein assay with bovine serum albumin as standard.

HLM as well as RLM and MLM showed a much higher affinity and higher intrinsic clearance for ring hydroxylation (N,N-diethyl-m-hydroxymethylbenzamide (BALC) formation) than for N-deethylation (N-ethyl-m-toluamide (ET) formation) from DEET. When mice were treated with the high dose (200 mg/kg/day) there was a significant

increase in the Vmax and intrinsic clearance of BALC and ET, indicating that DEET may induce its own metabolism.

Among the 15 different human P450 isoforms screened, only CYP1A2, 2B6, 2D6*1 (Val374) and 2E1 displayed detectable BALC metabolite production. The activity of CYP2E1 was significantly less than the activities of the other P450s producing the BALC metabolite. Production of the BALC metabolite was generally much higher than that of the ET metabolite. Isoforms producing detectable amounts of the ET metabolite included CYP3A4, 3A5, 2A6, and 2C19. These isoforms produced no detectable amounts of the BALC metabolite. CYP2C19 showed significantly higher activity than CYP3A4, 3A5, and 2A6. Kinetic studies indicated no significant differences in Km, Vmax, and CLint (intrinsic clearance, Vmax/Km) in production of the BALC metabolite between CYP1A2 and 2B6. Activity of the CYP2D6 isoform was too low for accurate kinetic determinations. Comparisons of male and female differences in metabolism of DEET were performed using pooled liver microsomes from two different suppliers. Microsomes from one included five pooled males and females, whereas those from the second included 10 individuals from each gender. In both cases, activity of females in the production of BALC and ET metabolites was greater than that of males. However, the authors suggested that as pooled samples were prepared in a non-random manner, the data are insufficient to demonstrate a definitive gender difference.

To further determine the importance of CYP2B6 and 1A2 in ring methyl oxidation of DEET and the importance of CYP3A4, 2C19, and 2A6 in N-deethylation of DEET, liver microsomes from three different individuals possessing varying levels of these isoforms were investigated with respect to their ability to metabolise DEET. The individual with high levels of both CYP2B6 and 1A2 (HG042) had significantly greater ability to produce the BALC metabolite than the other two individuals. In contrast, individuals with high levels of CYP1A2 (HG043) or CYP2D6 (HG095) had significantly lower ability to metabolise DEET to the BALC metabolite, indicating the importance of CYP2B6 in formation of this metabolite. The individual with high levels of CYP3A4 and 2A6 but low level of CYP2C19 (HG042) had the highest activity for production of the ET metabolite. The individual (HG043) with the highest level of CYP2C19 but low levels of CYP3A4 and 2A6 had significantly greater ability to produce the ET metabolite than the individual (HG095) with very low levels of these isoforms.

Experiments were conducted to examine the potential of DEET to induce enzymes involved in metabolism. Adult male CD-1 mice, (28 to 30g) were administered low (2 mg/kg/day), medium (20 mg/kg/day), and high (200 mg/kg/day) doses of DEET in $100\mu l$ of corn oil, given intraperitoneally daily for 3 days. Doses approximating LD_{10} values for phenobarbital (80 mg/kg/day) in 100 µl of water or 3-methylcholanthrene (20 mg/kg/day) in 100 µl of corn oil were also administered intraperitoneally, to separate groups of mice, daily for 3 days. Controls were given corn oil only or water. Microsomes were prepared from livers of fed mice on the 4th day as described above. The following substrates were used as indicators of the activities for the following isozymes: ethoxyresorufin *O*-deethylation (EROD) and methoxyresorufin *O*-demethylation (MROD) for CYP1A1/2, pentoxyresorufin *O*-dealkylation (PROD) for CYP2B10, and benzyloxyresorufin *O*-dealkylation (BROD) for CYP2B. Briefly, assays were initiated by the addition of an NADPH-regenerating system and incubated for 5 min at 37°C. Product formation for EROD, MROD, PROD, and BROD activities were determined by comparison with a standard curve generated withresorufin. No significant levels of induction were observed

for the two lower doses of DEET. In contrast, the high dose of DEET produced significant increases in BROD (3.5-fold) and in PROD activities (4.0-fold) indicating CYP2B induction.

Studies were also conducted to examine the possible effect of DEET, phenobarbital, and 3-methylcholanthrene to induce metabolism of chlorpyrifos in mice. No significant differences were observed in the dearylation of chlorpyrifos with any treatment. However, significant increases in chlorpyrifos desulfuration activity were observed with phenobarbital (5.5-fold) and the high dose of DEET (2.8-fold).

The possibility that chlorpyrifos or chlorpyrifos-oxon may inhibit DEET metabolism by human CYP2B6 was investigated by incubating 100 µM concentration of each substrate for 5 min before addition of DEET as a substrate. Chlorpyrifos preincubation resulted in 100% inhibition of the production of the BALC metabolite, whereas for chlorpyrifos-oxon, 58% inhibition was observed.

3.2.4.3 Summary of Human Metabolism Studies

Two studies have examined the metabolism of DEET following topical application to humans. In both the study of Selim *et al*. (1995) and Wu *et al*. (1979), major metabolites were found to be N-ethyl-3-carboxylbenzamide and N,N-diethyl-3-carboxylbenzamide. In the earlier study (Wu *et al*. 1979), the pattern of urinary metabolites was also reported to include glucuronide conjugates (oxidation product of the aromatic methyl group) and some unchanged DEET. A reasonable explanation for this could be the overloading of metabolic capabilities in this study, which was 700 times higher than that applied in the latter. The recent *in vitro* work by Usami *et al*. (2001) suggest that the main products of DEET metabolism are N,N-diethyl-m-hydroxymethylbenzamide (BALC) and N-ethyl-m-toluamide (ET).

3.2.5 Excretion

3.2.5.1 *In Vivo*

<u>Dermal</u>
Elimination of DEET in humans appears to be fast, indicating that bioaccumulation is unlikely. The study of Selim *et al*. (1995), demonstrated that over 99% of the radioactivity absorbed was eliminated in the urine after technical DEET or a 15% DEET solution in ethanol was dermally applied to human volunteers (for study details see section 3.2.2.1). Meanwhile, the elimination of DEET in the faeces was found to be minimal, accounting for less than 0.1% of the dermal dose.

3.2.6 Summary of Absorption, Distribution, Metabolism, Elimination Studies

A summary of ADME studies is set out below.

The dermal penetration and absorption of DEET has been extensively studied in laboratory animals using *in-vitro* and *in-vivo* methods. The percentage absorbed was dependent on the solvent used, concentration of DEET, method used to determine

absorption (e.g. recovery in urine following dermal application or comparison of kinetics following intravenous or dermal application) and extent of occlusion used. It is difficult to compare these results. In rats, dermal absorption values of up to 80% have been reported. A number of studies using human volunteers and *in-vitro* methods with human skin samples are available. These studies confirm the influence of solvent and formulation on absorption of DEET. The most appropriate study to use for risk assessment is the investigation undertaken for the EPA re-registration review (Selim et al 1995). Allowing for the extent of recovery, a value of 20% absorption was used for a 15% ethanolic solution of DEET and 12% for 100% DEET.

In a study submitted to the EPA and subsequently published (Schoenig *et al.*, 1996), nearly complete oral absorption of DEET was reported in male and female rats given oral doses of 100 mg/kg bw or 500 mg/kg bw. In this study, absorbed DEET was rapidly distributed and excreted mainly in the urine as metabolites. The predominant pathways of metabolism involved oxidation of the methyl group on the aromatic ring and N-deethylation of the amide moiety. The available studies in animals, which used dermal administration also, showed that absorbed DEET was excreted predominantly via the urine.

Two studies have examined the metabolism of DEET following topical application to humans. Selim et al (1995) treated volunteers with 12-15 mg of ^{14}C-|DEET. Absorbed DEET was excreted as metabolites in the urine. The available information suggests a qualitatively and quantitatively similar to that documented in rats following oral administration. In an earlier study (Wu et al 1979), the pattern of urinary metabolites was also reported to include glucuronide conjugates (of oxidation of the aromatic methyl group) and some unchanged DEET. A reasonable explanation for this latter difference is the apparently higher dose used in the study by Wu et al (ca 10.4g of a DEET formulation).

Overall it is concluded that a value of 20% dermal absorption could be used as a reasonable overall estimation for risk assessment. In addition the ADME of DEET in experimental animals and humans is similar.

Table 3.7: Absorption, Distribution, Metabolism, and Elimination of DEET

Species, Strain and Age	Number and Sex	Chemical Form and Concentration	Route/Dose	Exposure/ Observation Period	Results/ Comments	Reference
Mouse						
CD-1 (age n.p.)	5 M	DEET (conc. n.p.)	i.p. 0.5 g/kg (2.6pmol/kg)	Plasma sampling and HPLC analysis at 0.5, 1.0, 1.5, 2.0, 3.0 and 5h	Significant increase in ammonia levels in treated mice versus controls at 3 and 5 h. DEET-dosed animals became drowsy, then comatose, with complete recovery within 1-2 h. The level of consciousness of the animal could not be correlated to the level of plasma ammonia.	Heick *et al* (1988)
Rat						
Phenobarbital-induced liver microsomes from Wistar rats (age n.p.)	M	DEET (conc. n.p.)	*In vitro* 200 µM (38.3µg/ml)	Incubated at 37°C for 45 mins	Metabolism corresponding to benzylic combinations of these reactions, were detected in methyl t-butyl ether extracts via capillary GC. The two major metabolites had a mean yield of 69%.	Taylor (1996)
Phenobarbital-induced liver microsomes from 12 wk old Wistar rats	M and F	DEET (conc. n.p.)	*In vitro* 200 µM (38.3µg/ml))	Incubated at 37 °C for 2 h	DEET degraded more rapidly from incubations prepared from male rats than those from female rats, with half-lives of 10 and 15 min, respectively. The results suggest the presence of a sex Difference in the metabolism of DEET.	Yeung & Taylor (1988)

Abbreviations: conc. = concentration; d = day(s); GC = gas chromatography; h = hour(s); HPLC = high performance liquid chromatography; n = number; n.p. not provided; PEG = polyethylene glycol; wk = week(s); yr = year(s).

Table 3.7: Absorption, Distribution, Metabolism, and Elimination of DEET (Continued)

Species, Strain and Age	Number and Sex	Chemical Form and Concentration	Route/Dose	Exposure/ Observation Period	Results/ Comments	Reference
Rat (cont.)						
CD	M and F Group I: 4/sex Group II: 5/sex 6 dose grps Group III: 5/sex	^{14}C-labelled DEET 98.9%	Group I: single oral dose of 100 mg/kg (0.523mmol/kg) Group Il: single or repeated doses of 100 or 500 mg/kg (0.523 or 2.61 mmol/kg) orally, or 100 mg/kg (0.523 mmol/kg) dermally Group III: single oral dose of 500 mg/kg (2.61 mmol/ kg)	Group I: Sampling (blood) up to 24 h post-application Group II: Sampling (tissues and excreta) up to 168 h post-application Group III: (excreta) up to 72 h post-application	Oral administration: 85-91% dose recovered in urine and 3-5% in faeces. The fastest excretion rate was noted in the repeated low-dose group, followed by the single low-dose and the single high-dose groups. Dermal administration: 74-78% dose recovered in urine, 4-7% in faeces, and 6.5% on the surface of the skin. No unchanged parent compound was detected in urine after either route; 2 major urinary metabolites were identified.	Schoenig *et al.* (1996)
Excised abdominal skin from 4 wk old Cotton rats	Number and sex n.p.	DEET >98.5%	In vitro, dermal: 0.15 g (0.78μmol)/formulation Formulations: 12% DEET solutions with 40, 50 75 or 100% ethanol or PEG 400 gel emulsions containing 10 or 15% DEET and various polymers or humectants gel emulsions containing 7.5% DEET, various polymers, and PEG 400 or ethanol	Sampling and HPLC analysis occurred at 0.5, 2, 4, 6, 8 and 10h	40 and 50% aqueous ethanol solutions increased the steady-state penetration rate by 157 and 137%, respectively, while a 75% aqueous solution, neat ethanol, and PEG 400 decreased the rate by 67, 74, and 59%, respectively. Humectants had no significant effect. Polymers and 75% ethanol decreased rates up to 22.7%. A formulation incorporating PEG 400, Carbopol 940F, and Pemulen TR-2 was also effective in reducing DEET skin permeation	Qiu *et al.* (1998)

Abbreviations: conc. = concentration; d = day(s); GC = gas chromatography; h = hour(s); HPLC = high performance liquid chromatography; n = number; n.p. not provided; PEG = polyethylene glycol; wk = week(s); yr = year(s).

Table 3.7: Absorption, Distribution, Metabolism, and Elimination of DEET (Continued)

Species, Strain and Age	Number and Sex	Chemical Form and Concentration	Route/Dose	Exposure/ Observation Period	Results/ Comments	Reference
Rabbit						
New Zealand White (age n.p.)	8 M	DEET (>95%)	i.v. 0.1ml/kg of a 10% DEET solution in ethanol dermal 1g/kg (5µmol/kg) of 10% DEET in liposphere or ethanol solution	Blood and urine collected at various intervals up to 24h, then daily for 7d	The bioavailability of DEET from the ethanol solution was 45% and that from lipospheres was 16%. Approximately 74% of the i.v. dose was recovered in urine, versus 39 and 19%, respectively, of the dermal ethanolic and lipospheric formulations	Domb et al. (1995)
Dog						
Adult beagles (age n.p.)	4 M	DEET (>98.5%) Formulation: A: Off! SkintasticII® (7.125% DEET) B: Gel emulsion (7.5% DEET)	i.v.: 2.5mg/kg (0.013mmol/kg) into the cephalic vein. After a 2-wk washout, 6.0mg/kg (0.031 mmol/kg) given dermal: 15mg/kg (0.078mmol/kg) Formulation A applied to anterior dorsal region along backline. After a 2wk washout, formulation B was applied	Blood sampling at various intervals for 15min – 18h post-application	Linear pharmacokinetics was demonstrated after i.v. dosing. DEET underwent extensive extravascular distribution and rapid elimination The transdermal absorption of DEET was faster from formulation A than B. The bioavailabilities were 18.3% and 14.0%, respectively.	Qiu et al. (1996)
Pig						
Yorkshire (age n.p.)	9 F	^{14}C-labelled DEET (~98%)	Dermal 4µg/ cm2 (0.-02µmol/cm2)	48h after application	Increasing the rate of air flow over the skin from 60ml/min to 600ml/min significantly increased evaporation from the skin surface, decreased the residues in the upper skin layer, and decreased the penetration of DEET in the epidermis	Reifenrath et al. (1991)

Abbreviations: conc. = concentration; d = day(s); GC = gas chromatography; h = hour(s); HPLC = high performance liquid chromatography; n = number; n.p. not provided; PEG = polyethylene glycol; wk = week(s); yr = year(s).

Table 3.7: Absorption, Distribution, Metabolism, and Elimination of DEET (Continued)

Species, Strain and Age	Number and Sex	Chemical Form and Concentration	Route/Dose	Exposure/ Observation Period	Results/ Comments	Reference
Species Comparisons						
Sprague-Dawley rats and Rhesus (Macaca mulatta) (ages n.p,)	5-8 M rats and monkeys /group	^{14}C-labelled DEET, (>98%)	Single dose: 44 µg; (0.23µmol) on shaved dorsal region of rats and the forearm, forehead, and ventral and dorsal sides of the forepaw of monkeys Repeated doses: three 33µl doses applied at 2-h (rat) or 0.5-h (monkey) intervals. Dose occluded for 24 h, then washed.	Urine samples collected at 4 and 8 h (Day 1), then at 24h intervals for 7d	Absorption in rats was 36%. The extent and rate of absorption in the monkey were highly dependant upon the anatomic site, with penetration 14% from the forearm, 27% from the dorsal side of the paw, 33% from the forehead, and 68% from the ventral side of the forepaw. No significant difference was seen between the total percentage absorbed with single versus repeated doses for either species.	Moody *et al.* (1989)
Human and pig (strain and/or age n.p.)	Human abdominal or breast skin (sex n.p.) and pig skin (3-9, sex n.p.)	^{14}C-labelled DEET, >98% in the following formulations: A = silicone polymer B = acrylate polymer C = fatty acid D = proprietary polymer E = dimethyl phthalate Control = unformulated	dermal: 320-360 µg/cm^2 (1.67-1.88 µmol/cm^2) control: l0 µg/cm^2 (0.052µmol/cm^2)	Sampling occurred at various intervals up to 50 h	The evaporation and penetration of A B, and C were not significantly different from those of the control. D showed significantly less evaporation and less penetration than the control. For E the total evaporation and penetration of each repellent did not change, but the maximum evaporation and penetration rates were lower and extended over a longer period of time.	Reifenrath *et al.* (1989)

Abbreviations: conc. = concentration; d = day(s); GC = gas chromatography; h = hour(s); HPLC = high performance liquid chromatography; n = number; n.p. not provided; PEG = polyethylene glycol; wk = week(s); yr = year(s).

Table 3.7: Absorption, Distribution, Metabolism, and Elimination of DEET (Continued)

Species, Strain and Age	Number and Sex	Chemical Form and Concentration	Route/Dose	Exposure/ Observation Period	Results/ Comments	Reference
Species Comparisons (cont.)						
Rhino mice (10 mo old) Sprague-Dawley rats(5 wk old), hairless Crl: IAF/HA(hr/hr)BR Guinea pigs (9 mo), Yorkshire pig (8-9 wk old), Caucasian human (33 yr old)	n=4 (each skin type M mouse rats, and pig (back skin) F guinea pigs (back skin) F human (abdominal skin); M Testskin (human foreskin)	^{14}C-ring-labelled DEET, >98%	*In vivo* and *in vitro* application of 50 μL at the following dose rates: Species Doses (ug/cm^2)(μmol/cm^2) mouse 33.3 0.174 rat 38.7 0.202 gpig 12.5 0.0653 pig 19.4 0.1013 human 44.7 0.234 Testskin 27.9 0.146	*In vitro:* sampling of the receiver fluid at various intervals up to 48 h *in vivo* urinary sampling at 24-h intervals for 14 d	*In vitro:* the total percentage of absorption were as follows: Mouse (36%) Human (28%) Rat (21%) Pig (15%) Testskin (15%) Guinea pig (11%). The *in vitro* procedure under-estimated the observed *in viva* dermal penetration of DEET.	Moody & Nadeau (1993)
Sprague-Dawley rats (5 wk old), Hairless guinea pig (9 mo old), Caucasian human (40 yr old)	n=4 for each skin type M rats (back skin), F guinea pigs (back skin), F h~ (abdominal skin)	OFF!" (14.25% DEET) Deep Woodsy (23.75% DEET); Muskol® (95% DEET)	*In vitro:* 14.5-97.3 mg/cm^2 (0076-0.509 mmol/cm^2) *In vivo* (rat only): same doses as above	Automated sampling at various intervals up to 48 h; 24-h soap wash	*In vitro,* the total percentage of absorbed DEET for OFF!®, Deep Woods®, and Muskol®, respectively, were 50, 49, and 44% 46, 37 and 19% (rat), (guinea pig), and 48, 36, and 17% (human). Soap wash percentage recoveries increased for all species with the more concentrated DEET formulations. *In vivo,* DEET penetration through rat skin was significantly lower than *in vitro* penetration for each formulation.	Moody *et al.* (1995)

Abbreviations: conc. = concentration; d = day(s); GC = gas chromatography; h = hour(s); HPLC = high performance liquid chromatography; n = number; n.p. not provided; PEG = polyethylene glycol; wk = week(s); yr = year(s).

3.3 Acute toxicity, Irritancy and Sensitisation

3.3.1 Acute Oral

<u>Rat</u>

i) Verschoyle *et al*. (1992) carried out an acute oral toxicity study in rats. Female Lac:P Wistar derived rats 160-190g, were dosed by oesophageal intubation after overnight starvation. All rats were fed immediately after dosing. DEET was given as a mixture with arachis oil at doses of 0.4 to 5g/kg and at concentrations of 200 and 500mg/ml. Control animals were given an equivalent volume of appropriate solvent. In some cases, piperonyl butoxide was given as a 500mg/kg i.p. dose in arachis oil 30 minutes prior to challenge. LD_{50}'s were calculated by using four rats for each dosage group. LD50 values increased with age and were lower in females (667-3429 mg/kg) than males (891-3564mg/kg). Pre-treatment with piperonyl butoxide greatly increased toxicity, suggesting a metabolic factor for these age- and sex-related differences. The animals suffered ataxia, severe and often fatal prostration, with prolonged suppressed seizure activity. Pathology revealed a spongiform myelinopathy, largely confined to the cerebellar roof nuclei and accompanied by neuronal cytoplasmic clefts (Further details pertaining to the neurotoxicity seen in this study can be found in section 3.8).

ii) An Oral LD_{50} value in the female rat has been estimated at 2g/kg and 3 g/kg for male rats (Haight *et al*., 1979, cited by Robbins & Cherniack).

iii) A set of full guideline GLP studies conducted on typical production grade DEET by one of the major DEET manufactures have reported a rat oral LD50 of 1892mg/kg (Moore, 2000 (unpublished); cited in Schoenig & Osimitz, 2001)

<u>Dogs</u>

i) Six male beagle dogs weighing between 12 and 22kg were anaesthetised with sodium pentobarbital (26mg/kg i.v.). The dogs were heparinised with 150 U/kg of sodium heparin (i.v.). Catheters were placed in the external jugular vein and femoral artery for drug injections and pressure monitoring, respectively. On completion of surgical procedures a 15-20 minute period was allowed for readings to stabilise. Pre-dose values were made and the animals were then injected with 225mg/kg DEET (i.p.). The dose resulted in a significant decrease in both blood pressure and heart rate and also cardiac output. However, stroke volume and peripheral resistance was not altered (Leach *et al*., 1988).

ii) Mount *et al*. (1991) carried out an acute oral toxicity study in 2 male (hound breed, one 3 months old, the other 2 years old) and 2 female (mixed breed, one 8 months, the other 9 months) dogs. A commercial flea and tick repellent was used containing 198g of formulation of which approximately 24% was propellant. 9.0% of the formulation was DEET and 0.09% was 3-phenoxyphenyl) methyl-4-chloro-alpha-(1-methylethyl)benzene acetate (common name fenvalerate). Food was removed 12 hours prior to dosing. Doses of 118mg/kg DEET or 356mg/kg DEET were administered using a stomach tube followed by water to flush the tube. The dogs receiving the higher dose showed moderate or severe

clinical signs, including restlessness, muscle tremors, ataxia, hypersalivation, emesis, incoordination, depression and generalised seizures. These dogs recovered after 48 hours. The dogs receiving the lower dose showed minor or moderate clinical signs, including ataxia, restlessness and emesis. These dogs recovered after 2-4 hours.

Cats

Mount *et al*. (1991) carried out an acute oral toxicity study in 2 cats (1 male, 1 female). A commercial flea and tick repellent was used containing 198g of formulation of which approximately 24% was propellant. 9.0% of the formulation was DEET and 0.09% was 3-phenoxyphenyl) methyl-4-chloro-alpha-(1-methylethyl)benzene acetate (common name fenvalerate). Food was removed 12 hours prior to dosing. A dose of 89mg/kg DEET or 178mg/kg DEET was administered using a nasogastric tube, followed by rinsing the tube with water. Both cats experienced ataxia and hypersalivation between 5 and 10 minutes after exposure. The cat receiving the higher dose experienced more severe clinical signs including depression, hypersalivation and incoordination and generalised seizures. This cat was in a course of rapid deterioration before euthanasia was performed. The cat receiving the lower dose was asymptomatic at 26 hours.

3.3.2   Acute Dermal

Rat

i) The effect of DEET on the urinary excretion of 6β-hydroxycortisol, a marker of hepatic CYP3A induction, was investigated in rats (Abu-Qare & Abou-Donia, 2001b). Simultaneous experiments were carried out using permethrin and a combination of the two chemicals.

Male Sprague-Dawley rats (250-300g) were kept in a 12 hour light/dark cycle (temperature 21-23°C) and were provided with a free supply of feed and tap water. A single dose (0.1ml) of 400mg/kg DEET (dissolved in 70% ethanol) was applied with a micropipette (1ml/g) to an unprotected 1cm^2 area of pre-clipped skin in the back of each rat. A group of five rats was used for each time point. Five control rats were treated with an equal volume of 70% ethanol (1ml/kg) and kept under conditions similar to treated rats. After dosing, urine samples were collected from each group of 5 animals at 2, 4, 8, 16, 24, 48 and 72 hours. The samples were frozen until analysis by HPLC.

A single dose of 400mg/kg DEET caused a significant increase in the urinary excretion of 6b-hydroxycortisol at 24, 48, and 72 hours after dosing. There was no significant effect within 16 hours of treatment.

ii) The effect of DEET on the urinary excretion of 3-nitrotyrosine, a marker of oxidative stress, was carried out in rats (Abu-Quare et al., 2001). Simultaneous experiments were carried out using pyridostigmine bromide and permethrin and all three chemicals in different combinations. A single dermal dose of 400mg/kg DEET (in ethanol) was administered by micropipette to an unprotected 1cm2 area of pre-clipped skin on the back of each rat. A group of five animals was used for each time point. Five control rats were treated with equal volume of either water or ethanol and kept under similar conditions as treated rats. Urine samples were collected at 4, 8, 16, 24, 48 and 72 hours after dosing. The samples were frozen at –20°C until analysis by HPLC.

A single dermal dose of 400mg/kg DEET caused a statistically significant increase in the urinary excretion of 3-nitrotyrosine, 24 hours after dosing. Maximum induction was detected 48hours after dosing.

iii) A set of full guideline GLP studies conducted on typical production grade DEET by one of the major DEET manufactures have reported a rat dermal LD50 of >5000mg/kg (Moore, 2000; cited in Schoenig & Osimitz, 2001)

Dogs

Mount *et al*. (1991) carried out an acute dermal toxicity study in 2 male (various breeds, ages were 5 months and 1.5 years) and 4 female (various breeds, 5-9 months) dogs. A commercial flea and tick repellent was used containing 198g of formulation of which approximately 24% was propellant. 9.0% of the formulation was DEET and 0.09% was 3-phenoxyphenyl) methyl-4-chloro-alpha-(1-methylethyl)benzene acetate (common name fenvalerate). Food was removed 12 hours prior to dosing. Two dogs received a dose of 356mg/kg DEET, two received 1426mg/kg DEET and two received 1782mg/kg DEET. These doses were administered to the dogs by pouring the liquid amount upon the skin and dispensing the material over the hair to prevent runoff. The two dogs receiving 1426mg/kg DEET were used in a subsequent experiment when a dose of 7128mg/kg DEET was applied dermally.
The dogs receiving the highest dose of 7128mg/kg DEET were mildly affected showing signs of hypersalivation, restlessness, incoordination and depression. Recovery was after 19 hours. The same dogs receiving 1426mg/kg DEET showed no clinical signs at all. Dogs receiving 1782mg/kg DEET showed a minor clinical sign of restlessness. Dogs receiving lower dermal doses showed no clinical signs.

Cats

Mount *et al*. (1991) carried out an acute dermal toxicity study in 1 adult male cat and 7 female cats (various breeds, aged between 3 months and 3 years). A commercial flea and tick repellent was used containing 198g of formulation of which approximately 24% was propellant. 9.0% of the formulation was DEET and 0.09% was 3-phenoxyphenyl) methyl-4-chloro-alpha-(1-methylethyl)benzene acetate (common name fenvalerate). Food was removed 12 hours prior to dosing. Four cats received 1782mg/kg DEET, two received 3564mg/kg DEET and two received 7128mg/kg DEET.
The cats receiving the highest dose were severely affected. Clinical signs included hypersalivation, restlessness, depression, muscle tremors and seizures. Death occurred in both cats at 2 hours and 8 hours. Cats receiving 3654mg/kg DEET showed moderate and severe clinical signs, including hypersalivation, restlessness and depression. Three cats receiving 1782mg/kg DEET showed minor clinical symptoms and one cat showed mild clinical signs (muscle tremors and mild depression).

Rabbit

A dermal LD50 in the rabbit of 3g/kg has been reported (Carpenter *et al*., 1974, cited in Robbins & Cherniack, 1986)

3.3.3   Acute Inhalational

Rat

i) A 1979 study in which the U.S. Army conducted acute behavioural studies where rats were exposed to aerosols of DEET (Sherman, 1979) was reviewed in Robbins & Cherniack (1986). A battery of tests, which included balance-beam performance, passive and quick avoidance and endurance performance, after four hours exposure to DEET at concentrations of 0 mg/m3 (control), 2300 mg/m3 (low), 2900 mg/m3 (medium), and 4100 mg/m3 (high). No toxic signs were noted in the control, low-exposure or medium-exposure groups, while shaking, prostration and loss of balance were seen in the high exposure females and shaking in the high dose males. Decrements in performance were reported in a concentration-related pattern.

ii) A four-hour inhalational LC50 of 6 g/m^3 was reported in the rat. (Macko & Bergman, 1979, cited in Robbins & Cherniack, 1986)

iii) A set of full guideline GLP studies conducted on typical production grade DEET by one of the major DEET manufactures have reported a rat 4-hr LC50 of greater than 2.0mg/litre (Moore, 2000 (unpublished); cited in Schoenig & Osimitz, 2001)

3.3.4   Acute Intraperitoneal

Rats

Twenty male Sprague Dawley rats were anaesthetised with sodium pentobarbital (60mg/kg i.p.). A 15cm length of tubing was filled with heparinised saline and inserted into the left carotid artery to monitor blood pressure. Groups of 5 rats were treated with DEET (75%) at dosages of 225, 113 and 56 mg/kg or with a 25% ethanol vehicle. Blood pressure and heart rate fell within the first few minutes of injection of 225mg/kg DEET. The response was maximal between 10 and 15 minutes post-dosing. Neither 56mg/kg DEET nor the 25% ethanol vehicle caused any effect on blood pressure or heart rate (Leach *et al*., 1988).

