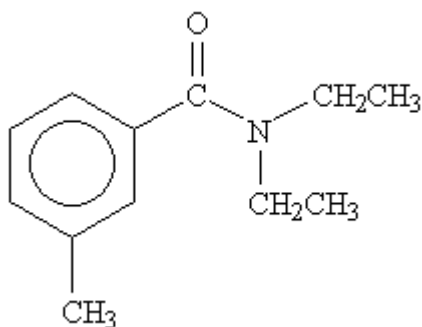# *N,N*-DIETHYL-*META*-TOLUAMIDE
(DEET)

RISK CHARACTERIZATION DOCUMENT

California Environmental Protection Agency
Department of Pesticide Regulation

September 2000

RCD 00-01

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION

Paul E. Helliker
Director

N,N-DIETHYL-*META*-TOLUAMIDE
(DEET)

RISK CHARACTERIZATION DOCUMENT

Medical Toxicology and Worker Health and Safety Branches

DEPARTMENT OF PESTICIDE REGULATION

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

May 23, 2000

N,N-DIETHYL-*meta*-TOLUAMIDE

SUMMARY

N,N-diethyl-*meta*-toluamide (DEET) was developed and patented by the U.S. Army in 1946 for use as an insect repellent by military personnel (U.S. EPA, 1980). In 1957, DEET was registered for use by the general public without any restriction on the amount or frequency of application. Its only registered use since that time has been as an insect repellent. The purpose of this current risk assessment is to address the potential adverse health effects for the general public and park and forestry workers who use DEET as an insect repellent.

DEET is of relatively low toxicity to animals, especially by the dermal route, but in large enough doses, it will produce signs of neurotoxicity primarily with acute inhalation or oral exposure. The mechanism behind the neurotoxicity is unclear, but there is evidence which suggests DEET may be acting indirectly through elevation of serum ammonia levels and/or directly through CNS excitation. At approximately 500 mg/kg by the oral or inhalation route, mild neurological effects were seen including reduced motor activity, changes in heat sensitivity, loss of balance, incoordination, reduced muscle tone, tremors, decreased heart rate and reduced blood pressure. At near lethal doses (> 1,000 mg/kg), twitching, convulsions or seizures, unconsciousness, prostration and neuropathological lesions in the brain were seen. In the acute dermal toxicity studies, a NOEL was established based on the lack of any systemic effects at 5,000 mg/kg. Dermal irritation was the most sensitive endpoint in laboratory animals after acute dermal exposure to DEET. The acute NOEL for dermal irritation was also 5,000 mg/kg. Since this effect is probably concentration dependent, the NOEL for dermal irritation was expressed as 25.8 mg/cm^2.

With subchronic exposure to DEET, generally mild neurological effects were seen probably because of lower dose levels. In a 13-week inhalation neurotoxicity study in rats, only reduced endurance was observed at the highest dose level tested, 360 mg/kg/day. In a 48-week oral neurotoxicity study in rats, only increased exploratory behavior was seen at the highest dose level tested, 196 (M) or 275 (F) mg/kg/day. No neurological effects were observed in the standard 13-week dermal toxicity studies in rats or micropigs exposed at doses up to 1,000 mg/kg/day. Several other systemic effects were seen in laboratory animals with subchronic exposure to DEET including reduced body weights and food consumption, changes in liver, kidney and testes weights, changes in clinical chemistry and hematological values, and microscopic lesions in the liver, kidneys, testes, stomach, bone marrow, thymus and skin. Many of the changes in the organ weights, clinical chemistry and hematological values were of uncertain toxicological significance because of a lack of correlation with any microscopic lesions. The microscopic lesions in the kidneys of male rats were shown to be due to the binding of DEET to α_{2u}-globulin and its subsequent accumulation in the kidneys. Since humans do not produce α_{2u}-globulin or a similar protein to which DEET can bind, this effect was not considered relevant to humans. Unlike acute exposure, the most common and sensitive systemic endpoint with subchronic exposure to DEET was the reduction in body weight. The subchronic NOEL for systemic effects was 300 mg/kg/day based on reduced body weights in male rats which had DEET applied topically to their backs for 90 days. As with acute dermal exposure to DEET, dermal irritation was the most sensitive endpoint with subchronic dermal exposure. The subchronic NOEL for dermal irritation was estimated to be 10 mg/kg/day or 61.5 μg/cm^2 by dividing the LOEL by a default uncertainty factor of 10.

Reductions in body weight and food consumption, changes in clinical chemistry values, and histopathological lesions in the lymph node, uterus and liver were seen in laboratory

animals after chronic oral exposure to DEET. The chronic NOEL was 100 mg/kg/day based on reduced body weights and food consumption and increased cholesterol levels in rats fed DEET in the diet for 1 to 2 years. There was no evidence of carcinogenicity in three species of laboratory animals, including mice, rats, and rabbits, after chronic exposure to DEET. Furthermore, there was no evidence of genetic toxicity in any of the available tests, except a dominant lethal assay which had equivocal results.

There was no evidence of increased sensitivity in infants and children to DEET from the guideline developmental and reproductive toxicity studies. However, evidence of increased sensitivity in infants and children was found in two neurotoxicity studies, including increased mortalities in 11 day old pups versus 47-56 day old pups and reduced body weight gain in pups after weaning. Children may also be at greater risk because of increased exposure to DEET. In a usage study conducted by the registrants, children had more DEET applied not only in terms of body weight, but also surface area. Over the past 30 years there have been at least a dozen cases of seizures in children after topical exposure to DEET. It is possible that the occurrence of these seizures in children after DEET exposure is coincidental since afebrile seizures are common in children. However, it seems unlikely that in cases where there is a clear history of excessive use that the association is coincidental. Even if all these case reports of seizures are actually due to DEET, the incidence is relatively low (less than one in a million) considering that approximately 17 million children use DEET each year.

There is evidence that suggests DEET interacts synergistically with other chemicals including fenvalerate, permethrin, pyridostigmine bromide and chlorpyrifos. Possible explanations for the enhanced toxicity of these combined exposures include: 1) facilitation by DEET of the transport of chemicals across skin or the blood brain barrier, 2) inhibition by cholinesterase inhibitors of non-specific esterases involved in the detoxification of DEET and 3) overloading of metabolic pathways. This evidence suggests that gardeners and agricultural workers who use DEET while working with insecticides may be at greater risk for toxicity from either DEET or the insecticide. However, there is insufficient information at this time to quantitate the risks.

Exposure dosages for DEET were estimated for the general population from a mail-in survey and a usage study conducted by the registrants. The absorbed daily dosages (ADDs) for DEET were estimated assuming the dermal absorption for humans was 8.4%. The average ADDs ranged from 840 µg/kg for female adults to 3,610 µg/kg for female children using the average dermal exposure. The high exposure was assumed to be 3 times higher than the average exposure based on survey data indicating the 95th percentile was approximately 3 times higher than the average exposure. The high ADDs ranged from 2,520 µg/kg to 10,830 µg/kg. The seasonal average daily dosages (SADDs) were estimated assuming DEET was applied 7.5 times during the peak months of June and July. The average SADDs ranged from 103 µg/kg/day for female adults to 443 µg/kg/day for female children. The high SADDs ranged from 309 µg/kg/day to 1,329 µg/kg/day. The annual average daily dosages (AADDs) was estimated assuming that 55% of the annual use of DEET occurred in the peak months of June and July. The average AADDs ranged 37 µg/kg/day to 130 µg/kg/day for female adults and female children, respectively. The high AADDs ranged from 111 µg/kg/day to 390 µg/kg/day. For workers, the acute exposure was assumed to be the same as the high ADD for adult males (3,060 µg/kg). The SADD for workers was estimated to be 729 µg/kg/day assuming DEET was applied once a day for 5 days per week during the peak months of June and July. The AADD was estimated to 222 mg/kg/day assuming that 55% of annual use of DEET occurred in June and July.

To evaluate the risk for potential dermal irritation from DEET, the average and high daily dermal concentration (DDC) were estimated. The average DDCs ranged from 69 µg/cm^2 for female adults to 192 µg/cm^2 for female children less than 12 years old. The high DDCs ranged from 207 µg/cm^2 to 576 µg/cm^2 for female adults and female children, respectively. The seasonal average dermal concentrations (SADCs) ranged from 8 µg/cm^2 for female adults to 24 µg/cm^2 for female children based on the average exposure. The high SADCs ranged from 24 to 72 µg/cm^2. The DDC and SADC for workers were 249 and 59 µg/cm^2.

There was no estimate of inhalation exposure from the use of aerosol products since there was no attempt in the usage survey to determine how much of the product was inhaled versus absorbed through the skin. Consequently, the exposure estimates are probably higher than estimated for individuals using aerosol products. However, this omission should not significantly underestimate exposure as the dermal exposure is much greater than inhalation exposure.

The risk for non-oncogenic health effects is expressed as a margin of exposure (MOE) which is the ratio of the NOEL from the animal study to the human exposure dosage. For systemic effects, an MOE of at least 100 is generally desirable assuming that humans are 10 times more sensitive than animals and that the most sensitive human is 10 times more sensitive than the least sensitive human. The MOEs for acute systemic effects in the general population were greater than 100 for all population exposure groups based on average or high-end acute exposure estimates. The MOEs for acute occupational exposure were also greater than 100. The MOEs for subchronic systemic effects were greater than 100 for all population subgroups based on average exposure estimates and for adults and juveniles based on high-end exposure estimates. Only the subchronic MOEs for children based on high-end exposure estimates were less than 100. The small number of case reports of seizures in children exposed to DEET suggests that the MOEs for children may not be adequate with heavy use. The MOEs for chronic systemic effects were greater than 100 for all population subgroups based on either the average or high-end exposure estimates. The subchronic and chronic MOEs for workers were greater than 100.

For dermal irritation, an MOE greater than 10 may be sufficiently health protective since humans are probably less sensitive than laboratory animals to dermal irritation. The MOEs for acute dermal irritation were greater than 10 for all population subgroups, including children and workers, when based on the average or high-end exposure estimates. On the other hand, the MOEs for subchronic dermal irritation were less than 10 for all population subgroups using either the average or high-end exposure estimates. The subchronic MOE for dermal irritation in workers was also less than 10. Risks for dermal irritation with repeated exposure may have been overestimated since the subchronic NOEL for this endpoint was estimated and humans are probably less sensitive than the animals. However, several literature reports of dermal irritation with high concentration formulations or heavy use suggest that the risk for dermal irritation is not negligible.

Contributors and Acknowledgments

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I. INTRODUCTION

A.   REGULATORY BACKGROUND

N,N-diethyl-*meta*-toluamide (DEET) was developed and patented by the U.S. Army in 1946 for use as an insect repellent by military personnel (U.S. EPA, 1980). In 1957, DEET was registered for use by the general public without any restriction on the amount or frequency of application. Its only registered use since that time has been as an insect repellent. The purpose of this current risk assessment is to address the potential adverse health effects for the general public who use DEET as an insect repellent.

B.   CHEMICAL IDENTIFICATION

DEET is an all-purpose insect repellent for use on skin, clothing, bedding and tents. It is as or more effective than other repellents including indalone, dimethyl phthalate, and ethylhexanediol (Robbins and Cherniack, 1986). DEET is effective against a wide range of pests including mosquitoes, biting flies, chiggers, fleas, ticks, stable flies, and leaches (U.S. EPA, 1980).   It has been suggested that DEET repels mosquitoes by interfering with sensory receptors they use to follow warm and moist air currents given off by warm-blooded animals (Wright, 1975). Gender-related differences in efficacy have been reported with an extended duration formulation of DEET (Golenda *et al.*, 1999). Females experienced less protection over time than did males. The *meta* isomer is comparable in effectiveness as the *ortho* and *para* isomers, although the *ortho* isomer is somewhat more toxic and the *para* isomer is slightly less toxic than the *meta* isomer (Robbins and Cherniack, 1986). DEET is of relatively low toxicity to animals, but in large enough doses, it will produce signs of neurotoxicity. The mechanism behind the neurotoxicity is unclear, but there is evidence which suggests DEET may be acting indirectly through elevation of serum ammonia levels and/or directly through CNS excitation. The possible mechanisms behind the neurotoxicity associated with DEET are discussed in more detail under Acute Toxicity in the Hazard Identification section.

C.   TECHNICAL AND PRODUCT FORMULATION

Currently there are over 160 products registered in California that contain DEET as an active ingredient. Approximately 90 of these products are registered by just three companies, S.C. Johnson & Son, Inc. (Off!), Spectrum Group, A Division of United Industries Corp. (Cutter) and Wisconsin Pharmacal Co. Inc. (Repel). However, the vast majority of the registrants (~30) have only 1-3 products registered in California. DEET and related isomers are the only active ingredients in approximately 70% of these products. Among the other active ingredients added to the DEET formulations, the most common were octyl bicycloheptene dicarboximide (synergist) and dipropyl isocinchomeronate (repellent). At least 14 products also contain sunscreen active ingredients, such as octyl methoxycinnamate and oxybenzone. Fenvalerate or esfenvalerate (insecticide/repellent) is currently used in 3 products registered by Hartz Mountain Corp. for cats and dogs. Although most of the products contain between 5 and 40% DEET, there are approximately 12 products that are 100% DEET. The products were usually sprays, lotions, gels, creams or sticks, but there were also DEET impregnated towelettes. Analysis of 26 DEET formulations ranging in concentration from 14 to 95% DEET, found *N*-nitrosodiethylamine (NDEA), which is potentially oncogenic in two of them at 0.05 ppm and 0.14 ppm (Wigfield and McLenaghan, 1990). Two other samples had trace levels of NDEA (between 0.02 and 0.05 ppm).

D.   USAGE

Based on a registrant's survey of its employees, the U.S. EPA estimated that approximately 38% of the general public used insect repellents of which 22% were sprays containing 15-20% DEET (U.S. EPA, 1980). Similar results were found in a more recent mail survey of approximately 8,000 households distributed throughout the contiguous United States that was also sponsored by a registrant (Boomsma and Parthasarathy, 1990). The sample households were nationally balanced to U.S. Census data based on head-of-household age, household income, geographic region, population density and household size. Approximately 37% of the people from these households used an insect repellent in 1989, although only 20% of participants in the Pacific region used an insect repellent in that year. It was estimated that approximately 30% of the population used DEET-containing repellents in 1989. Among DEET repellent users, 32% were adult men, 39% were adult women, 6% were children ages 12 to 17 years old, and 23% were children less than 12 years old. DPR has no data on the pounds used in California which is only reported for pesticides with agricultural uses; however, the number of pounds of DEET sold in California was approximately 104,082 lbs. active ingredient for 1995 (DPR, 1996).

E.   ILLNESS REPORTS

There were 11 cases of illnesses related to DEET exposure in DPR's Pesticide Illness Surveillance Program data from 1982 to 1994. Two of these cases involved systemic effects while the remainder involved skin (3 cases) and eye (6 cases) effects.   Hospitalization was not reported in any of these cases.   Ten of these cases were occupational incidents and only one case involving an allergic reaction was a non-occupational incident.

A number of cases of seizures and toxic encephalopathy following exposure to DEET have been reported. The earlier reports involved primarily girls, including two 18-month-old girls, a 3½-year-old girl, a 5-year-old girl and a 6-year-old girl (Gryboski *et al.*, 1961; Zadikoff, 1979; Heick *et al.*, 1980; Edwards and Johnson, 1987). Most of these girls had DEET sprayed onto their skin, clothes or bedding daily for two weeks to 3 months before developing symptoms. One of the 18-month-old girls had ingested a small amount of a 10% DEET product (Zadikoff, 1979). The concentration of DEET in the products varied from 10-20%. These girls exhibited signs of toxic encephalopathy including seizures and/or episodes of crying and tremors, between which they exhibited disorientation, agitation, slurred speech, ataxia, and lethargy. Two of the girls (5- and 6-year-old girls) died after developing symptoms and, in both cases, were found to have edema of the brain upon autopsy (Zadikoff, 1979; Heick *et al.*, 1980). It was later determined that the 6-year-old girl had a deficiency in the urea cycle enzyme, ornithine carbamoyl transferase. This genetic disorder is usually fatal in males, but of varying severity in females who are heterozygous. Her serum ammonia concentration rose to 1,852 µg/dl (normal 80-110 µg/dl) despite measures to decrease it. At autopsy, her brain was not only edematous, but also necrotic. Her liver was enlarged and microscopic examination revealed that the parenchymal cells were foamy and contained abundant glycogen.

Not all cases of seizures following DEET exposure involved young girls or excessive use. New York State Department of Health reported five cases, including 4 boys ages 3-7 and a 29-year-old man, in which seizures occurred after topical use of DEET (Oransky *et al.*, 1989). Four had fewer than 3 applications. The concentration of DEET in the formulations used varied (although the range was not reported). The onset of seizures was between 8 and 48 hours since the last application. There were two other independent reports of seizures after only a few topical applications of DEET. One of these cases involved an 8-year-old girl who had been

exposed to copious amounts of two DEET formulations containing 15 and 100% DEET over 3 days (Roland *et al.*, 1985). The other case involved a 5-year old boy who received only two applications on a single day (one of which was a 95% DEET formulation) before developing a major seizure (Lipscomb *et al.*, 1992). There were no deaths with any of these cases.

Seizures were also reported in 3 of 5 cases where large quantities of concentrated DEET (47.5% to 95%) products were ingested either intentionally or accidently (Tenebein, 1987). These cases included a 1-year-old girl, a 14-year-old girl, a 16-year-old girl, a 33-year-old woman, and a 26-year-old man. Both the woman and man died. In both of these cases, drugs or alcohol had also been consumed with the DEET and may have contributed to the fatal outcome.

U.S. EPA reported 3 other cases of seizures associated with topical application of DEET (U.S. EPA, 1998). These cases involved two children (a 3-year old girl and a 2-year old boy) and a 29-year old man. There was no information about the concentration of the DEET products used or how frequently it was applied except that the adult male had a seizure after only two applications. Both children survived; however, the adult male died after choking on his food when he had the seizure.

Other toxic effects have been reported in humans after exposure to DEET. Acute manic psychosis was reported in one 30-year old man who had treated a rash over a 3 week period by applying DEET daily over his entire body followed by a 60-90 minute sauna (Snyder *et al.*, 1986). After hospitalization and treatment with haloperidol for 6 days, the psychosis disappeared. Cardiovascular and neurological effects were reported in another case where a 61-year-old woman had applied copious amounts of sunscreen and DEET before going to do yard work (Clem *et al.*, 1993). Initially she fell to the ground, but did not lose consciousness. She was unable to speak and developed nausea, vomiting and diarrhea. Upon admission to the hospital her blood pressure was low and she had orthostatic hypotension. Her electrocardiogram showed marked sinus bradycardia. The patient returned to normal within several hours. In another case, a 34-year-old woman was taking chloroquine and using DEET daily during her pregnancy (Schaefer and Peters, 1992). Her male baby was born with antimongoloid slant of the palpebral fissures, hypertelorism, thin lips, poorly developed philtrum, a broad nasal bridge and impaired sensimotor coordination. Since chloroquine was used simultaneously with DEET, it is unclear what role, if any, DEET had in the development of these malformations.

A survey of park workers in the Everglades National Park was conducted to evaluate the occupational exposure to DEET and potential health effects (McConnell *et al.*, 1986). Among 143 workers who completed a self-administered questionnaire in the initial survey, there was a significant increase in the prevalence of symptoms of skin rash or blisters, chest pain or wheezing, depression, irritability, insomnia, difficulty in starting and stopping urinary stream, and muscle cramps among heavily exposed workers. Based on use reported in the initial questionnaire, the median exposure of workers was estimated to be 2.6 g/week ($\cong$0.4 g/day) while approximately 5% of these workers were estimated to apply 66.3 g/week ($\cong$9.5 g/day) or more. Twenty of these workers submitted urine samples for analysis of DEET concentration. The DEET level in urine correlated reasonably well (r = 0.70, p = 0.04) with the estimated exposure for that day, despite the small number of samples and the crude method used for estimating exposure. In the follow-up survey conducted a year later, 77 workers filled out questionnaires regarding exposure and symptoms, and then were subjected to a battery of neurobehavioral tests. The median 24-hour exposure estimated from the follow-up

questionnaire was 0.2 g/day ($\cong$1.4 g/week) with a 95th percentile of 11.5 g/day ($\cong$80.5 g/week). Based on the follow-up questionnaires, there was a significant increase in daytime sleepiness, memory problems, difficulty in concentrating, and absentmindedness among heavy users. No significant differences in the neurobehavioral tests were found.

A retrospective study of 9,086 calls regarding DEET exposure to poison control centers between 1985 and 1989 found that nearly two thirds of those exposed had only minor symptoms (such as mild gastrointestinal symptoms, drowsiness, skin irritation or first degree burn) that resolved rapidly (Veltri *et al.*, 1994). There were 66 patients whose symptoms were more pronounced or prolonged, although these symptoms were not described. Five patients (all male) experienced life-threatening effects, although there was insufficient information and follow-up in most of these cases to document them as a finding of major effect according to the criteria of the American Association of Poison Control Centers National Data Collection System. One 33-year old male died after ingesting 8 oz. of a DEET product of unknown concentration. He had a cardiac arrest from which he was resuscitated. He later developed seizures, cerebral edema, and disseminated intravascular coagulopathy. Most of the calls (61.4%) were regarding exposure to young children ages 2-5 years old who usually ingested the product. The number of calls per million packages of DEET sold almost doubled from 1985 to 1989. The number of patients referred to a health care facility also increased each year. The reason for the increase is uncertain, but it is not associated with an increase in the number or severity of symptoms since the rate of admissions to the hospital did not increase. Symptoms were more likely to occur with inhalation or ocular exposure.

Rabinovich (1966) reported skin or eye irritation in 4 of 85 people who applied a 40% DEET solution daily for a prolonged period of time (not specified). The reactions included contact dermatitis, exacerbation of seborrhea or acne vulgaris and conjunctivitis. There are several reports of skin irritation and sensitization for DEET. Blistering skin eruptions in the antecubital fossae (fold of the elbow) were seen in some U.S. soldiers in South Vietnam after application of a 75% DEET formulation that was issued (Lamberg and Mulrennan, 1969). Thirty-seven of 77 soldiers (48%) tested developed blisters or erosions in the antecubital fossae after it was applied overnight under a 1-inch gauze square and occluded with tape. Two different types of formulations (liquid and aerosol) with difference vehicles (ethanol and dichlorodifluormethane) were tested, but there was no difference reactions. Three men developed local necrosis requiring 3 weeks heal with scarring. None of these soldiers reacted to the DEET when tested on the upper inner part of the arm. Additional tests were done on groups of four soldiers who had reacted positively to DEET previously. These tests included 5% DEET in petrolatum, ethanol alone, and full strength DEET. None of the soldiers reacted to the 5% DEET or ethanol, but 3 of 4 reacted to the full strength DEET when applied to the antecubital fossae. Similar findings of marked erythema, blisters, ulceration and/or scarring in the antecubital fossae were seen in another 10 soldiers who had used a 50% DEET formulation a few hours earlier (Reuveni and Yagupsky, 1982). The repellent had been applied to the face, neck, upper part of the trunk and legs before sleep. Contact urticaria was observed in a 35-year old woman in response to DEET (Maibach and Johnson, 1975). This was confirmed by open patch test; however, the wheal-and-flare response was seen within 20 minutes of exposure. This response was passively transferred suggesting an immunological mechanism. Contact urticaria in a 4-year-old boy following a single exposure to DEET was reported by von Mayenburg and Rakoski (1983). When tested later at age 9 and 15 years old, a similar reaction was obtained.

F. PHYSICAL AND CHEMICAL PROPERTIES (U.S. EPA, 1980)

1. Common Name: DEET

2. Chemical Name: *N,N*-diethyl-*m*-toluamide

3. Trade Names: Muskol, Off, Repel, Metadelphene, MGK Diethyltoluamide, Ultrathon, Blockade, Skedaddle

4. CAS Registry No.: 134-62-3

5. Empirical Formula: $C_{12}H_{17}NO$

6. Structural Formula:
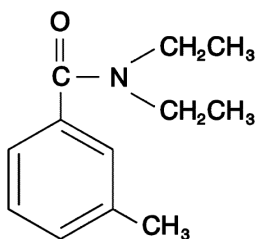
7. Molecular weight: 191.26

8. Specific Gravity: 0.990 - 1.000 at 25°C

9. Solubility: Water: 2-3 mg/ml at 25°C.
Organic solvents: Miscible in most petroleum hydrocarbons, alcohols and chlorinated solvents.
(Meinen and McKay, 1987)

10. Vapor Pressure: 1.67×10^{-3} mm Hg at 25°C

11. Color: Nearly colorless

12. Odor: Faint characteristic odor

13. Physical State: Liquid

14. Octonal/Water Coefficient: (log K_{ow}) 2.00 at 25°C (Meinen and McKay, 1987)

II. TOXICOLOGY PROFILE

A. PHARMACOKINETICS

Summary: By the oral route, DEET is quickly absorbed with approximately 90-100% absorption in rats. Dermal absorption of DEET is much slower and depends on the skin anatomy and physiology of the species, vehicles used, and protective coverings used. The average dermal absorption was approximately 40% in laboratory animals and 10% in humans. The main route of excretion is the urine. The two major urinary metabolites, *N,N*-diethyl-*m*-hydroxymethylbenzamide and *N*-ethyl-*m*-hydroxymethylbenzamide, have been found in both laboratory animals and humans.

Oral Absorption

Selim (1991a) administered ^{14}C-DEET by oral gavage to 5 rats/sex/group in a single dose at 100 or 500 mg/kg or in 13 consecutive daily doses at 100 mg/kg/day. In rats administered a single oral dose of DEET at 100 or 500 mg/kg, 85-88% and 4-5% of the administered dose was excreted in the urine and feces, respectively, within 7 days following administration. When rats were administered repeated oral doses of DEET at 100 mg/kg/day, urinary excretion increased slightly to approximately 90% while fecal excretion decreased slightly to 3%. The radioactivity in the feces was assumed to be due to biliary excretion because a similar amount was excreted in the feces with dermal application. Therefore, the oral absorption rate in rats was assumed to be 100%.

Dermal Absorption

Dermal absorption rates between 8 and 80% have been reported for DEET using laboratory animals including rats, mice, guinea pigs, rabbits and dogs (Table 1). The variable results are probably due to a number of factors including differences in coverings over the application site, differences in the vehicles used, and differences in the skin anatomy including application site and physiology between strains and species.

The dermal absorption of DEET apparently can be significantly influenced by the vehicle in which it is applied. The effect of the vehicle on dermal absorption was studied by Hannibal (1992) in rabbits. The plasma concentration of DEET was monitored after dermal application of two formulations, 10% DEET in ethanol and 10% DEET in Skedaddle®. The dermal absorption with the ethanol vehicle was significantly higher (42%) than with the Skedaddle® vehicle (15%). On other hand, there was little difference in the dermal absorption of undiluted DEET (6%) and a 15% DEET solution in ethanol (8%) when applied to human volunteers (Selim, 1992). Most of the available dermal absorption studies used ethanol as the vehicle (Schmidt *et al.*, 1959; Blomquist and Thorsell, 1977; Reifenrath *et al.*, 1980 & 1981; Snodgrass *et al.*, 1981; Moody *et al.*, 1989; Selim, 1992; Hannibal, 1992), although several studies used acetone (Feldman and Maibach, 1970; Moody *et al.*, 1989; Moody and Nadeau, 1993) and two studies did not use any vehicle (Selim, 1991a & 1992). Interestingly, DEET has been found to significantly enhance the dermal absorption of various drugs (e.g., hydrocortisone, benzocaine, ibuprofen, erythromycin) in an *in vitro* diffusion cell model using hairless mouse skin (Windheuser *et al.*, 1982). However, other investigators found the simultaneous application of DEET with permethrin or carbaryl to mouse, rat and/or pig skin *in vitro* reduced the absorption of permethrin or carbaryl, respectively (Baynes *et al.*, 1997). These investigators suggested that DEET may be enhancing the depot formation of lipophilic chemicals on the skin surface.

Table 1. Estimates of Dermal Absorption for DEET

Species, sex (n)	Dose (µg/cm^2)	Collection Period	Vehicle	Protective Covering	Urinary Excretion[a]	Fecal Excretion[a]	Recovery[b]	Estimated Absorption[a]	Ref.[c]
Mice, male (6)	33	2 d	ethanol	none	34	NA[d]	NA	34	1
Rat, male (3)	4	7 d	ethanol	non-occl.[e]	43.2±6.8	0.4±0.1	88.2±5.9	49.0	2
Rat, female (3)	4	7 d	ethanol	non-occl.	32.4±8.5	0.4±0.2	91.6±0.7	35.4	2
Rat, male (8)	10.5	7 d	acetone	non-occl.	NR[f]	NA	79±10[g]	36±8	3
Rat, male (5)	2.4	7 d	none	occlusive[h]	77.9±17.1	4.0±1.1	88.5±17.4	92.5	4
Rat, female (5)	2.4	7 d	none	occlusive	74.0±6.3	7.1±1.5	88.1±5.3	92.0	4
Rat, NA (4)	13	14 d	acetone	non-occl.	38.1±10.3	1.1±0.5	NA	39.2	5
Guinea pig, NR (2)	~1,000	9 d	ethanol	none	18,45	0.1,0.4	NA	18,45	6
Guinea pig, NR (4)	12.4	14 d	acetone	non-occl.	26.3±5.4	2.9±0.8	NA	29.2	5
Rabbit, female (6)	4	7 d	ethanol	non-occl.	36.1±10.7	2.3±1.2	93.6±5.9	38.6	2
Rabbit, male (6)	~39,000	24 hr	ethanol	non-occl.	NA	NA	NA	42[i]	7
Rabbit, male (6)	~39,000	24 hr	Skedaddle®	non-occl.	NA	NA	NA	15[i]	7
Dog (beagle), male (3)	4	7 d	ethanol	non-occl.	30.8±5.4	0.4±0.2	52.1±12.3	59.1	2
Dog (hairless), M/F (3)	4	5 d	ethanol	non-occl.	11.6±4.2	NA	NA	12.8±4.6	8
Dog (hairless), M/F (3)	320	5 d	ethanol	non-occl.	8.5±3.3	0.2±0.3	NA	9.4±3.6	8
Dog (hairless), M/F (3)	320	5 d	ethanol	non-occl.	NR	NR	90.6±2.7	7.9±2.5	9
Cattle, female (4)	1,000	24 hr	ethanol	none	NA	NA	NA	72.9±8.3[i]	10
Monkey, male (8), forehead	10.5	7 d	acetone	non-occl.	NR	NA	87±10[g]	33±11	3
Monkey, male (8), forearm	10.5	7 d	acetone	non-occl.	NR	NA	87±10[g]	14±5	3
Monkey, male (8), v. forepaw	10.5	7 d	acetone	non-occl.	NR	NA	87±10[g]	68±9	3
Monkey, male (8), d. forepaw	10.5	7 d	acetone	non-occl.	NR	NA	87±10[g]	27±3	3
Human, NR (4)	4	5 d	acetone	none	NR	NA	52.3	16.7±5.1	11
male (6) Human, female (1[j])	~733	2 d	ethanol	none	5.5, 3.8	NA	NA	4.6	1
Human, male (6)	500	5 d	ethanol	non-occl.	8.3±3.6	0.08±0.06	NA	8.4	12
	625	5 d	none	non-occl.	5.6±2.8	0.02±0.03	NA	5.6	12

[a] Percent of applied dose
[b] Percent of applied dose recovered in the urine after intravenous administration
[c] References: 1. Blomquist and Thorsell, 1977; 2. Snodgrass *et al.*, 1981; 3. Moody *et al.*, 1989; 4. Selim, 1991a; 5. Moody and Nadeau, 1993; 6. Schmidt *et al.*, 1959; 7. Hannibal, 1992; 8. Reifenrath *et al.*, 1981; 9. Reifenrath *et al.*, 1980; 10. Taylor *et al.*, 1994; 11. Feldman and Maibach, 1970; 12. Selim, 1992.
[d] NA = Not analyzed
[e] non-occl. = non-occlusive
[f] NR = not reported
[g] Percent of applied dose recovered in the urine after intramuscular administration
[h] Occlusive covering was a glass enclosure.
[i] The percent dermal absorption was calculated using the area under the plasma concentration curve (AUC) as follows:

% dermal absorption = (AUC$_{dermal}$)(dose$_{iv}$) / (AUC$_{iv}$)(dose$_{dermal}$) x 100

[j] One female was used for two experiments.