Mice

A study was carried out to investigate the effect of DEET on ammonia metabolism (Heik *et al*., 1988). Male CD-1 mice were exposed to 0.5 g/kg technical grade DEET in corn oil by intraperitoneal injection, blood samples were taken for up to 5 hours. Animals were reported to be drowsy then comatose but recovered by the end of the experiment. All animals exposed to DEET showed elevated ammonia levels, with peak values occurring between two and five hours. Ammonia levels did not correlate with the clinical symptoms. Results indicated that injections of DEET could acutely increase ammonia levels in normal mice and thus be hazardous to individuals with defective urea cycles.

3.3.5 Skin Irritancy

<u>Rabbit</u>

i) Mild erythema, desquamation and dryness of the skin have been observed in rabbits treated with 2 ml/kg of 50% DEET (1 g/kg) for 3 days (Ambrose *et al*., 1959; Wong & Yew, 1978, cited in Robbins & Cherniack, 1986).

ii) A set of full guideline GLP studies conducted on typical production grade DEET by one of the major DEET manufactures have been shown to produce moderate erythema and oedema to the skin of albino rabbits following a 4-hr occluded exposure. All skin irritation subsided within 7 days (Moore, 2000 (unpublished); cited in Schoenig & Osimitz, 2001)

3.3.6 Eye Irritancy

<u>Rabbit</u>

i) Ocular toxicity has been extensively studied by MacRae *et al*. (1984). Eyes of New Zealand albino rabbits weighing 2.5-3kg were examined with a Haag-Streit 900 slit lamp. Corneal thickness was measured on each eye by optical pachymetry. One drop of proparacaine HCl 0.5% was instilled onto each eye prior to topical application of the test substance. Innoculum volumes of 10, 30 or 100µl of 100% DEET, 100% ethanol, or 80% ethanol/20% DEET mixture were topically applied to the cornea. Six eyes were studied in each of the nine groups, and six additional eyes served as control and received a topical application of 30µl 0.9% NaCl. In order to apply the above liquids topically, the lids were retracted slightly, and the test substance was placed on the superior limbus. After one second, the lower lid was moved upward to facilitate equal distribution of the test substance. The eyes were not irrigated.
The eyes were graded by the Draize scoring method at 24, 48, 72 and 168 hours and corneal thickness measured at 1, 4, 24, 48, 72 and 168 hours after application of the test substance. A slit-lamp examination was also performed at the same time intervals. Conjunctival swelling, corneal cloudiness and iris reaction were graded with the McDonald-Shadduck scale. Each eye was stained at 24, 48, 72 and 168 hours with 1% fluorescein strips, and the excess stain washed out with balanced salt solution. The cornea was examined with a cobalt blue light and the corneal fluorescein-staining pattern noted. The percent of total corneal area, which stained with fluorescein, was estimated subjectively to the nearest 10% by the examiner. One masked observer performed the ocular examination and scoring for the above experiment.
No significant difference was seen in Draize scores among the three substances scores tested at 24 hours. At 72 hours, eyes treated with 100% DEET or the 80% ethanol/20% DEET mixture had significantly higher Draize scores than those treated with 100% ethanol. No difference was seen at 24 hours among the three test substances, when evaluating iris reaction, corneal cloudiness and conjunctival swelling. At 72 hours there was significantly less iris reaction, corneal cloudiness and conjunctival swelling in eyes treated with 100% ethanol when compared to 100% DEET or 80% ethanol/20% DEET. Measurements of corneal thickness and percent fluorescein staining of the cornea at 24 and 72 hours showed that 100% ethanol caused significantly less fluorescein staining and

corneal thickening than 100% DEET or 80% ethanol/20% DEET. By 168 hours, all the measured parameters returned to their pre-treatment values.

When 30μl of test substance was topically applied to the cornea, there was no significant difference among the three substances by Draize scoring, evaluation of iris contraction/corneal cloudiness/conjunctival swelling, percent fluorescein staining or corneal thickness. By 168 hours the ocular irritancy test showed complete reversal to pre-treatment condition. Control animals treated with 30μl of 0.9% NaCl demonstrated no toxicity.

When 100μl of test substance was topically applied to the cornea, 100% DEET or 80% ethanol/20% DEET caused a significantly greater amount of fluorescein staining at 24 hours than in corneas exposed to 100% ethanol. At 24 hours there was no significant difference among the three substances when eyes were evaluated by Draize scoring, iris reaction, corneal cloudiness, conjunctival swelling and corneal thickness. By comparison, at 72 hours there was significant difference among eyes treated with 100% ethanol, 100% DEET, or 80% ethanol/20% DEET in percent fluorescein staining and Draize scores. The other four methods of evaluation did not demonstrate significant differences in toxicity among the test substances.

Despite the volume applied, in all cases the eyes returned to normal by 168 hours. There was no evidence of irreversible ocular irritancy at any inoculum size with the substances tested.

The results of study were not presented in tabular form as usual for this type of study.

ii) Irritation of the conjunctival epithelium and corneal opacities occurred in rabbits exposed daily to four five second facial sprays of 71 % DEET and one direct application of one drop of 100 % DEET to the eye daily (Christensen *et al*., 1969, cited by Robbins & Cherniack, 1986).

iii) A set of full guideline GLP studies conducted on typical production grade DEET by one of the major DEET manufactures have reported that when instilled into the rabbit's eye, slight corneal opacity and slight to moderate conjunctive irritation in the form of redness, swelling and discharge were observed. All ocular irritation cleared within seven days (Moore, 2000 (unpublished); cited in Schoenig & Osimitz, 2001).

3.3.7   Summary of Acute Toxicity Studies

Data on acute toxicity, irritancy (skin/eye), and skin sensitisation of DEET are available in a number of studies (summarised below in Table 3.8). Limited data are available on individual formulations. DEET is of low acute oral (LD_{50} 2170-3664 mg/kg bw) and inhalation (LC_{50} 5.95 mg/l) toxicity in the rat and low acute dermal toxicity (LD_{50} 4280 mg/kg bw) in the rabbit. Based on a comparison of acute doses that result in 50% mortality, females appeared more sensitive than males and young animals appeared more sensitive to high acute oral exposures than adults. It is possible that these sex- and age-related differences might be due to metabolic differences. Clinical signs of toxicity at near lethal doses included ataxia, tremors, prostration, lack of balance, convulsions and hypersalivation.

Species, Strain, and Age	Number and Sex	Chemical Form and concentration	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Rat						
Lac:P Wistar derived, 7-9 wk old	M and F, numbers n.p.	DEET, 98%	Oral: 400-5000 mg/kg(2.09-26.14 mmol/kg)	Single dose, 8-d observation period	LD_{50} values increased with age and was greater in females than males. Doses of 2000-3000 mg/kg produced a severe rapid decrease in stimulus reactivity and muscle tone. Rats given 2500-4000 mg/kg died between 50 minutes and 24 hours, exhibiting progressive respiratory depression.	Verschoyle et al. (1992)
Sprague- Dawley, age n.p.	5 M/dose	DEET, 75%	i.p.: 56, 113, and 225 mg/kg(0.29, 0.591, and 1.18 mmol/kg)	Single injection, observation period n.p.	No effects observed at 56 mg/kg. Dose-related decrease in blood pressure and heart rate seen with higher doses.	Leach et al. (1988)
Dog						
Hound, Setter, Shepherd, Pitbull, and mixed breeds, 3 mo to 2 yr old	4 M, 6 F	DEET, 9%	Oral or dermal: 0.089-7.128 g/kg (0.46-37.26μmol/kg)	Single dose or application, observation period up to 72 h	Symptoms included ataxia, seizures, muscle tremors, hypersalivation, restlessness, depression, and incoordination.	Mount et al. (1991)
Beagles, age n.p.	6 M	DEET, 75%	i.p.: 225 mg/kg(1.18 mmol/kg)	Single injection, observation period n.p.	Significant decrease in blood pressure and heart rate	Leach et al. (1988)
Cat						
DSH and Siamese, 3 mo to 5 yr old	2 M, 8 F	DEET, 9%	Oral or dermal: 0.089-7.128 g/kg (0.46-37.26 mol/kg)	Single dose or application, observation period up to 72 h	Symptoms of toxicity included ataxia, seizures, muscle tremors, hypersalivation, restlessness, depression, and incoordination	Mount et al. (1991)

Table 3.8: Summary of Acute Toxicity Studies
Abbreviations: d = day(s); DSH = Domestic Short Hair; F = female; h = hour(s); i.p. = intraperitoneal injection; M = male; mo = month(s); n.p. = not provided; wk = week(s); yr = year(s).

DEET is a mild skin irritant in rabbits (Table 3.9). It is difficult to assess the eye irritancy data from the available study (MacRae *et al.*, 1984) in comparison to the criteria for classification under the dangerous Substances Directive (67/548/EEC as amended for 7th time 92/32/EEC). However, the evidence of reversal by 168 hours suggests that DEET did not induce serious damage to eyes but should be regarded as an eye irritant. It would be prudent to assume such a classification for all formulated products unless appropriate data are available to show otherwise.

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Concentration	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Rabbit						
New Zealand albino, age n.p.	Number and sex n.p.	DEET, 100%	Ocular: 10, 30, 100 µL	Single application, 168-h observation period	Signs of irritation included oedema of the nictitating membrane, lacrimation, conjunctivitis, purulent discharge, and occasional corneal cloudiness. Eyes returned to normal within 1 wk and no permanent damage was observed.	MacRae et al. (1984)
Strain and age n.p.	Number and sex n.p.	DEET, 71% and 100%	Facial spray (71% DEET) and ocular (one drop of 100% DEET)	4 days exposure	Irritation of the conjunctival epithelium and corneal opacities	Christensen *et al.* (1969) cited by Robbins & Cherniack (1986)

Table 3.9: Summary of Sensitisation and Irritation Studies
Abbreviations: d = day(s); DSH = Domestic Short Hair; F = female; h = hour(s); i.p. = intraperitoneal injection; M = male; mo = month(s); n.p. = not provided; wk = week(s); yr = year(s).

Study	Results	Reference
Oral LD_{50} rat	2170 to 3664mg/kg	U.S. Army Environmental Hygiene Agency, 1979
Dermal LD_{50} rabbit	4280mg/kg	U.S. Army Environmental Hygiene Agency, 1979
Acute inhalation LD_{50} rat	5.95mg/l	U.S. Army Environmental Hygiene Agency, 1979
Eye irritation – rabbit	Eye irritation and corneal opacity cleared by day seven and three, respectively	U.S. Army Environmental Hygiene Agency, 1979
Dermal irritation – rabbit	Minimal irritation – cleared by day seven	U.S. Army Environmental Hygiene Agency, 1979
Dermal sensitisation	Not a skin sensitiser	U.S. Army Environmental Hygiene Agency, 1979

Table 3.10: Summary of Acute Toxicity Values for DEET as Reported by the EPA

3.4   Short-Term Toxicity

3.4.1   Oral Toxicity

<u>Rat</u>

i) A 90-day oral dose-range finding study was carried out in Charles River CD® rats (Johnson, 1987a (unpublished); cited by Schoenig & Osimitz, 2001). Groups of rats (15/sex/dose) received DEET (technical grade) in the diet at doses of 0, 100, 500, 1000, 2000 and 4000mg/kg for 90 days. Due to rejection of the treated diets and subsequent body weight depression and mortality, the 4000mg/kg/day group was discontinued after 7 days. No treatment-related clinical signs or effects on haematology, clinical chemistry, or gross pathology were observed in the remaining treatment groups. At 500mg/kg or above, decreased body weights and food consumption were observed, and signs of inanition were evident at 2000mg/kg/day. Non-specific liver weight increases without correlative microscopic findings occurred in all treatment groups except the 100mg/kg/day females.

An increase in the incidence of renal lesions, which were characterised by granular casts, multifocal chronic inflammation, regeneration tubular epithelium and hyaline droplets were observed in the kidneys of all treated males. These renal lesions were considered to be reflective of α2u-globulin induced nephropathy.

A NOAEL or LOAEL cannot be established due to effects in all male rats

ii) A 90-day feeding study was carried out in CD®, Fischer and NCI Black-Reiter (NBR) male rats in order to establish an association between renal toxicity in male rats and the α2u-globulin mechanism of renal toxicity (Goldenthal, 1992 (unpublished); cited by Schoenig & Osimitz, 2001). Groups of male rats from each strain (10/group) received either a control diet or DEET-treated diet at a concentration that would lead to a dose level of 400mg/kg/day for 90 days. A limited set of parameters was evaluated, including clinical observations, body weight and food consumption measurements, kidney weight measurements, and gross and microscopic examination of the kidney. Hematoxylin and eosin and Mallory-Heidenhain stained slides of the kidney of each rat were examined microscopically

Treatment-related clinical findings in this study included slightly decreased body weights for CD and NBR treated rats as compared with their respective control groups. In addition, slightly decreased food consumption was observed for treated CD rats as compared with the CD control group. DEET treatment produced an increased in incidence of microscopic kidney lesions, including granular case formation (CD® male rats only), chronic inflammation, regenerative tubular epithelium and hyaline droplets in the CD and Fischer 344 rats but not in the NBR rats. The distribution of microscopic kidney lesions in the three strains of rats supports the correlation of DEET kidney toxicity in male rats with α2u-globulin nephrotoxicity.

A NOAEL or LOAEL cannot be established for this study due to only a single dose tested

iii) Testicular hypertrophy was reported in a study in which rats were fed a diet containing 48-531 mg DEET/kg/day for 200 days (Angerhofer & Weeks, 1980 (unpublished), reviewed by Robbins & Cherniack, 1986).

Hamster

A 90-day range finding study was carried out in Golden Syrian VAF/Plus® hamsters (Goldenthal 1989a (unpublished); cited by Schoenig & Osimitz, 2001). Groups of hamsters (15/sex/dose) received 0, 1000, 5000, 10000 or 15000ppm of DEET in the diet for 90 days (0, 61, 305, 624 or 940mg/kg/day). At 15,000ppm there were clinical signs, including labored breathing, decreased defecation, decreased activity, pale skin and mortality. At 5000ppm in males, there was a consistent drop in food consumption and body weight. These decreases were more marked at 10000 and 15000ppm in male and female hamsters. The testes appeared smaller and decreased testes weights were observed at 10000ppm and above. These observations were accompanied by an increased incidence of tubular degeneration in the testes and an associated accumulation of cellular lumenal debris in the epididymides. Blood potassium levels were elevated at 15000ppm. No other effects in the haematological or clinical chemistry parameters were observed. Renal lesions, as seen in the rat 90-day study were not observed.

The NOAEL for short-term toxicity in hamsters is 1000ppm (61mg/kg/day) and LOAEL was 5000ppm (305mg/kg/day).

Mouse

i) A 90-Day dose-range finding study was carried out in mice (Johnson, 1987b (unpublished); cited in Schoenig & Osimitz, 2001). Groups of Charles River CD-1 mice (15/sex/dose) received DEET at dietary concentrations of 0, 300, 1000, 3000, 6000 or 10000mg/kg/day body weight for 13 weeks. A marked decrease in the food intake and body weights was found in the 6000 and 10000mg/kg/day groups during the first week of the study. These groups were terminated at week two. weight depression and mortality, the 4000mg/kg/day group was discontinued after 7 days. No treatment-related clinical signs or effects on food consumption or gross pathology were observed in the remaining treatment groups. Decreased defecation early in the study and a decrease in body weight was seen animals in the 3000mg/kg/day dose group. A statistically significant increase in the liver weight was seen in 1000 and 3000mg/kg/day dosed mice of both sexes. A slight increase in liver weight was also seen in 300mg/kg/day dosed female mice. Multifocal hepatocellular hypertrophy was observed at high incidence in males and females at 3000mg/kg/day and at a lower incidence in females at 1000mg/kg/day. The increased liver weights and the corresponding hypertrophy were considered to be adaptive changes rather than an indication of systemic toxicity. No other gross or microscopic changes were observed. Haematology and clinical chemistry evaluations were not included in this study.

A NOAEL and LOAEL cannot be established due to effects at lowest dose

ii) To investigate the contribution of Pyridostigmine bromide (PYR), DEET, JP-8 Jet Fuel, and stress in accelerating or exasperating autoimmune disease, a strain of autoimmune prone mice, MRL Ipr/Ipr, were exposed for 14 days at age 12 weeks (Gilkeson et al., 2001, abstract only). At 18 weeks of age, immunological parameters in mice were assessed. No remarkable changes occurred in complete peripheral blood counts, spleen and thymus total cellularity and CD4/8 flow cytometry, except for a decrease in thymic CD4-/CDB+ cells after exposure to JP-8 only. Furthermore, macrophage nitrite production or splenic lymphocyte proliferation after exposure to concanavalin A or lipopolysaccharide was not significantly different. Proteinuria was also assessed in these mice at 14, 16 and 18 weeks. By 18 weeks of age, MRL-Ipr/Ipr female mice had an expected time-dependent increase in proteinuria. Following single exposure to either PYR or exercise stress, an increase in proteinuria was noted over controls. Single exposures to DEET or JP-8 minimally affected the development of proteinuria as compared to controls. Furthermore, mice exposed to the stress mixture (20 min of exercise + 15.5 mg/kg DEET + 2 mg/kg PYR) or JP-8 mixture (500 mg/kg JP-8 + 15.5 mg/kg DEET + 2 mg/kg PYR) demonstrated increased proteinuria over control values, but this change was not typically greater than levels achieved after single exposure to PYR or stress.

Rabbit

Rabbits were given 132-528mg/kg (0.690-2.76mmol/kg) DEET orally for 15 days (Haight et al, 1979, cited by Robbins & Cherniack, 1986). Those in the high-dose group showed decreased body weight and increased kidney weight. Furthermore, serum calcium levels

decreased while body cholesterol and triglyceride levels increased. No other signs of toxicity were observed.
A NOAEL and LOAEL cannot be established for this study

Dog

i) A two week palatability study was carried out (Goldenthal, 1994a (unpublished); cited in Schoenig & Osimitz, 2001). One male and one female dog received DEET in the diet at concentrations corresponding to 0, 300, 1000, 3000 and 10000ppm. Clear evidence of diet rejection was observed at a dietary concentration of 10000ppm. Findings with regard to palatability at dietary concentrations of 3000ppm or more were inconclusive.
A NOAEL and LOAEL cannot be established for this study

ii) An eight-week palatability study was carried out (Goldenthal, 1994b (unpublished); cited in Schoenig & Osimitz, 2001). Groups of Beagle dogs (2/sex/dose) received DEET in the diet at concentrations of 0, 300, 1000, 3000 or 6000ppm. During the first two weeks of the study, the highest dose male and female dogs rejected the diet. This treatment was withdrawn and after one week on the basal diet, the dogs received 4500ppm of test diet for two weeks. Subsequent to one week on the basal diet (week six), at week seven, the dose was reduced further from 4500ppm to 3000ppm. Control dogs received basal diet.
Under the conditions of this study, DEET did not produce any treatment-related clinical signs or effects on body weight, food consumption, haematology, clinical chemistry, organ weights, or gross or microscopic pathology at the dietary concentrations of 3000ppm or less. Dogs receiving the highest concentration, whose diets were reduced in subsequent weeks (i.e. 6000/4500/3000ppm DEET) suffered food rejection, which led to decrease body weight and thin appearance. Food consumption by the dogs in this group was much lower when offered diet at 3000ppm DEET during study week 8 compared to that of the dogs offered diet at 3000ppm throughout the study, indicating that these dogs developed an aversion to the taste when exposed to the higher doses. Based on the results of this study, it was determined that DEET does not produce toxicity in digs when administered in the diet at concentrations up to 3000ppm (approximately 75g/kg/day), ehilr diets containing greater than 3000ppm are not palatable to the beagle dog
A NOAEL and LOAEL cannot be established for this study

iii) A third palatability study was three weeks in duration and evaluated dietary concentrations of DEET of 0 and 4000ppm (Goldenthal, 1995a (unpublished); cited in Schoenig & Osimitz, 2001). Two male and two female dogs were evaluated at each level. Depressed food consumption, decreased body weight, decreased defecation, thin and/or dehydrated appearance and emesis were observed in the 4000ppm treatment group. These results confirmed that the highest concentration of DEET in the diet that is palatable to dogs is 3000ppm (approximately 75mg/kg/day).
A NOAEL and LOAEL cannot be established for this study

iii) A two-week oral dosing study was carried out using gelatin capsules (Goldenthal 1994c (unpublished); cited by Schoenig & Osimitz, 2001). DEET was administered daily as a single bolus dose, at levels of 0, 62.5, 125, 250 and 500mg/kg/day. One male and one female dog were evaluated at each dose level. Food was allowed *ad libitum*. No treatment-related effects on body weight or food consumption were observed. At 250 and

500mg/kg/day emesis, ptyalism and nodding and twitching of the head and neck were observed occasionally. The male dog at 500mg/kg also exhibited ptosis, ataxia and convulsions. Clinical signs were observed shortly after dosing and the affected animals fully recovered shortly after their occurrence. No treatment-related clinical signs were observed at the two lower dose levels.

A NOAEL and LOAEL cannot be established due to insufficient subjects

iv) An eight-week oral dosing study was carried out using gelatin capsules (Goldenthal, 1995b (unpublished); cited by Schoenig & Osmitz, 2001). DEET was scheduled to be administered daily as a single bolus dose at levels of 0, 75, 125, 175 and 225mg/kg/day for a period of eight weeks. Two male and two female dogs were evaluated at each dose level. Food was allowed *ad libitum*. At dose levels of 125mg/kg/day or more, emesis, ptyalism, abnormal biting and scratching and abnormal head movements were observed in one or more animals in each group during the first five days of the study. Ataxia and ptosis also were observed in some dogs at 175 and 225mg/kg/day. In addition, convulsions were observed following the first dose in a female dog in the 225mg/kg/day dose group and following the third dose in a male dog in the 125mg/kg/day dose group. Clinical signs occurred shortly after dosing, after which recovery was observed. Because of the unexpected nature and severity of the clinical signs, this study was terminated after five days.

The NOAEL for toxicity in this study is 75mg/kg/day and the LOAEL is 125mg/kg/day.

v) An eight-week oral toxicity, dose-range-finding study was carried out in dogs. (Goldenthal, 1997 (unpublished); cited by Schoenig & Osimitz, 2001). Groups of Beagle dogs (2/sex/dose) received DEET in a gelatin capsule at dose levels of 0, 50, 100, 200 or 400mg/kg/day. However, due to previous findings that a single bolus dose is not a suitable method for administration, the dosing procedure was modified. Dogs were allowed access to food 1 hour prior to dosing to prevent dosing on an empty stomach. In addition, the daily dose was equally divided into one morning and one afternoon dose. No treatment-related clinical signs or effects on body weight, food consumption, haematology, clinical chemistry, organ weights, or gross and microscopic pathology were observed at dose levels of 50 and 100mg/kg/day. Clinical observations including abnormal head movements and ptyalism were observed at 400mg/kg/day. Ptyalism also was observed occasionally at 200mg/kg/day. These effects generally were observed within 1 hour of dosing and were considered to be related to the administration of DEET. Additional treatment-related effects observed at 400mg/kg/day, included decreased body weight gain in dogs of both sexes, decreased food consumption for females, and a slight decrease in cholesterol levels for males.

A NOAEL and LOAEL cannot be established for this study due to insufficient subjects

vi) A one-year repeated dose study was carried out (Schoenig et al., 1999). Groups of beagle dogs (4/sex/dose) received DEET in a gelatin capsule at dose levels of 0, 30, 100 or 400mg/kg/day for one year. The daily dose was divided equally into two doses administered in the morning and afternoon following a 1 hour period of food availability. Parameters evaluated were clinical signs, body weight and food consumption measurements, haematology, clinical chemistry, urinalysis, ophthalmology, organ weights

and gross and microscopic pathology. Treatment-related effects were observed only in the 400mg/kg/day group and included emesis, ptyalism, decreased body weights and decreased food consumption for both males and females. One male in the 400mg/kg/day group also exhibited occasional ataxia, tremors, abnormal head movements and convulsions. These clinical signs generally occurred shortly after dosing and were followed by complete recovery before the next dose. Other treatment-related effects observed in the 400mg/kg/day group included transient reduction in haemoglobin and haematocrit levels, increased alkaline phosphatase levels (males only), decreased cholesterol levels, and increased potassium levels (males only)

The NOAEL for short-term toxicity in dogs is 100mg/kg/day and the LOAEL is 400mg/kg/day.

3.4.2 Dermal Studies

<u>Rat</u>

i) A 90-day dermal toxicity study was carried out (Johnson, 1987c (unpublished) cited in Schoenig & Osimitz, 2001). Groups of Charles River CD® rats (15/sex/dose) received DEET (technical) at doses of 0, 100, 300 or 1000mg/kg/day. The 1000mg/kg/day dose level represented the maximum dose of DEET that could be applied dermally without significant runoff. Treatment related effects included dermal irritation, body weight depression in males at the 1000mg/kg/day dose level, and renal lesions that were observed in all males at all dose levels. Microscopically, these lesions were described as granular cast formation, multifocal inflammation, regenerative tubular epithelium and hyaline droplets. These lesions were accompanied by increased kidney weights and slightly increased urea nitrogen levels at 13 weeks at the 300 and 1000mg/kg/day dose levels. The dermal irritation was confirmed microscopically in the form of acanthosis and/or hyperkeratosis. Increased liver weights also were observed in male and female rats in the 1000mg/kg/day dose level. No treatment-related clinical signs or effects on food consumption, haematology, or opthalmology were observed in any of the treatment groups. As in the case in the 90-day oral toxicity study, the renal lesions observed in the male rats were attributed to α2u-globulin nephropathy.

A NOAEL or LOAEL cannot be established due to effects in all male rats

ii) A 90-day dermal toxicity study was carried out in castrated male rats (Goldenthal, 1989b (unpublished) cited by Schoenig & Osimitz, 2001). This study was carried out to investigate the cause of the increase in the incidence of hyaline droplets in the renal tubules of DEET-treated CD® male rats seen in the oral and dermal toxicity studies. Groups of castrated male CD® rats (15/group) received DEET by dermal application at dose levels of 0 or 1000mg/kg/day for 90 days. In addition, non-castrated CD® rats were administered DEET dermally at a dose level of 1000mg/kg/day. Microscopic examination of the kidney revealed renal lesions in both the castrated and non-castrated rats that were treated with DEET. The incidence and severity of the lesions were greater in the non-castrated group. No a2u-globulin was observed in the kidneys of the castrated control groups.

A NOAEL or LOAEL could not be established (single dose tested)

iii) Abnormal sperm morphology and altered sperm motility have been reported in a number of rodent studies, but the significance of these findings are questionable. Russian

investigators were first to report two to five-fold increases in abnormal sperm after exposure to DEET, but later studies have failed to confirm this finding. Sperm head abnormalities and altered motility were reported in rats after daily dermal exposure to doses of 100 and 1000 mg/kg for 1.5 and 6 months (Gleiberman *et al.*, 1976 (in Russian), reviewed by Robbins & Cherniack, 1986). Insufficient details were reported to evaluate the significance of these findings.

iv) A dermal study in rats reported an absence of morphological abnormalities (details not reported) following exposure to doses of 100, 300, or 1000 mg DEET/kg/day for nine weeks (Lebowitz *et al.*, 1983, cited by Robbins & Cherniack, 1986).

Pigs

A 90-day dermal toxicity study was carried out in micropigs® (Goldenthal, 1991 (unpublished) cited in Scoenig & Osimitz, 2001). This study was carried out to determine if a renal lesion related to an increase in hyaline droplet formation and the presence of α2u-globulin in the kidney tubules observed in male rats of previously conducted 90-day dermal and oral toxicity would occur in micropigs®.

DEET was dermally applied to two groups of micropigs® (4/sex/dose) at dose levels of 0 (water), 100, 300 or 1000mg/kg/day body weight for 13 weeks (1000mg/kg/day was a maximum dose which could be applied without significant runoff). The results indicated that DEET did not produce any mortality or changes in body weights, haematological and biochemical parameters, gross pathology, and organ weights. At the skin application site, gross pathology showed that the DEET-treated animals had an increase in the incidence of desquamation and/or dry skin. Histopathology showed an increase in the incidence of acanthosis and/or hyperkeratosis at the skin application sites.

Under the conditions of this study, DEET did not produce any renal lesions in micropigs®. It also did not cause any renal lesions in hamster that received DEET in dietary concentrations up to 15,000 ppm (940mg/kg/day). These findings indicate that the renal lesions produced by DEET were specific to male rats. This study met the data requirements for a non-rodent 90-day dermal toxicity study. There was insufficient information to set an NOAEL or LOAEL.

A NOAEL or LOAEL cannot be established for this study

Rabbit

i) Rabbits exposed dermally to a commercial insect repellent containing DEET (purity and dose n.p.) for 14 days had increased foldings and indentations on the skin surface, epidermal thinning, and cystic dilations of the dermis containing eosinophilic fluid (Wong & Yew, 1978, cited in Robbins & Cherniack, 1986).

A NOAEL or LOAEL cannot be established for this study

ii) A 90-day dermal study in rabbits was performed for the US EPA. Animals received a daily dose of 1.3g DEET/day. Increased kidney weights and marked pathological changes were reported, but the study was not used in the EPA Pesticide Registration Standard as it lacked growth curves, blood chemistries and individual animal data. (USEPA, 1980, cited in Robbins & Cherniack, 1986))

A NOAEL or LOAEL cannot be established for this study

3.4.3   Inhalational Studies

Rats

i) The U.S. Army conducted sub-chronic behavioural tests on rats exposed to aerosols of DEET (Sherman, 1979). Rats were exposed to 0, 250, 750 and 1500 mg/m3 DEET for six hours/day, five days/week, for 13 weeks. Tests included tactile sensitivity, passive and quick avoidance tests, endurance performance and balance beam performance. The only concentration low enough to be within the range of normal use (250mg/m^3) was not significantly different from the controls for most tests until the sixth week of exposure. (Sherman, 1979 (unpublished), cited by Robbins & Cherniack, 1986).
A NOAEL or LOAEL cannot be established for this study

ii) A small increase in abnormally shaped sperm was observed in rats after inhalational exposure to 1500 mg/m^3 for six hours/day, five days/week, for 13 weeks. The investigators noted their data were difficult to interpret due to the small size of the increases (Macko & Bergman, 1979 (unpublished), reviewed by Robbins & Cherniack, 1986).
A NOAEL or LOAEL cannot be established for this study

iii) Rats were exposed to an aerosol mist of DEET for two one-hour periods daily for 30 days. Tracheitis and pulmonary oedema were observed, but the high rate of murine pneumonia in both controls and exposed animals confounded the results. (Christensen *et al*., 1969, cited by Robbins & Cherniack, 1986)
A NOAEL or LOAEL cannot be established for this study

iv) A slight epistaxis was seen in rats during daily exposure to a saturated vapour of DEET (1 ml of 85-95% DEET/14,000 1 in 40 hours), exposed for 8 hours/day, five days/week for 7 weeks. No other effects were noted (Ambrose *et al*., 1959, cited by Robbins & Cherniack, 1986).
A NOAEL or LOAEL cannot be established for this study

Dogs

 In 13-week inhalation studies, periodic vomiting was observed in dogs exposed to 750-1500 mg/m^3 and an exudate from the nose and eyes was observed in rats exposed to 1500 mg/m^3 (Macko & Bergman, 1979 (unpublished), reviewed by Robbins & Cherniack, 1986).
A NOAEL or LOAEL cannot be established for this study

3.4.4   Summary of Short-Term and Sub-Acute Studies

In male rats, kidney lesions including hyaline droplet formation and granular cast accumulation in the renal tubules, chronic inflammation and tubular epithelial regeneration were observed following sub-chronic oral and dermal dosing. This renal pathology was shown to be associated with an accumulation of α2u-globulin in renal tubules that occurs uniquely in rats. Since human do not produce α2u-globulin, this syndrome of kidney lesions is not considered in the setting of no observed adverse effect levels (NOAELs) in the toxicity studies.

Kidney weights were also variably affected in other species (e.g. mice, rabbit). This was generally associated with possible kidney pathology at high doses. This could be due to a non-adverse, adaptive response and was therefore not considered in the setting of NOAELs. A summary of short term and sub-acute studies is presented below.

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Concentration	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Rat						
CD®, age n.p.	15 F/dose 15 M/dose	DEET, 98.3%	**Oral:** 100, 500, 1000, 2000mg/kg (0.52, 2.60, 5.20, 10.40nmol/kg)	Daily doses for 90 days, 90-day observational period.	Increase in incidence of death at highest dose. Increase in incidence of renal lesions in all treated males. **NOAEL not established**	USEPA (1998)
CD®, age n.p. Fischer NBR	10 M/species	DEET, 98.3%	**Oral:** 400mg/kg	Daily doses for 90 days, 90-day observational period	CD® rats formed renal lesions and hyaline droplets Fischer rats renal lesions, no hyaline droplets No renal effects seen **Non-guideline**	USEPA (1998)
Castrated CD® rats, age n.p.	15 M/group	DEET, conc. n.p.	**Dermal:** 1000mg/kg	Daily doses for 90 days,	Castration did not protect against hyaline droplet formation or other kidney lesions **NOAEL not established**	USEPA (1998)
Strain and age n.p.	10 M, 10 F.	DEET, conc. n.p.	**Inhalation:** 250, 750, and 1500 mg/m^3 (1.31, 3.92, and 7.841 mmol/m^3; 31.9, 95.9, and191.7 ppm)	Exposed 6 hours/day, 5 days/week, for 13 weeks, 13-week observation period	Considerable increase in weight and size was observed; no significant signs of toxicity were seen. **NOAEL not established**	Sherman (1979)
Hamster						
Strain and age n.p.	15/sex/dose	DEET, conc. n.p.	**Oral:** 1000, 5000, 10000 or 15000ppm (61, 305, 624, 940mg/kg)	Daily doses for 90 days, 90-day observational period	Decrease in food consumption and body weight at 5000ppm (305mg/kg). Also increases in testicular lesions. **NOAEL = 61mg/kg**	USEPA (1998)
Mice						
CR CD-1	15/sex/dose	DEET, conc. n.p.	**Oral:** 300, 1000, 3000, 6000, 10000mg/kg	Daily doses for 13 weeks, 13-week observational period	Decrease in food intake and body weights at 3000mg/kg. Increase in liver weights at 300mg/kg **NOAEL not established**	USEPA (1998)
Rabbit						
New Zealand white, age n.p.	6 M/group	DEET, 95%	Oral: 132, 264, and 528 mg/kg (0.690, 1.38, and 2.76 mmol/kg)	15-d exposure and observation period	Rabbits in the high-dose group showed decreased body weight and increased kidney weight. Serum calcium levels decreased while cholesterol and triglyceride levels increased. No other signs or symptoms were observed in any group. **NOAEL not established**	Haight et al. (1979) cited by Robbins & Cherniack (1986)
Albino, species and age n.p.	Number and sex n.p.	DEET in a commercial repellent, conc. n.p.	Dermal: dose n.p.	Applied to ears for 14 d, observation period n.p.	Increased foldings and indentations on skin surface, thinning of the epidermis, and cystic dilations (containing eosinophilic fluid) of the dermis were observed. **NOAEL not established**	Wong and Yew (1978)

Table 3.11: Summary of Short-Term and Sub-Acute Toxicity Studies
Abbreviations: conc. = concentration; d = day(s); F = female; h = hour(s); M = male; mo = month(s); n.p. = not provided; s.c. = subcutaneous injection; wk= week(s).

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Concentration	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Dog						
Dogs, beagle, age n.p.	4 M and 4 F/group		**Oral**: 30, 100, or 400 mg/kg (0.16, 0.523, or 2.09 mmol/kg)	Daily in capsules for 1gelatin yr; 1-yr observation period	Vomiting, reduced haemoglobin and haematocrit levels, increased alkaline phosphatase (M only); decreased cholesterol, increased potassium in the high dose group. One M dog in high dose group exhibited ataxia, tremors, and/or convulsions on several occasions. **NOAEL = 100mg/kg/day**	Schoenig *et al.* (1999)
Dogs, beagle, age n.p.	2/sex/dose	DEET conc. n.p.	**Oral**:0, 300, 1000, 3000, 6000ppm (8.4, 28.6, 93.3, 19.5mg/kg/day)	Daily in the diet for 8 weeks	Food rejection at highest dose. No toxicity seen at dietary concentration s of 3000ppm or less **NOAEL not established**	USEPA (1998)
Dogs, beagle, age n.p.	2/sex/dose	DEET conc. n.p.	**Oral**: 0, 50, 100, 200, 400mg/kg	Daily from gelatin capsules for 8 weeks	Decrease in testis/epididymis weight in 400mg/kg/day males as well as decrease in cholesterol. Decrease in weight gain in 400mg/kg/day male and female **NOAEL not established**	USEPA (1998)
Pig						
Micropigs®, age n.p.	4/sex/dose	DEET, conc. n.p.	**Dermal**: 100, 300, 1000mg/kg	Daily doses for 13 weeks.	No renal lesions seen. **NOAEL not established**	USEPA (1998)

Table 3.10 (cont.): Summary of Short-Term and Sub-Acute Toxicity Studies
Abbreviations: conc. = concentration; d = day(s); F = female; h = hour(s); M = male; mo = month(s); n.p. = not provided; s.c. = subcutaneous injection; wk= week(s).

3.5   Genotoxicity

3.5.1   Mutagenicity Tests

3.5.1.1   Ames Assay

i) DEET was tested over a concentration of 28 to 8333μg/plate with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 (San & Schadly, 1989 (unpublished) cited by Schoenig & Osimitz, 2001). Each strain was tested in the presence and absence of metabolic activation by a rat liver S9 system induced with Aroclor 1254. The results indicated that DEET was not mutagenic in this test system.

ii) DEET did not induce reverse mutations in five strains of *Salmonella typhimurium* with or without metabolic activation, or induce gene conversions in *Saccharomyces cerevisiae* D4 without activation. In a survey of mutagenic potential of pesticides using the bacterial plate assay method, 50% DEET gave a negative result (Robbins and Cherniack, 1986).

iii) DEET (10-3333μg/plate; 0.052-17.42μmol/plate) was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA97 or TA98 with or without induced rat or hamster liver S9, when tested in a pre-incubation assay (Zeiger *et al*. (1992).

3.5.1.2   Chromosomal Aberration Assay

A chromosomal Aberration Assay was conducted in Chinese hamster ovary cells (Putman and Morris, 1989 (unpublished) cited in Schoenig & Osimitz, 2001). The assay was performed in the presence and absence of metabolic activation by a rat liver S9 system induced with Aroclor 1254. In the absence of metabolic activation, the concentrations of DEET evaluated were 0.063, 0.125, 0.2, 0.5 and 1µl/ml while, with activation, the concentrations of DEET evaluated were 0.032, 0.063, 0.125, 0.25 and 0.5µl. No increase in chromosomal aberrations was observed with or without metabolic activation.

3.5.1.3   Unscheduled DNA Synthesis (UDS) Assay

An unscheduled DNA synthesis assay was conducted using primary rat hepatocytes (Curren, 1989 (unpublished) cited in Schoenig & Osmitz, 2001). DEET was tested at concentrations of 0.003, 0.01, 0.03, 0.1 and 0.2µl/ml in an initial assay. In a confirmatory assay, DEET concentrations of 0.03, .1, 0.2 and 0.3µl/ml were evaluated. No increase in DNA synthesis was observed in either assay.

3.5.2   Other Studies

Rat

There is one report of a study to investigate the urinary excretion of the oxidised base 8-hydroxy-2-deoxyguanine, used as a biomarker for DNA oxidative damage, following dermal application of DEET in 70% ethanol (Abu-Qare & Abou-Donia, 2000). A single dose of 400mg/kg was applied directly to shaved skin on the back of individual rats. Ethanol (70%) was used as control. The effect of DEET alone and DEET plus permethrin (1.3mg/kg) were investigated.

Time course studies were carried out using groups of 5 rats and urine samples were collected at 2, 4, 8, 16, 24, 48 and 72 hours following application. Increasing levels of 8-hydroxy-2-deoxyguanosine were measured in the urine at up to 24hours post dose, both with DEET and ethanol, but the values were somewhat higher (about 2 fold) with the DEET compared to the ethanol control. Permethrin on its own gave similar levels to those of the control. The values reported for 24-hour elimination of 8-hydroxyguaninosine were 1701±321ng in the animals given DEET compared to 1009±341 min those given ethanol.

The authors concluded that the results indicated that dermal administration of DEET could generate free radical species and hence cause oxidative damage to DNA in rats. However, the increase was not large compared to the control substance and the significance of these data are unclear. No conclusions from this study can be drawn from this study regarding the mutagenicity of DEET.

Mouse

A dominant lethal study was performed in mice using single doses of corn oil (5 mg/kg, control), triethylenemelamine (TEM, 10 mg/kg, positive control) and DEET (600 mg/kg). No dominant lethal effects were observed, but a slight statistically significant reduction in the number of implants was noted in pregnant females and was attributed to aspermia or reduced sperm motility (USEPA, 1980).

Lower Eukaryotic Systems

Exposure of *Tradescantia paludosa* pollen mother cells in early prophase I to 1-3 sprays of the commercial insect repellent OFF!®, did not induce micronuclei in later stages of pollen maturation (early tetrad stage) (Ma *et al*., 1984)

3.5.3 Summary of Genotoxicity Studies

Genotoxicity studies are summarised in Table 3.11 below. DEET was not mutagenic *in-vitro* in *Salmonella typhimurium* (in presence/absence of exogenous metabolic activation), did not induce clastogenicity in CHO cells *in-vitro*, and did not induce Unscheduled DNA Synthesis in rat hepatocytes *in-vitro*. These data were provided for the EPA review.

DEET was cited to give negative results in regulatory studies submitted to the EPA to investigate clastogenicity in CHO cells, and UDS in hepatocytes. No conclusions could be drawn from the oxidative damage mentioned.

DEET did not appear to have any structural alerts for mutagenicity and it is negative in the Salmonella assay. As DEET also shows no evidence of carcinogenicity in adequate animal studies, it is unlikely to have significant mutagenic potential.

Although there is the possibility of high human exposure to DEET, the 3 *in vitro* tests are sufficient in number and quality to adequately assess DEET's mutagenic potential. In addition as there is adequate negative carcinogenicity data in rats and mice, it is unlikely that an additional *in-vivo* study is required. In conclusion, DEET does not appear to be mutagenic.

3.6 Oral Long-Term Toxicity and Carcinogenicity

<u>Rat</u>

A combined two-year chronic toxicity and carcinogenicity study was carried out (Schoenig *et al.* 1999). Groups of Charles River CD® rats (60/sex/dose) received DEET (98.3% purity) in the diet at dose levels of 0, 10, 30 or 100mg/kg/day for males and 30, 100 or 400mg/kg/day for females. Two control groups were run concurrently. The animals were treated for two years. Experimental evaluations included survival, clinical observations for toxicity, body weight, food consumption, haematology, organ weights, and gross and microscopic pathology. Ophthalmology, clinical chemistry and urinalysis were also included. Histopathology was conducted in a full set of tissues in the control and high-dose groups. Lungs, livers, kidneys and gross lesions of rats in the low- and mid-dose groups were also examined microscopically.

No treatment-related clinical signs of toxicity were observed, there was no effect on survival, nor was there any treatment-related effects in the parameters of ophthalmology (conducted pre-test and at study termination), haematology, urinalysis, organ weights, or gross and microscopic pathology.

In the 400mg/kg/day female rats, there were progressive and statistically significant decreases in body weights, a decrease in food consumption, and a statistically significant increase (25-50%) in cholesterol levels at various intervals. No compound-related increases in non-neoplastic or neoplastic lesions were seen that were attributable to the test material. No toxicity was seen in any dose groups of male rats.

Based on the results of this study, the NOAEL for the chronic toxicity of DEET in females is 100mg/kg/day and the LOAEL is 400mg/kg/day (based on decreased body weights and food consumption and increased cholesterol levels in female rats). The NOAEL for the chronic toxicity of DEET in males is 100mg/kg/day (i.e. the highest dose tested or HDT).
The NOAEL for this study is 100mg/kg/day and the LOAEL 400mg/kg/day

<u>Mouse</u>

i) A 78-week carcinogenicity study was carried out (Schoenig et al., 1999). Groups of Charles River CD® -1 mice (60/sex/dose) received DEET at dietary concentrations of 0, 250, 500 or 1000 mg/kg/day for 78 weeks. Experimental evaluations included survival, clinical observations for toxicity, body weight, food consumption, haematology, organ weights, and gross and microscopic pathology. Histopathology was conducted in a full set of tissues in the control and high-dose groups. Lungs, livers, kidneys and gross lesions of mice in the low- and mid-dose groups were also examined microscopically.

No treatment-related clinical signs of toxicity were observed. There was no effect on survival and no treatment-related effects on haematology or gross pathology were seen. A statistically significant decrease in mean body weight, body weight gains, and food consumption for both male and female mice was seen in the 1000mg/kg/day group. An increase in liver weights noted in male and female mice at 500 and 1000mg/kg/day, were considered to be adaptive in nature, since no corroborative microscopic findings were observed. The NOAEL for systemic toxicity was established at 500mg/kg/day and a LOAEL at 1000mg/kg/day. No evidence of carcinogenicity was found.
The NOAEL for this study is 500mg/kg/day and the LOAEL 1000mg/kg/day (based on systemic toxicity).

ii) A tumour induction study was performed in mice (50 animals per group) using cutaneous doses of 1-20 mg DEET in acetone, twice a week for life (40-120 weeks in mice). No statistical differences in tumour incidence between the treated animals and untreated controls were reported. DMBA was used as a positive control. The EPA reviewed this study and judged to be inadequate for various reasons including the lack of clinical data reported. (Stanbaek 1977, cited by Robbins & Cherniack, 1986)
No NOAEL or LOAEL was established due to insufficient information

<u>Rabbits</u>

A tumour induction study was performed in rabbits (five animals per group) using cutaneous doses of 1-20 mg DEET in acetone, twice a week for life (20-80 weeks). No statistical difference in tumour incidence between treated animals and untreated controls was reported. DMBA was used as a positive control. The EPA reviewed this study and judged to be inadequate for various reasons including - no clinical data reported, no pathology of the rabbit tumours and early and abrupt deaths of rabbits. (Stanbaek, 1977, cited by Robbins & Cherniack, 1986).
No NOAEL or LOAEL was established due to insufficient information

3.6.1 Summary of Long-Term Toxicity/Carcinogenicity Studies

The available information is summarised in Table 3.12 below. In long term, repeat-dosing toxicity studies, as indeed the short-term studies, the most common treatment-related effects were reduced body-weight gain and food consumption. Long term exposure did not seem to significantly increase toxicity, and also there is no evidence of treatment related induction of tumours in animals.

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Concentration	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Rat						
Rats, CD, age n.p.	60 M and 60 F/group	DEET, 98%	Oral: 10, 30 or100 mg/kg (M) (0.052, 0.16, or 0.523 mmol/kg); 30, mg/kg (F) (0.16, 0.523, or2.09 mmol/kg)	Daily in diet for 2 yr; 2-yr observation period	Decreased body weight and food consumption, and increased serum cholesterol (F only) in high the dose group. **NOAEL = 100mg/kg/day**	Schoenig et al. (1999)
Mice						
Mice, CD-1, age n.p.	60 M and 60 F/group	DEET, 98%	Oral: 250, 500, or 1000 mg/kg (1.31, 2.61, or 5.23 mmol/kg)	Daily in diet for 18 mo; 18-mo observation period	Decreased body weight and food consumption in the high dose group. **NOAEL = 500mg/kg/day**	Schoenig et al. (1999)
Mice, Swiss, 7 wk old	50 F/group	DEET, 10, 50, and 100%	Dermal: 0.02 ml (0.02 g; 0.1 mmol)	Twice weekly on the back, up to160-wk observation period	Tumours of various organs, including skin, ovary, and mammary gland were observed;, Incidence was not increased compared to controls. **No NOAEL established**	Stenbäck (1977) cited by Robbins & Cherniack (1986)
Rabbits						
Rabbits, New Zealand, 8 wk old	5 M or F/group	DEET, 10, 50, and 100%	Dermal: 0.02 ml (0.02 g; 0.1 mmol)	Twice weekly on the left ear, up to160-wk observation period	No tumours or mortality were observed in either treated animals or controls. **No NOAEL established**	Stenbäck (1977) cited by Robbins & Cherniack (1986)

Table 3.12: Summary of Long-Term Toxicity/Carcinogenicity Studies
Abbreviations: conc. = concentration; n.p. = not provided; M = male; F = female; yr = year(s); mo = month(s); wk = week(s).

3.7 Reproductive Toxicity

Rat

i) A two-generation reproduction study was carried out (Schardein, 1989 (unpublished) cited in Schoenig & Osimitz, 2001). Groups of Sprague-Dawley rats (28/sex/dose) received DEET at dietary concentrations of 0, 500, 2000 or 5000ppm for two consecutive generations. The F0 parental generation were administered treated or control diet for at least 80 days prior to mating. Twenty-eight male and 28 female offspring per group from the F1 generation were selected randomly to become the parents of the F2 generation. These animals were treated for at least 93 days prior to mating. For both parental groups, treatment was continued through gestation and lactation. Parental toxicity in the form of decreased body weight and food consumption was noted for males and females in the F0 and F1 generations at 5000ppm, and decresed body weight was noted for males in the F0 generation at 2000ppm. A slight increase in hair loss was observed in the F0 and F1 females at 5000ppm. In the F1 adult males kidney effects were seen, including mottling, chronic inflammation, the presence of hyaline droplets, granular cast formation, and tubular regeneration. These kidney effects occurred in dose-related manner in all treatment groups and were characteristic of α2u-globulin nephrotoxicity. No other treatment related effects were seen in the parental generations at 500ppm.
Neonatal toxicity as evidenced by a significant reduction in the body weights of pups in both generations was noted for males and females in the 5000ppm group. No treatment

related effects were observed in pups at 2000ppm or less. No treatment related effects on reproduction or fertility were observed at any of the dose levels evaluated in this study. The reproductive toxicity NOAEL was 5000ppm (or 500mg/kg/day based on the conversion, 10ppm = 1mg/kg/day), which is the Highest Dose Tested (HDT).
The NOAEL for reproductive toxicity for this study was 500mg/kg/day

ii) A developmental toxicity study was carried out (Schoenig *et al*., 1994). Groups of 25 mated female CD® rats received DEET by gavage, at doses of 0, 125, 250, or 750mg/kg/day from days six to fifteen of gestation. In the high-dose dams (750mg/kg/day), clinical signs such as hypoactivity, ataxia, decreased muscle tone, foot splay, perinasal encrustation, and perioral wetness were observed. Some of these signs were suggestive of neurotoxicity in this dose group because none of these signs were seen in controls. Some of these clinical signs were seen only sporadically in the other treated groups. In the high-dose dams, there was an increase in mortality rate, a reduction in body weight gain and food consumption and an increase in mean liver weights.

A slight increase in percent post-implantation loss and a statistically significant decrease in mean foetal body weight/litter was seen in the high dose group. No additional compound-related effects were found.

Based on the increase in the clinical signs, reduced body weights and food consumption, increased mortality rate, and an increase in mean liver weight, the NOAEL for maternal toxicity was 250mg/kg/day and the LOAEL was 750mg/kg/day. The NOAEL for developmental toxicity was 250mg/kg (based in a statistically significant decrease in mean foetal body weight/litter at 750mg/kg/day).
The NOAEL for maternal toxicity for this study was 250mg/kg/day and the LOAEL 750mg/kg/day. The NOAEL for developmental toxicity was 250mg/kg/day.

iii) The potential of DEET to cause reproductive and developmental effects has been investigated in Sprague-Dawley rats exposed to daily subcutaneous (sc) injections of undiluted DEET (Wright *et al*., 1992). Pregnant females were dosed from day 6-15 of gestation, in a dose finding study, doses of 0.5-1.2 ml DEET/day were used. All animals at the highest dose died and deaths occurred in all other groups except 0.5 ml/day. In the main study animals received 0 or 0.3 ml/day, half were killed on day 20 and the rest were allowed to litter, offspring were observed for 14 days. No significant effects on gestational or developmental parameters or foetal toxicity were observed. Neurotoxicity was seen in the treated females in the form of abnormal gait.

As part of the same study, effects of DEET on male reproduction were examined in a fertility and dominant lethal study. Male rats were dosed sc daily for 5 days/week with doses of 0.3-1.8 ml DEET, for 9 weeks. All animals in the 1.8ml group died and deaths occurred in all other groups except 0.3 ml, gait abnormalities were also seen in adult males. Males from the 0.3 and 0.73 ml DEET groups were mated with untreated females, half of these were killed at 12-14 days gestation for dominant lethal effects and the rest allowed to litter. No evidence of adverse effects was seen in the foetuses or offspring of DEET-exposed males. Histopathology of the testes revealed no adverse effects.
No NOAEL for developmental toxicity was established for this study

Rabbit

A developmental toxicity study was carried out in rabbits (Schoenig *et al.* 1994). Groups of presumed pregnant female New Zealand white rabbits (16/group) received DEET by

gavage, at doses of 0, 30, 100, 325mg/kg/day body weight from gestation day six through day eighteen. Under the conditions of this study, DEET did not produce any compound-related maternal toxicity or developmental toxicity. The NOAEL for maternal and developmental toxicity was 325mg/kg/day (i.e. the HDT). The results indicate that the test animals could have tolerated higher dose levels. The report failed to present any explanation for dose selection.

Supplemental data was added to this study as requested by the EPA. This consisted of a dose-range finding developmental toxicity study in rabbits. In this supplemental data, groups of timed-pregnant NZ white rabbits (5/dose group) received DEET (98.7%) by gavage at dose levels of 0 (corn oil), 62.5, 125, 250, 500 or 1000mg/kg/day from gestation days six through eighteen. The results indicated that at doses of 250mg/kg/day or above there was a non-dose-related increase in the incidence of rapid respiration in the maternal animals. The incidence increases seen at 250 and 1000mg/kg/day were statistically significant. At 1000mg/kg, clinical signs of hypoactivity, ataxia, and prostration were also seen.

Deaths were found in 500 and 1000mg/kg/day groups. The individual necropsy data showed that the animals that died at 500 and 1000mg/kg/day groups all had sloughing and/or ulceration of the stomach lining. In contrast, the survivors did not show any gross pathology of the stomach. At doses of 500mg/kg and above, the corrosive effect of DEET to the gastric lining appeared to be linked to the death of these animals. No evidence of treatment-related developmental toxicity was reported in any of the treatment groups. In the surviving litters, there was no evidence of pre- or post- implantation loss in treated groups as compared to the controls. Based on these results, the investigator of the study recommended 0, 30, 100 and 325mg/kg/day to be employed for the definitive developmental toxicity study in rabbits.

The results of the definitive study, in which no maternal or developmental toxicity was observed at doses of 325mg/kg/day were consistent with the results of the dose range-finding study, in which toxic effect was not seen in animals which were treated at doses below 500mg/kg/day. In reviewing the definitive and the dose range-finding studies together, the results indicate that the highest possible dose, which would not result in stomach ulceration and death for a gavage developmental toxicity study in rabbits, would probably be approximately 400mg/kg/day. The difference between 400mg/kg/day and 325mg/kg/day in a toxicological study is not marked. The highest dose tested in the definitive rabbit developmental toxicity study (325mg/kg/day) has been adequately high to assess the maternal and the developmental toxicity of DEET. Little would be gained by conducting an additional study to establish a more precise NOAEL and LOAEL, especially in light of the severe maternal toxicity noted at 500mg/kg/day and above in the dose range-finding study.

A NOAEL of 325mg/kg/day for developmental toxicity can be established (i.e. the HDT).

3.7.1 Summary of Developmental Toxicity Studies

No adverse reproductive toxicity was observed in rats over 2 generations, however parental toxicity, such as reduced body-weight gain and food consumption, was observed in the high dose males and females. Effects in young rats were restricted to reductions in body-weight gain occurring in the high dose group only during the lactation period. No teratogenicity or age-related sensitivity was observed in either rat or rabbit developmental

studies. Minor developmental effects were only seen at maternally toxic dose levels. Studies are summarised below

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Concentration	Route/Dose	Exposure/ Observation Period	Results/Comments	Reference
Rats						
Charles River CD, age n.p.	25 F/dose	DEET, ≥98.3%	Gavage: 125, 250, and 750 mg/kg (0.653, 1.31, and 3.92 mmol/kg)	Exposure on gd 6-15, sacrificed on gd21	Reduced fettle weight observed at 750 mg/kg. No treatment-related effects, were observed. **NOAEL = 250mg/kg/day**	Schoenig *et al.* (1994)
Sprague-Dawley, age n.p.	35-37 F	DEET, 97-98%	s.c.: 0.30 ml/kg(1.6 mmol/kg)	Exposure on gd 6-15, half of the animals sacrificed on gd 20 and the remaining half observed on pd 3, 9, and 14	No evidence of reproductive/ developmental toxicity reported. Sporadic malformations and variations observed, but not significantly different from controls. No signs of adverse effects on reproductive success in rats exposed *in utero* . **NOAEL not established**	Wright et al. (1992)
	20 M/dose		s.c.: 0.30 and 0.73 ml/kg (1.6 and 3.8 mmol/kg)	Exposure 5 d/wk for9 wk, observation period n.p.	No effects on sperm or evidence of induced dominant lethal mutations. No effect on survival of foetuses sired from male treated rats or on neonatal growth/development. **NOAEL not established**	
Rabbits						
New Zealand white, age n.p.	16 F/dose	DEET, ≥98.3%	Gavage: 30, 100 and 325 mg/kg(0.16, 0.523, and1.70 mmol/kg)	Exposure on gd 6-18, sacrificed on gd29	No treatment-related effects, observed. **NOAEL=325mg/kg/day**	Schoenig et al. (1994)
New Zealand white, age n.p.	20 F/dose	DEET, 95%	Dermal: 50, 100, 500 and 1000 mg/kg (0.26, 0.523, 2.61, 5.227 mmol/kg)	Daily exposure on gd 1-29, sacrificed on gd 30	No reproductive or teratological effects observed	Angerhofer and Weeks (1981) cited by Robbins & Cherniack, 1986)

Table 3.13: Summary of Development Toxicity Studies
Abbreviations: conc. = concentration; CNS = central nervous system; d = day(s); F = female; gd = gestational day(s); M = male; n.p. = not provided; pd =postnatal day(s); s.c. = subcutaneous injection; wk = week(s); yr = year(s).