A.   PHARMACOKINETICS (cont.)

Another factor that may have significantly affected the absorption of DEET is the protective covering, if any, that was used to cover the application site. While most studies used some type of non-occlusive covering (Reifenrath *et al.*, 1980 & 1981; Snodgrass *et al.*, 1981; Moody *et al.*, 1989; Hannibal, 1992; Moody and Nadeau, 1993; Selim, 1992), several did not use any covering (Schmidt *et al.*, 1959; Blomquist and Thorsell, 1977), and one used a glass enclosure glued to the skin surrounding the application site (Selim, 1991a). The glass enclosure appears to have significantly enhanced dermal absorption which was estimated to be approximately 90%. In most of the other animal studies, the dermal absorption ranged between 30% to 40%. A significant portion of DEET was found in the rinses of the non-occlusive coverings which suggested some of the DEET was being lost due to evaporation and sloughing of the skin.

There appear to be some differences between species in the dermal absorption of DEET. In a study conducted by Snodgrass *et al.*, (1981), the amount excreted in the urine of rats, rabbits, and dogs was similar (38, 36, and 31% of the applied dose, respectively). However, after correcting for incomplete urinary excretion (i.e., total radioactive recovery) which was significantly lower in dogs (52%), the dermal absorption rate in dogs appears to be significantly higher (60%). The dermal absorption in cattle also appears to be fairly high (73%), although some of the difference may be due to estimating absorption using the area under the plasma concentration curve rather than urinary excretion (Taylor *et al.*, 1994). The dermal absorption in mice and guinea pigs may be slightly lower (26-36%) than rats and rabbits; however, it is uncertain if there is a real species difference because there was no adjustment for incomplete urinary excretion in the studies for these species and in general, there was a large interindividual variation in the studies. Variation in dermal absorption based on anatomical site was also observed in a study with rhesus monkeys (Moody *et al.*, 1989). Dermal absorption varied from 14% (forearm) to 68% (ventral forepaw). There also appears to be a considerable difference in the dermal absorption of DEET between some dog breeds (10% for hairless dogs vs. 60% for beagle dogs). This difference may be due to differences in skin physiology between hairy and hairless animals. The greater number of hair follicles and more complex duct networks in hairy animals could result in greater diffusion through these appendageal shunts during the initial stage of absorption (Snodgrass *et al.*, 1981). There also appears to be a difference in the metabolism of DEET between these dog breeds based on the large difference (52% vs. 91%) in recovery of radioactivity in the urine after intravenous injection. The hairless dog appears to be more similar to humans in its dermal absorption of DEET (~10%), but the beagle dog may be more similar to humans in its metabolism of DEET based on the recovery of radioactivity in the urine after intravenous injection (~50%).

The dermal absorption study conducted by Selim (1991a) appears to be the closest to meeting Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) guidelines of all the dermal absorption studies. However, it has several limitations as far as its usefulness for risk assessment. They did not include a group that was administered DEET intravenously to correct for incomplete urinary excretion and they used an occlusive covering (glass enclosure) over the application site. The occlusive glass enclosure probably significantly increased the absorption of DEET by increasing the hydration of the stratum corneum (Dugard, 1983). The occlusive covering may have also enhanced absorption by reducing evaporation because DEET has a moderately high vapor pressure (1.7×10^{-3} mm Hg at 25°C). Given the use of the occlusive covering, it is not surprising that the dermal absorption in this study was significantly higher than in other studies. Since the application site in the dermal toxicity studies for DEET were either covered with a non-occlussive material or not at all, this study was excluded in estimating a dermal absorption. In addition, the dermal absorption of DEET with the Skedaddle®

A.   PHARMACOKINETICS (cont.)

formulation as the vehicle was not used since this vehicle is not normally used in the dermal toxicity studies for DEET and it was shown that the dermal absorption of DEET was lower with this vehicle than with ethanol. Therefore, the dermal absorption of DEET in animals was estimated using the average from the other rat and rabbit studies. The average dermal absorption was 39% which was rounded to 40%. The dermal absorption for humans was assumed to be 8.4% based on the study conducted by Selim (1992) in which DEET was applied to 6 male volunteers in an ethanol vehicle. For a more detailed discussion of the selection of the human dermal absorption value, see the exposure assessment document by Sanborn (1999).

Distribution

The half-life of DEET was calculated in several studies where it was administered intravenously. Feldman and Maibach (1970) estimated the half-life for DEET in humans to be 4 hours based on the urinary excretion. Snodgrass *et al.* (1981) estimated the half-lives to be 30 and 35 minutes in rabbits and dogs, respectively, based on the disappearance of radioactivity from the blood. The peak blood level was determined to be at 15 minutes in rabbits which had blood samples taken regularly during the first 24 hours. The dissimilarities in the estimated half-lives for humans and dogs suggests that, despite similar elimination rates, the distribution and/or metabolism in humans may not be as rapid as other species.

The tissue distribution of DEET was examined in several animal studies. Autoradiography was used to examine tissue distribution in mice administered DEET either intravenously or dermally (Blomquist *et al.*, 1975; Blomquist and Thorsell, 1977). With intravenous injection, the highest concentrations of radioactivity during the first 24 hours were found in liver, kidney, lacrimal gland, nasal mucosa, urine, and bile. No radioactivity was detected on days 4 and 16. When DEET was administered dermally, the highest tissue concentrations were similar to when it administered intravenously except that the skin at the application site also had a high concentration. Six days after dermal application, the radioactivity was only detected at the application site and in the urine. Thirty-six days after dermal application, significant radioactivity was still detected at the application site, but no other tissues. The radioactivity was confined to the epidermis and hairs. Based on these findings, the investigators suggested that the skin may be acting as a depot resulting in the slow release of DEET into the blood. These investigators also administered DEET intravenously to 2 pregnant female mice (Blomquist *et al.*, 1975). The radioactivity in the fetuses was very low, although the uterine lumen outside the yolk sac villi had fairly high radioactivity levels. The investigators suggested that this may be due to excretion from the fetus through the villi.

Snodgrass *et al.* (1981) measured the radioactivity in selected tissues (brain, lungs, liver, kidneys, spleen, testes, skin, muscle, fat, and bone) 7 days after intravenous and dermal administration of DEET to rats, rabbits, and dogs. In general, the highest residues were found in the spleen, liver, lung, kidney, and skin in all three species with both routes of exposure. Snodgrass *et al.* also determined the tissue distribution of DEET in pregnant rabbits. No detectable residues were found in fetuses on gestation day 29 after repeated dermal application on gestation days 1-29. When pregnant rabbits were given a single intravenous injection of DEET on gestation day 15, their fetuses had the lowest tissue concentrations (approximately 6 times lower than blood levels of the parent) of all tissues when examined at 1, 3, 6, and 24 hours post-dosing.

A.   PHARMACOKINETICS (cont.)

Selim (1991a) determined the time to peak blood levels in 4 rats/sex for 24 hours after administration of ^{14}C-DEET at 100 mg/kg by either the oral or dermal route (Table 2). With oral administration, gender-related differences in peak blood levels were seen with the peak blood levels at 30 minutes in males and 2 hours in females. With dermal administration, peak blood levels were between 3 and 4 hours for both sexes. There was approximately a 18-fold difference in the peak blood levels with oral and dermal administration. DPR estimated the area under the curve (AUC) which represents the body burden using the trapezoid method (Ritschel, 1992). A comparison of the AUC's with oral administration to those with dermal administration indicates that the body burden is significantly higher with oral administration, particularly in females. The AUC with oral exposure was approximately 4.5 times higher than the AUC with dermal exposure when the sexes were combined.

Selim (1991a) also examined the tissue distribution in rats which were administered a single oral or dermal dose of ^{14}C-DEET and sacrificed at peak blood levels. With oral administration, male and female rats were sacrificed at 30 minutes and 2 hours, respectively. Most of the radioactivity was absorbed at the time of sacrifice based on the average amount of radioactivity remaining in the gastrointestinal contents and tissue (M: 53%; F: 6%). The tissues with residues higher than the plasma included the stomach, kidneys, small intestine, liver, fat, sciatic nerve, and lungs (females only). With dermal administration, both males and females were sacrificed 3-½ hours after dosing. Only 5-17% of the dose had been absorbed at the time of sacrifice based on the amount of radioactivity recovered in the skin rinse and the skin. The tissues with residues higher than the plasma included kidney, small intestine, liver, stomach, fat, large intestine, sciatic nerve, sexual organs (M- prostate, seminal vesicles; F-uterus, ovaries), and bone (females only).

Five studies were conducted to provide a comparison of blood levels of DEET at the no-effect levels for systemic effects in animals and at the 95th percentile exposure level in humans. In one study, plasma levels of DEET were examined in 24 Sprague-Dawley rats/sex administered a single dose of DEET (>97% meta isomer) by oral gavage at 200 mg/kg/day (Laveglia, 1998a). Three rats/sex was euthanized at 8 time intervals during the first 4 hours after dosing and plasma levels were measured. There was significantly variability in this study in the plasma levels in both sexes during the first 90 minutes after dosing. After confirming that this was not due to an error in the analysis of DEET, the investigator suggested that the differences may be due to variability in the site within the stomach to which these small volumes (~50 µl) were administered or differences in the stomach contents resulting from possible differences in water consumption prior to dosing. The investigator suggested that the higher values at 15 and 30 minutes after dosing more likely reflect the actual DEET blood profile. The plasma levels appeared to plateau within 45 minutes after dosing in both sexes. The mean DEET concentration during the 4 hour period was 9.58 and 13.61 µg/ml in males and females, respectively. The AUC was not calculated since DEET plasma levels did not return to baseline.

A similar study was conducted in which plasma levels were monitored in 40 Sprague-Dawley rats/sex that were administered DEET (> 97% meta isomer) by oral gavage at 200 mg/kg (Goldenthal, 1999). Five rats/sex were euthanized at 8 time intervals from 30 minutes to 48 hours after dosing and plasma levels of DEET were measured. The peak plasma levels were observed 30 minutes after dosing. The mean peak plasma concentrations were 7.06 and 15.28 µg/ml in male and female rats, respectively. The elimination of DEET was biphasic with rapid absorption from the stomach, distribution in the body within 2 to 4 hours, and elimination from the plasma within 12 hours. The mean AUCs were 22.91 and 79.18 µg·hr/ml in male and female rats, respectively.

A.   PHARMACOKINETICS (cont.)

Table 2. Blood Levels in Rats After Oral and Dermal Administration of Radiolabeled DEET [a]

Time (hrs)	Oral		Dermal	
	Males (dpm[b]/0.1 ml)	Females (dpm/0.1 ml)	Males (dpm/0.1 ml)	Females (dpm/0.1 ml)
0.25	4571	2414	11	9
0.5	6891	3484	96	85
1	5638	3251	297	218
2	2484	4659	333	206
3	1313	4218	300	255
4	737	3144	311	241
5	411	1781	289	214
6	274	1047	293	217
8	252	707	262	194
10	245	706	257	192
12	196	620	227	188
24	134	441	180	173
AUC[c]	16,481	29,533	5,641	4,565

[a] Selim (1991a)
[b] Concentration of DEET in blood represented as radioactivity (dpm= disintegrations per minute) per ml
[c] AUC = Area Under Curve

Plasma levels of DEET were also followed in 20 Sprague-Dawley rats/sex after a single and 5 consecutive dermal applications of DEET (> 97% meta isomer) at 1000 mg/kg/day (Laveglia, 1998b). Four rats/sex were euthanized at 5 time intervals during the 24 hours following the first or fifth application and plasma levels measured. With a single dermal dose, the mean time to peak plasma levels was 4 and 8 hours in males and females, respectively. With 5 consecutive dermal doses, the mean peak time was 8 hours in both sexes. The mean peak plasma levels in males decreased from 5.58 µg/ml after a single dose to 4.83 µg/ml after 5 consecutive doses. A similar pattern was seen in females with the mean peak plasma levels decreasing from 26.60 µg/ml after a single dose to 17.2 µg/ml after 5 consecutive doses. The mean AUC was calculated to be 89.5 and 368.2 µg·hr/ml in males and females, respectively, with a single dermal dose. After 5 consecutive doses, the mean AUC was slightly higher in males (100.8 µg·hr/ml) when compared to a single dose, but it was lower in females (134.8 µg·hr/ml). The mean half-life was 6.9 and 2.2 hours in males and females, respectively, with a single dose. The mean half-life was slightly higher in both sexes (8.7 and 2.9 hrs for males and females, respectively) after 5 consecutive doses.

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A.	PHARMACOKINETICS (cont.)

	Plasma levels of DEET were examined in 4 beagle dogs/sex after administration of DEET (97.48% meta isomer) in gelatin capsules at 75 mg/kg/day for 4 consecutive daily doses (Badalone, 1997). Blood samples were collected at 12 time intervals on the first and fourth day of dosing. The mean time to peak plasma concentration was around 30 minutes in both sexes. The mean peak concentration was 18.3 and 14.0 µg/ml in males and females on day 1. The mean peak concentration was lower on day 4 at 14.2 and 12.5 µg/ml for males and females, respectively. The mean half-life in both sexes was approximately 28 minutes on both days 1 and 4. The mean AUC were 15.9 and 13.4 µg·hr/ml in males and females, respectively, on day 1. On day 4, the mean AUC were slightly lower at 11.2 and 10.2 µg·hr/ml for males and females, respectively.

	Plasma levels of DEET were also monitored in human volunteers (3 males and 3 females) after dermal application of DEET (97.5% meta isomer) for 4 consecutive days (Ohayon *et al.*, 1997). Females and males were administered DEET at 3 and 4 g/day, respectively, to one arm and both legs. Blood samples were collected at 17 time intervals on day 1 and 4 of dosing. In females, the mean peak plasma concentration decreased from 430 µg/ml on day 1 to 254 µg/ml on day 4. The mean peak plasma concentration in males also decreased from day 1 (618 µg/ml) to day 4 (487 µg/ml). The mean half-life was 5.9 and 6.4 hours in male and females on day 1, respectively. On day 4, the mean half-life was slightly increased in both sexes at 6.7 and 9.0 hours for males and females, respectively. The mean AUC in males decreased from 5.11 µg·hr/ml on day 1 to 3.76 µg·hr/ml on day 4. A similar decrease in the mean AUC was seen in females from day 1 (3.07 µg·hr/ml) to day 4 (2.10 µg·hr/ml).