3.8   Neurotoxicity Studies

There have been a few studies that have investigated the neurotoxic effects of DEET, sometimes in relation to other neurotoxic agents such as permethrin. The majority of these studies have been carried out in rats, however some have been carried out in the hen. Summaries of these papers are given below.

3.8.1   Acute: Oral

Rat

i) Verschoyle *et al*. (1992) carried out an acute oral toxicity study in rats. In general, female Lac:P Wistar derived rats 160-190g, 7-9 weeks of age were used. Dosing was by oesophageal intubation after overnight starvation except for pipernyl butoxide was given by intraperitoneal injection. All rats were fed immediately after dosing. DEET was given as a mixture with arachis oil at doses of 0.4 to 5g/kg and at concentrations of 200 and 500mg/ml. Control animals were given an equivalent volume of appropriate solvent. In some cases, piperonyl butoxide was given as a 500mg/kg i.p. dose in arachis oil 30

minutes prior to challenge. LD$_{50}$'s were calculated by using four rats for each dosage group.

The oral toxicity of DEET and the effect of sex and age are shown in the table below. DEET showed decreasing toxicity with increasing age and was more toxic to females than males. The authors suggest that this in relation to the findings of clinical poisonings in young girls (see section 3.10.2) may be due to an inherent age- and sex- related toxicity in addition to more rapid skin absorption or increased surface area to body weight ratio in the young. Toxicity was greatly increased in the presence of pipernyl butoxide (a p450 inhibitor) suggesting a metabolic factor in the age- and sex differential since DEET is detoxified by the P450 linked microsomal system.

Age of rat in days	Sex	LD$_{50}$ mg/k g	95% Confidence limits
11	Female	667	(559-796)
25	Female	1259	(950-1668)
31-35	Female	1414	(937-2134)
47-56	Female	3429	(2613-4500)
11	Male	891	(794-1000)
25	Male	2000	(1312-2327)
31-35	Male	1782	(1365-2327)
47-56	Male	3564	(2951-4305)

Table 3.14: Summary of Oral Toxicity
Reported by Verschoyle *et al.* 1992

Motor and EEG changes

Electroencephalogram (EEG) and auditory evoked potential recordings were also made (electrodes were implanted 7 to 10 days prior to dosing and locations were confirmed histologically). Auditory stimuli were 1 msec tone bursts of 40 kHz delivered at 0.8 Hz. Rats were freely moving and EEG/auditory evoked responses to 113-dB stimuli were collected continuously during daily 2-hr recording sessions. One complete intensity curve over the range 47 to 119 dB was also recorded during each session.

At doses of 2 to 3g/kg (n = 64) but not below 1.5g/kg (n = 26), DEET produced a severe rapid decrease in reactivity and muscle tone (as assessed by palpitation). Time to onset varied with dose between 7 and 60 minutes and by 25 to 65 minutes the flexor withdrawal reflex was lost. Rats given 2.5 to 4g/kg died between 50 minutes and 24 hours with progressive respiratory depression.

Although numbers of rats were not given, the authors reported that the general picture of CNS depression was interrupted initially by occasional head shakes and by spontaneous myoclonic twitching (triggered by auditory or tactile stimuli), similar to partially controlled seizures, starting between 27 and 302 minutes depending on dose level. Of 22 rats surviving doses of 2 or 2.5g/kg, 3 failed to recover muscle tone or righting reflex over 24 hours, and 3, despite recovering brisk reflexes and being able to right themselves, remained clearly ataxic, while 16 recovered. Three rats with implanted electrodes were given DEET, two at 2.5g/kg and one at 3g/kg. Marked slowing of the EEG and development of regular 2 to 5Hz activity accompanied the loss of muscle tone. In the rat administered 3g/kg, activity further declined to an isoelectric baseline shortly before death at 100 minutes. A prominent feature in all three rats was the development of spike discharges, which were frequently accompanied by the head and body twitches already described and persisted for 5 hours in one rat at 2.5g/kg DEET and 24 hours in the other.

Systemic changes were unremarkable after DEET. Two rats dying 70 and 82 minutes after 3g/kg and two rats surviving 2g/kg showed an initial fall in blood pressure from 106 $\pm$ 2mm Hg to 86 $\pm$ 4mm Hg at 40 minutes which paralleled the CNS depression. This fall continued and was either unchanged at 24 hours or declined steadily until death. Respiration was well sustained right until death with an arterial pO2 of 125 $\pm$ 11mmHg at 60 minutes. Rectal temperature showed only a small fall just prior to death.

Auditory evoked responses were monitored in both the auditory cortex and cerebellum in the three DEET-dosed rats used for EEG recording. In each case the spontaneous EEG spike discharges, which developed on giving DEET, were preceded and accompanied by the development of a large abnormal biphasic component at 24/32msec. The cerebellar records all remained unchanged

Neuropathology

Following delayed removal (2 hr at 4°C), the brain was sliced coronally into blocks 3-4 mm thick for step-serial sectioning (1 in 20) and 6-µm sections were stained with hematoxylin and eosin. In three animals given DEET and killed at either 24 or 48 hr the spinal column was decalcified, several transverse cervical and lumbar blocks were embedded, and 10µm sections were stained with hematoxylin and eosin. For combined light and electron microscopy the fixative employed was 6.5% glutaraldehyde in 0.14 M cacodylate buffer (pH 7.2). Coronal brain slices, 1-2 mm thick, were cut to include the cerebellar cortex and roof nuclei, vestibular nuclei, reticular formation, caudate nucleus and putamen, and frontal and parietal cortices. Slices were post-fixed in 1 % OsO_4 and after dehydration embedded in Epon-Araldite. Semithin sections (1.0 µm) were stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate.

The quality of perfusion fixation was judged as good in all animals because of the uniform colour and form consistency of the brain and the absence of blood in vessels. In control animals the staining of all cellular elements was uniform with no evidence of histological artefacts indicating that the delayed removal had no effect on brain histology. Light microscopy showed no histological changes in 8 rats given from 1 to 3g/kg DEET, 6 of which had shown no abnormal behaviour. In the remaining 23 animals two main types of changes were seen. The first consisted of vacuolation of myelin sheaths of fibres largely restricted to the cerebellar roof nuclei where it was bilaterally symmetrical. However, in two rats a few vacuolated fibres were also seen in the vestibular nuclei of one and the reticular formation of the other. Within the damaged fibres the axons appeared normal apart for very occasional focal vacuoles. These spongy lesions, first seen 2 hours when they were of slight to moderate severity, had become severe in 6 animals by 24 hours. The second type of change appeared as single or multiple, clear, cytoplasmic clefts on neurones diffusely distributed throughout the brain, but were most frequent in the vestibular, cerebellar roof, and cochlear nuclei, the reticular formation, superior olives, red nuclei, and frontal and parietal cortices. Roof nucleus lesions and clefts were classified at grades of severity ranging from + to +++ over the dose range of 1 to 3g/kg at 2 to 48 hours. There was no obvious dose response over this range: 2/2 rats at 1g/kg, 11/16 rats at 2g/kg and 7/10 rats at 3g/kg showing lesions. All rats with lesions were either severely prostrate or ataxic, and all 18 rats with neuronal clefts showed semi-prostration (3 animals) or prostration. Although 3 of 6 rats developing ataxia alone failed to show any neurological changes, all animals prostrate at the time of killing had developed clefts. One animal that showed semi-prostration in the early stages of intoxication, but appeared

normal when killed at 24 hr, had no evidence of clefts or any other histological abnormalities. The appearances of the clefts remained unaltered at all survival times examined and with the three fixatives and two embedding media employed.

In addition to the above, typical ischaemic cell change characterised by pyknosis of nucleus and cytoplasm was present bilaterally in a few neurones of the caudate nucleus and putamen of nine animals and several microvacuolated neurones were present particularly in layer V of the frontal and parietal cortex of two of these. Scattered hyperchromatic and fragmented oligodendrocytes were occasionally seen in the corpus callosum, centrum semiovale, and the caudate nucleus and putamen of six rats. In semithin plastic sections of seven animals, several neurones in the vestibular and cerebellar roof nuclei were surrounded by well-stained axon terminals made conspicuous by pale staining of the juxtaposed neuroglial investment.

Electron microscope examination, confined to the roof nuclei of animals killed at 24 hours, showed extensive oedematous swelling of the inner loop of the myelin and splitting of the innermost myelin lamellae occurring at the intraperiod line. In the axon cylinders the only changes were occasional compression by the swollen inner loops, margination of mitochondria and, very rarely, large focal vacuoles. Moderate swelling of dendritic processes frequently occurred. No changes were observed in the soma of oligodendrocytes and astrocytes and blood vessels appeared normal. There was however a withdrawal of ensheathing astrocytic feet from around a few neurones with consequent enlargement of the extracellular space bringing adjacent synaptic boutons into prominence.

While some of the neurones exhibiting cytoplasmic clefts appeared to be otherwise normal, several showed a loss or dispersal of the organised arrays of rough endoplasmic reticulum (Nissl bodies) accompanied by a decrease in the background density of the cytoplasm. The haphazardly distributed, usually curved clefts almost invariably had one edge bounded by a slightly undulating membrane that was frequently studded with ribosomes. The other edge was less well defined and consisted of disrupted, attenuated cytoplasm, fragments of which were scattered within the electron-lucent lumen of the cleft.

ii) A single-exposure acute neurotoxicity study was carried out using Charles River CD rats (Schoenig *et al*., 1993). Data from the study were accepted by the EPA and used in their RED report. Animals were approximately 12 weeks old and weighed approximately 330g (males) and 242g (females) at the time of DEET administration. Forty male and 40 female rats were selected randomly and assigned to 4 treatment groups each containing 10 animals/sex. Following an overnight fast, the test article was administered in the undiluted form by gavage at dose levels of 0 (control), 50, 200 and 500mg/kg body weight. Animals in the control group were administered a non-toxic material (white mineral oil) having similar physical properties to those of DEET, at a dose volume equal to that given in the high-dose animals. The animals were maintained for approximately 2 weeks after a single administration of the test article. The rats were observed for overt signs of toxicity at least twice daily throughout the study and detailed clinical observations and body weight and food consumption measurements were made weekly. All animals were euthanised and examined externally and internally for grossly evident morphological changes.

Treatment at any dose had no effect on survival, body weights, food consumption, or the incidence of gross lesions. No attempt was made to document signs of toxicity between the time the animals were dosed and the start of the neurobehavioural evaluations.

Neurobehavioral Evaluations

All animals from each group were tested by the in-life neurobehavioral evaluations outlined below. Evaluations were conducted 1 hr, 24 hr. and 14 days following dose administration. For each task, a detailed schedule was constructed so that an equal number of rats of each sex from each treatment group were evaluated each day and at approximately the same time each day. All observations were conducted without knowledge of treatment group by the laboratory personnel who performed the observation and/or recorded the data.

Functional observational battery: Observational indices

Animals were observed for gross signs of nervous system dysfunction using a functional observational battery. Animals were observed individually for a minimum period of 1 min in an acrylic open field enclosure measuring 50.8 X 50.8 X 20.3 cm. The behaviours evaluated were tremors, convulsions, lacrimation, unusual respiration, urination/defecation, vocalisation, piloerection, and pupil size. These were scored by type and intensity where appropriate. The occurrences of other signs such as diarrhoea or increased salivation were recorded, if observed. Any abnormal body position, locomotor activity, or incoordination was noted, if it occurred. Additional observations included the occurrence and relative intensity of specific bizarre or stereotypic behaviour, including unusual head movement, compulsive biting or licking, self-mutilation, circling, retropulsion, and spatial disorientation.
No effects related to DEET exposure were observed.

Quantitative neuromuscular and sensory indices

Each animal was tested for nociceptive response (thermal response) by determining the time (60 sec maximum) it took to display licking of the paws when placed on a hot plate at a temperature of 52±0.2°C. Each animal was tested for neuromuscular function by assessment of forelimb and hindlimb grip strength using a grip bar attached to force-displacement transducers with a maximum working range of 2 kg.
Thermal response time was increased for animals in the 500mg/kg dose group at the 1 hour post exposure evaluation. There were no effects on thermal response time at the 24 hour or 14 day post-exposure evaluations that could be attributed to treatment with DEET. There were no treatment-related effects on forelimb or hindlimb grip strength.

Motor activity.

Motor activity of each animal was assessed in an open field enclosure (40.6 X 40.6 X 30.5 cm) using a photobeam activity device designed to monitor movement within the field. Beam sets oriented along horizontal planes were positioned approximately 2.5 and 17.8 cm above the floor of the field in order to monitor horizontal movement and vertical movement, respectively. Automated analysis of photobeam interruptions generated a variety of variables for possible evaluation. The following activity parameters were

selected for evaluation: horizontal activity (total number of horizontal beam interruptions), vertical activity (total number of vertical beam interruptions), total distance travelled (cm), movement time (sec), rest time (see), number of movements (number of beam interruptions of at least one second separated by at least one second), vertical time (sec), number of vertical movements (number of times vertical beams were interrupted separated by at least 1 sec), stereotypy time (number of repeated beam interruptions), number of stereotypic movements (number of same beam, repeated interruptions), and clockwise revolutions and counter clockwise revolutions (sequential beam interruptions of a minimum of 5.1 cm per side and through an angle of 360°).

Animals were placed in the darkened testing room while in animal caging for a 10-min adaptation period preceding testing. Animals were subsequently transferred to the motor activity enclosures and activity was recorded for eight consecutive 5-min intervals to allow for examination of both exploratory and baseline activity levels These intervals were pre-established in the testing laboratory as those demonstrating stabilised activity approaching asymptotic levels for the final 20% of the session.

Motor activity was generally unaffected by treatment with DEET. However, there was a statistically significant decrease in vertical activity counts and vertical time (compared to control) observed for both males and females in the 500mg/kg dose group at the 1 hour post-exposure evaluation. Vertical activity counts were decreased during the mid-portion of the test session while vertical time was generally decreased throughout the test session. Therefore the authors suggest that the No Observed Adverse Effect Level is below 500mg/kg (i.e. 200mg/kg).

iii) A neurobehavioural study was carried out by Van Haaren *et al*. (2001). Groups of 12 male and 24 female Sprague-Dawley rats (approximately 70 days of age) were acclimatised to reversed light-dark cycles (housed in groups of three) and allowed *ad libitum* access to food and water for 1 week. After this period, food restriction was applied (16g/day/male and as 12 g/day/female).

The animals learned Fixed Ratio (FR) and Fixed Interval (FI) reinforcement schedules using a combined FR/FI learning scheme. Animals were tested in an Operant conditioning chamber where food was delivered when tasks were successfully completed. The FR schedule testing used food delivery after 50 presses of the food delivery lever after a light stimulus. The FI schedule used light stimulus and food delivery after a 2-minute delay using lever pressing. Animals were tested in their dark hour periods.

Animals were given single oral doses of DEET by gavage in mineral oil. A dose response experiment using 0, 50, 200 and 500 mg/kg bw was used. Concurrent studies with pyridostigmine bromide (PB) and permethrin and combinations of these chemicals were also undertaken. It is presumed from details given in the methods that animals were tested 30 minutes post dose. Each animal served as its own control and was dosed at all dose levels using an ascending and descending order. Tests were undertaken on different days.

FR response rates decreased dose-dependently after DEET administration and more so in female rats than in male rats. Post-hoc analyses showed that only 500mg/kg DEET decreased response rates significantly compared to vehicle administration. FI response rates also decreased after DEET administration, but differences in sexes was not observed. FI response rates were significantly lower following 500mg/kg DEET relative to the behavioural effects of vehicle administration.

iv) A study was carried out to investigate the effects and potential interactions of pyridostigmine bromide, permethrin and DEET (Hoy *et al.*, 2000). Groups of 9 male or female (either pro or met estrus) Sprague-Dawley rats (200-250g) were given single oral doses of DEET (using mineral oil as a vehicle) by gavage at 50, 200 or 500 mg/kg bw. Animals were tested for locomotor activity 30 minutes post dosage. Tests (2h duration) were undertaken in an open field arena using video cameras to record activity (speed m/min) and position. The animals had been acclimatised to a reverse 12h light-dark cycle. The authors report that serum levels of DEET were measured 5d or more after the locomotor test. Comparisons of single-drug administrations versus vehicle administrations for all gender categories turned up a significant effect in only 2 out of 36 cases, i.e. met estrus females (N=9) that received 500mg DEET/kg had a reduced speed. Little other data is reported for DEET administration other than when administered at 200mg/kg, DEET was found at a much higher concentration in the serum of both pro- and metestrus females than in males.

3.8.2   Acute: Dermal

Rat

The effect of 70% DEET (in ethanol) on rat brain cytochrome c was investigated (Abu-Qare & Abou-Donia, 2001a). Sprague-Dawley rats (250-300g) were kept in a 12hour light/dark cycle and were provided with a free supply of food and tap water. A single dose of 400mg/kg DEET was applied with a micropipette (1ml/kg) to an unprotected $1cm^2$ area of pre-clipped skin on the back of each rat. A group of 5 animals was used for each time point. Control animals were treated with equal amounts of 70% ethanol (1mg/kg). Animals were anaesthetised with halothane and sacrificed by heart exsanguination at time intervals of 0.5, 1, 2, 4, 8, 16, 48 and 72 hours after dosing. Rat brain was removed, blotted dry and washed in saline, then stored at –70°C prior to analysis by HPLC. Results indicated that a single dose of DEET did not produce a statistically significant effect on the release of cytochrome c from brain mitochondria over the time course of treatment.

3.8.3   Sub-Chronic: Oral

Rat

A Chronic Exposure Neurotoxicity Study was also carried out (Schoenig *et al.*, 1993). Charles River CD rats were used. These rats originated as F2 offspring from a two-generation rat reproduction study conducted on DEET at dietary concentrations of 0 (control), 500, 2000 and 5000ppm (unpublished study). The 5000ppm dietary concentration represents approximately a dose of 225mg/kg. Two males and two females were selected from all F2 litters of each dose group after weaning and maintained on their respective diets for approximately 9 months. At the end of the 9 months, 20 litters from each group were selected randomly. One male and one female rat were selected from each litter (total of 120 rats) for use in this study. At this time, animals were approximately 40 weeks old and weighed 665g (males) and 345g (females). Treatment continued until neurobehavioral evaluations were completed (approximately 48 weeks of age).

No effect on survival or clinical signs was reported in any of the treatment groups. Decreases in mean body weights were observed in all measurement intervals for all DEET

treatment groups of males and females compared to the mean for the control groups. The authors suggest that this finding in low and medium dose animals was a result of random selection, as body weight effects had only been observed in the high treatment group (5000ppm) in a previous study.

Neurobehavioral Evaluations

All animals from each group were tested by the in-life neurobehavioral evaluations outlined below. For each task, a detailed schedule was constructed so that an equal number of rats of each sex from each treatment group were evaluated each day and at approximately the same time each day. All observations were conducted without knowledge of treatment group by the laboratory personnel who performed the observation and/or recorded the data.

Functional observational battery and motor activity

Tests were carried out essentially as described above in the acute neurotoxicity study (Schoenig *et al*. 1993).

No effects related to DEET exposure were observed in the behavioural or clinical observations made during the functional observational battery or the thermal response or limb grip strength measurements. There was an increase in horizontal activity observed for male and females rats dosed at 5000ppm rats at the beginning of the evaluation session (this effect was transient, occurring early during the test session). Mean motor activity for the 5000ppm treatment group was not different from the mean activity for the control groups for the remainder of the test sessions. Increases in counter-clockwise revolutions that resembled the effects observed for horizontal activity were also observed early in the test session for males and females in the high dose treatment group.

Swim Maze

Vision, motor co-ordination, and learning were evaluated for each animal in a swimming M maze outfitted with a movable wire mesh escape ramp and signal lights at either end of the maze. The time required for a successful escape, the number of incorrect channel entries, and the first response direction were recorded. Testing was carried out in two phases: an acquisition phase consisting of 10 swimming trials per day for two successive days to the lighted side of the maze as the goal, and a reversal phase consisting of 20 trials per day for two successive days, the goal being the dark side. The goal side in both phases was varied according to a predetermined randomised sequence pattern: Minimum intertrial intervals of at least 1 min were allowed between successive daily trials. The dimensions of the maze were approximately 58.4 cm wide. 43.2 cm deep, and 40.6 cm high and it was filled with water at a temperature of 20-22.2°C to a depth of 70.3 cm. Testing was conducted in a dark, quiet room following a l0-min dark adaptation period.

No treatment-related effects were found on acquisition response time, errors or accuracy of initial choice or on reversal response time or errors. A statistically significant decrease in the percentage of correct initial choices on reversal was found (significant main effect of dose). This was not considered to be an indication of DEET-related effect as there was a lack of a dose response.

Acoustic Startle Habituation

Habituation to the acoustic startle response was tested for each rat in an acrylic box with inside dimensions measuring 22.9 x 30.5 x 21.6 cm. Each box contained a startle platform, an associated load cell and loudspeaker. Following a 5min adaptation period in the startle box, each animal was subjected to a 20msec burst of a 110dB noise (background 70 dB) from a random noise generator. Each animal received 51 trials separated by intertrial intervals of 7.1 to 12.9 seconds. A recording window of 120 msec, beginning at the onset of the 110dB noise, was used for all measurements. Maximum and mean response amplitudes (Vmax, and Vmean respectively) and the time to maximum response amplitude (Tmax) were recorded. No treatment-related effects were found

Passive avoidance.

Passive avoidance was conducted as a one-trial step-through to darkness learning paradigm with 24- and 48-h post-training testing as assessments of memory. Each rat was tested in a computer-operated Omnitech shuttlebox system modified by the employment of an opaque chamber on one side. Males were tested in one box and females were tested in another, to avoid gender-specific olfactory disruptions. Testing consisted of one trial per day per rat for a period of 3 min each on three consecutive days. Entry to the dark side was aversively reinforced on Day 1 with a 0.8 mAmp footshock of 3sec duration. No shock was administered on Day 2 or 3 of testing. Because of the potential interpretational difficulties associated with one-trial learning procedures, an additional group (sham) was included as a control for non-associative factors. This was an extra group of animals drawn from the two-generation reproduction study controls. The sham group animals were tested along with all other groups except that the footshock reinforcement administered in the dark compartment to the primary study groups following initial entry was omitted from this group. No treatment-related effects were found.

Neuropathology

Following the in-life neurobehavioral evaluations, one male and one female from each of 10 litters (total of 80 rats) were selected at random from each group for neuropathological evaluation. All other animals were euthanised by carbon dioxide asphyxiation. Animals selected for neuropathological evaluation were euthanised following anaesthesia by intraperitoneal injection of sodium pentobarbital. The order of euthanisation was one litter from each group and then repeating. Each rat was examined for external abnormalities, including palpable masses and the thoracic and abdominal cavities and organs were examined grossly. The following tissues were harvested and fixed, following in situ perfusion with 3% paraformaldehyde and 0.03% glutaraldehyde in cacodylate buffer at pH 7.4:

forebrain	spinal cord at
centre of the cerebrum	cervical swelling (C,-C.)
midbrain	lumbar swelling (L,-L,)
cerebellum	Gasserian ganglia
pons	dorsal root ganglia
medulla oblongata	(C3-C6, L1-L4)
proximal sciatic nerve	dorsal and ventral root fibres
sural and tibial nerves	(C3-Cs, L1-L4)

Both sciatic nerves with tibial, plantar, and sural extensions were dissected free from the carcass to a point below the hock. The brain, Gasserian ganglia, and spinal cord were removed after representative cervical and lumbar dorsal root ganglia with corresponding dorsal and ventral roots were dissected free.

Representative cross and longitudinal areas of cervical and lumbosacral cord were taken for hematoxylin-eosin (H&E) staining. Sagittal sections of brain were also taken including the cerebrum, medulla, pons, and cerebellum for H&E sections. Both sciatic nerves (proximal) were sampled so that both cross and longitudinal sections of left and right sciatic nerves were processed for H &E staining. Extra sections of each nervous system tissue were made and stained with luxol fast blue, gallocyanin, and Bielchowsky's stain. The following tissues were post-fixed in osmic acid and processed to 1µm epoxy sections stained with toluidine blue: tibial nerve (cross and longitudinal sections) and cervical and lumbar dorsal root ganglia with corresponding dorsal and ventral root fibres. Sural nerve fibres were used for teased nerve preparation. Microscopic examination of the prepared tissues was performed for all animals in the high-dose and control groups.

Subtle neurological degenerative lesions such as myelin bubbles and myelin phagocytosis were observed in nervous tissue from control as well as from treated animals indicating mild peripheral nervous impairment. The authors suggested that this resulted from housing in wire-bottom caging. No treatment related gross or microscopic alterations were seen.

3.8.4 Sub-Chronic: Dermal

<u>Rat</u>

i) A study of the effects of daily dermal application of DEET on sensorimotor performance, blood brain barrier, and blood-testis barrier was carried out in rats (Abou-Donia *et al.*, 2001a). Male Sprague-Dawley rats (200-350g) were randomly assigned to control and treatment groups and housed at 21-23°C with a 12-h light/dark cycle. They were supplied with Purina certified rodent chow and tap water ad libitum. The rats were allowed to adjust to their environment for a week before treatment.

For dermal application of the chemicals, $1in^2$ of the back of the neck was shaved. The chemicals were applied on the shaved area to give the desired concentration of test compounds in 0.2ml vehicle. Groups of 10 rats received a daily topical dose of 4, 40 or 400mg/kg DEET (0.1x, 1x and 10x) in 70% ethanol. The 1x dose of DEET was based on an estimate of exposure that may have occurred to army personnel during the Gulf War. Control animals received an equal volume of the vehicle. Neurobehavioural evaluation was undertaken at days 30, 45 and 60 of treatment

General observations of animals treated with 4, 40 or 400mg/kg DEET in ethanol was not different from controls and no difference was observed in the weights of treated animals as compared with control.

Blood brain barrier studies

For blood brain barrier (BBB) permeability studies, 24 hours after the last treatment, subgroups of 5 animals were anaesthetised and then injected in the tail vein under anaesthesia with [3H] hexamethonium iodide (10µCi, mixed with cold hexamethonium iodide) to give a final dose of 0.7mg/kg (1µCi/kg). After 10 min, animals were

anaesthetised with ketamine/xylazine, blood was collected from the heart with heparinised syringes, and the animals were sacrificed by decapitation. Brains and testes were removed and placed in ice-cold normal saline. Brain regions were dissected in ice into cortex, brainstem, mid-brain and cerebellum. Following dissection, the brain regions and testes were snap frozen in liquid nitrogen. Plasma was separated from whole blood by centrifugation. Plasma, brain regions, and testes were stored at $-20°C$ for later analysis.

For the determination of [3H]hexamethonium iodide uptake in tissues and plasma, a weighed amount was subjected to oxygen combustion using a tissue oxidiser. Total radioactivity present in tissues and plasma was determined in triplicate using a scintillation spectrometer.

A decrease uptake of [3H]hexamethonium iodide was observed in the brainstem of the animals treated with DEET at 1x and 10x dose (40 and 400mg/kg bw/day respectively) when compared to the control group indicating reduced BBB permeability. DEET treatment alone also caused a decrease in blood-testis barrier permeability.

Behavioural tests

A battery of standardised behavioural tests was employed. These tests were designed to measure sensorimotor reflexes, motor strength, and co-ordinated gait. All behavioural testing was performed by trained observers who were blind to animal treatment status and was carried out in a soundproof room with subdued lighting (less than 10% lumen/m^2, ambient light). Rats were handled for 2 min daily for 5 days during the week prior to behavioural testing.

Postural reflexes

Rats were held gently by the tail, 1 m above the floor, and were observed for forelimb extension. Rats with consistent forelimb flexion were further assessed by placing each on a large sheet of plastic-coated paper that could be gripped with forepaws. With the tail held, gentle lateral pressure was applied behind the rat's shoulder until the forelimb slides several inches. The manoeuvre was repeated five times in each direction. Scoring was as follows. Grade 0 was given to rats without evidence of consistent forelimb flexion when held above the floor; grade 1, to rats with consistent forelimb flexion; and grade 2, to otherwise grade 1 rats that do not resist lateral pressure on at least three of five trials in either direction.

There was no effect due to DEET.

Limb Placing

Visual, tactile, and proprioceptive forelimb placing responses were examined. For visual placing, rats were held in the examiners' hands 10 cm above the tabletop with forelimbs hanging free. The rats were then slowly tilted toward the table. For tactile placing, the dorsal and then lateral portions of the forepaws were touched to the table edge. Proprioceptive placing was tested by pushing the forepaw onto the table edge. Care was taken to avoid the vibrissae touching the table. Scoring was as follows. For each test, grade 0 was assigned if the placing response was immediate; grade 1 if the placing response was slow or delayed; and grade 2 if the placing response did not occur within 2 seconds.

There was no effect due to DEET.

Orienting to Vibrissae Touch

The rat was placed atop an inverted polycarbonate cage and allowed 1 min for habituation. Its vibrissae were then touched with a cotton-tipped swab. Grade 0 was assigned if the rat oriented to the side of the probe on at least two of three trials from each side, and grade 1 if the rat failed to orient on at least two of three trials on either side.
There was no effect due to DEET.

Grip Time

Rats' forepaw grip time was assessed by having them hang from a 5mm-diameter wood dowel gripped with both forepaws. Time to release their grip was recorded in seconds.
For the forepaw grip test, there was a significant effect of DEET in all treatment groups. Dose x time interactions were not significant as all groups were affected from the first time point.

Beam Walking and Beam Score

The testing apparatus was a 2.5 x 122-cm wooden beam elevated 75.5-cm above the floor with wooden supports. A 20 x 25 x 24cm goal box with a 9.5-cm opening was located at one end of the beam. A switch-activated source of bright light (75W tungsten bulb) and a source of white noise (sound pressure levels of 41dB at 8000Hz, 58dB at 4000Hz, 56dB at 2000Hz, 56dB at 1000Hz, 58dB at 500Hz, and 52dB at 250Hz at the centre of the frequency at each octave band) were located at the start end of the beam and served as avoidance stimuli. The rats were first trained to traverse the beam with a series of three approximate trials (i.e., rats were first placed at the entrance to the goal box, then at the mid-portion of the beam, and finally at the start end of the beam). After behavioural training, rats underwent a craniotomy and the cerebral cortex lesioned. After recovery, the rat was placed at the start end of the beam, near the sources of light and noise. Beam-walking ability was measured with a seven-point scoring: 1, the rat was unable to place the affected hind paw on the horizontal surface of the beam; 2, the rat placed the affected hind paw on the horizontal surface of the beam and maintained balance for at least 5 seconds; 3, the rat traversed the beam while dragging the affected hind paw; 4, the rat traversed the beam and at least once placed the affected hind paw on the horizontal surface of the beam; 5, the rat crossed the beam and placed the affected hind limb on the horizontal surface of the beam to aid less than half its steps; 6, the rat used the affected hindpaw to aid more than half its steps; and 7, the rat traversed the beam with no more than two footslips. In addition, the latency until the animal's nose entered the goal box (up to 90 seconds) was recorded for the final trial. Rats that fell off the beam were assigned latencies of 90 seconds.
There was a significant effect of DEET dose as well as a significant effect of DEET dose x time interaction for beam walk score. At the 30-day time point the 0.1x dose of DEET differed significantly from the controls.
For beam walk time there was a significant effect of DEET dose but no dose x time interaction. At the 30 day time point the 0.1x dose of DEET differed significantly from the controls.

Inclined Plane

The rats were placed on a flat plane in the horizontal position, with the head facing the side of the board to be raised. The board was slowly rotated to the vertical position. Two trials were performed for each testing session. The angle at which the rat begins to slip downward was recorded. The results of two trials were averaged at each time point.
There was a significant effect of DEET dose. At the 30 day time point the 0.1x dose of DEET differed significantly from the controls

ii) In a similar series of experiments, carried out by Abou-Donia *et al*. (2001b), a deficit in locomotor and sensorimotor performance was observed in rats following exposure to DEET. Other experiments were simultaneously carried out involving pyrodostigmine and permethrin and all three chemicals in different combinations.
Groups of five male Sprague-Dawley rats weighing 225-250g were randomly assigned to control and treatment groups and housed at 21-23°C with a 12-hour light/dark cycle. They were supplied with food and water *ad libitum*.
The rats were allowed to adjust to their environment for a week before starting the treatment. A one-inch square area on the back of the neck was pre-shaved and DEET (40mg/kg/day in 70% ethanol) was applied for 45 days. For the control group of rats, 70% Ethanol was applied for 15 days. Sensorimotor testing, including postural reflexes, limb placing, orientating to vibrissae touch, beam walking and beam score, and incline plane, were undertaken on days 30 and 45. These behavioural studies were carried out as described above (Abou-Donia *et al*., 2001a). The authors also undertook to measure brain cholinesterase and plasma cholinesterase using the Ellman method and to measure ligand binding to nicotinic and muscarinic acetylcholine receptors in brain regions.
There were no overt clinical signs of toxicity recorded throughout the study except for occasional diarrhoea in rats receiving DEET. There was no effect of DEET on postural reflexes, limb placing or vibrissae touch. DEET also did not affect beam walk score or beam walk time that had been seen in the previous report, although DEET did reduce incline plane performance and forepaw grip time. Treatment with DEET significantly increased brainstem acetylcholinesterase activity and cortex choline acetyltransferase activity. DEET also caused a significant increase in ligand binding for muscarinic but not nicotinic receptors in the cortex.

iii) Further work by Abdel-Rahman *et al*. (2001) considered the histopathological effects of a sub-chronic dermal application of DEET. Groups of five male Sprague-Dawley rats received dermal doses of DEET at 40mg/kg bw/day for 7 days per week for 60 days (in 70% ethanol). 24 h after last dose the animals were anaesthetised with pentobarbital, and perfused through the heart with saline followed by 4% paraformaldehyde and 0.15% glutaraldehyde in Tris buffer. The brains were removed, post-fixed and embedded in paraffin. Four micrometre coronal sections were cut through different brain regions and sections stained with H&E. The authors undertook measurement of the density of healthy (surviving) and dying neurones and immunohistochemistry with microtubule associated protein 2 (MAP-2) to assess neurodegeneration. The authors also undertook immunohistochemistry with glial fibrillary acidic protein (GFAP) to assess upregulation of GFAP-immunopositive structures.
A significant reduction in the density of healthy (or surviving neurones) was reported in the motor cerebral cortex, the dentate gyrus, the CA1 and CA3 subfields of the

hippocampus and the cerebellum. A significant number of degenerating neurones (eosinophilic) were documented in these brain regions. The neurone loss in the motor cortex and the CA1 subfield was corroborated by a significant decrease in MAP-2. Analysis of GFAP indicated a significant hypertrophy of astrocytes in hippocampus and cerebellum of all treated groups.

iv) Histopathological alterations in the brain of adult male rats were evaluated following a daily dermal dose of DEET (40 mg/kg in 70% ethanol) for 60 days (Abdel-Rahman *et al.*, 2001, abstract only). Simultaneous experiments with permethrin (0.13 mg/kg in 70% ethanol) or a combination of the two chemicals was also carried out for 60 days. Control rats received a daily dermal dose of 70% ethanol for 60 days. Animals were perfused and brains were processed for morphological and histopathological analyses following the above regimen. In animals receiving DEET, degenerating (eosinophilic) neurones were diffusely observed in distinct regions of the motor and somatosensory cortex, the hippocampus, and the cerebellum. Analysis of glial fibrillary acidic protein immunoreactivity revealed significant hypertrophy of astrocytes in animals treated with DEET. Thus, sub-chronic dermal application of DEET may lead to diffuse neuronal cell death in cerebral cortex, hippocampus and cerebellum of the adult brain. These neurotoxic effects were more pronounced when DEET and permethrin were applied together.

3.8.5   Sub-Chronic: Sub-Cutaneous

Hen

i) A study was designed to determine the toxicity produced by exposure of 5 hens, 5 days/week for 2 months to 500mg/kg/day DEET (neat), sub-cutaneous; (Abou-Donia *et al.*, 1996). Simultaneous experiments were carried out to examine the effects of pyrostigmine bromide and chlorpyrifos and all chemicals in different combinations. 24 hours following administration of the last dose, treated and control hens were anaesthetised using 0.25% halothane and then euthanised by decapitation. The spinal cord and sciatic nerve were excised immediately and immersed in 4% phosphate-buffer formalin and post-fixed for at least a week in the same fixative. Cross-sections of cervical and thoracic spinal cord and longitudinal sections of proximal and distal sciatic nerve were stained for hematoxylin and eosin and periodic acid Schiff's method. In addition, selected tissues were stained with Marsland and Glee's silver stain. Tissues were assessed with a step down approach by first comparing sections from the control groups and then subsequently examining treatment groups. Severity scores for the spinal cord were based on enlarged axons in the ventral, lateral, and columns in cervical and thoracic regions (0, no detectable differences from control; 1, mild increase in the frequency of enlarged axons; 2, moderate; 3, severe). Severity scores for the sciatic nerve also ranged from 0 to 3 and were based on the combined frequency of prominent enlarged axons in the proximal and distal segments.

Treatment with DEET caused immediate rapid shallow breathing and inactivity, which recovered within 24 hours of dosing daily. There was no detectable change in spinal cords of hens treated with DEET. In two animal treated with DEET, there was a small but detectable increase in the frequency of enlarged axons in the sciatic nerve.

No NOAEL or LOAEL was established due to insufficient information

ii) In a similar set of experiments to those described above, Abou-Donia *et al.* (2001c) investigated the neurotoxicity of 500mg/kg DEET administered 5 days per week for 2 months. Simultaneous experiments were carried out using pyridostigmine bromide and permethrin and all chemicals in different combinations. Groups of five healthy, adult leghorn hens were housed in temperature-controlled rooms (21-22°C) with a 12 hour light/dark cycle. They were supplied with food and water *ad libitum*. A dose finding study was initially carried out and 500mg/kg/d DEET (neat) was chosen as the treatment dose as it was found to cause the minimum changes in clinical parameters.

Clinical evaluation included walk pattern, leg movement, flying, body tremor and ability to enter the home cage. Other observations such as diarrhoea, salivation, or pupil constriction were recorded. Animals treated with DEET developed rapid shallow breathing and tendency toward inactivity shortly after dosing but recovered within 24 hours of dosing. DEET treated hens had significantly less weight at termination. Histological assessment was as described above. Some animals treated with DEET exhibited minor neurological changes that consisted of a small increase in the frequency of slightly enlarged axons. Treated animals also exhibited a slight inhibition of plasma butyl cholinesterase activity.

No NOAEL or LOAEL was established due to insufficient information.

3.9 Summary of Neurotoxicity Studies

The data from Verschoyle *et al.* (1992) indicate that the predominant signs of toxicity in rats given oral doses of DEET resulting in lethality were CNS depression with myoclonic twitching seen in some animals (triggered by auditory or tactile stimuli). Recovery in any surviving animal was slow. Also reported was evidence of histological changes in animals given lethal doses in the CNS predominantly consisting of vacuolation of myelin sheaths of fibres in the cerebellar roof nuclei and cytoplasmic clefts, which were diffusely distributed throughout the brain. Verschoyle *et al.* (1992) also suggest that female rats are more susceptible to DEET than male rats, and also suggest an effect of age (decreasing toxicity with increasing age). These age- and sex- related effects might have relevance for the interpretation of other neurotoxicity studies. The work also showed that inhibition of metabolism increased the acute lethality of orally administered DEET in adult animals. These data support the view that liver metabolism decreases the toxicity of DEET.

The paper of Schoenig *et al.*, (1993) reports a well carried out acute neurotoxicity study. It is possible that the decrease in vertical activity seen at 500mg/kg DEET in this study indicates threshold level effects. The authors themselves suggest that effects observed at 500 mg/kg bw do not reflect selective mammalian neurotoxicity, but were consistent with general acute toxicity. It should be noted this acute neurotoxicity study was submitted to the EPA, who considered that it satisfied their data requirements for an acute screening study. In addition, the EPA RED document (US EPA, 1998) suggested that the rats also showed signs of piloerection and increased vocalisation at 500mg/kg DEET. Furthermore, the report also states that a decrease in vertical activity was observed during the first 15 minutes of the evaluation carried out one hour after dosing at 200mg/kg. However, no effect was seen in any test animals at 50mg/kg. Therefore, it was suggested that the NOAEL should perhaps be 50mg/kg and the LOAEL at 200mg/kg. These results

were extensively debated by the EPA's Office of Pesticide Programs (OPP) Health Effects Division Toxicity Endpoint Selection (TES) Committee. This committee concluded that the decrease in vertical activity seen at 200mg/kg was an isolated and transient effect and the toxicological significance of this finding was not certain. FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) Scientific Advisory Panel (SAP) also supported these conclusions. Therefore, the EPA decided on an NOAEL for this study of 200mg/kg and an LOAEL at 500mg/kg.

Although the sub-chronic neurotoxicity screening study (Schoenig *et al.*, 1993) was not designed as such, the duration of exposure and the parameters examined met the EPA data requirements for a 90-day neurotoxicity study. Furthermore, the study is able to provide an abbreviated assessment of functional development following *in utero* and postnatal exposure to DEET. However, because the animals were not evaluated until they were adults, the effect of treatment with DEET on the ontogeny (i.e. the history of the development of an individual) of functional development was not assessed. However, the authors report no histological changes in the CNS for this study, using appropriate methods for fixation of tissues by *in-situ* perfusion and histology. Unfortunately, a morphometric evaluation of the CNS was not undertaken in this study. Although a transient increase in motor activity was seen in the first 5-15 minutes of the functional observational battery, these effects were not accompanied by any histological changes in the CNS. However, effects on motor activity should not be discounted. A NOAEL can therefore be derived from this study as 2000 ppm (95mg/kg bw/day).

Neurobehavioural studies from other groups are also reported. In one such study, carried out by Van Haaren *et al.* (2001), rats (70 days old) were acutely dosed with DEET and effects on a Fixed Ratio and Fixed Interval reinforcement schedules measured. In agreement with Schoenig *et al.* (1993), only a dose of 500mg/kg DEET affected behaviour, however there were no differences due to sex. Unfortunately, in this study it is unclear whether animals were subject to dosing at more than one dose level, as it is noted that a second dosage of DEET to female rats was reported. Is should be noted that this study reports no sensorimotor function tests in young animals and it is possible that the NOAEL would be lower in such animal.

The study of Hoy *et al.* report very few results regarding animals dosed with DEET alone. A dose of 500 mg/kg bw DEET to met-estrus rats resulted in statistically significant reduced speed in the locomotor test. It is noted that serum concentrations of DEET (following dosing of 200 mg/kg bw) were significantly higher in met- and pro-estrus females compared to males. This might explain why an effect on locomotor activity was only seen in females. There is no clear reason why serum concentrations should have been higher in female rats. Schoenig *et al.* did not report a sex difference in kinetics in rats dosed with 100 mg/kg bw in corn oil. Data from Schoenig *et al.* following acute dosing would indicate higher bioavailability in male rats compared to females over a 25-hour period. Overall the finding of reduced locomotor activity in this study at 500 mg/kg bw is consistent with the reduced motor activity reported by Schoenig *et al.* in rats at the same dose level.

The papers of Abou-Donia *et al.* also report neurotoxicity studies in the rat. However, it is not clear if the different papers report different studies or use the same data set (Abou-Donia *et al.* 2001 and Abdel-Rahman *et al.* 2001). Abou-Donia *et al.*, 2001a and 2001b

both describe a study where DEET was applied dermally and neurobehavioural effects measured. In the former, beam walk performance was affected after 30 days and more significantly after 60 days, when a dose of 40mg/kg was applied. In the latter, the same dose was used and the same neurobehavioural tests were carried out, however similar effects were not observed after 30 days or indeed 45 days, although other neurobehavioural tests were affected. However, it should be noted these studies are somewhat in disagreement with Schoenig *et al.* who also observed neurobehavioural changes due to DEET but at a higher dose. Of course, neurobehavioural effects do depend somewhat on the observer, therefore a more appropriate endpoint would be neuropathological observations.

The dermal studies carried out by Abou-Donia may have more significance with reference to the actual use of DEET in humans, however low doses of DEET were used in 70% ethanol. These doses were equivalent to and below those reported in the EPA evaluation of DEET as being relevant to normal use of DEET products (ca 9-12 mg/kg/d for adults, 21 mg/kg/day for 13-17y, and 37.6mg/kg/d for <12y). The use of 70% ethanol as a solvent might have resulted in substantial absorption of DEET.

In the studies where neuropathology was examined, it should be noted that the evaluation of such subtle changes requires expert judgement. The appropriate use of morphometric analysis and immunohistochemical staining for effects on glial cells assisted the identification of subtle lesions that may have been overlooked in the Schoenig studies. Variability due to the different strains of rat used between the two groups is not expected to be the cause in this disagreement. Unfortunately, no histopathology data is available from the Abou-Donia studies for 30 or 45 days of dermal treatment and it is therefore difficult to evaluate whether there was a correlation between effects on sensorimotor performance and histological changes in the CNS.

It should also be noted that similar effects were seen with DEET on motor function and CNS histology that had been reported with permethrin and pyrodostigmine and combinations of these chemicals. It is extremely difficult to resolve why these structurally and toxicologically dissimilar chemicals should produce a similar pattern of CNS toxicity.

3.10 Evidence of Toxicity from Human Exposure

There have been some reports of toxicity in humans through use of insect repellent products. These are divided into exposure that has resulted in dermal toxicity (section 3.10.1), toxic encephalopathy (section 3.10.2), psychosis (section 3.10.3), reproductive toxicity (section 3.10.4) and finally any other effect (section 3.10.5). These case reports have been classified using the following criteria.

	Classification	Criteria
1.	Confirmed	There are clinical symptoms and signs typical of exposure to the cited pesticide formulation combined with either - (i) corroborating medical and (where appropriate) biochemical evidence or (ii) evidence of over exposure
2.	Likely	The balance of evidence based on reported exposure circumstances, clinical symptoms and signs or biochemical evidence (where appropriate) is consistent with ill health due to exposure to the cited pesticide formulation.
3.	Open	(i) The reported ill health is consistent with the known potential ill health effects of the cited pesticide formulation given the reported exposure circumstances, but the implied association cannot be entirely discounted in the light of current knowledge. or (ii) The evidence is consistent with pesticide exposure being the cause of the reported ill health but alternative explanations, e.g. pre-existing disease are also present.
4.	Unrelated	There is strong evidence (e.g. evidence about exposure or from medical reports) that the reported ill health is not pesticide related.
5.	Insufficient Information	The available data are insufficient, incomplete or conflicting and therefore the case cannot be classified for one or more of these reasons.

Table 3.15: Criteria for the classification of case reports concerning DEET

3.10.1 Dermal Toxicity

Case Report 1:   (von Mayenburg & Rakoski, 1983)
At the age of 4 years a male patient developed acute contact urticaria after application of DEET (Autan product) to the entire skin. After 10 minutes, a generalised erythema with severe pruritis developed. At the same time, small wheals developed close together on the skin. After cleansing the skin, the application was repeated 1 hour later with similar results, which again subsided quickly. A few days later the application was repeated at a circumscribed site and a wheal and erythema reaction was elicited. The same symptoms have been observed in the same child at the age of 9 and 15 years and have been demonstrated to be via an immunological mechanism.
Classification: 1

Case Report 2   (Wantke *et al.*, 1996)
Shortly after being bitten by mosquitoes, an insect repellent was applied to the legs and forearms of a 4-year-old boy. Minutes later, generalised itch and urticaria developed and the boy wheezed and coughed for about 30 minutes. After application of topical anti-histamine the wheals disappeared by the following morning. Results of closed patch test revealed persistent non-specific erythematous reactions against the insect repellent, as well as other substances including ethanol. This was an indication of the high sensitivity of the boy's skin. Distinct wheal and flare reactions were also seen to histamine and saline in skin prick testing, which indicated a non-specific cutaneous hyperactivity.
Classification: 3

Case Report 3:   (Lamberg & Mulrennan, 1969)
A bullous eruption appeared sporadically among U.S. military personnel in South Vietnam during the summer months of the late 1960s. The eruption presented itself in a stereotyped manner in the antecubital fossae and healed slowly with scarring. The cause of this eruption had been thought to be a vesicating insect, although it may have been due to DEET-containing insect repellents.
The two main characteristics of the eruption were its distinctive course and its restriction to the antecubital fossae. It was always noted in the morning as a reddened tender area, which evolved during the next 24 hours into blisters on a tender base. The blisters remained intact for one to three days and left an eroded purulent base, which persisted for two to three weeks. The erosions were disabling and most of the men were unable to extend their arms due to pain. Several cases of bilateral involvement were found. In all cases, the men had slept in a field the night the eruption developed and had used an insect repellent (75% DEET and 25% ethanol or dichlorodifluoromethane). Most cases developed definite and permanent scarring. None of the men recalled an insect on their arms but during the same period of time other blistering eruptions were seen in military personnel, which were typical for the linearly placed bullae of vesicating insects of the genus *Paederus*.
Further testing was carried out. One patient was tested by placing a gauze pad soaked with the repellent in the antecubital fossa of the arm, which was wrapped in flexion. He was tested with his arm flexed on the premise that this was possibly how he slept before when blisters were formed. After 18 hours occlusion, blisters exactly like those seen clinically had appeared
Further studies were carried out in 63 volunteer military personnel at DaNang. Fifty-one of these were tested with a liquid preparation (75% DEET and 25% ethanol) and 12 were tested with an aerosol preparation (75% DEET and 25% dichlorodifluormethane) while they remained on closed air-conditioned hospital wards recovering from minor wounds or convalescing from minor medical problems. The men were tested both on the antecubital area and the inner part of the upper arm. Twenty-nine of the sixty-three men developed a reaction at the antecubital area with no differences between the results from the liquid or aerosol preparations. Three men developed local necrosis requiring three weeks to heal with scarring. None of the 52 men patch tested on the upper arm showed any reaction, even after 48 hours.

A different group of 10 men were tested in the United States in the same way and using the same preparations as above. Six of these subjects developed blisters or erosions in the

antecubitals, whilst none had any reaction in the upper part of the arm. In two, local necrosis and late scarring developed.

Classification: 2

Case Report 4:   (Reuveni & Yagupsky, 1982)

Ten soldiers, aged between 18 and 20 were treated for acute dermatitis. A history of the local use of an insect repellent containing 50% DEET, between 18 and 24 hours beforehand, was obtained in all cases. The repellent had been applied to the uncovered skin of the face, neck, upper part of the trunk, and legs before sleep. All of the patients denied a history of allergy or thermal burns. In each case there was the sudden onset of burning sensation and erythema of the antecubial fossa of one or both arms. On subsequent examinations, the erythema had progressed to haemorrhagic blister formation. The bullae drained spontaneously after one to two days and in some cases left deep ulcerations. The lesions were observed on both arms in only two patients.

Four patients were referred to a dermatologist for consultation. A diagnosis of allergy of unknown cause was made in two of them, burns in one case, and eczema in the other. In two cases a permanent scar remained. One case is described in detail.

A healthy 19-year-old soldier was examined because of the sudden appearance of a red burning area in the right antecubital fossa. The night before, he had applied the DEET repellent to his face, neck, and arms. On examination, the right antecubital area appeared red, warm and painful. No abnormal findings were noted in other body area including the additional sites to which the repellent had been applied. The patient was referred to a dermatologist who made the diagnosis of allergic dermatitis due to an unknown agent. Triamcinolone acetonide and oral doses of promethazin hydrochloride were recommended.

Despite treatment, blisters subsequently developed in the affected site. Two days later, haemorrhagic contents were discharged from the bullae, leaving an ulcerated area. Therapy was discontinued. The ulcer healed progressively. In this particular patient, however, an unusual complication developed. On examination four weeks later, scar formation and mild flexion contracture of the antecubital fossa were observed. Surgical correction of the contracture was required.

Classification: 2

Case Report 5:   (Miller, 1982)

A 42-year-old-woman with no prior history of atopia developed pruritis then angioedema after touching a companion who had just sprayed himself with insect repellent containing 52% DEET. Generalised pruritis rapidly developed and progressed to generalised angioedema. On arrival at hospital she was nauseated and losing consciousness. Her blood pressure was 70/40mmHg. She was treated symptomatically with epinephrine, diphenhydramine and corticosteroids. A week later, again after exposure to DEET, periorbital oedema developed.

Controlled challenge with DEET resulted in pruritis and urticaria spreading from the site of administration. The only other ingredient, isopropyl alcohol did not elicit a reaction

Classification: 1

Case Report 6:   (Amichai *et al.*, 1994)
A 20 year old male soldier developed skin eruptions and a burning sensation after applying an insect repellent containing 33% DEET. On examination, 3 livid patches, with areas of erosions and blisters were seen in the right antecubital fossa. A diagnosis of irritant contact dermatitis was made based on anamnestic and clinical data. After 10 days of treatment with topical corticosteroids, the lesions disappeared. The patient has since used the same product, but by avoiding the flexural areas, there has been no recurrence of skin lesions
Classification: 5

Case Report 7:   (Maibach & Johnson, 1975)
Another woman reported severe pruritis caused by DEET. Both 48- and 96-hour patch tests for delayed hypersensitivity were negative, but an open patch test was highly positive within one hour and resolved over two days. Passive immunologic transfer was positively demonstrated in two controls, suggesting an immunologic mechanism.
Classification: 2

Case Report 8:   (Vozmediano *et al.* 2000)
In Spain, a 16 year old woman, whose medical records included disproportional reactions to insect bites, presented on various occasions with soapy cutaneous reactions, accompanied by oedema and severe pruritis. She claimed they were becoming increasingly severe and related to the application of Autan®, of which she was a regular user. The ingredients of this insect repellent are 20% diethyltoluamide (DEET; active ingredient), polyethylene glycol (fixer), alcohol (96°) with indicator and fragrence 78041. Autan® showed positive results in open tests, as did diethyltoluamide at 100% and 1/100. Diethyltolumide at 1/1000 and the rest of the components showed negative results. Negative results were also obtained for epicutaneous tests to the standard battery of the Spanish Group for the Research of Contact Dermititis (GEIDC) and other toluamides (diethyl-p-toluamide, toluenediisocyanate, toluenesulfonamide and toluenediamine sulfate).
Classification: 1

3.10.1.1 Summary of Dermal Toxicity

From the case reports of dermal toxicity, it would seem that DEET is sometimes associated with skin reactions. However, there is limited evidence (only in two reported cases) that suggests that urticarial reactions to DEET may be allergic in nature (due to specific immunological responses). According to the classification criteria, it would be suggested that most of these cases would be defined as likely or confirmed cases of DEET related poisening.

3.10.2 Toxic Encephalopathy

A number of cases of toxic encephalopathy were reported following exposure to DEET. In most cases, exposure appears to have been extreme, and in some cases has led to fatality. Those cases and reports involving children have been considered in detail.

Fatal

Case report 9:   (Zadikoff, 1979)

A five-year-old girl was admitted to hospital with a ten-day history of progressively worsening headaches. Three days prior to admission she was noted to be unduly agitated and mildly disorientated. On the day of admission her speech was slurred, she was severely confused, and she later developed ataxia and had a generalised convulsion. She had been given aspirin on two occasions for headache during the previous ten days. She was afebrile from the onset of the illness and had no rhinitis, pharyngitis, or otitis. There were no rashes. There was no history of measles or other childhood illness, trauma, or allergy. Her development was normal until this episode.

The girl had been sprayed daily for almost three months with Mylol (Boots Company, South Africa), an insect repellent containing 10% DEET. The parents followed the instructions on the label and ensured that there was no contact with mucous membranes.

On admission the child was extremely agitated, restless and irritable, with constant involuntary movements involving the head trunk and limbs. There was athetosis with wildly thrashing movements, which alternated with short periods of quiet and episodes of marked shaking and crying or screaming. She responded purposefully to noxious stimuli but not to other attempts at communication. There was no focal neurologic deficit and the eyegrounds were normal. Pupils were equal and reactive to light. Deep tendon jerks appeared normal. There was no nystagmus. The respiratory rate was 28/minute, blood pressure 110/65mm Hg, and pulse 135/minute. Shortly after admission she had a generalised convulsion which was controlled with intravenous administration of diazepam.

A lumbar puncture was performed but the pressure could not be measured because of the child's continued movements. The CSF contained 30 neutrophils and 135 lymphocytes/mm3 with protein 18mg and glucose 60mm/dl. No viruses, bacteria, parasites, or fungi were isolated from the CSF or blood. Hemogloblin was 14gm/dl, WBC count 13,600/mm3 with 60% neutrophils, 38% lymphocytes, and 2% monocytes. Platelets were normal. Blood urea was 24mg/dl, sodium 137mEq/l, potassium 3.9mEq/l, calcium 5.1mEq/l and glucose 72mg/dl. Blood lead concentration was 19µg/dl, and copper within normal limits (60µg/dl). Thyroid and liver function studies were normal.

The child was maintained on intravenous fluids and was treated for meningitis (the authors state that this diagnosis was unlikely) with ampicillin 400mg/kg/day intravenously, but there was no improvement in her condition. She continued to have convulsions once or twice every day, and these proved difficult to control. She was treated with phenobarbital, diazepam, diphenylhydantoin, clonazepam, nitrazepam, and paraldhyde in varying doses and combinations during the 24 days she was in hospital, with little control of either her involuntary movements or the convulsions, which became more severe and regular toward the end of her hospitalisation. Haloperidol was also given without any success.

The child's state of consciousness improved during the first few days in hospital, although she continued to be in an almost constant state of agitation with wild flinging movements of her limbs, episodes of opisthotonos, and crying involuntarily. During brief periods of quiet she was able to indicate with a slurred "yes" or "no" in answer to questions that she wanted to remain still but could not do so. Swallowing became a problem – she could not

clear saliva spontaneously, and developed right upper lobe pneumonia, which cleared with physiology and antimicrobial agents.