	Biotransformation

	The urinary metabolites from the rat pharmacokinetic study conducted by Selim (1991a) were analyzed (Selim, 1991b). Four distinct metabolite peaks and two zones of multiple polar metabolites were isolated. Only two of the 4 distinct metabolites were identified and described as metabolite A and B. Metabolite A (*m*-[*N,N*-diethylamino)carbonyl]benzoic acid or *N,N*-diethyl-*m*-hydroxymethylbenzamide) was the product of oxidation of the methyl substituent on the aromatic ring to a carboxylic acid group. Metabolite B (*m*-[*N*-ethylamino)carbonyl]benzoic acid or *N*-ethyl-*m*-hydroxymethylbenzamide) appears to be the product of N-dealkylation of the ethyl substituent on one of the amide moieties of metabolite A. Metabolite A and B represented 46-59% and 10-17%, respectively, of the radioactivity in all treatment groups. The remaining peaks or zones represented 1 to 14% of the applied dose.

	The *in vitro* metabolism of DEET by rat liver microsomes was examined (Taylor, 1986; Yeung and Taylor, 1988; Constantino and Iley, 1999). The major metabolites identified were *N,N*-diethyl-*m*-hydroxymethylbenzamide and *N*-ethyl-*m*-toluamide. Other minor metabolites identified included *N*-ethyl-*m*-hydroxymethylbenzamide, *m*-toluamide and *N,N*-diethyl-*m*-formylbenzamide. These investigators suggested that these metabolites were the products of monooxygenase-mediated reactions involving benzylic hydroxylation or N-deethylation or combinations of both. The microsomes from male rats metabolized DEET significantly faster than those from female rats. In both cases the rate of metabolism leveled off after 90 minutes of incubation. The estimated metabolic half-lives were 10 and 15 minutes for males and females, respectively. The difference in the metabolic rates between males and females could account for some or all of the difference in the blood levels between male and female rats.

A.   PHARMACOKINETICS (cont.)

Urinary metabolites of DEET were also examined in human volunteers (Selim, 1992). The major metabolites identified in human urine were *m*-[*N,N*-diethylamino)carbonyl]benzoic acid and *m*-[*N*-ethylamino)carbonyl]benzoic acid which represented 24.0-42.4% and 7.6-25.5%, respectively, of the applied dose in 24-hour urine samples with either the undiluted DEET or a 15% DEET solution in ethanol. Wu *et al.* (1979) also examined the urinary metabolites in a male volunteer which had applied 10.4 g of unlabeled DEET over 75% of his skin surface (~0.711 mg/cm^2). The unchanged parent compound was isolated in the urine during the first 18 hours. They also identified the hydroxylation products, *N*-hydroxyethyl-*N*-ethyl-*m*-toluamide glucuronide conjugate and benzylic alcohol, and the oxidation product, *m*-carboxyl-*N,N*-diethyl-benzoylamide. The difference in the metabolic pattern observed in the Selim (1992) and Wu *et al.* (1979) studies may be related to a 1000-fold difference in the dose levels applied (12-15 mg vs. 10 g).

No data were available on the toxicity of these tissue or urinary metabolites.

Excretion

The major route of excretion in rats with oral or dermal administration of DEET was the urine (Selim, 1991a). After a single or 13 consecutive oral doses at 100 mg/kg, 75-85% of the radioactivity was excreted in the urine in the first 12 hours. However, after a single oral dose at 500 mg/kg, only 34-50% of the radioactivity was excreted in the urine. The excretion was also delayed following dermal application of DEET at 100 mg/kg, with approximately 60% of the radioactivity excreted between 12 and 72 hours after dosing. Approximately 3-5% of the administered dose was excreted in the feces with either route of administration suggesting that biliary excretion was involved. With both dermal and oral exposure, less than 0.02% of DEET was excreted as CO_2 (Lin and Selim, 1991).

B.   ACUTE TOXICITY

Summary: There were numerous acute toxicity studies available for DEET, primarily for the formulations. DEET appears to be more toxic by the oral and inhalation route, than the dermal route. A No-Observed-Effect Level (NOEL) of 5,000 mg/kg was established for systemic and local effects with dermal exposure to DEET. A NOEL could not be established in the available oral and inhalation studies. The Lowest-Observed-Effect Level (LOEL) for technical grade DEET by the oral route was 1,000 mg/kg based on hunched posture, hypoactivity, piloerection, reduced fecal volume, ataxia, tremors and prostration. Effects observed at higher doses included ataxia, tremors, convulsions, loss of balance, reduced blood pressure and heart rate, prostration, petechial hemorrhages in the lungs, mottled liver, kidneys and spleen, and congested kidneys. Similar effects were observed with inhalation exposure. The LOEL for inhalation exposure was 2,020 mg/m^3 (323 mg/kg[1]) based on irregular respiration, hunched posture, hypoactivity, and red ocular discharge. DEET appears to be more toxic in young rats than older rats. No sensitization was observed with technical grade DEET. The effects observed with the formulations varied depending on both the dilution and toxicity of inert and active ingredients. There is evidence that DEET has a synergistic effect on the toxicity of several chemicals.

B.   ACUTE TOXICITY (cont.)

Systemic Effects

Oral

The acute oral toxicity of DEET is summarized in Table 3. Although the meta-isomer is the primary isomer, all the formulations contained a small percentage of other isomers. There appears to be an age-related decrease in sensitivity to DEET in rats (Verschoyle *et al.*, 1992). The LD_{50} values in females increased from 667 mg/kg at 11 days to 3429 mg/kg at 47-56 days while in males they increased from 891 mg/kg at 11 days to 3564 mg/kg at 47-56 days. The effects observed after a single oral dose of technical grade DEET include lethargy, hunched posture, piloerection, loss of balance, ataxia, convulsions, tremors, bloody tears, labored breathing and prostration. Gross pathological findings included petechial hemorrhages in the lung and intestines, mottled livers, kidneys and spleen, transparent, injected, fluid-filled stomachs with pink or white pylorus, and congested kidneys. A NOEL was not established in any of these studies. In one study by Weil (1973), only lethargy and labored breathing were observed at 1,250 mg/kg, the lowest dose tested. In a study by Macko and Weeks (1980), prostration, ataxia, and body tremors were observed at 1,000 mg/kg, the lowest dose tested. Similar findings were reported in a third study by Moore (2000a) where hunched posture, hypoactivity, reduced fecal volume, and piloerection were seen at the LOEL, 1,000 mg/kg. The study by Moore (2000a) was found acceptable to DPR toxicologists based on FIFRA guidelines.

Since the number of DEET formulations registered in California is so large, not all of the acute toxicity data for the formulations is summarized in this section. Instead, data for several representative formulations were included. The clinical signs observed with oral exposure to the formulations containing DEET as the only active ingredient were very similar to those observed with the technical grade material, except for mydriasis and hypothermia which were also seen (Thompson, 1980). The gross pathological findings were similar except for raised white pinpoint areas in the lung and thickened areas or nodules in the forestomach (Thompson, 1980; Doyle and Smith, 1984a). The only additional clinical sign seen with formulations containing DEET and other active ingredients was catalepsy which was observed in the formulation containing DEET and fenvalerate (Platt, 1988). Blanching of the liver, kidneys, and spleen was also observed with this formulation (Platt, 1988).

Dermal

The acute dermal toxicity for DEET is summarized in Table 4. The lowest lethal dose for technical grade DEET by the dermal route was 3,200 mg/kg (Macko and Weeks, 1980). The systemic effects observed with dermal application of technical grade DEET included slow respiration, rolling eyes, lethargy, rear limbs extended, and hematoma. Local effects included erythema and edema which were observed at lower doses than the systemic effects. Gross pathological lesions were observed in the lungs, spleen, kidneys, and liver. In a study conducted by Weil (1973), DEET was applied to abraded or intact skin of two rabbits/dose (sex not reported) under polyethylene sheeting (exposure duration not reported). Slow respiration and rolling eyes was observed at 8000 mg/kg with intact skin. Gross pathological lesions were observed in this study, including congestion of the lungs, spleen, and kidneys, and pale and mottled liver. However, the dose levels at which these lesions were observed were not reported. Only local effects (erythema and edema) were seen in rabbits at the lowest dose tested, 2000 mg/kg, with both abraded and intact skin. In another study conducted by Macko and Weeks (1980), DEET was applied to the clipped skin of 4 New Zealand White rabbits/sex/dose and covered by a plastic cuff for 24 hours. One male rabbit exhibited clinical

B. ACUTE TOXICITY (cont.)

Table 3. Summary of Acute Oral Toxicity for DEET

Species	Sex	LD_{50} (mg/kg)	References[a]
Technical Grade (85 - 100%)			
Rat	M	1,800 - 3,237	1-4
	F	1,750 - 2,420	1,3-4
DEET Only Formulations (15-44%)			
Rat	M	4,801 (44%)	5
	F	3,873 (44%)	
	M/F	3,198 (40%)	6
	M/F	>5,000 (35%)	7
	M	5,410 (25%)	8
	F	2,510 (25%)	
	M/F	>5,000 (15%)	9
DEET Formulations with Other Active Ingredients[b] (9-25%)			
25% DEET[c], 5% OBD, 2.5% DPI			
Rat	M	4,870	10
	F	2,670	
10% DEET, 7.5% OMC, 5% OB			
Rat	M/F	> 5,000	11
9% DEET, 0.09% Fenvalerate			
Rat	M	9,250	12
	F	5,420	

[a] References: 1. Ambrose *et al.*, 1959; 2. Weil, 1973; 3. Macko and Weeks, 1980; 4. Moore, 2000a; 5. Doyle and Smith, 1984a; 6. Levine, 1990a; 7. Randen, 1986a; 8. Thompson, 1980; 9. Kukulinski and Locke, 1984a; 10. Gabriel, 1990a; 11. Wnorowski, 1995a; 12. Platt, 1988.
[b] The other active ingredients include octyl bicycloheptene dicarboximide (OBD), dipropyl isocinchomeronate (DPI), octyl methoxycinnamate (OMC), oxybenzone (OB) and fenvalerate.
[c] The formulation was tested for acute oral toxicity without the propellant which was 25% of the formulation.

signs at the lowest dose tested, 1800 mg/kg. Although the clinical signs observed in this study were reported to be lethargy, rear limbs extended and large hematoma on ventral side, the incidence was not broken down by dose level. In a more recent study, no systemic or local effects were seen in male or female Sprague-Dawley rats (5/sex) when DEET was applied

B. ACUTE TOXICITY (cont.)

Table 4. Summary of Acute Dermal Toxicity for DEET

Species	Sex	LD_{50} (mg/kg)	References[a]
Technical Grade (85 - 100%)			
Rabbits	NR	3,180	1
	M	4,268	2
	F	4,223	
	NR	>2,000	3
Rat	M/F	>5,000	4
DEET Only Formulations (15-75%)			
Rabbits	M	4,340 (75%)	2
Rats	M	4,154	
	F	4,903	
Rabbits	M/F	>2,000 (15-44%)	5-10
DEET Formulations with Other Active Ingredients[b] (9-25%)			
25% DEET[c], 5% OBD, 2.5% DPI			
Rabbits	M/F	>2,000	11
10% DEET, 7.5% OMC, 5% OB			
Rabbits	M/F	>2,000	12
9% DEET, 0.09% Fenvalerate			
Rabbits	M/F	>2,000	13

[a] References: 1. Weil, 1973; 2. Macko and Weeks, 1980; 3. Ambrose *et al.*, 1959; 4. Moore, 2000b 5. Doyle *et al.*, 1984; 6. Levine, 1990b; 7. Randen, 1987; 8. Costello and Gilman, 1982; 9. Thompson, 1980; 10. Kukulinski and Locke, 1984b; 11. Gabriel, 1990b; 12. Wnorowski, 1995b; 13. Rothstein, 1986a.

[b] Other active ingredients include octyl bicycloheptene dicarboximide (OBD), dipropyl isocinchomeronate (DPI), octyl methoxycinnamate (OMC), oxybenzone (OB) and fenvalerate.

[c] The formulation was tested for acute dermal toxicity without the propellent which was 25% of the formulation.

undiluted to their clipped backs (application site approximately 2 inches by 3 inches) at 5000 mg/kg, the only dose level tested (Moore, 2000b). The application site was covered for 24 hours with a semi-occlusive wrapping (gauze covered by Durapore® tape). This study was found acceptable based on FIFRA guidelines. The NOEL established in this study is higher than the LOELs in the two previous studies; however, an occlusive covering was used in the two previous studies which probably increased dermal absorption significantly. Another factor that may have contributed to the different results was species differences in sensitivity, although

B.   ACUTE TOXICITY (cont.)

Macko and Weeks (1980) did not find a major difference in the dermal LD_{50} values in rats and rabbits exposed to a 75% DEET formulation.

The systemic signs observed with the DEET formulations were primarily diarrhea and slight body weight loss. Diarrhea was observed with one formulation containing 25% DEET, 5% octyl bicycloheptene dicarboximide, and 2.5% dipropyl isocinchomeronate (Gabriel, 1990b). Erythema and/or edema were seen with most formulations. Desquamation, fissuring, and subcutaneous hemorrhaging were also observed with two formulations containing 25-40% DEET as the only active ingredient (Thompson, 1980; Levine, 1990b). Diffuse, pitted surface of the kidney and gas/liquid-filled gastrointestinal tract were the only treatment-related gross lesions reported with formulations (Thompson, 1980; Gabriel, 1990b).

<u>Inhalation</u>

Fewer studies were available for the acute inhalation toxicity of DEET and its formulations (Table 5). In a 4-hr acute inhalation study conducted by the U.S. Army, the clinical signs observed included unconsciousness, labored breathing, slight convulsions, nasal discharge, ataxia, ruffled hair, tremors, red urine, and death (Macko and Bergman, 1980). No gross pathological lesions were found. At the lowest dose tested, 3700 mg/m^3 (592 mg/kg[1]), ataxia, labored breathing, red urine, nasal discharge and death were observed. In a recent 4-hr

Table 5. Summary of Acute Inhalation Toxicity for DEET

Species	Sex	LC_{50} (mg/m^3)	References[a]
Technical Grade (100%)			
Rats	M/F	5,950 (4-hr, whole body)	1
	M/F	>2,020 (4-hr, whole body)	2
DEET Only Formulations (15%)			
Rats	M/F	2,330-5,500 (4-hr, whole body)	3
DEET Formulations with Other Active Ingredients[b] (9-25%)			
25% DEET[c], 5% OBD, 2.5% DPI			
Rats	M/F	>7,810 (4-hr, whole body)	4
9% DEET, 0.09% Fenvalerate			
Rabbits	M/F	>19,020 (4-hr)	5

[a] References: 1. Macko and Bergman, 1980; 2. Moore, 2000c; 3. Kukulinski, 1984; 4. Hershman, 1990; 5. Rothstein, 1986b.
[b] Other active ingredients include octyl bicycloheptene dicarboximide (OBD), dipropyl isocinchomeronate (DPI).
[c] The formulation was tested for acute inhalation toxicity without the propellant which was 25% of the formulation.

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[1]   Estimated assuming a respiratory rate of 0.16 m^3/kg/4 hrs for a rat (Zielhuis and van der Kreek, 1979).

B.   ACUTE TOXICITY (cont.)

inhalation study conducted by Moore (2000c), no deaths were observed at the only dose level tested 2,020 mg/m^3 (323 mg/kg[1]). The clinical signs were observed, including irregular respiration, hunched posture, hypoactivity, and red ocular discharge. No gross pathological lesions were seen. This study was found acceptable based on FIFRA guidelines.

The requirement for an inhalation study was waived by U.S. EPA and DPR for several formulations because most of the particles in the aerosol were not respirable (greater than 16 microns in diameter) or the formulation was a lotion or cream that would not be expected to be inhaled with normal use. Among the available inhalation studies for formulations, the only clinical signs observed were prostration, inactivity/sluggishness, wet/unkempt/ruffled fur, closed eyes, and nasal staining, (Kukulinski, 1984; Hershman, 1990). No gross lesions were seen in any of these studies.

Based on transient decreases in blood pressure observed by Ambrose *et al.* (1959) after intravenous injections of DEET, Leach *et al.* (1988) investigated the cardiovascular effects in rats administered DEET intraperitoneally at 56, 113 or 225 mg/kg. At 113 and 225 mg/kg, both the heart rate and blood pressure were reduced. No effect on heart rate and blood pressure were observed at 56 mg/kg. The cardiovascular effects of DEET were reduced in rats pretreated with 5 mg/kg atropine or 10 µg/kg acetylcholine. DEET had no effect in rats on the responsiveness to several autonomic and cardiovascular drugs including epinephrine, norepinephrine, and histamine. The hemodynamic effects of DEET were also examined by these investigators in dogs administered the compound intraperitoneally at 225 mg/kg. There was not only a reduction in blood pressure and heart rate at this dose, but a decrease in cardiac output. Slight ECG changes were observed including changes in the P-R and Q-T intervals. The NOEL for these cardiovascular effects were 56 mg/kg in rats. A NOEL could not be established for these effects from the dog study.

Local Effects

The ocular irritation potential for various DEET formulations is summarized in Table 6. After a 24-hour exposure, technical grade DEET caused slight to moderate corneal opacity, slight iritis, and moderate to severe conjunctivitis in rabbits (Ambrose *et al.*, 1959; Macko and Weeks, 1980; Moore, 2000d). The corneal opacity and iritis cleared in 5-7 days in all studies, but in some studies the conjunctivitis persisted after 7 days. The study by Moore (2000d) was found acceptable to DPR toxicologists based on FIFRA guidelines. The ocular irritation observed with the formulations varied significantly and was not necessarily related to the DEET concentration. The mildest reaction was mild corneal opacity and conjunctivitis which cleared in 72 hrs with a formulation that contained 10% DEET, 7.5% octyl methoxylcinnamate (OMC) and 5% oxybenzone (OB) (Wnorowski, 1995c). The most severe reaction was observed with a formulation that contained only DEET as the active ingredient (Thompson, 1980). The ocular effects with this formulation included peeling corneal epithelial damage and pannus which persisted after 7 days, corneal neovascularization that was still present on day 21, slight iritis that cleared by day 14, and slight to moderate conjunctivitis with purulent discharge that persisted after day 21. In a study designed to evaluate the effect of the nictitating membrane on the ocular response to DEET, the membrane was removed from one eye of rabbits 2-3 weeks prior to testing (Meyer *et al.*, 1987). Bilateral application of DEET resulted in higher scores during the first 72 hours in the intact eyes due to conjunctival congestion. However, after 1-3 weeks the eyes without the membrane had higher scores due to corneal cloudiness and pannus (probably from altered tear function). When the eyes were irrigated after

B.   ACUTE TOXICITY (cont.)

Table 6. Summary of Eye Irritation Potential of DEET

Species	Sex	Results	References[a]
		Technical Grade (100%)	
Rabbit	NR[b]	Moderate-Severe Irritation	1-3
		DEET Only Formulations (15-44%)	
Rabbit	M/F	Severe Irritation (44%)	4
	M/F	Severe Irritation (40%)	5
	M/F	Severe Irritation (35%)	6
	NR	Corrosive (25%)	7
	M/F	Moderate Irritation (15%)	8
		DEET Formulations with Other Active Ingredients (9-25%)[c]	
		25% DEET, 5% OBD, 2.5% DPI	
Rabbit	NR	Moderate Irritation	9
		10% DEET, 7.5% OMC, 5% OB	
Rabbit	M/F	Slight Irritation	10
		9% DEET, 0.09% Fenvalerate	
Rabbit	F	Severe Irritation	11

[a] References: 1. Ambrose *et al.*, 1959; 2. Macko and Weeks, 1980; 3. Moore, 2000d; 4. Wingard and Smith, 1984; 5. Busch, 1985a; 6. Randen, 1986b; 7. Thompson, 1980; 8. Kukulinski and Locke, 1984c; 9. Moore, 1992; 10. Wnorowski, 1995c; 11. Rothstein, 1986c.
[b] NR = Not reported
[c] Other active ingredients include octyl bicycloheptene dicarboximide (OBD), dipropyl isocinchomeronate (DPI), octyl methoxycinnamate (OMC), oxybenzone (OB) and fenvalerate.

application, the scores were higher in the intact eyes during the entire observation time (probably due to less effective rinsing).

The dermal irritation potential of various DEET formulations is summarized in Table 7. Technical grade DEET caused slight to moderate erythema at 1 to 72 hours post-exposure and slight edema at 1 to 24 hours post-exposure (Macko and Weeks, 1980; Moore, 2000e). The study by Moore (2000e) was found acceptable to DPR toxicologists based on FIFRA guidelines. The dermal irritation was cleared by 7 days. Like the ocular irritation, dermal irritation varied significantly between the formulations. The other ingredients, either active or inert, in the formulations appeared to significantly affect the dermal irritation since there was no consistent decrement in the response as the concentration of DEET decreased. The strongest dermal reactions (moderate irritation) to the formulations were with two DEET only formulations that contained either 40% DEET or 15% DEET (Busch, 1985b; Kukulinski and Locke, 1984d). On the other hand, no dermal irritation was observed with a formulation containing 25% DEET and

B.   ACUTE TOXICITY (cont.)

Table 7. Summary of Dermal Irritation Potential of DEET

Species	Sex	Results	References[a]
Technical Grade (100%)			
Rabbit	NR[b]	Slight-Moderate Irritation	1-3
DEET Only Formulations (15-44%)			
Rabbit	M/F	Slight Irritation (44%)	4
	M/F	Moderate Irritation (40%)	5
	M/F	Slight Irritation (35%)	6
	M/F	No Irritation (25%)	7
	M	Moderate Irritation (15%)	8
DEET Formulations with Other Active Ingredients (9-25%)[c]			
25% DEET, 5% OBD, 2.5% DPI			
Rabbit	NR	Slight Irritation	9
10% DEET, 7.5% OMC, 5% OB			
Rabbit	M/F	Slight Irritation	10
9% DEET, 0.09% Fenvalerate			
Rabbit	F	No Irritation	11

[a] References: 1. Ambrose *et al.*, 1959; 2. Macko and Weeks, 1980; 3. Moore, 2000e; 4. Kreuger and Smith, 1984; 5. Busch, 1985b; 6. Randen, 1986c; 7. Thompson, 1980; 8. Kukulinski and Locke, 1984d; 9. Romanelli, 1990; 10. Wnorowski, 1995d; 11. Rothstein, 1986d.
[b] NR = Not reported
[c] Other active ingredients include octyl bicycloheptene dicarboximide (OBD), dipropyl isocinchomeronate (DPI), octyl methoxycinnamate (OMC), oxybenzone (OB) and fenvalerate.

another formulation containing 9% DEET and 0.09% fenvalerate (Thompson, 1980; Rothstein, 1986d).

The dermal sensitization potential of the DEET formulations is summarized in Table 8. No dermal sensitization was observed in guinea pigs or humans with any formulation (including technical grade DEET).

Synergism

There is some evidence that suggest DEET may act synergistically with other chemicals. In 1987, 266 calls were received by the Illinois Animal Poison Information Center regarding cats with clinical signs of toxicosis that had been exposed to a product containing 9% DEET and 0.9% fenvalerate (Dorman *et al.*, 1990). The most common clinical signs in these cats were tremors, hypersalivation, ataxia/incoordination, vomiting, depression, seizures,

B.   ACUTE TOXICITY (cont.)

Table 8. Summary of Dermal Sensitization Potential of DEET

Species	Sex	Results	References[a]
Technical Grade (100%)			
Guinea Pig	M/F	No Sensitization	1-3
DEET Only Formulations (15-44%)			
Guinea Pig	F	No Sensitization (44%)	4
	M/F	No Sensitization (40%)	5
Human	NR	No Sensitization (35%)	6
Guinea Pig	M/F	No Sensitization (25%)	7
Human	M/F	No Sensitization (25%)	8
Guinea Pig	M	No Sensitization (15%)	9
DEET Formulations with Other Active Ingredients (9-25%)[c]			
25% DEET[d], 5% OBD, 2.5% DPI			
Guinea Pig	NR	No Sensitization	10
10% DEET, 7.5% OMC, 5% OB			
Guinea Pig	M/F	No Sensitization	11
9% DEET, 0.09% Fenvalerate			
Guinea Pig	M	No Sensitization	12

[a] References: 1. Macko and Weeks, 1980; 2. Ambrose *et al.*, 1959; 3. Moore, 2000f; 4. Doyle and Smith, 1984b; 5. Kreuzmann, 1990; 6. Randen, 1986d; 7. Hughes and Kreuzmann, 1985; 8. Wilson and Gabriel, 1980; 9. Kukulinski and Locke, 1984e; 10. Romanelli, 1990b; 11. Wnorowski, 1995e; 12. Nitka, 1986.
[b] NR = Not reported
[c] Other active ingredients include octyl bicycloheptene dicarboximide (OBD), dipropyl isocinchomeronate (DPI), octyl methoxycinnamate (OMC), oxybenzone (OB) and fenvalerate.
[d] The formulation was tested for dermal sensitization without the propellent which was 25% of the formulation.

hyperactivity/ hyperexcitability, anorexia, hypothermia, mydriasis, disorientation, dyspnea, and vocalization. Twenty deaths were reported. The clinical signs appear to be due to a combination of effects observed with the two active ingredients. Death was observed in 2 cats administered 40% of a 7 oz. can of product/kg (equivalent to 7.13 g DEET/kg and 71.3 mg fenvalerate/kg) dermally (Mount *et al.*, 1991). One of 2 cats that received 20% of a can/kg (3.56 g DEET/kg; 35.6 mg fenvalerate/kg) dermally became moribund at 22 hours. Dermal application resulted in only mild signs at these same dose levels in dogs. The investigators indicated the severity of the clinical signs paralleled the serum DEET concentrations; however, the dermal dose of DEET at which these cats died, 7.13 g/kg, was on the same order of magnitude as the reported dermal LD_{50} values in rats (Table 4). Instead this data suggests that the toxicity of fenvalerate may have been enhanced by DEET since the dose of fenvalerate administered dermally to cats that died, 71.3 mg/kg, was significantly lower than the reported

B.   ACUTE TOXICITY (cont.)

dermal LD_{50} of 2500 mg/kg in rabbits cited by the investigators. However, since the investigators did not administer the test compounds separately to dogs and cats, it is uncertain if the apparent difference in toxicity is due to species differences or a synergistic effect. Cats were more sensitive than dogs to this product in this study. In a study discussed previously under the pharmacokinetics section, DEET increased the dermal absorption of several drugs examined which could result in an increase in their toxicity (Windheuser *et al.*, 1982).

The potential synergistic effects of DEET, permethrin and pyridostigmine bromide (PB) were examined in male rats (McCain, 1995). All three of these chemicals were given to military personnel during the Persian Gulf War in 1991 and was investigated as a possible cause for the commonly known "Gulf War syndrome." The LD50 values were initially calculated for each compound when administered separately by oral gavage. Rats were then administered each compound by oral gavage at their LD_{16}, LD_{30}, LD_{50}, LD_{70}, and LD_{84} concentration (DEET: 1946, 2628, 3664, 5109 or 6896 mg/kg; permethrin: 279, 511, 1000, 1953 or 3576 mg/kg; PB: 46, 53, 61, 100 or 126 mg/kg) in combination with the other two chemicals at their respective LD_{16} concentration. Greater than expected mortality was observed in rats administered all three compounds where the additive value was less than 100%.   The differences were significant where low mortality was expected. Rats were also administered various combinations of two of the three compounds at their respective LD_{16} concentrations. The observed toxicity when PB was combined with either DEET or permethrin was greater than expected by a factor of 2 to 3. However, when DEET and permethrin were combined, the observed toxicity was less than additive (by a factor of 3). The investigator suggested one possible mechanism for the enhanced toxicity between DEET and PB was enhanced oral absorption of PB by DEET. However, this mechanism would not necessarily apply when PB is given by the oral route and DEET by the dermal route as it was in the Persian Gulf. The investigator suggested the enhanced toxicity between PB and permethrin may be due to reduced detoxification of permethrin from either the inhibition of non-specific esterases or overloading of metabolic pathways. No explanation was given for the less than additive toxicity between DEET and permethrin. The general population is unlikely to be exposed to DEET in combination with PB, but it is possible for people to apply both DEET and permethrin simultaneously since a tick repellent containing permethrin is now available to the general public for use on human clothing. People may treat their clothes with the permethrin repellent and then apply DEET to their exposed skin surfaces.

The synergistic effects of DEET, permethrin and PB were also examined in hens by Abou-Donia and Wilmarth (1996). Groups of 5 hens were given chemicals individually or simultaneously 5 days/wk for 2 months as follows: DEET (neat) injected subcutaneously at 500 mg/kg/day, permethrin (corn oil) injected subcutaneously at 500 mg/kg/day, and PB (water) administered by gavage at 5 mg/kg/day. The combined treatments included DEET + permethrin, DEET+ PB, permethrin + PB, and DEET + permethrin+ PB. Animals were scored for clinical signs, locomotor dysfunction, tremor, histopathological examination of the spinal cord and sciatic nerve, and cholinesterase (ChE) activity in the plasma and brain. A slight increase in the scores for clinical signs was seen when either PB or DEET were given alone. A slight increase in the score for neuropathological lesions was also seen when either permethrin or DEET were given alone. When two or more chemicals were combined there was an increase in the score for all the parameters, except for the neuropathological lesions which were unaffected by PB + permethrin. The scores for the various combination regimens increased in the following order: PB + permethrin < permethrin + DEET < PB + DEET < PB + DEET + permethrin. Plasma ChE activity was significantly reduced when PB (17% of controls) or DEET (83% of controls) were given alone. The investigators attributed the significant plasma ChE

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B.   ACUTE TOXICITY (cont.)

inhibition with DEET to its structural similarity to phenyl carbamates. Significant plasma ChE inhibition was also seen when two or more chemicals were combined, although the inhibition with all 3 chemicals was not greater than with PB alone. Brain ChE activity was not inhibited with any treatment regimen. The investigators attributed the increase in scores when PB was combined with DEET and/or permethrin to the inhibition of esterases involved in their detoxification. In support of their hypothesis, the investigators suggested that the neuropathological findings in hens exposed to the combination of all 3 chemicals were similar to findings in other studies where near lethal doses of permethrin or DEET were given. However, this hypothesis does not explain the increase in scores when permethrin and DEET were given simultaneously. The increase in scores when permethrin and DEET were combined is also in contrast to the findings of McCain where combined exposure to DEET and permethrin in rats resulted in a less than additive lethality. Like the McCain study, comparison of the findings from the Abou-Donia et al. study to the exposure scenario for soldiers in the Persian Gulf is limited because of the route of administration of DEET and permethrin. The investigators apparently selected subcutaneous injection over topical application to accurately account for the absorbed dose. However, Baynes *et al.* (1997) reported that simultaneous topical application of DEET and permethrin resulted in reduced absorption of permethrin (see the Pharmacokinetics section).