The child deteriorated steadily and died 24 days after admission, her seizures being more intractable and her agitation worse. At autopsy, the brain showed generalised oedema with intense congestion of the brain and meninges. There was no evidence of meningitis. Cerebral blood vessels showed swelling of endothelial cells. There was no perivenous or other demyelination and no perivascular infiltrate.

Classification: 3

Case report 10: (Heick *et al*., 1980)

A six-year-old girl was admitted to hospital with a four-day history of lethargy, mood changes, and nightmares. On the day prior to admission she stopped eating, had colicky abdominal pains, and vomited. On the day of admission she complained of headaches and was ataxic and disoriented. Before and during the onset of the illness the child had used a spray containing 15% DEET on at least 10 occasions on extensive areas of skin. She had used DEET previously as a lotion of lower concentration and never in the amount used in this occasion. There was no evidence of exposure to other toxic chemicals.

She had a life-long aversion to protein and had had two episodes, which may have been attacks of hyperamonemia. During the neonateal period she had a bout of vomiting attributed to cow milk intolerance. The second episode occurred one year prior to her fatal illness and lasted three days – she was lethargic and sleepy by day, restless at night, and had attacks of unresponsiveness, vacant staring, and upward eye rolling. When she developed ataxia she was admitted to hospital in another city, but by the time that she was examined the only abnormal feature was lethargy. During her six-day hospital stay she behaved normally, but an electroancephalogram showed gross slowing bilaterally, most marked in the posterior region of the left hemisphere. She was treated with diphenylhydantoin and two weeks later the EEG showed improvement. Diphenylhydantoin therapy was continued. There had been no exposure to DEET immediately prior to that episode.

At the time of her final admission the child appeared ill and disorientated. The weight was 17.4kg, height 110cm, temperature 36.6°C, pulse 66 beats per minute, and blood pressure 108/68mm Hg. The abnormal physical findings were periumbilical tenderness, moderate ataxia of gait and finger to nose movements, and brisk knee and ankle reflexes. The laboratory findings included normal blood levels of glucose, calcium and urea. The WBC count was 8000/mm3 with 39% neutrophils, 49% lymphocytes and 12% monocytes. The CSF glucose concentration was 60mg/dl, protein 20mg/dl, and WBC 1/mm3. For the first 72 hours in hospital the patient was permitted clear fluids only. Diphenylhydantoin therapy was continued. She improved gradually and on the third day a normal diet was reintroduced but sparingly eaten.

In the evening of the fourth hospital day she became agitated, started screaming, and became combative. Her behaviour was ascribed to an emotional disturbance and she was treated with 425mg chloral hydrate twice during the evening. Her condition deteriorated and she became progressively disorientated. The CSF contained red blood cells, 7/mm3, glucose 90mg/dl and protein less than 20mg/dl. Her liver was now palpated 4 cm below the right costal margin. The SGOT was 43 IU, bilirubin concentration less than 1mg/dl, prothrombin time 13 seconds (control 11), and the partial thromboplastin time 37 seconds (control 38). The serum ammonia concentration was 647µg/dl and the urine contained orotic acid 2.2mg/mg creatinine (normal – less than 10µg/mg creatinine). A differential

diagnosis of Reye syndrome or congenital OCT (ornithine carbamoyltransferase) deficiency was made.

On the fifth day in hospital she could not be roused, had occasional decerebrate posturing, and the pupils were dilated but reactive to light. Generalised convulsions developed, which were controlled with paraldehyde intramuscularly and pentabarbital intravenously. The trachea was intubated and she was maintained by assisted ventilation. Dexamthesone and 20% mannitol solution were given intravenously to treat cerebral oedema. Enemas were given, and MgSO4 and neomycin were administered by gastric tube. Peritoneal dialysis was begun 11 hours after the onset of coma. Despite these measures the serum ammonia concentration rose to 1852µg/dl, SGOT 165IU, SGPT54, and alkaline phosphatases 228IU. The orotic acid excretion was 3.6mg/mg creatinine. EEG's on the seventh and eighth hospital data were flat, indicating cerebral death and supportive therapy was discontinued eight days after admission.

Necropsy showed a soft, oedematous brain weighing 1460g. The cerebellar tonsils were necrotic and portions of necrotic cerebellum had herniated down around the spinal cord. Microscopic sections showed axonic neuronal change. Alzheimer type II astrocytes were present in the putamen. Overall cell preservation was poor, as would be expected after two days of life on a respirator.

The liver was enlarged (642g). Its gross appearance was normal. Microscopic examination showed that most liver parenchymal cells were foamy and contained abundant parenchymal glycogen in periodic acid-Schiff stain (PAS and PAS diastase stains). Centrilobular liver cells were vacuolated and contained fat dispersed in fine droplets on oil red 0 stain of frozen sections. Electron microscopic examinations of a liver biopsy taken just before death showed mild mitochondrial pleomorphisms, possible loss of dense bodies, and minor abnormalities of cristae. The smooth endoplasmic reticulum was distended and contained an unidentified material. The appearance of the liver suggested a non-specific hepatic injury, and was not that of Reye syndrome.

Virology tests showed no viruses were found in cell culture of specimens of stool, liver, and cerebrospinal fluid on two occasions. There was no evidence of infection by parainfluenza, adenovirus, herpes, influenza A or B, measles or mumps in the complement fication tests on serum taken on the second and sixth days after admission.

Tests on the post-mortem liver tissue indicated that the OCT activity was 10% of the normal adult mean. Phthallyl alcohol was identified as an unusual constituent by gas-liquid chromatography and mass spectrometry.

A follow-up to obtain family information revealed that the parents had another girl three years after the death of the patient. At eight months old the sister was apparently normal. The urea cycle enzyme activities in a biopsy of the mother's liver were all in the low normal range except for that of OCT, which was below normal (35% of the normal adult activity). This level was considered to be consistent with the diagnosis of a carrier of OCT deficiency.

Classification: 3

Case Report 11:   (Pronczuk de Garbino & Laborde 1983)
A 17 month-old female child was admitted to an Uruguayan hospital with acute encephalopathy of unknown origin. During the three weeks prior to admission she had received frequent topical applications of a lotion containing an unknown concentration of

DEET. Her condition rapidly deteriorated with a fatal outcome that prevented a complete toxicological study. Surgical and medical causes were eliminated and no micro-organisms were isolated from the CSF, DEET toxicity was strongly suspected to be the cause.

Classification: 3

Non-Fatal

Case Report 12: (Zadikoff, 1979)

An 18-month-old girl was admitted to hospital after ingesting an unknown quantity of a 10% DEET product (Mylol liquid) the previous day. She was extremely irritable and opisthotonic with abnormal movements and shaking and crying spells on admission. She appeared unaware of her surroundings. She had been well except for an upper respiratory infection the week before this episode. Family history was negative. On examination, no localising neurologic deficit could be found and she responded purposefully to noxious stimuli. Head control was poor. It was difficult to assess muscle tone due to continuous movement but was thought to be normal. Muscle flex reflexes were depressed. The remainder of the examination was normal.

Cerebrospinal fluid was abnormal with 25 lymphocytes/mm3, protein 28mg/dl, and sugar 62mg/dl. No bacteria, fungi, or viruses were isolated from the spinal fluid or blood. Blood urea, sodium, potassium, chloride, calcium, magnesium, lead, and sugar values were normal. Urine was normal, with no organisms being isolated and no aminoaciduria. Electroancephalogram, skull radiograph, and brain scan were normal.

She improved slowly during the six weeks she was in hospital – her movements became more controlled, and she was no longer irritable. Head control was not yet normal, but her gait, although ataxic became progressively steadier. Tendon reflexes remained depressed. She was discharged to a hospital nearer her home for further convalescence.

Classification: 2

Case Report 13: (Gryboski *et al*., 1961)

A $3^1/_2$ year-old girl was admitted with the chief complaint of "shaking and crying spells" of 1 day's duration. She had been well until the previous day, when in the morning she was found on the floor. It was thought that she might have fallen although she appeared well. Behaviour was normal throughout the day until the evening when she was again found sitting on the floor. After this time, however, she walked with her back extended and her head inclined to the right. She appeared unusually sleepy and her speech was slurred. A few minutes later the 1st "shaking and crying spell" was noted in which she cried in a tremulous voice, the face became flushed and the arms and legs stiffly extended, with flexion of the fingers and dorsiflexion of the toes, while she sat up stiffly. This episode lasted for approximately 2 minutes, after which she lay back, became quiet, but was apparently confused, appearing awake but not recognising her parents. She spoke incoherently and responded inappropriately.

Similar episodes occurred every 5 or 10 minutes, lasting up to 2 minutes each, throughout the night and following day. She gradually became more lethargic and less responsive between the episodes.

There had been no childhood diseases, trauma, allergy or bleeding disorder. Physical and mental development was normal. She had walked at 10 months and talked in sentences at 18 months of age. Childhood immunisations (3 diptheria-pertussis-tetanus injections, 3 poliomyelitis vaccine injections and smallpox vaccination) had been completed during the

1st year, and there had been no recent injections. The appetite was normal, and there was no known ingestion of a toxic substance or drugs.

Because mosquitoes were numerous about the house, the child, her bedding and nightclothes had been sprayed every evening with "OFF" during the 2 weeks before the illness. A total amount of 180ml (6 ounces) had been sprayed. 'There was no evidence of insect bites.

The family history was not significant. There was no relative with convulsive disorder or neurologic disease. Other members of the family were well.

Physical examination revealed a well-developed girl weighing 14 kg, who was having spells as previously described. She seemed to be in pain at these times. Episodes occurred spontaneously every 5 minutes, but became more frequent with stimulation by pain or noise. Between attacks she was awake but did not recognise her parents. Speech was slurred and its content inappropriate. She walked and climbed, but without apparent purpose. There was a recent bruise, 1 cm in diameter, on the left temple. The pupils were equal at 5 mm in diameter and reacted well to light and in accommodation. The retinal vessels seemed normal, and the disk margins were well defined. Deep tendon reflexes were symmetrical but hyperactive. The plantar responses were extensor. No abnormalities of the cranial nerves were detected, and there was no nystagmus or other positive cerebellar signs. Trousseau, Chvostek and peroneal signs were negative. The mucous membranes of the nose and throat were normal. No abnormality of the heart and lungs was detected. The abdomen was soft, with normal bowel sounds, and no masses were palpated. There was no gross lymphadenopathy.

The temperature was 99°F (37.2°C) by rectum, the pulse 100 per minute, and respirations 36. The blood pressure was 160/ 130.

The haemoglobin was 12.4g/100 ml, the packed red-cell volume 38%, and the white-cell count 10,000, with 74% neutrophils (including 2 non-segmented forms), 24% lymphocytes, 1% monocytes and 1% basophils. The stained blood smear showed adequate numbers of platelets and normochromic, normocytic red blood cells, with no evidence of basophilic stippling. The urine was clear, yellow and neutral, with a specific gravity of 1.030. Protein and sugar were not present, but the nitroprusside test for acetone was strongly positive. Centrifuged and uncentrifuged urinary sediments were negative. The Sulkowitch test was positive (++). No coproporphyrins were present as determined by the Watson-Schwartrz modification of the Ehrlich determination.

Lumbar puncture on the day of admission revealed an opening pressure of 110mm of water. The cerebrospinal fluid was clear and colourless, and contained a protein of 75mg and a sugar of 50mg/100 ml and 1 lymphocyte/mm3. A stained smear was negative for bacteria.

Nose and throat cultures grew out normal flora, and bacteria cultures of the blood and spinal fluid were sterile. Viral cultures of the throat, blood, stool and cerebrospinal fluid were negative.

The blood urea nitrogen was 11mg, the sugar 124mg, the calcium 12mg, and the phosphorus 4.5mg per 100ml. The carbon dioxide was 19.9millimols, the chloride 108milliequiv., the sodium 142milliequiv., and the potassium 4.5milliequiv. per litre. The alkaline phosphatase was 8.3 Bodansky units per 100ml.

Fluids were administered intravenously as 100ml of equal parts of physiologic saline solution and 5% glucose over a 4-hour period, followed by a solution of 5% glucose containing sodium (40milliequiv), potassium (35milliequiv), lactate (20milliequiv),

chloride (40milliequiv) and phosphate (15milliequiv.) per litre, at a rate of 1500ml per 24 hours.

Sixty milligrams of amylobarbitone (amobarbital) intravenously was effective in quieting the patient for 2 hours, after 1g of calcium gluconate given intravenously had had no apparent effect. Intravenous administration of amylobarbitone (30 to 40mg) repeated every 4 to 6 hours through the night, controlled activity. The blood pressure declined to 130/90 and remained stable.

The patient slept throughout the 2nd hospital day requiring no sedation, but by evening she became restless and exhibited brief stiffening of the extremities; 100 mg of sodium diphenylhydantoin was administered rectally, and 30mg of amylobarbitone intravenously. She remained quiet throughout the night.

She awoke on the morning of the 3rd hospital day responsive and alert, but with occasional tremors of the extremities. Intravenous therapy was discontinued, and she took fluids by mouth in adequate volume. She walked at first on a broad base, but by evening the gait was normal and speech no longer slurred.

By the 4th hospital day the deep tendon reflexes had become normal, and Babinski signs were no longer elicited. Lumbar puncture on this day revealed an opening pressure of 90mm of water. The cerebrospinal fluid was clear and colourless, with protein of 18mg and sugar of 77 mg per 100 ml and chloride of 127milliequiv per litre. No cells were present, and viral and bacterial cultures were again sterile. Electroencephalography was not performed.

The patient was discharged, apparently well, 3 days after admission. She continued to be well 15 months after the initial illness.

Classification: 3

Case Report 14:   (Roland *et al*., 1985)

An 8-year old girl was referred to hospital because of recurrent seizures and behaviour change. Four days prior to hospitalisation she had begun to apply copious amounts of a 15 % DEET preparation (OFF!®). Two days later a raised, erythematous, pruritic rash developed, mainly on the face and extremities, i.e. where the lotion had been applied. At the same time altered behaviour was noticed with unusual restlessness, but no headache vomiting, photophobia or neck stiffness.

The next day the child started to use Muskol® repellent (~100% DEET) as substitute. During that night she had a brief generalised convulsion, with clonic movements of all limbs. Five hours later she had a second seizure and was rushed to the local hospital, where she was noted to be drowsy and vomiting. A third seizure was witnessed in the emergency department. She was given a loading dose of phenytoin intravenously and transferred to another hospital for further investigation and management.

Two years earlier she had been treated for eoccidioidomycosis, diagnosed by means of lymph node biopsy. Amphotericin B had been administered at full dosage along with ketoconazole. She had since remained completely well. Her mother mentioned that she had "sensitive skin" and allergic rhinitis related to pollens and grasses. Otherwise she had no history of previous seizures or trauma. Birth and early development had been normal.

The family history was unremarkable, with no evidence of seizure disorder or other neurologic disease. The other family members were well.

The child was alert and oriented, but irritable. She was afebrile. Her pulse rate was 110 beats/min, her respiratory rate 28/min and her blood pressure 130/70 mm Hg. She had an erythematous, coalescent, maculopapular rash over the face and extremities, with sparing

of the trunk. There was no evidence of insect bites. A neurologic examination yielded nothing abnormal.

Blood specimens revealed a leukocyte count of 8.4 X 10^9/L (62% neutrophils, 20% lymphocytes, 9% eosinophils and 9% monocytes) and normal levels of glucose, electrolytes, calcium, magnesium, phosphate, ammonia, urea nitrogen and creatinine. Liver function studies had normal results. The serum phenytoin level was appropriate. The urine contained blood and occasional hyaline casts; screening for toxic drugs had negative results. Chest x-ray films were normal. The cerebrospinal fluid (CSF) had normal levels of protein (0.25g/L) and glucose (64mg/L) [3.6mmol/L] and no cells. Blood and CSF cultures for bacteria and fungi yielded no microorganisms.

The child's electroencephalogram was abnormal, with poorly organised, slow background activity (frequency 5 to 7 Hz) and frequent multifocal δ, θ and bifrontal sharp waves. Hyperventilation, photic stimulation and drowsiness did not elicit any further abnormalities.

The child was observed in hospital while receiving maintenance phenytoin therapy. Over the ensuing 2 days the restlessness settled and the rash faded. No further seizures occurred; the anticonvulsant medication was stopped, and the child remained well.

Classification: 2

Case Report 15:   (Lipscomb *et al*., 1992)

A healthy five-year-old boy experienced a major motor seizure during an afternoon nap at a day camp. There was no history of fever, trauma, seizures or vomiting. An examination one-year before the seizure detected mild delay in gross motor skills and co-ordination with behavioural difficulties in group settings and good reading skills. His only drug exposure was the virtual total application that morning of the insect repellent preparation containing 95 % DEET (Muskol®) and a later exposure to another DEET-containing preparation (OFF!®). The patient continued convulsing in the emergency department and was treated with diazepam prior to respiratory arrest. Mechanical ventilation was required until next morning. Laboratory tests were unremarkable, as were lumbar puncture, computed tomography scan and blood cultures. Skin decontamination was performed and two 25g doses of activated charcoal in sorbitol were given. An EEG on the third day indicated a diffuse encephalopathy. Occasional epileptiform discharges were present in the right posterior head region. A urine sample collected between nine and 30 hours after hospitalisation contained 0.003ìg/ml DEET. He had no further seizures and a repeat EEG three weeks later was normal.

Classification: 3

Case Report 16:   (Oransky, 1989)

In June-August 1989 generalised seizures with a temporal association with topical use of DEET were reported in four boys aged 3-7 years and one 29-year-old man in new York and Connecticut. Only limited details were available of these cases. Four had had fewer than three applications (concentrations not stated), seizures occurred within 8-48 hours of the last application. All had few prodromal symptoms and recovered quickly. They all had unremarkable medical histories with no previous neurological problems. All had normal non-focal neurological examinations after the event and four had normal laboratory examinations and normal CT and/or NMRI investigations. One case developed urticaria before the seizure and one other developed urticaria in reaction to phenytoin administered during treatment.

Classification: 2

Case Report 17:   (Tennenbein, 1987)

A 14 year old native Canadian girl was witnessed to ingest the contents of a 50ml bottle of insect repellent (95% DEET, 5% related toluamides). Within 30 minutes she was unconscious whereupon she was taken to the nearest hospital 2 hours away. On arrival, her spontaneous respiration rate was 30/min; pulse rate was 90beats per minute; and systolic blood pressure was 60mmHg. She was hypertonic with positive Babinski signs in both feet. She was intubated and transferred to a hospital two hours away. En route she required vigorous crystalloid and colloid infusions to maintain a blood pressure of 70/40 to 80/40mmHg

On arrival her pulse rate was 100 beats per minute; respiration rate was 52/min and irregular. She responded to painful stimuli and had pupils that were fixed and dilated. Mechanical ventilation was instituted. Approximately 90 minutes later she had a generalised seizure that was successfully treated with diazepam and phenobarbital sodium. Tremors persisted for the next two hours. She gradually improved over the next 30 hours, at which point she was extubated.

Extensive clinical investigations uncovered no other aetiology for her illness. Her convalescence was complicated with a left lower-lobe pneumonia and a blood culture positive for *Streptococcus Pneumoniae* in day 2. At discharge ten days after admission she was physically and neurologically normal. Ten months later it was believed that she had no neurological sequelae.

Classification: 1

Case Report 18:   (Tennenbein, 1987)

After consuming several bottles of beer, a 16-year old girl was found with an empty 50ml bottle of insect repellent (95% DEET, 5% related toluamides) and three other empty cosmetic bottles, all alcohol based. On arrival at hospital, her respiration rate was 20/min; pulse rate, 100 beats per minute; and blood pressure 90/50mmHg. She was comatose, flaccid, and unresponsive to painful stimuli. There were no corneal, blink, gag, or deep-tendon reflexes. Her stomach was lavaged after her trachea was intubated. She was given charcoal and a saline cathartic. Forty-five minutes after arrival she responded to painful stimuli. After another 45 minutes she answered simple questions. Over the first three hours her blood pressure gradually rose to 100/70mmHg, where it stabilised. She was functioning normally at 30 hours after admission when she discharged herself against medical advice.

Extensive laboratory investigations uncovered no other aetiology for her illness. Her blood alcohol level was 28mmol/L (130mg/dL) and low volatile organic compounds were detected in both the urine and gastric contents. This is consistent with, but not specific for the presence of DEET. No drugs were found in the blood, urine or gastric contents.
Classification: 2

Case Report 19:   (Tennenbein, 1987)
A 1-year-old girl was found limp and unresponsive but with normal colour. An empty 50ml bottle of insect repellent (47.5% DEET, 2.5% related toluamides) and a feeding cup with strong odour of insect repellent was found nearby. It was discovered that the girls 3½ year old brother had emptied the contents of the 50ml container into the cup and fed it to his sister. The bottle was estimated to be half full. On the way to hospital, the child had a brief (less than one minute) seizure. In the hospital, she responded poorly to physical stimulation. Her stomach was aspirated and was transferred to another hospital, however, on route, she experienced brief intermittent paroxysms of stiffening.
On arrival (approximately 90 minutes after ingestion), her heart rate was 120 beats per minute; respiration rate, 24/min; temperature, 36°C; blood pressure, 108/70mmHg; weight 10kg. Her level of consciousness was depressed but she responded to stimulation. She was hypertonic with intermittent opisthotonic spells. Administration of diazepam and phenytoin sodium reversed these findings. Activated charcoal and a saline cathartic were administered via a nasogastric tube. During her 40-minute stay in the emergency department, she experienced four more brief (less than one minute) clonic spells.
Five hours after transferring to intensive care, she had one more clonic episode that lasted for 40 seconds. However, her level of consciousness improved and she was noted to be alert and responsive two hours later (approximately 8 hours after ingestion). Extensive clinical investigations uncovered no other aetiology for her illness. The following 20 hours in the hospital were uneventful, after which she was discharged.
Classification: 1

Case Report 20:   (Edwards & Johnson, 1987)
An 18½-month old girl with progressive weakness and ataxia was hospitalised. The patient had been healthy until nine days before admission when the parents noted that she was walking as if she were drunk. The gait demonstrated was wide-based ataxic without staggering. The problem persisted without deterioration over the next several days. Six days before admission pharyngitis and otitis media were diagnosed and treatment with amoxicillin was begun. The child's condition did not improve and on the day of admission she began having bizarre movements of the arms, head and legs. She had uncontrollable coarse tremors, and upon admission she was unable to stand or crawl. The parents noted increased drooling, however there was no ptosis or facial drooping. The parents also noted the development of left esotropia. The patient was having no breathing or swallowing difficulties.
On questioning the parent, it was ascertained that the patient had been sprayed with a DEET-containing insect repellent (20% DEET, Deep Woods OFF!), daily for approximately three months before admission. The parents also said that the child often licked her skin after application.
On physical examination the patient was very agitated and crying, with prominent coarse tremors and opisthotonic posturing. The patient was afebrile. Her pulse rate was 120

beats/minute, and her systolic blood pressure was 110mmHg. A neurological examination showed no abnormalities.

Serum laboratory values obtained on admission showed an elevated white blood cell count of 16,900/cu mm (24% neutrophils, 69% lymphocytes, 1% bands, 6% monocytes) and normal concentrations of electrolytes and glucose. Liver-function tests were within normal limits. The cerebrospinal fluid (CSF) contained normal concentrations of glucose (59mg/dL) and protein (17mg/dL) and contained red blood cells (42/cu mm) and white blood cells (14/cu mm; 66% lymphocytes, 34% histiocytes). A Gram's stain of the CSF was negative. Qualitative analysis of the urine by gas chromatography was positive for diethyltoluamide.

On admission the patient was maintained on intravenous fluids and diazepam to control agitation. The patient's condition improved minimally during hospitalisation. Coarse tremors continued, with increased crying spells, opisthotonos and nystagmus. Eight days after admission, treatment with corticotrophin was begun in an attempt to manage opsoclonus-myoclonus. The patient was discharged on these medications 11 days after admission, to be followed by her paediatrician. At this time the patient could sit with assistance and feed herself; however, she continued to experience myoclonic jerking, opsoclonus, and pronounced irritability. The patient's symptoms slowly resolved over the next three months with no apparent neurological sequelae.

Classification: 1

Case Report 21:   (Briassoulis *et al*., 2001)

An 18-month-old boy, who had been previously healthy, presented to a community health centre with generalised seizures and respiratory difficulties. During the morning, the child was noted to be drowsy, irritable, and vomiting. Soon he had generalised convulsions and respiratory difficulty and at that point was rushed to the health centre. More seizures were witnessed and he was given diazepam rectally and a loading dose of phenytoin intravenously. Pupils were equal and reactive to light. There was no focal neurologic deficit and the eye grounds were normal. He continued to have generalised seizures and respiratory difficulties. His pulse rate was 110 beats/min and SaO_2 was 72%. He became comatose and responded only to painful stimuli. His trachea was intubated and mechanical ventilation was instituted. Following intubation and stabilisation, the patient was transferred to another institution for further management.

Family history was negative, with no evidence of seizure disorder or other neurologic disease. The other family members were well. Patient's development was normal until this episode. Immunisations were up-to-date. Past medical history was uncontributory. There was no history of seizures, trauma, or other childhood illness. However, the family had camped in the countryside and the last night, because mosquitoes were numerous about the tent, the child had been applied with Autan, an insect repellent, containing 17.6% DEET (Bayer AG, Germany).

On arrival, his pulse rate was 160 beats/min, his blood pressure 100/60 mm Hg, and temperature 37°C. He was hypertonic with intermittent opisthotonic spells. Pupils were equal and reactive to light. There were no rashes. A chest roentgenogram and an electrocardiogram were normal. Admission biochemical and blood tests were unremarkable. Calcium, urea nitrogen, creatinine, and ammonia were normal. Arterial blood gases were normal (pH 7.37, pO_2 19 kPa (144mm Hg), PCO_2 4.8kPa (36mm Hg), HCO_3 18mmol/l, SaO_2 99%). Blood specimens revealed a leukocyte count of 10.4 x 10^9/l (56% neutrophils, 36% lymphocytes, 8% monocytes). The cerebrospinal fluid (CSF) had

normal levels of protein < 20mg/dl, glucose 50mg/dl, and two cells. Blood and CSF cultures for bacteria and fungi yielded no microorganisms. Virus titre determination in the serum and polymerase chain reaction of blood samples for the detection of viral genome was negative, as were mycoplasma titres. The laboratory investigation of metabolic disease was also negative. The child's electroencephalogram (EEG) had slow background activity without discharges (postictal). Computed tomography scan (CTS) of the brain was normal. Next day, the patient was extubated. He was responsive and alert and neurologically normal.

The following 7 days in the hospital were uneventful, after which he was discharged home. EEG after a week was normal, and the child remained normal. The patient was followed in the outpatient clinic and continued to do well. He continued to be well 9 months after the initial illness.

Classification: 2

Case Report 22:   (Petrucci & Sardini, 2000).
During the night, a 10kg, 3-year-old girl was found unresponsive and vomiting. The mother had been applying amounts of insect repellent (20% DEET in alcoholic solution, Autan®) to the girl's skin. The girl was reported to having ingested a volume of the insect repellent prior to sleeping. At hospital, three hours after ingestion, the girl appeared comatose with no verbal response, had weak withdrawal of the limbs to painful stimuli, spontaneously breathing at 28 breaths per minute with a Glasgow Coma Score (GCS) of 6 (1 eye response, 1 verbal response, 4 motor response). Her pupils were reactive to light, but left horizontal nystagmus was present. Pulse rate was 126 beats per minute, and her systolic blood pressure was 95mmHg. Cervical rigidity and Kernig's sign were absent, and no skin lesions were observed. The patient was afebrile.

The mother provided a half-empty, 20ml bottle of insect repellent, which she had opened 2 days before. It was estimated that about 800mg (4ml of 20ml 20% DEET solution) had been ingested. The girl was transferred to an intensive care unit where she became hypotonic with opisthotonic episodes, followed by three brief (less than 1 minute), generalised seizures with clonic movements of the facial muscles. These were successfully treated with diazepam and assisted ventilation with 100% O_2. Blood specimens revealed normal leukocyte count and levels of glucose, electrolytes, calcium, urea nitrogen, ammonium and creatine. Liver function studies and ECG were unremarkable. A head CT scan did not demonstrate any lesions. The level of consciousness improved over the next 4 hours, and the child was alert and responsive 10 hours later. A full neurological examination yielded nothing abnormal, and she was discharged following and uneventful 24-hour stay.

Classification: 2

3.10.2.1 Summary of Case Reports of Toxic Encephalopathy

The cases involving DEET -related encephalopathy are summarised in the table below:
Examination of these reports reveals that two cases (17 & 18) involved teenagers, not children, and were attempted suicides/parasuicides. One of the reports recorded in case report 16 involved a 29-year-old male, and case report 19 involved the deliberate (but unintentional) poisoning of a young child. The remaining 12 case reports involved seven girls (three cases were fatal) and five boys aged between one and eight years old who

were mostly subjected to excessive doses and/or prolonged periods of exposure. According to the classification criteria, most case reports would be considered as likely or confirmed reports of DEET poisoning, due to excessive and/or prolonged exposure.

It should be noted that of the fatal cases, all were young females, which could indicate an age and sex susceptibility to DEET. This is in line with the findings of Verschoyle *et al.* (1992) who found that the LD_{50} in rats was dependent on age and sex.

Case Report	Sex and Age of Individual	Chemical form and concentration	Route/Dose	Exposure	Results/Comments	Reference
Fatal						
9	F, 5 years	DEET, 10%	Dermal: Dose n.p.	Sprayed daily for 3 months	Abnormal movements and intractable seizures. Death after 24 days	Zadikoff (1979)
10	F, 6 years	DEET, 15%	Dermal: Dose n.p.	Daily skin exposure for 10 days	Reye-like syndrome that included lethargy, mood changes, nightmares, vomiting, colicky abdominal pain, headaches, ataxia, disorientation, convulsions, coma and death 8 days after exposure	Heik *et al.* (1980)
11	F, 17 months	DEET Conc. n.p.	Dermal: Dose n.p.	Frequent topical applications of DEET - containing lotion	Acute encephalopathy. Condition rapidly deteriorated leading to death.	Pronczuk de Garbino & Laborde (1983)
Non-Fatal						
12	F 18 months	10% DEET	Ingestion Dose n.p.	Single ingestion	During six weeks hospitalisation, movement and gait improved but head control remained abnormal and deep tendon reflexes depressed	Zadikoff (1979)
13	F, 3½ years	DEET	Dermal:	27 ml for 2 weeks. 180 ml of 15% DEET to skin, night/ bedclothes daily over 2 weeks	Encephalopathy, which included symptoms of tremors, crying spells, confusion, slurred speech, stiffening of extremities and staggering gait	Gryboski *et al.* (1961)
14	F, 8 years	DEET, 15% and 100%	Dermal:	Copious application of 15% DEFT for 2 days followed by application of 100% DEET for one day	Encephalopathy, which included symptoms of tremors, crying spells, confusion, slurred speech, stiffening of extremities and staggering gait. Recovery after 3 days.	Roland *et al.* (1985)
15	M, 5 years	DEET, 95% in Muskol®. % in OFF!® n.p.	Dermal: Dose n.p.	Single application of Muskol® followed by application of OFF!®	Seizures. A urine sample collected 9-30 h after exposure revealed a DEET concentration of 0.003 ìg/ml, (method of analysis n.p.).	Lipscomb et al. (1992)
16	5 M, 3-7 years and 29 years	DEET conc. n.p.	Dermal: Dose n.p.	< 3 topical applications	Seizures 8 to 48 h after application. Physical exams and laboratory tests were normal. One patient developed urticaria before his seizure.	Oransky et al. (1989)
17	F, 14 yr. old	DEET, 95% 5 % related toluamides	Oral: 50ml	Single exposure	Unconsciousness, hypertonia, dilated pupils, and tremors	Tenenbein (1987)

Table 3.16: Summary of Case Reports of Toxic Encephalopathy

Case Report	Sex and Age of Individual	Chemical form and concentration	Route/Dose	Exposure	Results/Comments	Reference
Non-Fatal (cont.)						
18	F, 16 yr. old	DEET, 95% 5 % related toluamides	Oral: 50ml	Single exposure	Comatose state: no corneal, blink, gag, or deep-tendon reflexes. Patient showed a positive response to treatment and was normal 30 h later.	Tenenbein (1987)
19	F, 1 yr	DEET, 47.5%	Oral: 12.5 - 25 ml	Single exposure	Unresponsiveness, seizure, and hypertonia. Patient showed a positive response to treatment with activated charcoal and a saline cathartic and was normal 20 h later.	Tenenbein (1987)
20	F, 18 ½ months	DEET, 10%	Dermal/ Oral Dose n.p.	Sprayed daily with 20% DEET for 3 months	Progressive weakness and ataxia, uncontrollable coarse tremors, increased drooling. Symptoms resolved over three months	Edwards & Johnson, (1987)
21	M, 18 month	DEET, 17.6%	Dermal: Dose n.p.	Single exposure	Drowsiness, irritability and vomiting leading to generalised convulsions and respiratory difficulty and coma.	Briassoulis *et al.*, (2001)
22	F, 3 yrs	DEET, 20%	Dermal/Oral Dose 800mg	Repeated exposure over 1 to 2 days.	Vomiting leading to a comatose state. Eventually hypotonic with opisthotonic episodes with short generalised seizures. Discharged after 24 hours.	Petrucci & Sardini, (2000)

Table 3.16: Summary of case reports of toxic encephalopathy (continued)

3.10.3 Psychosis

Case Report 23: (Snyder *et al.*, 1986)
Three weeks prior to admission, a previously healthy 30 year old man began daily applications of the insect repellent DEET (70% solution). The man used the insect repellent for the purpose of self-medication for a rash, as he believed that it had cured a similar rash four years previously. In addition, application was followed by a 1-2 hour period in a light-bulb heated box. Sedation and incoherence were noted for short periods following each application session. Aggressiveness and psychotic ideation led to hospital admission where he displayed psychomotor hyperactivity, rapid and pressured speech, tangentiality, flight of ideas, and grandiose delusions. Treatment was begun with haloperidol. Clinical improvement was complete within six days, atypical for classic endogenous mania. DEET and metabolites were identified in the urine more than 2 weeks after the last drug application.
Classification: 3

Case Report 24: (Hampers *et al.*, 1999)
A 27 year old, previously healthy man was admitted to hospital with acute onset of confusion and combativeness. While fishing on a very hot (>34°C) and humid afternoon he had applied 25% DEET insect repellent (Off! Deep Woods Spray) to his arms, neck and legs several times prior to the onset of symptoms. Earlier in the day he had applied

Off! Deep Woods Sunscreen Lotion (DEET 20%). He had not used any other DEET-containing products in the preceding two weeks. He first experienced parasthesisas over his limbs and face. He then developed auditory hallucinations ("a loud radio") and became progressively more confused, disorientated, and agitated. He was brought to the hospital one hour after onset of symptoms. On presentation to the hospital, the patient was tremulous, disorientated, and combative. Examination of skin was unremarkable. Neurological examination was non-focal, but the patient was diffusely hypertonic with prominent tremors of his upper and lower extremities. After heavy sedation, he required endotracheal intubation and mechanical ventilation. Over 24 hours he responded to supportive therapy and was weaned from the ventilator. By the third hospital day his mental status had returned to normal, although he complained of headaches.

Classification: 3

Case Report 25: (Leo *et al*., 2001).

The family of a 41-year-old man presented him to hospital with lethargy, headaches, nausea, vomiting, and abdominal pain. He insisted that a sample of vomit brought with him contained worms, despite reassurances from medical staff who found no abnormalities in the contents of his vomit. He reported poor sleep and appetite in recent weeks.

His family indicated that he first made a passing reference to fleas in his apartment 6 weeks earlier, when he mentioned that he sought out an exterminator. At the time, no one had been particularly concerned about the allegation of the flea problem, although in retrospect, no one could recall much evidence to suggest that fleas were present. The man did not own any pets and could not account for why he thought that there were fleas in his apartment.

Family members prompted hospitalisation after the man became increasingly withdrawn and seclusive, his self-care progressively declined, and he kept minimal food in his house. Much of his food and bedding had been discarded for fear that they had become infested with fleas. When approached with this information, he conceded to these beliefs, although he could not account for why he thought this. He believed fumigation had been unsuccessful, which lead to the repeated application of DEET-containing insect repellent on his food, his mattress and himself. More than 20 empty cans of insect repellents were discovered throughout his house. Despite these attempts, the man was convinced that the fleas were not eliminated and insisted that that they were entering his body through his nose and mouth, laying eggs, which would then become the worms that he reported were in his vomit. The repetitive use of the insect repellent accounted for physical symptoms.

Aside from sleep and appetite disturbances, no other significant symptoms of depression or mania were apparent. His mood was slightly depressed, but he attributed this to physical symptoms. His thoughts were goal directed. He denied experiencing auditory or tactile hallucinations (e.g. formication). He denied having any grandiose, paranoid, or persecutory beliefs. Cognitively, there was no evidence of delirium or significant deficits in higher executive functions.

Medical work-up failed to reveal any metabolic abnormalities or evidence of sepsis. Computerised tomography of the head failed to demonstrate any abnormalities. Neuropsychological testing failed to reveal significant cognitive deficiencies suggestive of organicity.

Anti-psychotic medication was not prescribed. The patient's symptoms began to abate within days, and within 2 weeks the psychosis was completely resolved. He resumed usual levels of self-care and had returned to baseline functioning.
Classification: 3

3.10.4 Reproductive Toxicity

Case Report 26:   (Schaefer & Peters, 1992)
A 34-year-old mother of a healthy boy had an uneventful second pregnancy and delivered without complications a boy of 3250g 5 days after the estimated date of birth. During the pregnancy, the mother had been working in Africa and continuously took prophylactic chloroquine against malaria and applied a lotion containing 25% DEET to her arms and legs once or twice a day. It should be noted that chloroquine has been shown to cause teratogenic effects
Normal head circumference, antimongoloid slant of the palpebral fissures, hypertelorism, thin lips, poorly developed philtrum, and a broad nasal bridge were documented. A CT brain scan revealed slight dilation of the ventricles. EEG was normal, as were analyses of chromosomes and screening for inborn errors of metabolism. The family history revealed no genetic disorders.
During the first months of life, an increasing statomotor retardation, muscular hypotonia, central hearing loss and strabismus were observed. These symptoms in connection with a disorder of sensorimotor integration and the morphologic changes did not fit into the pattern of any known genetic disease.
Classification: 4

Case Report 27:   (Hall *et al.* 1975)
This paper contains two case reports within one family. Two male cousins, whose mothers are sisters but with unrelated fathers, were born within two weeks of each other and both had congenital heart disease, which included coarctation of the aorta. The boys' conceptions were calculated to have occurred eight days apart. The pregnancies were uncomplicated except that at about eight weeks from the last menstrual period, all four parents went on a camping trip together (elevation about 3,000 feet). Due to bothersome flies and mosquitoes, all used large amounts of insect repellents and insecticides containing N-octyl bicycloheptene dicarboximide, piperonyl butoxide, allethrin, pyrethrins, diethyl toluamide and 2-2 dichlorovinyl dimethyl phosphate. The commercial products used were OFF Insect Repellent, Raid Insect Spray, Raid Mosquito Coil and Shell Vapona Insect Strip. This was the only time during the pregnancy that the mothers were together. There were apparently no other unusual occurrences during the camping trip, the pregnancies or the deliveries. Neither mother reported any illness in the first trimester.

The first infant was a 3,560g white male born ten days prior to expected delivery caesarean (EDC) to a 25-year-old woman and a 24-year-old man who already had a healthy $2^1/_2$ year old son. The mother was in good health with no cardiac problems. The father had a murmur and enlarged heart as a child. His physical examination and cardiac status were entirely within normal limits at the time of the birth of the patient. There was a paternal uncle who died in infancy of multiple congenital anomalies. The mother's

father's brother had had a heart murmur when young, which he had outgrown. The mother had used an IUD prior to conception. The baby initially did well and no murmur was noted in the nursery. At about 4 weeks of age, he began to have difficulty feeding and lost weight over the ensuing month. He was admitted in congestive failure at 2 months of age. Cardiac catheterisation, pressure measurements and cineangiography revealed a 2.5:1 shunt through a ventricular septal defect, a poorly contractile left ventricle and a moderate preductal coarctation of the aorta just distal to the left subclavian artery. He was digitalised and has remained compensated. The remainder of his physical examination was normal.

The second infant was a 3,232g male born three days after the EDC to a 28-year-old woman, the sister of the mother of the infant in case 1, and a 31-year-old man. There had been no previous children or abortions. The mother had been on birth control pills until just prior to conception. No other family history of congenital malformations or of cardiac problems was obtained. At birth, a murmur was noted. He had a haematocrit of 75% and became symptomatically hypoglycaemic over the first 24 hours with blood glucose down to 23mg/100 ml. He was treated with phlebotomy and intravenously administered glucose. Over the next week, he went into congestive failure but responded to digitalisation. Cardiac catheterisation at 1 month of age showed aortic valvular stenosis with poststenotic dilatation, a poorly contractile hypertrophied left ventricle and a moderate to severe coarctation of the aorta just distal to the left subelavian artery. At age 38 clays, he had sudden collapse while being played with in a crib. Cardiopulmonary resuscitation failed. Post-mortem examination revealed preductal coarctation of the aorta (without a web-like enfolding of the aortic media, but rather a uniformly narrow segment between the subclavian artery and ductus arteriosus), stenotic hypoplastic aortic valve, biventricular subendocardial fibrosis and moderate islet hypertrophy of the pancreas (probably explaining the neonatal hypoglycaemia).

Classification: 3

3.10.5 Other Effects

Case Report 28: (Tennenbein, 1987)
A 33-year-old-woman with a long history of a unipolar-depressive illness was found unconscious and breathing irregularly. Empty prescription bottles of chlorpromazine hydrochloride, hydralazine hydrochloride and an empty 50ml bottle of insect repellent (95% DEET, 5% related toluamides) were found nearby. Ingestion was thought to have occurred approximately one hour prior to discovery. She was taken to hospital where she was found to be deeply comatose with a pulse rate of 80- beat per minute and a blood pressure of 80/50mm Hg. Emesis with the strong odour of insect repellent was apparent. Her trachea was intubated and she underwent gastric lavage and activated charcoal administration and was transferred to another hospital. On arrival she was comatose and pulseless. She was resuscitated and admitted to the intensive care unit, where she required vigorous crystalloid, colloid, and a catecholamine infusion to maintain a systolic blood pressure of 40 to 60mmHg. Hemodialysis and charcoal hemoperfusion were carried out. During the first 24 hours there was evidence of generalised seizure activity. On the second day, the radiological finding of intramural bowel gas prompted a laparotomy that revealed massive generalised bowel infarction incompatible with life. She died later that

day. The following DEET tissue levels were obtained: gastric lavage returns, 0.54mmol/l (10mg/dl); blood, 0.88mmol/l (16.8mg/dl); post-mortem blood, 0.58mmol/l (11.2mg/dl); and liver, 0.92mmol/kg (17.7mg/dl). Results of the drug screen were normal.
Classification: 4

Case Report 29:   (Tennenbein, 1987)
A 26 -year-old man after a bout of drinking, was found dead with an empty 50ml bottle of DEET (95 % DEET, 5% related toluamides). Tissue levels reported were, blood 1.25 mmol/l (24mg/dl); vitreous, 0.78 mmol/l (15mg/dl); urine, 0.52 mmol/l (10mg/dl). His blood ethanol level was 28mmol/l (130mg/dl) and his urine contained cannabinoids.
Classification: 5

Case Report 30:   (Clem *et al*., 1993)
A 61-year-old woman developed light-headedness and presyncope after coating herself with sunscreen and spraying on a DEET-containing insect repellent. There was no loss of consciousness, however she reported that she was unable to speak and developed nausea, vomiting and explosive diarrhoea. At hospital, her BP varied from a palpable systolic of 70 to 100/60mmHg, and she had orthostatic hypotension and was observed to be in obvious distress with noticeable shaking chills. She denied chest pains or palpitations. She also denied any recent upper respiratory infection, abdominal pain, melena or hematemesis.
On examination, she was found to have relative hypotension and orthostatic change in BP. Heart rate was 70 beats/minute and respiration rate was 18 breaths/minute. The rest of her physical examination was unremarkable except her skin was warm to the touch. An electrocardiogram showed marked sinus bradycardia (heart rate 44 beats/minute), but was otherwise normal. A repeat ECG one hour later revealed sinus rhythm at a rate of 64 beats/min and no abnormalities. The patient became completely asymptomatic and had a stable BP several hours after admission. She was discharged the following day in her usual state of health.
Classification: 5

Case Report 31:   (Fraser *et al*., 1995)
A 19-year-old woman with a history of psychiatric disorders was admitted to the emergency department within 1 hour after ingestion of 15-25ml of the insect repellent Muskol®. On admission the patient was drowsy and had a blood pressure of 90/60mmHg, a heart rate of 100 beats/minute, and was intubated to protect the airways. She was transferred to an intensive care unit. An electrocardiogram recorded shortly after admission indicated right and left atrial enlargement, diffuse STT abnormalities, and a normal QT interval. Within 12 hours, the EKG returned to normal, and by 24 hours, there were no cardiac abnormalities. The patient was extubated and transferred to a different hospital for psychiatric assessment. The routine serum and urine drug screening procedure was negative. DEET was qualitatively identified by GC-MS.
Classification: 2

3.10.6 Reports from U.S. Poison Control Centres

A retrospective analysis of calls to poison control centres resulting from exposure to the insect repellent DEET was made in the U.S. from 1985 to 1989 (Veltri *et al.*, 1993). In total, the number of human exposures was 9,086. Nearly two thirds of those exposed had no adverse effects or only experienced minor symptoms that resolved rapidly. Symptoms were more likely to occur if the product was ingested. Examples of reports are given below.

A 17-year-old male apparently saturated his clothing with an insect repellent containing 17.9% DEET. The patient arrived at hospital stumbling and may have passed out or had a seizure prior to arrival. The poison control centre undertook a follow-up with the medical facility about 13 hours after the call and at that time the patient had been discharged. No anti-convulsants were administered. No other follow-up was obtained.

A poisons centre was called by a health care professional at the home of a 33-year-old male who may have inhaled the fumes from an insect repellent containing DEET while he was applying the product. The product was estimated to be 6 years old. The patient's exposure to DEET had occurred about 1 week prior to the call. About one week prior to the patient's exposure (two weeks prior to the call), he was diagnosed as having Raynauds Disease (no information was provided about who made the diagnosis or how the diagnosis was made).
At the time, the patient was reportedly numbed from the neck down, dizzy, light-headed and he was vomiting. The patient's blood pressure was reported to be 60/40mmHg with a heart rate of 37bpm. Mast trousers were put on the patient at the scene and the patient was transported to a local hospital. The poison control centre did a follow-up about 12 hours later at which time the patient was reported as doing fine.

A Poisons centre was contacted about an adult male who had sprayed his entire body with an insect repellent containing 20.9% DEET. The patient was complaining of a strange feeling in his facial muscles and difficulty with speech. In the hospital the patient was observed to have a dystonic reaction, which responded to diphenylhydramine therapy. Upon further questioning it was noted that the patient had taken prochlorperazine earlier in the day.
It was unclear whether the symptoms the patient was experiencing are consistent with exposure to DEET since dystonias are a common side effect of phenothiazine drugs such as prochlorperazine.

A 33-year old man intentionally ingested 8oz of an insect repellent containing DEET (concentration unknown). One to two hours later he experienced a cardiorespiratory arrest from which he was apparently resuscitated. The patient was transported to a hospital where he was intubated and placed on a ventilator. On day 2 of hospitalisation he became hyperglycaemic (blood glucose 250mg/dl), developed status epilepticus and disseminated intravascular coagulopathy. His seizure disorder responded to therapy with phenytoin. His condition deteriorated, he developed cerebral oedema and died nine days after the ingestion.

A more recent review of reports to the US American Association of Poison Control Centres between 1993 and 1997 has been published. The authors evaluated information pertaining to 20,764 exposures involving insect repellents containing DET. Nearly 70% of cases reported no symptoms related to exposure to DEET. It was reported that 26 subjects experienced major effects (13 were adults). Evidence of neurotoxicity was documented in four out of the 13 adults and 6 out of the remaining 13 individuals (three infants, seven children and three teenagers). Two individuals (a 26-year-old male and a 34-year-old female) died. There was little information documented regarding these two individuals. Both had applied DEET topically. There was a report of seizure in the 26-year-old male but it is possible that aspiration of food contents may have been the cause of death as this was identified at autopsy.

3.10.7 Report from US National Park Services

US National Park Service employees in the Florida Everglades, who use DEET regularly in the summer, anecdotally reported episodes of confusion and decreasing sweating while using DEET (McConnell *et al.*, 1985; cited by Robbins & Cherniack, 1986). Responses to a neurobehavioral questionnaire of 143 of these workers indicated a significant increase in certain neurological signs - muscle cramping, insomnia, irritability and depression among those who had an estimated dermal exposure to 4.25 g or more in an average week. Skin rash, blisters and difficulty in starting or stopping micturition were also significantly higher in this group.

3.10.8 Summary of Human Toxicity

An overview of all the published case reports detailing adverse effects documented in individuals exposed to DEET is presented in Table 3.17 below. Thirty-one case reports were found. These predominantly came from the U.S.A. The effects reported have to be considered in the context of the large proportion of the US population regularly using DEET containing insect repellents. There are no formulation details available for these case reports and thus effects of co-formulants cannot be excluded. Eight reports involved dermal effects (although several involved more than one individual), 14 reported CNS toxicity (a total of 18 cases), 3 predominantly psychosis, 2 reported adverse effects on reproduction and 4 reported a range of effects including CNS toxicity and dermal effects (4 cases).

With regard to the dermal exposure cases, there was evidence of severe skin reactions amongst 29/63 individuals in case report 3. It is uncertain what role co-formulants played in these reactions and no information on formulation details are available. The findings from this case report have not been reproduced on such a scale in other reports and are not consistent with the large-scale use of DEET products by millions of people every year. The evidence is suggest that DEET products under normal conditions of use do not cause irritancy but may, in some circumstances where exposure is high and/or prolonged, give rise to severe irritation of the skin.

Table 3.17: Summary of Case Reports of Human Toxicity

Case Number	Sex and Age(s) of Individual(s)	Chemical form and Concentration	Route/Dose	Exposure	Results/Comments	Reference
Dermal Toxicity						
1	Male, 4 years	DEET, conc. n.p.	Dermal Dose n.p.	Single application to entire skin	After 10 minutes, generalised erythema with sever pruritis. Symptoms demonstrated to be via a immunological mechanism	von Mayenburg & Rakoski (1983)
2	Male, 4 years	DEET, conc. n.p.	Dermal Dose n.p.	Single application to legs and forearms	Within minutes, generalised itch and urticaria developed with wheezing and coughing for 30 minutes. Diagnosis was a non-specific cutaneous hyperactivity	Wantke *et al* (1996)
3	1 Male,	DEET, 75%	Dermal Dose n.p.	Single application to the antecubital fossa. Site was covered and the arm flexed.	After 18 hours occlusion, blisters on a tender base that remained intact for 1-3 days. Erosions were left for 2-3 weeks, that were disabling	Lamberg & Mulrennan (1969)
	63 Males, age n.p.	DEET, 75%	Dermal Dose n.p.	Single application of liquid and aerosol preparations to the antecubital fossa.	29 men developed a reaction at the antecubital area. No effect of type of preparation. 3e men developed local necrosis requiring three weeks to heal with scarring.	
	10 Males	DEET, 75%	Dermal Dose n.p.	Single application of liquid and aerosol preparations to the antecubital fossa.	6 men developed blisters or erosions at the antecubital fossa. 2 of these men developed local necrosis and scarring	
4	10 Males, 18-20 years	DEET, 50%	Dermal Dose n.p.	Single application to face, neck, upper trunk, and legs	Sudden inset of burning sensation and erythema of the antecubital fossa of one or both arms, lasting for 2 days. Ulcerations were left in some cases. Diagnoses were made in four subjects of allergy, burns or eczema.	Reuveni & Yagupsky (1982)
5	Female, 42 years	DEET, 42%	Dermal Dose n.p.	Contact with companion who had sprayed himself	Generalised pruritis rapidly developed and progressed to generalised angioedema	Miller (1982)
6	Male, 20 years	DEET, 33%	Dermal Dose n.p.	Single application	Skin eruptions and burning sensations were reported. Areas of erosions and blisters were seen in the right antecubital fossa. Diagnosis was made of irritant contact dermatitis.	Amichai *et al.* (1994)
7	Female, age n.p.	DEET, conc. n.p.	Dermal Dose n.p.	Open patch test after found to be sensitive to DEET	Test was highly positive within one hour, resolving in two days. Suggestion of immunological mechanism	Maibach & Johnson (1975)
8	Female, 16 years	DEET, 20%.	Dermal: Dose n.p.	Open patch test after found to be sensitive to DEET	Soapy cutaneous reactions accompanied by oedema and severe pruritis after using insect repellent. Test was highly positive for DEET (100% and 1/100) Authors suggest immunological mechanism	Vozmediano *et al.* (2000)

Abbreviations: conc. = concentration; d = day(s); F = female; h = hour(s); M = male; min = minute(s); n.p. = not provided; wk = week(s); yr = year(s).

Table 3.17: Summary of Case Reports of Human Toxicity (cont.)

Case Number	Sex and Age(s) of Individual(s)	Chemical form and Concentration	Route/Dose	Exposure	Results/Comments	Reference
Toxic encephalopathy						
9	Female, 5 years	DEET, conc. n.p.	Dermal: Dose n.p.	Sprayed daily for 3 months	Abnormal movements and intractable seizures, unresponsive to treatment. Death after 24 hours	Zadikoff (1979)
10	Female, 6 years	DEET, 15%	Dermal: Dose n.p.	Daily skin exposure for 10 days	Reye-like syndrome that included lethargy, mood changes, nightmares, vomiting, colicky abdominal pain, headaches, ataxia, disorientation, convulsions, coma and death 8 days after exposure	Heik *et al.* (1980)
11	Female, 17 months	DEET Conc. n.p.	Dermal: Dose n.p.	Frequent topical applications of DEET-containing lotion	Acute encephalopathy. Condition rapidly deteriorated leading to death.	Pronczuk de Garbino & Laborde (1983)
12	Female, 18 months	10% DEET	Oral: Dose n.p.	Single ingestion	During six weeks hospitalisation, movement and gait improved but head control remained abnormal and deep tendon reflexes depressed	Zadikoff (1979)
13	Female, 3½ years	DEET, 15%	Dermal: Dose	27 ml for 2 weeks. 180 ml to skin, night/bedclothes daily over 2 weeks	Encephalopathy, which included symptoms of tremors, crying spells, confusion, slurred speech, stiffening of extremities and staggering gait. Recovery after 3 days.	Gryboski *et al.* (1961)
14	Female, 8 years	DEET, 15% and 100%	Dermal:	Copious application of 15 % DEFT for 2 days followed by application of 100% DEET for one day	Brief generalised convulsions, with clonic movements of all limbs. Erythematous, coalescent maculopapular rash on face and extremities. Recovery over two days	Roland *et al.* (1985)
15	Male, 5 years	DEET, 95% in Muskol®. % in OFF!® n.p.	Dermal: Dose n.p.	Single application of Muskol® then application of OFF!® later in the day	Seizures. Mechanical ventilation was required. Occasional epileptiform discharges were present in the right posterior head region EEG normal three weeks later	Lipscomb *et al.* (1992)
16	5 Males, 3-7 years and 29 years	DEET Conc. n.p.	Dermal: Dose n.p.	< 3 topical applications	Seizures 8 to 48 h after application. Physical exams and laboratory tests were normal. One patient developed urticaria before his seizure.	Oransky *et al.* (1989)
17	Female, 14 yr. old	DEET, 95% (5% related toluamides)	Oral: 50ml	Single exposure	Unconsciousness, hypertonia, dilated pupils, and tremors. Slow recovery over 30 hours	Tenenbein (1987)
18	Female 16 yr. old	DEET, 95% (5 % related toluamides)	Oral: 50ml	Single exposure	Comatose state: no corneal, blink, gag, or deep-tendon reflexes. Patient showed a positive response to treatment and was normal 30 h later.	Tenenbein (1987)
19	Female, 1 yr	DEET, 47.5% (2.5% related toluamides)	Oral: 12.5 – 25 ml	Single exposure	Unresponsiveness, seizure, and hypertonia. Patient showed a positive response to treatment with activated charcoal and a saline cathartic and was normal 20 h later.	Tenenbein (1987)

Abbreviations: conc. = concentration; d = day(s); F = female; h = hour(s); M = male; min = minute(s); n.p. = not provided; wk = week(s); yr = year(s).

Table 3.17: Summary of Case Reports of Human Toxicity (cont.)