A similar study was conducted by Abou-Donia *et al.* (1996) in which the synergistic effects of combined exposure to DEET, PB, and chlorpyrifos were examined. The number of animals and dosing regimen was the same as the previous study except that 10 mg/kg/day of chlorpyrifos (corn oil) was injected subcutaneously instead of permethrin. The scoring for neurotoxic effects was also the same as the previous study. Chlorpyrifos alone produced mild cholinergic signs and a slight increase in neuropathological changes. Combined exposure to two chemicals resulted in an increase in scores for all parameters, with the largest increase for chlorpyrifos + PB. When all 3 chemicals were given simultaneously the scores were even higher. Plasma ChE activity was reduced with all dosing regimens ranging from 83% of controls when DEET was given alone to 13% of controls when all three chemicals were given simultaneously. Brain ChE activity was only reduced in animals given chlorpyrifos or some combination with it and ranged from 67% of controls for chlorpyrifos alone to 24% of controls for all 3 chemicals. Significantly reduced brain neuropathy target esterase (NTE) activity (71-73% of controls) was only found when chlorpyrifos was given simultaneously with at least one other chemical.

Chaney et al (1998a) administered DEET (100, 200, 400 or 700 mg/kg) and PB (2, 3, 4 or 5 mg/kg) separately and combined (200 mg/kg DEET and 2, 3 or 4 mg/kg PB) by intraperitoneal injection to male ICR mice. Although the combined exposure resulted in a synergistic increase in lethality, the incidence of seizure were no greater than that observed for DEET alone. Pretreatment with various anticonvulsant drugs (diazepam, dilantin, phenobarbital or dextrorphan) did not reduce the incidence of seizures with either DEET or PB, although some of the drugs prolonged the time of onset of the seizures or the time to death. Pretreatment with anticholinergic drugs such as atropine and atropine methyl nitrate protected again lethality caused by PB and PB + DEET, but did not protect against seizures from DEET or PB + DEET (Chaney, 1999). Mecamylamine which is a nicotinic ganglionic antagonist protected against both seizures and lethality of PB, but not DEET or PB + DEET.

Chaney et al. (1998b) also administered DEET (200 mg/kg) and PB (1, 2 or 3 mg/kg) separately and combined by intraperitoneal injection in male rats. In this study, they examined the effect of these treatments on heart, diaphragm, blood and whole brain ChE activity. DEET

## B.	ACUTE TOXICITY (cont.)

alone did not affect ChE activity in any of these tissues. Pyridostigmine alone inhibited ChE activity in all tissues except the brain. The lack of ChE inhibition in the brain is as expected since PB is a quaternary ammonium compound which should not be able to penetrate the blood-brain barrier. Significant inhibition of whole brain ChE activity (60% of controls) was observed when DEET and PB (3 mg/kg) were given simultaneously. The investigators suggest that DEET may be facilitating the transport of PB across the blood-brain barrier at high doses of PB.

## C.	SUBCHRONIC TOXICITY

Summary: The subchronic toxicity of DEET has been examined in 12 different studies using different species and routes of exposure. An increased incidence of hyaline droplets, regenerative tubules, granular casts, and chronic inflammation in the kidneys were observed in male rats which was indicative of α_{2u}-globulin nephropathy. The presence of α_{2u}-globulin in the hyaline droplets was confirmed in a study in which kidney tissue sections were stained with an immunocytochemical technique. Another study demonstrated that male NBR rats, which do not produce α_{2u}-globulin, do not develop these renal lesions when exposed to DEET. Recent reviews of α_{2u}-globulin nephropathy have concluded that this sex and species-specific effect is probably not relevant as far as human toxicity because humans do not produce α_{2u}-globulin (Borghoff *et al.*, 1990; Flamm and Lehman-McKeeman, 1991; Hard *et al.*, 1993). Humans produce proteins related to α_{2u}-globulin in the kidney, but only at concentrations at least 2 orders of magnitude lower than male rats (< 0.05 mg/day/g kidney).   Therefore, α_{2u}-globulin nephropathy in male rats was not considered an adverse effect in selecting NOELs for evaluating human health risks from the use of DEET. Increased liver weights were observed in a number of the subchronic studies, but were rarely associated with any gross or histological changes. The associated pathological findings in the liver included accentuated lobulation or round edges, diffuse multilobular mottling or foci, and hepatocellular hypertrophy. These liver changes suggest there was induction of microsomal enzymes which was considered an adaptive response rather than an adverse effect. Changes in testicular weights were also seen in several studies; however, due to the inconsistent direction of change and associated histopathological findings, the toxicological significance of these effects were uncertain. The adverse effects observed with subchronic exposure to DEET included clinical signs, reduced body weights and food consumption, reduced kidney weights and cytoplasmic vacuolization of the kidney tubules (dogs), erosion of the stomach, hypocellularity of the bone marrow, thymic atrophy and hemorrhage and dermal irritation. The lowest systemic LOEL by the oral route was 200 mg/kg/day in a 8-week study in which dogs exhibited salivation, vomiting, and relaxed nictitating membrane. The lowest systemic LOEL by the dermal route was 1,000 mg/kg/day in a 90-day study in which male rats had a reduction in body weights (9%). The NOEL was 300 mg/kg/day for both of these studies. The LOEL for dermal irritation was 100 mg/kg/day. A NOEL was not established for this endpoint.

Inhalation-Rat

Ten Sprague-Dawley rats/sex/dose were exposed to DEET (95% meta, 5% other isomers) aerosol at nominal concentrations of 0, 250, 750 or 1,500 mg/m^3 (0, 60, 180 or 360 mg/kg/day[2]) for 6 hours/day, 5 days/week for 13 weeks (Macko and Bergman, 1980). There

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[2]	Estimated assuming a respiratory rate of 0.24 m^3/kg/6 hrs for a rat (Zielhuis and van der Kreek, 1979).

C.   SUBCHRONIC TOXICITY (cont.)

were no deaths. Rats at 750 and 1,500 mg/m^3 had disheveled and discolored fur after exposure. The toxicological significance of these signs is uncertain, but DPR toxicologists assumed that the animals were not grooming themselves because they did not feel well. Red exudate was also observed around the eyes and noses of rats at 1,500 mg/m^3. There was no treatment-related effect on body weights, serum chemistry, hematology or histopathology. At study termination, there was a significant increase in the mean relative kidney weight (M: 15, 17, 14%) and the mean relative liver weights (M: 8, 13, 15%; F: 3, 4, 14%) at 250, 720 and 1,500 mg/m^3, respectively. The increase in liver weights was considered adaptive in nature by DPR toxicologists based on the pathological evidence of liver hypertrophy in other studies suggesting there was induction of microsomal enzymes (Johnson 1987a&b). Therefore, the NOEL was 250 mg/m^3 (60 mg/kg/day) based on disheveled and discolored fur. This study had several deficiencies including no air flow or chamber temperature determinations and no individual animal data were presented.

Diet-Mouse

Groups of 15 Charles River CD-1® mice/sex/dose were administered DEET (98% meta, 0.3% other isomers) in the diet at 0, 300, 1000, 3000, 6000 or 10000 mg/kg/day for 90 days (Johnson, 1987a). Mice at 6000 and 10000 mg/kg/day rejected the diet and were sacrificed after week 3. Accentuated lobulation and diffuse multilobular mottling of the liver and erosion of the stomach mucosa were observed macroscopically in several animals at 6000 and 10000 mg/kg/day. There was no effect on food consumption or macroscopic findings up to 3000 mg/kg/day. Decreased defecation (M: 8/15; F: 14/15) and reduced mean body weights (M: 10%; F: 11%) were observed at 3000 mg/kg/day. Absolute kidney weights were reduced in both sexes at 3000 mg/kg/day (M: 11%; F: 14%). Since the relative kidney weights were not significantly lower, the investigators attributed the lower absolute weights to the reduced body weights. The absolute and relative liver weights were increased in males at 1000 mg/kg/day and higher and in females at all dose levels. Hepatocellular hypertrophy was observed microscopically in the liver of both sexes at 3000 mg/kg/day and in females at 1000 mg/kg/day. The increased liver weights and pathological changes in the liver suggest there was induction of microsomal enzymes which was considered an adaptive response rather than an adverse effect by DPR toxicologists. Consequently, the NOEL was 1000 mg/kg/day based on decreased defecation and reduced body weights. This study was range-finding study for the chronic oncogenicity study and, therefore, did not evaluate all the parameters normally investigated in a guideline study. The parameters not evaluated included ophthalmology, hematology, clinical chemistry, and complete histopathology.

Diet-Hamster

Groups of 15 Syrian VAF/Plus hamsters/sex/dose were fed DEET (98.0% meta, 0.3% other isomers) in the diet at 0, 1000, 5000, 10000 or 15000 ppm (M: 0, 61, 304, 611 or 943 mg/kg/day; F: 0, 61, 305, 636 or 942 mg/kg/day) for 90 days (Goldenthal, 1989a). Four males and 4 females at 15000 ppm died or were sacrificed *in extremis*, most during study days 12-18. One male at 5000 ppm also died, but its death was not considered treatment-related by the investigators. Clinical signs were observed in both sexes at 15000 ppm, including labored breathing (M: 3/15; F: 3/15), decreased defecation (M: 4/15; F: 2/15), hypoactivity (M: 2/15; F: 1/15), pale skin (M: 3/15; F: 3/15), tremors (M: 1/15; F: 1/15) and hunched posture (M: 2/15; F: 2/15). One female at 5000 ppm had labored breathing and decreased defecation at week 5. One male at 5000 ppm also had decreased defecation at week 13. Since these signs were not seen at 10000 ppm, it is unclear if these signs at 5000 ppm were treatment-related. Mean body

weights were significantly reduced in males at 5000 ppm (10%) and in both sexes at 10000 ppm (M: 10%; F: 13%) and 15000 ppm (M: 18%; F: 15%). Sporadic, but apparent dose-related reductions in mean food consumption were seen at 5000 ppm (M: 6.5%), 10000 ppm (M: 7.6%; F: 7.8%) and 15000 ppm (M: 13%; F11%).

A significant increase in serum potassium levels (M: 16%; F: 10%) were found at 15000 ppm. Two females at 15000 ppm had stomach erosion. Macroscopic examination revealed smaller testes and epididymides in males at 10000 ppm and higher (controls: 4/15; 1000 ppm: 4/15; 5000 ppm: 3/15; 10000 ppm: 7/15; 15000 ppm: 5/15). Males at 10000 and 15000 ppm had a decrease in absolute and relative testes weights (10000 ppm: 41% - absolute, 34% - relative; 15000 ppm: 44% - absolute, 31% - relative), although only the differences in the absolute weight were statistically significant. An increased incidence of testicular tubular degeneration was observed at 10000 ppm and higher. The number of animals affected were 4/15, 5/15, 6/15, 12/15, and 11/15 at 0, 1000, 5000, 10000, and 15000 ppm, respectively. The degeneration was usually bilateral and was characterized by degenerating tubular epithelial cells within the seminiferous tubules. The epididymides were small with luminal cellular debris in many of these animals. Among the animals examined, 3/11, 4/4, 3/3, 7/7 and 6/11 were affected at 0, 1000, 5000, 10000, and 15000 ppm, respectively. Both sexes had an increase in relative brain weights at 10000 ppm and higher. At 15000 ppm, males also had an increase in relative kidney and liver weights. The increase in liver weights was considered adaptive by DPR toxicologists based on pathological evidence of liver hypertrophy in other studies suggesting there was induction of microsomal enzymes (Johnson 1987a&b). The toxicological significance of the increased kidney weights in hamsters is uncertain in the absence of any histological findings. The NOEL was 1000 ppm (61 mg/kg/day) based on the significant reduction in the mean body weight (10%) and food consumption (6.5%) in males at 5000 ppm. This study met the FIFRA guidelines.

Diet-Rat

Ten albino rats/sex/dose were fed DEET (85-95% meta isomer) in the diet at 0, 100, 500, 1000, 5000 or 10000 ppm (M: 0, 5.6, 31.1, 59.2, 333, or 652 mg/kg/day; F: 0, 8.3, 38.4, 81.2, 379 or 821 mg/kg/day) for approximately 200 days (Ambrose *et al.*, 1959). A slight reduction in the mean body weights (~10%) was seen in both sexes at 10000 ppm by study termination. Three rats of each sex died during the study, but the incidence did not appear treatment-related since none occurred at the highest dose. There was no effect on food consumption. The relative weights of the testes was increased (14, 21, and 23%) in males at 1000, 5000 and 10000 ppm, respectively. The increase in testes weights was not considered adverse by DPR toxicologists since it was not associated with any histological lesions. There was also an increase in relative kidney weights (M: 13%; F: 12%) at 10000 ppm. Increased kidney weights have usually been associated with α_{2u}-globulin nephropathy in males. The toxicological significance of the increased kidney weights in females is uncertain in the absence of histological findings. The mean relative liver weights were elevated at 5000 ppm (M: 20%) and 10000 ppm (M: 24; F: 33%). The increase in liver weights was considered adaptive by DPR toxicologists based on pathological evidence of liver hypertrophy in other studies suggesting there was induction of microsomal enzymes (Johnson 1987a&b). Ten animals had some hemorrhages in the lungs, but the incidence was not dose-related. Only rats at 5000 and 10000 ppm were examined histologically. No compound-related lesions were found. The NOEL was 5000 ppm (M: 333 mg/kg/day; F: 379 mg/kg/day) based on decreased body weight (~10%). This study had several deficiencies including no clinical observations, hematology, urinalysis or individual animal data.

C.   SUBCHRONIC TOXICITY (cont.)

Diet-Rat

DEET (98.0% meta, 0.3% other isomers) was administered in the diet to 15 Charles River CD® rats/sex/dose at 0, 100, 500, 1000, 2000 or 4000 mg/kg/day for 90 days (Johnson, 1987b). Due to mortalities and body weight depression during the first week, all the remaining animals at 4000 mg/kg/day were sacrificed. Mortalities occurred in 1 and 4 females at 500 and 2000 mg/kg/day, respectively. The cause of death is uncertain in all 5 cases because no histopathological examination was done. However, the investigators suggested that the death in the female at 500 mg/kg/day in week 11 was related to a nasal injury received several weeks earlier and two deaths at 2000 mg/kg/day were related to complications with blood collection in week 13. Various clinical signs were noted in animals of both sexes at 2000 mg/kg/day including decreased defecation (M: 15/15; F: 15/15), firm areas on the ventral abdomen (M: 7/15; F: 5/15), high carriage (M: 6/15; F: 4/15) and raised tail (M: 5/15; F: 3/15) during locomotion. Decreased defecation was also observed in all females and one male at 1000 mg/kg/day. Firm areas on the ventral abdomen were observed at all dose levels; however, the toxicological significance of this finding at the lower doses is uncertain since 2 of the female control animals also exhibited this sign. In addition, some effects were only observed in the females at 2000 mg/kg/day including hunched posture (8/15), labored breathing (9/15), cool to the touch (3/15), and eye partially closed (6/15). Mean body weights were reduced at 500 (F: 10%), 1000 (M: 14%; F: 16%) and 2000 (M: 40%; F: 27%) mg/kg/day. Mean food consumption was also reduced at 500 (F: 11%), 1000 (M: 17%; F: 18%), and 2000 (M: 33%; F: 30%) mg/kg/day when expressed on g/animal/day; however, when converted to g/kg/day the food consumption was comparable to controls due to the reduced body weights in these groups.

Several significant differences in the hematological values were noted including decreased lymphocytes at 1000 (M: 18%) and 2000 mg/kg/day (M: 21%), increased erythrocytes (F: 12%) and hematocrit values (F: 7%) at 2000 mg/kg/day and reduced mean cell volume (F: 5%) and mean cell hemoglobin (F: 6%) at 2000 mg/kg/day. A few significant differences in clinical chemistry values were seen at 2000 mg/kg/day (males: decreased phosphorus (13%); females: increased potassium (17%) and bilirubin (300%) and decreased total protein (14%), albumin (15%), globulin (15%) and glucose (80%). The investigators suggested that the changes in the hematological and clinical chemistry values were probably due to decreased food or water consumption.

There were significant increases in the relative organ weights for adrenal glands (M: 41% at 2000 mg/kg/day), brain (M: 6, 17, and 59% at 500, 1000 and 2000 mg/kg/day, respectively; F: 16, 20 and 30% at 500, 1000, and 2000 mg/kg/day, respectively), liver (M: 40, 28, 37, and 66% at 100, 500, 1000, and 2000 mg/kg/day, respectively; F: 30, 43, and 66% at 500, 1000, and 2000 mg/kg/day, respectively), kidneys (M: 16, 16, and 37% at 500, 1000, and 2000 mg/kg/day, respectively; F: 10% at 1000 mg/kg/day), and testes (M: 20 and 61% at 1000 and 2000 mg/kg/day, respectively). Some of these increases may be due to the body weight reductions. A reduction in a few absolute organ weights were seen at 2000 mg/kg/day including, the brain weights in males (5%) and the ovary weights in females (26%). There were several possible treatment-related increases in macroscopic findings in males including granular kidneys and livers which had rounded edges, accentuated lobulation and/or tan or white foci. However, the incidence of granular kidneys and livers with rounded edges did not exhibit a clear dose-response relationship since the incidence was highest at 500 mg/kg/day. No dose-related microscopic lesions were found in the liver, adrenal gland or testes. There was a dose-related increase in microscopic lesions in kidneys of males, including granular cast formation, multifocal chronic inflammation, hyaline droplets, and the presence of renal tubular

C. SUBCHRONIC TOXICITY (cont.)

epithelium. The increased kidney weights, gross and histological lesions in the kidneys of males were consistent with α_{2u}-globulin nephropathy and were not considered relevant to humans by DPR toxicologists based on another study which demonstrated the hyaline droplets contained α_{2u}-globulin (Goldenthal, 1989b). The toxicological significance of the increased kidney weights in females is uncertain in the absence of histological findings. The increased liver weights and macroscopic findings in the liver were interpreted as evidence of microsomal enzyme induction by DPR toxicologists and, therefore, were considered adaptive responses rather than adverse effects . The increase in relative adrenal and testes weights was also not considered toxicologically significant by DPR toxicologists in the absence of any pathological findings. Therefore, the NOEL was 100 mg/kg/day based on reduced body weights (10%) and food consumption (11%) in females at 500 mg/kg/day. This study was range-finding study for the rat chronic toxicity/oncogenicity study and, therefore, did not evaluate all the parameters normally investigated in a guideline study. The parameters not evaluated included ophthalmology and complete histopathology.

Diet-Rat

Three different strains of male rats (Charles River CD®, Fischer 344, and NBR) were fed DEET (98.0% meta, 0.3% other isomers) at 0 or 400 mg/kg/day (10 rats/strain/dose) for 90 days to examine the renal toxicity (Goldenthal, 1992). Male NBR rats do not produce the α_{2u}-globulin as do male CD® and Fischer 344 rats. To confirm the presence of hyaline droplets, kidney sections were stained with a Mallory-Heidenhain stain in addition to a hematoxylin and eosin stain. Only the kidneys were examined microscopically. There was a high incidence of hyaline droplets, regenerative tubules, chronic inflammation, and granular casts in the Charles River CD® and/or Fischer 344 rats, but not in the NBR rats. Since the kidneys were not analyzed specifically for α_{2u}-globulin, these data suggest, but do not confirm, that the renal toxicity is due to the accumulation of α_{2u}-globulin in the cells lining the renal tubules. A NOEL could not be established for this study. This purpose of this study was to compare the severity of kidney lesions in different strains of male rats and, therefore, did not evaluate many of the parameters recommended in the FIFRA guidelines for subchronic toxicity studies.

Gavage-Rabbit

DEET (95% meta, 5% other isomers) was administered to 6 male New Zealand White rabbits/dose in propylene glycol by oral gavage at 0, 132, 264 or 528 mg/kg/day for 15 days (Haight *et al.*, 1980). There was a significant reduction (~20%) in the mean body weights by day 15 at 528 mg/kg/day. The mean relative kidney weights were increased (28%) at 528 mg/kg/day, but there was no corresponding histopathological changes. A suggestive dose-related increase in fatty changes in hepatocytes was observed. Among the animals examined, 0/4, 0/5, 1/6, and 2/3 were affected at 0, 132, 264, and 528 mg/kg/day, respectively. No other compound-related lesions were observed. Several statistically significant changes in serum clinical chemistry values were seen at 528 mg/kg/day. These changes included a 14% decrease in the mean calcium level, a 386% increase in the mean cholesterol level, and a 196% increase in the mean triglyceride level. The changes in triglyceride and cholesterol levels were considered toxicologically significant based on the fatty changes in the liver at 165 and 528 mg/kg/day. The NOEL was 132 mg/kg/day based on the fatty changes in the liver. This study did not meet the FIFRA guidelines for subchronic studies based on an inadequate exposure period, no females, no hematology, no ophthalmological examination, and an incomplete histopathological examination.

C.   SUBCHRONIC TOXICITY (cont.)

Capsule-Dog

Two beagle dogs/sex/dose were administered DEET (98.0% meta, 0.3% other isomers) in gelatin capsules twice a day at 0, 50, 100, 200 or 400 mg/kg/day for 8 weeks (Goldenthal, 1995a). Abnormal head movements (M: 2/2; F: 1/2) and trembling (F: 2/2) were observed at 400 mg/kg/day. Relaxed nictitating membrane was observed in males at 200 (1/2) and 400 (2/2) mg/kg/day and one control animals. Vomiting was seen at 100 (F: 1/2), 200 (M: 1/2; F: 2/2), and 400 (M: 2/2; F: 2/2) mg/kg/day, but it was also observed in the controls (F: 2/2). Excessive salivation was observed at 50 (M: 2/2), 100 (M&F: 2/2), 200 (M&F: 2/2) and 400 (M&F: 2/2) mg/kg/day. The salivation at 50 and 100 was not considered toxicologically significant because it was usually slight and it was only observed in one of eight dogs in the chronic dog study at 100 mg/kg/day (Goldenthal, 1994). Reduced mean body weights (3% - females only) and food consumption (M: 7%; F: 47%) were seen at 400 mg/kg. Serum cholesterol levels were decreased by 14% in males at both 200 and 400 mg/kg relative to their baseline values. The toxicological significance of the decreased cholesterol levels is uncertain since they were not associated with any gross or histological lesions. There was also a decrease in the mean absolute testes and epididymides weights (25%) in males at 400 mg/kg; however, the toxicological significance of these weight changes is also uncertain since there was no associated gross or histological changes in these tissues and there were only two dogs per group. The NOEL was 100 mg/kg/day based on excessive salivation, vomiting, and relaxed nictitating membrane. This study was range-finding study for a dog chronic toxicity study and, therefore, fewer animals were exposed over a shorter period of time than recommended in FIFRA guidelines.

Diet-Dog

DEET (98.0% meta, 0.3% other isomers) was fed to 2 Beagle dogs/sex/dose at 0, 300, 1000, 3000, and 6000 ppm (M: 0, 8.4, 29, 93 or 20 mg/kg/day; F: 0, 9.7, 30, 92 or 12 mg/kg/day) for 8 weeks (Goldenthal, 1995b). The high dose level was reduced to 4500 ppm at week 4 due to rejection of the diet and was further reduced to 3000 ppm at week 7 due to continued rejection of the diet. Due to the continued rejection of the diet by the dogs in the high dose group, their actual compound consumption was less than the 3000 ppm group. Diarrhea, decreased defecation and vomiting were common in the treatment groups, but it was also observed in a few control animals. Decreased activity was also observed in both sexes in the high dose group.   At study termination, reduced mean body weights (M: 27%; F: 36%) and food consumption (M: 30%; F: 80%) were observed at the high dose level which did not diminish despite reducing the dosage. The mean absolute weights were lower for liver/gall bladder (M: 34%), heart (M: 32%; F: 34%), and kidneys (M: 17%; F: 15%) at the high dose level. Males in this high dose group also had lower mean absolute spleen weights (61%). The mean relative (to body) brain weights were increased (M: 22%; F: 46%) at the high dose. Cytoplasmic vacuolization of the kidney tubules, hypocellularity of the rib bone marrow, thymic atrophy and hemorrhage were observed microscopically in both sexes at the high dose level. The investigators suggested that the changes in absolute organ weights was due to the body weight reductions. DPR toxicologists did not consider the changes in the heart and spleen weights to be toxicologically significant due to the lack of associated gross or histopathological changes. A NOEL was not selected for this study since it is unclear if effects seen at high dose level were due to the test compound or inadequate food consumption due to the rejection of the diet at the this dose level. This study was a range-finding study for a dog chronic toxicity study and, therefore, fewer animals were exposed over a shorter period of time than recommended in FIFRA guidelines.

C.   SUBCHRONIC TOXICITY (cont.)

Dermal-Rat

DEET (98.0% meta, 0.3% other isomers) was applied dermally at 0, 100, 300 or 1000 mg/kg/day undiluted to the clipped backs (uncovered) of 15 Charles River CD® rats/sex/dose for 5 days/week for 13 weeks (Johnson, 1987c). There was no effect on general behavior in any treatment group. Mean body weights were significantly reduced (9%) at 1000 mg/kg/day in males at study termination (Table 9). DPR toxicologists assumed the body weight reduction in males was toxicologically significant because it was statistically significant and the reduction was approaching the benchmark of 10% which is generally considered toxicologically significant when establishing a maximum tolerated dose (MTD) level. Dermal irritation in the form of red and scabbed areas was observed in both sexes at all dose levels. When examined microscopically, acanthosis and/or hyperkeratosis was observed at the application site in all treatment groups of both sexes. There is a possibility that the body weight reduction in males at 1000 mg/kg/day was due to the severe dermal irritation at this dose level. However, since the dermal irritation was seen in both sexes in apparent equal severity, DPR toxicologists assumed these effects were unrelated.

Several lesions were observed microscopically in the kidney of males at 100 mg/kg/day and higher including renal cast formation (granular), multifocal inflammation in the renal cortex, regenerative tubules in the renal cortex, and hyaline droplets. The possibility exists that the body weight reduction in males was due to the α_{2u}-globulin nephropathy. However, there did not appear to be a correlation in the severity of the renal lesions and the severity of the body weight reductions in the males at 1000 mg/kg/day. Furthermore, in a 90-day feeding study conducted by this same investigator with the same strain of rats, body weight reductions were seen in females only at 500 mg/kg and in both sexes at 1000 and 2000 mg/kg/day (Johnson, 1987b). In another 90-day feeding study using 3 different strains of male rats, the NBR rats (which do not produce α_{2u}-globulin) had similar body weight reductions to the Sprague-Dawley rats (3.4% and 4.4%, respectively) at 400 mg/kg/day (Goldenthal, 1992). Therefore, DPR toxicologists assumed that the reduction in body weights in males exposed to DEET dermally at 1000 mg/kg/day was unrelated to the renal lesions.

An increased incidence of hyaline casts and chronic inflammation were observed in the female rats exposed dermally at 1000 mg/kg/day, although the difference was only statistically significant for the chronic inflammation. These lesions were usually unilateral and only trace to mild in severity. The investigators considered these lesions common and naturally occurring. They attributed the hyaline casts in females to protein leakage from the glomerulus caused by natural disease. This is in contrast to the granular casts observed in male kidneys which the investigators attributed to tubular epithelial necrosis and sloughing. A similar increase in these lesions was not seen in female rats in the 90-day feeding study conducted by this same investigator (Johnson, 1987b). Consequently, DPR toxicologists did not consider the renal lesions in the females to be treatment-related. Relative kidney weights were significantly higher at 300 (M: 18%) and 1000 mg/kg/day (M: 26%; F: 15%). The absolute kidney weights were also significantly elevated in males at 1000 mg/kg/day (15%). The increased kidney weights and lesions in the kidney of males are consistent with α_{2u}-globulin nephropathy and were not considered relevant to humans by DPR toxicologists based on another subchronic study for DEET (Goldenthal, 1989b) where it was demonstrated that the hyaline droplets contained α_{2u}-globulin.

Hepatic vacuolar change was observed microscopically in males all dose levels, but it did not exhibit a dose-related trend. Relative liver weights were significantly increased at 100 (F: 14%), 300 (M: 15%; F: 16%), and 1000 mg/kg/day (M: 29%; F: 39%). Absolute liver weights

C.   SUBCHRONIC TOXICITY (cont.)

Table 9. Effects of DEET on Rats Exposed Dermally for 13 Weeks[a]

	Dose Level (mg/kg/day)			
	0	100	300	1000
MALES				
Body weights (g), wk 13	534±38	509±33	509±38	484±43**
Dermal Observations				
Red	0/15[++]	13/15***	14/15***	12/15***
Scabbed	0/15[+++]	2/15	3/15	9/15***
Microscopic Lesions				
Skin, acanthosis	0/15[+++]	12/15***	15/15***	15/15***
Skin, hyperkeratosis	0/15[+]	3/15	0/15	4/15*
Organ Weights				
Liver (relative)	4.12±0.54	4.16±0.39	4.74±0.71**	5.32±0.53**
Kidney (relative)	8.03±0.86	8.76±1.00	9.48±1.36**	10.08±1.09**
Clinical Chemistry				
BUN[b]	14.3±2.1	15.7±1.9	16.3±2.5*	16.3±1.6*
Glucose	110±11	101±12	104±12	98±7**
ALAT[c]	34±7	30±4	42±44	30±6
FEMALES				
Body weights (g), wk 13	312±24	310±25	311±19	304±23
Dermal Observations				
Red	0/15[+++]	11/15***	13/15***	14/15***
Scabbed	1/15[+]	7/15**	11/15***	8/15**
Microscopic Lesions				
Skin, acanthosis	0/15[+++]	13/15***	15/15***	15/15***
Skin, hyperkeratosis	0/15[++]	4/15*	0/15	5/15*
Kidney, inflammation	0/15[++]	1/15	0/15	4/15*
Kidney, hyaline Casts	0/15[+]	1/15	0/15	3/15
Organ Weights				
Liver (relative)	4.04±0.54	4.60±0.37*	4.67±0.49**	5.61±0.62**
Kidney (relative)	8.64±0.98	9.34±0.89	9.30±1.30	9.92±0.83**
Clinical Chemistry				
BUN[b]	17.0±4.5	16.1±3.9	16.6±1.5	16.5±2.8
Glucose	108±11	106±12	104±10	99±12
ALAT[c]	31±4	35±10	29±6	28±4*

[a]   Johnson, 1987c
[b]   BUN = Blood urea nitrogen
[c]   ALAT = Alanine aminotransferase
[+],[++],[+++]   Significant trend based on the Cochran-Armitage trend test at $p < 0.05$, 0.01, and 0.001, respectively (Gart *et al.*, 1986).
*,**,***   For continuous data, the mean was significantly different from the control group based on the Dunnett's test at $p < 0.05$, 0.01, and 0.001, respectively. For quantal data, the incidence was significantly different from the control group based on the Fisher's exact test at $p , < 0.05$, 0.01, and 0.001, respectively.

were also significantly increased at 300 (F: 14%) and 1000 mg/kg/day (M: 24%; F: 36%). However, the increased absolute and relative liver weight did not correlate with the incidence of the hepatic vacuolar changes. The increased liver weights was considered adaptive by DPR toxicologists based on pathological evidence of liver hypertrophy in other studies suggesting there was induction of microsomal enzymes (Johnson 1987a&b).