Case Number	Sex and Age(s) of Individual(s)	Chemical form and Concentration	Route/Dose	Exposure	Results/Comments	Reference
20	Female, 18 ½ months	DEET, 10%	Dermal/Oral Dose n.p.	Sprayed daily with 20% DEET for 3 months	Progressive weakness and ataxia, uncontrollable coarse tremors, increased drooling. Recovery over 3 months	Edwards & Johnson, (1987)
21	Male, 18 month	DEET, 17.6%	Dermal: Dose n.p.	Single exposure	Drowsiness, irritability and vomiting leading to generalised convulsions and respiratory difficulty and coma.	Briassouolis e al., (2001)
22	Female, 3 years	DEET, 20%	Dermal/Oral Dose 800mg	Repeated exposure over 1 to 2 days.	Vomiting leading to a comatose state. Eventually hypotonic with opisthotonic episodes with short generalised seizures. Discharged after 24 hours.	Petrucci & Sardini, (2000)
Psychosis						
23	Male, 30 years	DEET, 70%	Dermal: Dose n.p.	Daily applications followed by 1-2 hours in a light-bulb heated box	Sedation and incoherence for short periods after use. Aggressiveness/psychotic ideation. Recovery after 6 days	Snyder et al (1986)
24	Male, 27 years	DEET 25%	Dermal: Dose n.p.	Application to arms, neck and legs during humid afternoon	Parasthesisas over his limbs and face. Auditory hallucinations confusion, disorientation and agitation. Mechanical ventilation was required. Recovery after 3 days	Hampers et al., (1999)
25	Male, 41 years	DEET, conc. n.p.	Dermal Dose n.p.	Excessive application., details n.p.	Lethargy, headaches, nausea, vomiting, and abdominal pain. Poor sleep and appetite. Delusions were also noted	Leo et al. (2001)
Reproductive Toxicity						
26	Female, 34 years (gave birth to a male)	DEET, 25%	Dermal Dose n.p.	Application to arms and legs twice a day. (Chloroquine also taken)	Baby was born with antimongoloid slant of the palpebral fissures, hypertelorism, thin lips, poorly developed philtrum, and a broad nasal bridge. Increasing statomotor retardation, muscular hypotonia, central hearing loss and strabismus were observed with development	Schaefer & Peters (1992)
27	Female, 25 years (gave birth to a male)	DEET, conc. n.p.	Dermal Dose n.p.	Not determinable	After 4 weeks, loss of weight due to difficulty feeding. Admitted with congestive failure after 4 months. Diagnosed with coarctation of aorta. Family history of heart problems.	Hall et al (1975)
	Female, 28 years (gave birth to a male)	DEET, conc. n.p.	Dermal Dose n.p.	Not determinable	Heart murmur at birth. After a week, congestive failure. Severe coarctation of the aorta was diagnosed. After 38 days e collapsed and cardiopulmonary resuscitation failed.	
Other Effects						
28	Female, 33 years	DEET, 95%	Oral: 50ml	Possible ingestion with chlorpromazine and hydralazine	Unconsciousness, irregular breathing, comatose, pulseless, seizure during first 24 h after exposure, bowel infarction, and death on second day.	Tennenbein (1987)
29	Male, 26 years	DEET, 95%	Oral: 50ml	Ingested with alcoholic drinks	Death. Urine level was 0.52 mmol/l. Drug screening was positive for cannabinoids.	
30	Female, 61 years	DEET, conc. n.p.	Dermal: Dose n.p.	Spraying on insect repellent	Light-headedness, nausea, vomiting, diarrhoea, tremors, chills, and hypotension. Patient returned to normal 24 h later.	Clem et al. (1993)
31	Female, 19 yr. old	DEET, 95%	Oral: 15-25 ml	Ingestion of Muskol®	Cardiac abnormalities, including right and left atrial enlargement 2-h post-ingestion- Patient normal 24 h later.	Fraser et al. (1995)

Abbreviations: conc. = concentration; d = day(s); F = female; h = hour(s); M = male; min = minute(s); n.p. = not provided; wk = week(s); yr = year(s).

4 RISK ASSESSMENT

This section concerns the health risks to users of the insect repellent containing DEET. It intends to give a brief overview of the risk assessments and decisions pertaining to DEET that have been made in the USA and Canada (section 4.1), where the use of DEET is widespread. Finally, this section details issues and concerns that need to be considered for an appropriate risk assessment in the UK (section 4.2).

4.1   Approaches for the Risk Assessment of DEET

USA

From the literature over the past 35 years, the EPA identified case reports where 14 people reported having a seizure as a result of being exposed to DEET. Twelve of these individuals were children and two were adults. After analysing these incidents, the EPA were not able at the time to conclude that the seizures were directly related to DEET exposure. However, the EPA could also not definitely conclude that they were not DEET-related. Due to the fact that anecdotal reports of seizures are difficult to interpret, the EPA considered that one possible explanation for the seizures was coincidence. The EPA suggest that seizures coinciding with DEET should not be unexpected, given an estimated 15,000-20,000 afebrile seizures in children (ages zero-19 years) estimated annually and an estimated 17 million children using DEET 10 times a year.

In addition, The EPA considered the data from the acute and sub-chronic neurotoxicity screening study in rats, which indicated changes in horizontal and vertical activity on treatment with DEET (Schoenig *et al*., 1993). The Toxicity Endpoint Selection (TES) Committee determined that the possible neurotoxic effect in both studies was not robust enough to provide an adequate basis for risk assessment. Reasons given for this decision were that the increase in horizontal activity seen in the 225mg/kg/day (2000ppm) dose of the sub-chronic toxicity test was transient with significance only at the first measuring period, and that the magnitude of the effect was small. Furthermore, the effect was not accompanied by any clinical signs or histopathological changes.

In the EPA's final report, it was decided that based on the currently available information, the use of DEET as an insect repellent did not pose a significant health risk to the general U.S. population. This decision was based on the fact that DEET was not believed to be acutely toxic nor carcinogenic, significantly developmentally toxic nor mutagenic at the doses tested and that the available data did not support a direct link between exposure to DEET and reported seizure incidences.

However, it was also noted by the EPA that DEET is widely used among the U.S. population, including children and it is one of the few residential-use insect repellents that is applied directly to the skin. Furthermore, it has been thought to be associated with incidents of seizure. Therefore, the EPA believed that it was prudent to require improved label warnings and restrictions for DEET products (see section 1.2.3). The EPA believed that such common sense measures would be especially protective of children and other individuals who may be more sensitive to chemical substances.

Finally, in an effort to monitor and understand DEET poisonings, the Chemical Specialties Manufacturers Association (CSMA) set up a DEET registry through an independent research company. Agreements were made with a number of Poison Control Centers (PCCs) to collect information and follow-up on any serious DEET-related cases. The collected information is intended to establish whether any seizure type effects may be related to DEET use (EPA, 1998).

Canada

The Canadian Pest Management Regulatory Agency have carried out a separate risk assessment where consumer exposure potential was estimated using survey data and a usage study (Re-evaluation Decision Document RRD2002-01, Pest Management Regulatory Agency, Canada). Two exposure scenarios were identified for all populations, i.e. acute (occasional use) and intermediate (prolonged use) dermal exposure. In addition, a third scenario was identified for toddlers, aged 6 months to 2 years, which consisted of the possibility of a non-dietary oral exposure resulting from the transfer of residues from the skin to the mouth during hand-to-mouth activities. Age-related susceptibility was not regarded as a significant concern for DEET.

For the acute dermal risk assessment, the Canadian Pest Management Regulatory Agency considered that no appropriate acute dermal study adequately assessed neurotoxic parameters was available. Therefore, the NOAEL from the neurotoxicity study in rats following a single oral dose was used. For the intermediate-term dermal risk assessment, the NOAEL of 300mg/kg bw/d from the 90-d rat dermal toxicity study was used. For the acute incidental (non-dietary) oral exposure in children aged 6 months to 2 years, the end point from the neurotoxicity study in rats following a single oral dose was again used. In all cases, a standard 100-fold margin of exposure (MOE) was required, which accounted for extrapolation between species (10-fold) and variability within the human population (10-fold).

Due to the high exposure estimates resulting from the mode of use (i.e., direct application to skin), the Canadian Pest Management Regulatory Agency used a novel approach to refine the risk assessment for DEET. The acute oral NOAEL was converted to a dermal equivalent NOAEL by comparing pharmacokinetic data from rats following oral and dermal dosing. Although there was considerable variability in potential conversion factors that could be derived depending on the data selected, a conservative approach using the lower oral to dermal peak plasma ratios developed on the basis of measured levels of parent DEET was considered the most appropriate. Male and female ratios were pooled resulting in a final conversion factor of approximately 5. This value was multiplied by the NOAEL of the acute oral neurotoxicity study to derive a dermal equivalent in the acute risk assessment. In addition, it was noted that numerous studies estimating the dermal absorption of DEET in humans and rats have demonstrated that there may be approximately a 5-fold difference in DEET dermal absorption in rats (38.5% on average) and humans (7.5% on average). This additional 5-fold correction was factored into both the acute and intermediate risk assessments.

Using this approach and the dermal exposure levels calculated for adults and children, the acute risk assessment, as reported by the Canadian Pest Management Regulatory Agency, resulted in acceptable MOEs for adults for all concentrations of DEET products for both single and multiple applications per day. For children, acceptable MOEs were obtained for products up to 35% DEET for a single application per day. However, only products up to 10% DEET were acceptable for multiple applications per day.

The Canadian Pest Management Regulatory Agency considered a separate risk assessment for toddlers. This assessment required the combination of exposure from two routes, both dermal and oral (non-dietary ingestion associated with hand-to-mouth contact). The dermal risk assessment was performed in the same manner as for adults and children (i.e. by using a factor of 5 to correct for differences in route and a factor of 5 to correct for differences in rat and human dermal absorption). For the risk assessment of the non-dietary route of exposure, the NOAEL derived from the acute oral neurotoxicity study was used with the estimated oral exposure to calculate an MOE. The risks associated with the dermal route of exposure were combined with that associated with the oral (hand-to-mouth) route of exposure, as the toxicological end point for each route was the same. Based on a single application per day, the Canadian Pest Management Regulatory Agency showed that adequate MOEs can be achieved for approximately 6–8 hand-to-mouth events for products containing up to 10% DEET. When products with concentrations of 15% or greater were examined, inadequate MOEs are obtained based only on the dermal assessment (i.e., not considering any hand-to-mouth events). When multiple dermal applications within a day are considered, inadequate MOEs are obtained for all concentrations.

DEET may also be used on a daily basis for a prolonged period. The MOEs for intermediate dermal exposure for various DEET products for children and adults were calculated using the 90 day dermal rat NOAEL of 300 mg/kg bw/d and correcting for the difference in dermal absorption between rats and humans using the dermal absorption correction factor of 5. Based on this approach, acceptable MOEs are only obtained for adults with products having concentrations of 30% DEET and less. For children, acceptable MOEs are only obtained for products containing 10% or less DEET. For toddlers, inadequate intermediate-term MOEs were obtained for all concentrations of DEET products.

Based on this risk assessment, the Canadian Pest Management Regulatory Agency placed restrictions on DEET products. For adults, due to inadequate MOEs for prolonged use, products should be restricted to concentrations of 30% and less. For children (2–12 years), only products containing 10% DEET or less should be used. Finally, 10% may be used for toddlers between the ages of 6 months and 2 years, however as intermediate-term MOEs for this age range were inadequate at all concentrations of DEET, all products should state that application should not be more than once a day. A risk assessment was not performed for children under 6 months of age, since it is assumed that non-chemical measures can be utilised to protect this population from biting pressures. Consequently, all products registered in Canada should carry the statement: "DO NOT USE on infants less than 6 months of age."

As insufficient data were provided to determine exposure to DEET when wearing DEET impregnated clothing, an MOE cannot be determined. Exposure to these products, however, should not result in a greater potential exposure than products of the same concentration applied directly to the skin. As such, the same recommendations should be applied to these products.

4.2   Considerations for the Risk Assessment of DEET

The current review highlights thirty-one published human case reports concerning DEET exposure. Although predominantly from the US, it is important to note that the effects reported have to be considered in the context of the large proportion of the US population regularly using DEET containing insect repellents. Several case reports concerned effects on the skin, usually through irregular use. The evidence suggests that DEET products under normal conditions of use do not cause irritancy but may, in some circumstances where exposure is high and/or prolonged, give rise to severe irritation of the skin and in a very few cases skin sensitisation. In addition, a further nine published reports all referred to evidence of adverse effects in single individuals. Three referred to psychosis as the predominant clinical effect, two referred to adverse effect on reproduction and four to a range of other clinical effects. The chances that exposure to DEET was responsible for the reported clinical effects in these cases are either remote or inconclusive.

More importantly, the current review draws attention to eleven published reports, which contain evidence of CNS toxicity in 18 individuals. Fifteen of these individuals were between the ages of 1 and 8. The remaining three individuals were aged 14, 16 and 29 years. Signs of severe CNS toxicity seen in these 18 individuals included seizures, unconsciousness/coma, tremors, convulsions, depressed reflexes and ataxia. There was evidence of deliberate ingestion in five individuals, although all of these individuals recovered. Dermal exposure predominated in 14 individuals, of which three deaths occurred. Two of the deaths may have been associated with exposure to DEET. Evidence of Reye's syndrome in the third fatality was observed at post mortem and therefore symptoms were unlikely to be associated with DEET exposure. All of the remaining 14 individuals recovered, most within a few days of admission to hospital. The available information on dermal exposure varied between the different reports and is therefore difficult to assess for use in risk assessment. For some of these individuals dermal exposure was described as prolonged (daily applications for 1-3 months), frequent and/or high. However, for others the exposures reported varied from a single exposure to repeated exposures over several days. From these case reports, it would seem that there is some evidence to suggest that exposure to DEET may result in severe CNS toxicity in a few individuals, and predominantly in children. What follows is a discussion of issues that need careful consideration for an appropriate risk assessment of DEET.

In order to carry out an appropriate risk assessment, an appropriate study should be chosen which not only reflects similar exposure conditions, but also a similar endpoint. There are a number of neurotoxicity studies available. The studies of Vershoyle *et al*. (1992) suggest that the effects of DEET are highly dependent on the age and sex of the animal. DEET has a higher order of acute toxicity in young female rats. This is consistent with the observations in humans where most reported cases of toxicity involved young girls.

The age and sex dependency of the effects may result from differences in metabolic capacity, particularly cytochrome P450 profiles. Additional uncertainty factors may also be involved.

The chronic dermal study carried out by the group of Abou-Donia (Abdel-Rahman *et al.*, 2001) employed a route and duration (7 days per week for 60 days) of exposure that would be relevant to human usage. This study demonstrated that the administration of DEET at a dose level of 40mg/kg bw/day resulted in neuropathological changes. However, these findings contrast with those reported by Schoenig *et al.* (1993), who reported no histopathological effects in rats treated with up to 500mg/kg bw DEET by oral administration. For example, in the latter study, Schoenig *et al.* (1993) report spongiform myelinopathy and neuronal cytoplasmic clefts following oral administration of DEET at high doses. These lesions were not seen in the dermal studies carried out by Abou-Donia *et al.* One possible explanation could be that the spongiform change in the brain was an acute response to DEET that was likely to disappear on repeated dosing and that damaged neurones may have been removed rapidly and thus not have been present at later stages.

Inconsistent outcomes were observed during neurobehavioural testing depending on the duration of treatment with DEET. For instance, Abou-Donia *et al.* (2001a) reported significant effects on beam-walking, beam-walking time and grip strength when DEET was tested at 4, 40 and 400mg/kg DEET for 60 days. These changes were not reproduced in another study, carried out by the same group, in which a dose of 40mg/kg bw/day was administered for 45 days (Abou-Donia *et al.* 2001b). However, the neurobehavioural effects of DEET alone were more subtle than when it was administered as part of a mixture with pyridostigmine bromide and/or permethrin. There is some evidence to suggest that neuronal changes in response to DEET can have a late onset and thus may not always correlate with neurobehavioural outcomes. It should also be noted that the similar findings with three chemicals that are structurally different (i.e. DEET, pyridostigmine bromide and permethrin) suggest a potential methodological problem with the studies. For this reason, there would be a need for these findings to be replicated before they can be further considered in risk assessment. In addition, it is impossible to compare neuropathological changes in rats with changes seen in human post-mortem brain, where only two reports exist.

The DEET Joint Ventures group have suggested an alternative and pragmatic approach to risk assessment using pharmacokinetic data to extrapolate from animals to humans (Schoenig & Osimitz, 2001). Blood level studies were designed to define the profile of systemic exposure that occurs under different exposure scenarios (Schoenig & Osimitz, 2001). DEET plasma profiles were plotted following single and four daily oral doses of DEET at the NOAEL for acute neurotoxicity in dogs and rats. These data were subsequently compared to a plasma profile obtained from human volunteer data (three male and three female) who were exposed dermally to a 100% DEET solution over 8 hours. This plasma profile was measured following one application and also after four daily applications.

However, there are problems with regard to an adequate risk assessment, when comparing these peak plasma DEET levels in animals and human volunteers. For example, DEET was washed off the human volunteers by showering 8 hours after dermal application. In practical use, people may reapply DEET, which could possibly lead to higher peak plasma levels. Furthermore, although toxicokinetic data are available from studies in sensitive

animal species and for humans, an uncertainty factor would still be required for interspecies variability to take into account potential differences in toxicodynamics. In addition, the number of human volunteers is small that an uncertainty factor would also be required to take into account inter-human variation. Although a reduced uncertainty factor (from the traditional value of 100) may be appropriate, careful consideration is needed when deciding a suitable figure. It should be noted that the DJV suggest, on the basis of the observed 33-fold difference in overall mean peak plasma levels that this is equivalent to a 300-fold safety factor (Schenig *et al*. 2001)

Finally, it is difficult to undertake an assessment of the likely dermal exposure following the use of topically applied insect repllents containing DEET available within the UK. Exposures would depend on types of product used (e.g. aerosol, pump sprays, lotions), the concentration of DEET in the product, the recommended use pattern as outlined on the product label (including recommendations for multiple applications within one day), and the duration of product use (e.g. application on one day or daily application over several days/weeks). No data are available on the use patterns of DEET topical insect repellent products in the U.K. Such information may only be obtained when the formal regulatory review under the Biocide Product Directive is initiated.

5 DISCUSSION

This paper is a review of all the available toxicological literature for *N,N*-diethyl-*m*-toluamide (DEET) up to October 2002. DEET seems to be most effective and is the best studied insect repellent currently available to the general public. It is used in many countries, not only to prevent nuisance bites from mosquitoes and other biting insects but also to aid in lowering disease transmission from these pests. DEET has been available for use worldwide for 40 years. It is marketed in the United Kingdom in a variety of formulations and concentrations including aerosol and pump-spray products intended for application to skin as well as for treating clothing. However, there are currently no data on usage patterns in the UK.

ADME of DEET

The literature shows that dermal penetration and absorption of DEET has been extensively studied using *in vitro* and *in vivo* methods in experimental animals. The percentage absorbed seems to be dependent on the solvent used, concentration of DEET, method used to determine absorption (e.g. recovery in urine following dermal application or comparison of kinetics following intravenous or dermal application) and extent of occlusion used. Hence there is difficulty comparing these results. In rats, dermal absorption values of up to 80% were reported in one study, however there are no data from studies in animals using repeated dermal administration of DEET.

A number of studies using human volunteers and *in vitro* methods with human skin samples were available. These studies confirm the influence of solvent and formulation on absorption of DEET. One study estimated the mean absorption of DEET applied to the skin for 8 hours as being 8.4% for a 15% ethanolic solution of DEET and 5.6% for 100% DEET, allowing for extent of recovery. It is possible that absorption could be significantly above these values when copiously applied by individuals to repel insects and also depending on the formulation of the insect repellent product containing DEET.

Data are available from a toxicokinetic study using single and repeated dermal administration of undiluted DEET to human volunteers (four applications at 50mg/kg bw) and rats (five applications at 1000mg/kg bw) as well as oral administration to rats (single dose of 200mg/kg by gavage) and to dogs (75mg/kg bw/day by administration in gelatin capsules over 5 days). These studies had been designed to provide plasma time course data for DEET at the 95th percentile of human use, the oral NOAELs reported for neurotoxicity in rats and dogs and the limit dose for dermal application in rats. The results showed that oral administration at the doses used resulted in a rapid peak plasma level; 15-45 minutes in rats and 30 minutes in dogs. A much slower absorption was evident following dermal administration with peak plasma levels reported at 8 hours in humans and 4-8 hours in rats. It should be noted that the human plasma level only reduced after showering at hours, which may have limited the assessment of peak plasma levels. There were no apparent differences between the plams time course for DEET following single or repeated dermal administration in humans or rats. Quantitative comparisons of the peak plasma levels were 33x higher in dogs and 16-34x higher in rats given oral doses compared to dermal administration in humans.

The available evidence from metabolism studies in rats following oral dosing and from human volunteers following topical application of DEET suggests that absorbed DEET was metabolised and excreted in a similar manner in rats and humans. The predominant pathways of metabolism appear to involve oxidation of the methyl group on the aromatic ring and N-deethylation of the amide moiety. The available studies in animals, which used dermal administration also, showed that absorbed DEET was excreted predominantly via the urine.

In one recently published study, results from in-vitro metabolism experiments using liver microsomes from humans, rats and mice provide confirmatory evidence that the route of metabolism is qualitatively similar in humans and rodents. Additional *in-vitro* experiments using individual human cytochrome P450 isozymes have established that CYP2B6 is the principle cytochrome P450 involved in metabolism of DEET by ring hydroxylation. Studies using phenotyped human liver microsomes show that individuals with the highest levels of CYP2B6, 3A4, 2C19 and 2A6 have the greatest potential to metabolise DEET. These data, in part, may help to explain inter-individual differences in response to DEET.

Toxicity of DEET

DEET is of low acute oral, dermal and inhalation toxicity in experimental animals in studies that used adult animals and were designed to quantify mortality. It should also be noted that there is evidence in rats to show that young animals and particularly females are more sensitive than adult animals to the acute lethal effects of DEET (Vershoyle *et al.*, 1992).

DEET is also shown to be a mild skin irritant in rabbits, but not a skin sensitiser in guinea pigs. Technical grade DEET has given some evidence for eye irritation in rabbits. It is difficult to assess the data from the available study in comparison to the criteria for classification under the dangerous Substances Directive (67/548/EEC), but evidence of reversal by 168 hours suggests no serious damage to eyes but DEET should perhaps be regarded as an eye irritant.

A number of effects seen at high doses in the available toxicology studies are not relevant to risk assessment. These include effects on body weight gain associated with reduced food intake. It is not clear whether the effect on body weight/food consumption is due to palatability or an effect on the treated animal. The effects on kidney histology (hyaline formation) seen in CD rats and Fischer rats in 90 day studies at 400 mg/kg bw/day seem to be a result of $\alpha 2u$-globulin accumulation in renal tubules that occurs uniquely in rats. These were not seen in sub-chronic studies in other species or in a 2-year study in CD rats and are therefore not considered relevant. Other kidney effects seen were as a result of high doses. Additionally, the effects on spermatogenesis seen in hamsters at 624 mg/kg/day were not found in other species.

There was no evidence of toxicologically significant effects on reproduction in rats, or on development in rats and rabbits. DEET does not have any structural alerts to suggest mutation potential and this is supported by negative results in a bacterial mutation assay in *Salmonella typhimurium*. There was also evidence that DEET was not carcinogenic in experimental animals.

Neurotoxicity of DEET

A number of studies have shown some indication of neurotoxicity. The predominant signs of toxicity in rats given oral doses DEET which resulted in lethality between 50 minutes up to 24 hours post dose were CNS depression with myoclonic twitching seen in some animals (triggered by auditory or tactile stimuli). Recovery in surviving animals was slow. There was evidence of histological changes in animals given lethal doses in the CNS predominantly consisting of vacuolation of myelin sheaths of fibres in the cerebellar roof nuclei and cytoplasmic clefts, which were diffusely distributed throughout the brain.

The findings of decreased vertical motor activity in the functional observational battery in adult rats given an oral dose of DEET of 500 mg/kg bw by gavage was consistent with a neurotoxic effect. Reduced motor activity in Sprague-Dawley rats following a single oral dose of 500 mg/kg bw (in mineral oil) was also reported in a separate published study. Evidence for reduced completion of reinforcement learning schedules in Sprague-Dawley rats has been documented in a study where rats were given a single oral dose of 500 mg/kg bw. The NOAEL reported in these studies was 200 mg/kg bw, however it is noted that no sensorimotor function tests in young animals have been carried out and it is possible that the NOAEL could be lower in such animals.

Other published studies have been specifically designed to investigate potential neurotoxicity by using repeat dose regimens, however there was no clear difference between the results obtained following dietary administration to rats or dermal application (in 70% ethanol) to the shaved skin of rats.

The route of application and duration of the studies from Abou-Donia's laboratory were relevant to the risk assessment of the use of DEET as an insect repellent. The use of 70% ethanol as a solvent would likely to have resulted in substantial absorption of DEET. The use of morphometric analysis and immunohistochemical staining for effects on glial cells assisted the identification of subtle lesions that were not apparent from the Schoenig studies. Unfortunately, no histopathology data were available from the Abou-Donia studies for 30 or 45 days of dermal treatment and therefore any correlation between effects on sensorimotor performance and histological changes in the CNS cannot be determined. Interestingly, effects similar to those observed with DEET on motor function and CNS histology were reported with permethrin and pyrodostigmine and combinations of these chemicals. It is not possible to resolve why these structurally and toxicologically dissimilar chemicals should produce a similar pattern of CNS toxicity.

Human Case reports

Thirty-one published human case reports were found. These predominantly came from the US. It is important to note that the effects reported have to be considered in the context of the large proportion of the US population regularly using DEET containing insect repellents.

Seven published reports concerned effects on the skin. Five of the reports concerned individual cases where moderate to severe skin irritation were reported and in one individual evidence of skin sensitisation following an open patch test. The two other reports concerned US military personnel. Evidence of severe skin reactions were reported in some individuals stationed in South Vietnam during late 1960s who used insect repellent preparations containing 75% DEET. Further evidence of severe skin reactions

were reported in 10 US military personnel who used a preparation containing 50% DEET in the 1980s. It is uncertain what role co-formulants played in these reactions. The findings from these reports have not been reproduced on such a scale in reports concerning use of insect repellents by non-military personnel in the USA and are not consistent with the large number of people using DEET products in the USA every year. The evidence suggests that DEET products under normal conditions of use do not cause irritancy but may, in some circumstances where exposure is high and/or prolonged, give rise to severe irritation of the skin and in a very few cases skin sensitisation.

Fourteen published reports concerned CNS toxicity. In total there was evidence of CNS toxicity in 18 individuals. The age range was 1 year up to 29 years. However 15 of these individuals aged between 1-8 years and the remaining three individuals were aged 14, 16 and 29y. Signs of severe CNS toxicity seen in these individuals included tremors, seizures, convulsions, depressed reflexes and ataxia. There was evidence of deliberate ingestion as the predominant route of exposure in 5 individuals. All of these individuals recovered. There were 13 individuals where dermal exposure predominated. Three deaths occurred, of which two are likely to be associated with exposure to DEET. All of the remaining 10 individuals recovered mostly within a few days of admission to hospital. Recovery in two individuals took 3 weeks and 3 months respectively. The available information on exposure varies between the different reports. For many of these individuals dermal exposure was prolonged, and/or high. However for some individuals, the exposures reported varied from a single exposure to repeated exposures up to 10 days and could be relevant to the normal use of DEET insect repellents. Therefore, it seems there is sufficient evidence to suggest that exposure to DEET has resulted in severe CNS toxicity in a few individuals, and predominantly in children. However, the small number of reported cases of severe CNS toxicity has to be considered in the context of the large number of people using DEET containing products and that the risk of such effects may be extremely remote.

A further eight published reports all referred to evidence of adverse effects in single individuals. Three referred to psychosis as the predominant clinical effect, one referred to adverse effect on reproduction and four to a range of other clinical effects. The chances that exposure to DEET was responsible for the reported clinical effects in these cases are either remote or inconclusive.

Data from the six U.K. National Poisons Information Service (NPIS) centres were sought for a four year period 1 January 1997-31 December 2001. Information on over 1 million calls to the NPIS was available. Overall, there was evidence to demonstrate potential for localised effects (skin/eye irritation) following accidental exposure in a small number of cases. However, there were no reports of severe CNS toxicity in children. A single case of deliberate ingestion leading to coma was reported in one case involving an adult male.

Risk Assessment

In order to carry out an appropriate risk assessment of DEET, there are a number of approaches and issues that need to be considered. The various approaches that may be employed have been described previously (section 4). The traditional method of defining toxicologically relevant endpoints from exisiting animal studies and comparing the No

Adverse Effect Level (NOAEL) to an estimate of human exposure has been carried out by the US EPA (1998). The problems involved with defining this endpoint have also been discussed previously. The US EPA's final view was that DEET had been shown not to be a teratogen, a reproducive toxin or an oncogen at maximum tolerated doses by the more rigorous oral route of administration. Furthermore, there was not enough evidence for the EPA to consider neurotoxicity as an appropriate endpoint. Therefore, the US EPA was of the opinion that these types of effects are not toxicity endpoints for concern for DEET (US EPA, 1998). In this review, neurotoxic effects have been highlighted, particularly from the Schoenig *et al*. (1993) studies, as being of significance and possibly of relevence to risk assessment. The result of the US EPA evaluation was that although no formal risk assessment was carried out the EPA believed that it was prudent to require improved label warnings and restrictions for DEET products (see section 1.2.3)

The Canadian Pest Management Regulatory Agency carried out a risk assessment for acute dermal exposure to DEET using data from the neurotoxity study in rats (single oral dose) to identify the most relevant toxicity endpoint. An NOAEL derived from a 90-day dermal rat study was used to assess intermediate dermal exposure. Since some data was being used from a different route of administration (i.e. oral), conversion to a dermal equivalent NOAEL was achieved by comparing pharmacokinetic data from rats following oral and dermal dosing. A final conversion factor of 5 was multiplied by the NOAEL of the acute oral neurotoxicity study. In addition, a 5-fold correction was factored into the risk assessments due to an estimated 5-fold difference in DEET dermal absorption in rats compared to humans. Based on this risk assessment the Canadian Pest Management Regulatory Agency placed restrictions on DEET products (see section 1.2.3).

An alternative risk assessment approach as suggested by the DEET Joint Ventures group is to use additional pharmacokinetic data to aid the extrapolation from animals to humans. DEET plasma profiles in dogs and rats can be compared to plasma profiles obtained from human volunteer data in order to estimate an extrapolation factor. This approach, although seemingly pragmatic, has inherent problems including the fact that uncertainty factors would still be required for interspecies and interhuman variation. Although a reduced uncertainty factor (from the traditional value of 100) may be appropriate, careful consideration is needed when deciding a suitable figure. It should be noted that the DJV suggest, on the basis of the observed 33-fold difference in overall mean peak plasma levels that this is equivalent to a 300-fold safety factor (Schoenig *et al*. 2001).

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