Males had an increase in blood urea nitrogen (14%) at both 300 and 1000 mg/kg/day. The toxicological significance of the elevated BUN levels is uncertain, but it probably is related to the α_{2u}-globulin nephropathy in males. There was also a significant decrease in the glucose levels (M: 11%) and alanine aminotransferase activity (F: 10%) in the serum at 1000 mg/kg/day. The toxicological significance of these clinical chemistry changes was also uncertain since they did not correlate with the lesions in the liver. The systemic NOEL for this study was 300 mg/kg/day based on reduced mean body weights (9%) in males. The NOEL for local effects was less than 100 mg/kg/day based on the dermal irritation in both sexes. This study was acceptable to DPR toxicologists based on FIFRA guidelines.

Dermal - Rat

Groups of 15 castrated male Charles River CD® rats/dose had DEET (98.0% meta, 0.3% other isomers) applied to their shaved backs (uncovered) at 0 or 1000 mg/kg for 5 days/week over 13 weeks (Goldenthal, 1989b). An additional group of 15 uncastrated male CD® rats also had DEET applied to their shaved backs at 1000 mg/kg/day. Erythema was observed at the application site in both castrated and uncastrated treated males. Castrated treated males had slightly lower mean body weights (~7%) than the castrated control males, but significantly lower mean body weights and food consumption than uncastrated treated males (20% and 15%, respectively). The relative kidney weights of the castrated treated males were significantly higher than castrated controls; however, they were still significantly lower than uncastrated treated males. Microscopic examination of the kidneys revealed hyaline casts, hyaline droplets, inflammation, regeneration, and granular casts in both castrated and uncastrated treated males, although they were more prevalent in the uncastrated males. Sections of the kidney that were stained with two stains, Mallory-Heidenhain stain and an immunocytochemical stain, confirmed the presence of hyaline droplets and α_{2u}-globulin, respectively. A NOEL could not be established for this study. The purpose of this study was to determine the effect of castration on the severity of kidney lesions and, therefore, did not evaluate the parameters normally recommended in the FIFRA guidelines for subchronic toxicity studies.

Dermal-Pig

DEET (98.0% meta, 0.3% other isomers) was applied to the shaved skin (uncovered) of 4 micropigs®/sex/dose at 0, 100, 300, or 1000 mg/kg/day for 5 days/week over 13 weeks (Goldenthal, 1991). There was no effect on clinical signs, body weights, food consumption, or organ weights. An increase was seen clinically in the incidence of desquamation and dry skin at all doses (Table 10). As dose increased, the time of onset of these signs decreased while the severity increased. At necropsy, dry skin was the only macroscopic treatment-related finding. When the skin was examined microscopically, hyperkeratosis and acanthosis were observed. Ulcer, inflammation, and epidermal exudate were also observed microscopically in additional sections of skin from the application site that had gross lesions. Only hyperkeratosis was observed at the lowest dosage. The incidence of the dermal effects was analyzed statistically by DPR toxicologists with both sexes combined since the number of animals per group were small and the responses were similar. Some minor differences in response were

C.   SUBCHRONIC TOXICITY (cont.)

Table 10. Incidence of Dermal Effects in Micropigs® Fed DEET for 90 Days[a]

	Dose Level (mg/kg/day)			
	0	100	300	1000
Clinical Observations				
Desquamation	1/8[++b]	8/8***	8/8***	8/8***
Dry Skin	5/8	8/8	8/8	8/8
Microscopic Lesions				
Hyperkeratosis	0/8[+++]	3/8	5/8*	7/8***
Acanthosis	0/8[+++]	0/8	0/8	2/8

[a]   Goldenthal, 1991
[b]   Males and females were combined due to the small number of animals. Similar responses were seen in both sexes except the severity of desquamation was greater in females and the incidence of hyperkeratosis and acanthosis was higher in males.
[++, +++]   Significant trend based on a dose-weighted chi-square test at $p < 0.01$ and 0.001, respectively.
[*, ***]   Significantly difference from the control group based on the Fisher's exact test at $p < 0.05$ and 0.001, respectively.

seen between the sexes in that the severity of desquamation was greater in females, but the incidence of hyperkeratosis and acanthosis was higher in males. The LOEL for local effects was 100 mg/kg, the lowest dose tested, based on desquamation, dry skin, and hyperkeratosis. A NOEL was not established for local effects. Although it would be more appropriate to express the NOEL/LOEL for dermal irritation in mg/cm^2 since it is a local effect, there was insufficient information about the size of the application site to convert the dosage. The systemic NOEL was equal to or greater than 1000 mg/kg/day, the highest dose tested. This study met FIFRA guidelines.

D.   CHRONIC TOXICITY/ONCOGENICITY

Summary: The chronic toxicity and/or oncogenicity of DEET was evaluated in four species by the oral or dermal route. A NOEL of 100 mg/kg/day was established in two species based on reduced body weights (rats and dogs), reduced food consumption (rats), clinical signs (dogs), increased cholesterol levels (rats), increased alkaline phosphatase (dogs), and histological changes in mandibular lymph node, uterus, and liver (dogs). Three of the studies met FIFRA guidelines. No dose-related increase in tumors was found in any of the chronic studies including the male rats. An increase in renal tumors in male rats has been observed with a number of chemicals that cause α_{2u}-globulin nephropathy (Borghoff *et al.*, 1990; Flamm and Lehman-McKeeman, 1991; Hard *et al.*, 1993). The increase in renal tumors was attributed to an increase in cell death and subsequent regeneration. Since the dose levels for the male rats in the chronic study were low enough to not produce any evidence of α_{2u}-globulin nephropathy, it is not surprising that an increase in renal tumors was not observed in the rat chronic toxicity study for DEET. However, even if renal tumors had been observed in male rats after chronic exposure to DEET, this oncogenic effect is probably not relevant to humans since they do not produce α_{2u}-globulin. Humans produce proteins related to α_{2u}-globulin in the kidney,

D. CHRONIC TOXICITY/ONCOGENICITY (cont.)

but only at concentrations at least 2 orders of magnitude lower than male rats (< 0.05 mg/day/g kidney).

Diet-Mouse

Groups of 60 Charles River CD-1® mice were fed DEET (98.0% meta, 0.3% other isomer) at 0 (2 control groups), 250, 500 or 1000 mg/kg/day for 78 weeks (Goldenthal, 1990). Since there was no difference in the treatment of animals in the two control groups they were combined for statistical analysis. There was no dose-related effect on the clinical signs, survival, hematological values, and pathological findings. Reductions in mean body weights were statistically significant at 250 (M: 4%), 500 (M: 4%; F: 3%) and 1000 mg/kg/day (M: 6%; F: 6%). Significant reductions in the mean food consumption (expressed on a g/animal/day basis) was also reduced at 250 (M: 4.1%), 500 (M: 4.1%) and 1000 mg/kg/day (M: 6.1%; F: 4.0%). DPR toxicologists did not consider the reductions in body weights and food consumption to be toxicologically significant because they were only around 5%. Absolute liver weights were significantly increased at 500 (M: 10%; F: 5%) and 1000 mg/kg/day (M: 16%; F: 12%). The increase in relative (to body) liver weights at 500 (M: 16%; F: 9%) and 1000 mg/kg/day (M: 27%; F: 12%) was even more pronounced. A reduction in the absolute and relative (to brain) kidney weights was seen at 250 (A: 10%; R: 11%) and 1000 mg/kg/day (A: 11%; R: 11%) in males. There was a significant increase in hyperplastic nodules of the liver in both sexes at 1000 mg/kg/day when compared to controls (Table 11). An increase in bile duct hyperplasia was also seen in males that was significant by trend analysis, but not by pair-wise comparison. Although there was no increase in liver tumors, these pre-neoplastic lesions were considered treatment-related and adverse by DPR toxicologists. The NOEL was 500 mg/kg/day based on the increase in hyperplastic nodules in the liver and increased liver weights. This study was found acceptable to the DPR toxicologists for filling a data requirement under the California Birth Defects Prevention Act of 1984 (SB950) based on FIFRA guidelines.

Table 11. Pre-Neoplastic Lesions in the Liver and Bile Duct in Mice Fed DEET in the Diet for 78 Weeks.

Lesion	Dose Level (mg/kg/day)			
	Control	250	500	1000
MALES				
Hyperplastic liver nodule	13/120[+] (11%)	9/60 (15%)	12/60 (20%)	13/60* (22%)
Bile duct hyperplasia	0/120[++] (0%)	1/60 (2%)	1/60 (2%)	3/60[*] (5%)
FEMALES				
Hyperplastic liver nodules	2/120[++] (2%)	0/60 (0%)	1/60 (2%)	5/60* (8%)

* Significantly different from controls based on Fisher's exact test at p< 0.05.
[+],[++] A significant trend based on the Cochran-Armitage trend test at p < 0.05, and 0.01, respectively (Gart *et al.*, 1986).

D.   CHRONIC TOXICITY/ONCOGENICITY (cont.)

Diet-Rat

Groups of 60 CD® rats/sex/group were fed DEET (98.0% meta, 0.3% other isomers) in the diet at 0 (2 control groups), 10, 30, or 100 mg/kg/day to males and at 0 (2 control groups), 30, 100, or 400 to females for 2 years (Goldenthal, 1995c). There was no treatment-related effect on clinical signs, hematology, urinalysis, ophthalmology or pathological findings. The mean food consumption was reduced (7.5%) in females at 400 mg/kg/day throughout the study, although they were sporadic after week 72. Females at 400 mg/kg/day also had reduced mean body weights (13%) throughout the study (Table 12). The reductions in body weight and food consumption were both seen during the first week of exposure, so that it is uncertain if the body weight reductions are due to the reduction in food consumption. However, the reduction in body weights is more severe than the reduction in food consumption suggesting other factors may also be involved. A significant increase in the mean cholesterol levels (43%) was found in females at 400 mg/kg/day sacrificed at 6, 12, and 18 months, but not at study termination. Although the increase in cholesterol levels were not associated with any histological changes in the liver in this study, they were associated with fatty liver in a study where rabbits were administered DEET by oral gavage for 15 days (Haight *et al.*, 1980). Consequently, DPR toxicologists considered the increased cholesterol levels toxicologically significant. Significant increased relative liver weights were also seen in females at 400 mg/kg/day (30%), but were considered adaptive in nature by DPR toxicologists based on lack of histological lesions in the liver of this study and evidence of liver hypertrophy in other studies suggesting there was induction of microsomal enzymes (Johnson 1987a&b). Due to the lower dose levels for males, no effects were observed including renal toxicity. An increase in renal tumors in male rats has been observed with a number of chemicals that cause α_{2u}-globulin nephropathy (Borghoff *et al.*, 1990; Flamm and Lehman-McKeeman, 1991; Hard *et al.*, 1993). The increase in renal tumors was attributed to an increase in cell death and subsequent regeneration. Since the dose levels for the male rats in this study were low enough to not produce any evidence of α_{2u}-globulin nephropathy, it is not surprising that there was no increase in renal tumors. However, even if renal tumors had been observed in this study, this oncogenic effect is probably not relevant to humans since they do not produce α_{2u}-globulin. Humans produce proteins related to α_{2u}-globulin in the kidney, but only at concentrations at least 2 orders of magnitude lower than male rats (< 0.05 mg/day/g kidney). The NOEL was 100 mg/kg/day based on the reduced mean body weights (13%) and food consumption (7.5%), and increased mean cholesterol levels

Table 12.   Effects in Female Rats Fed DEET in the Diet for 2 Years[a]

	Dose Level (mg/kg/day)			
	0	30	100	400
Body Weight, wk 104 (g)	540±148	495±117	529±99	435±95*
Food Consumption, wk 72 (g/animal/day)	22.7±4.3	23.1±4.2	22.3±3.5	20.0±3.4**
Cholesterol, 18 mos (mg/dl)	72±19	81±19	83±19	105±27**
[a] Goldenthal, 1995c				
*,** Significantly different from the control group by the Dunnett's test at p<0.05 and 0.01, respectively.				

D. CHRONIC TOXICITY/ONCOGENICITY (cont.)

(43%) in females. The study was acceptable to DPR toxicologists based on the FIFRA guidelines.

Capsule-Dog

Four beagle dogs/sex/dose were given DEET (98.0% meta, 0.3% other isomers) in gelatin capsules in two daily doses at 0, 30, 100, or 400 mg/kg/day for 1 year (Goldenthal, 1994). Vomiting, excessive salivation, tremors, and decreased defecation were observed in both sexes at 400 mg/kg/day (Table 13). One male at 400 mg/kg/day also had ataxia, abnormal head movements, and convulsions throughout the study. Two females at 100 mg/kg/day had tremors on several occasions, but the investigators suggested that the tremors were not treatment-related since they occurred prior to dosing. The clinical signs were usually seen shortly after dosing and were typical of signs observed with acute exposure suggesting that these signs may be due to the bolus dosing. Dogs of both sexes at 400 mg/kg/day had reduced mean body weights through week 26 (M: 10%; F: 13%), although the differences were not statistically significant. Both sexes at 400 mg/kg/day also had reduced mean food consumption (M: 32%; F: 25%) during the first week or two of the study. A reduction in the mean hemoglobin values (M: 10 & 11%; F: 12 & 15%) and hematocrit values (M: 11 & 12%; F: 11 & 16%) was seen at 400 mg/kg/day at 6 and 12 months. Females at 400 mg/kg/day also had increased platelet counts (50 and 71%) at these times. The reductions in the hemoglobin and hematocrits were suggestive of anemia. It is unclear if the anemia is due to blood loss, increased fragility of erythrocytes or bone marrow suppression. Thymic hemorrhage and hypocellularity of the bone marrow were observed in a dog subchronic toxicity study where DEET was administered in the feed, but not when DEET was administered in capsules (Goldenthal, 1995a&b). The toxicological significance of the increased platelet count in females is less certain. At 400 mg/kg/day, the mean cholesterol levels were reduced at 6 (M: 33%; F: 35%) and 12 months (M: 35%), although the differences were only significant in males. Males at 400 mg/kg/day had increased alkaline phosphatase activity (49%) and potassium levels (23%) at 6 months. The toxicological significance of these clinical chemistry changes is uncertain since they did not correlate with any pathological lesions. There were no treatment-related macroscopic lesions. Microscopically, a dose-related increase brown pigment (possibly hemosiderin) in the mandibular lymph node was observed in males, although only small amounts (trace to mild) of the pigment were seen. Two females and one male at 400 mg/kg/day had mononuclear infiltration of the liver. Mild glandular epithelial hyperplasia of the uterus was observed in females at 100 and 400 mg/kg/day. The investigators attributed the uterine hyperplasia to "normal hormonal physiological variation." However, it seems unlikely that 3 of 4 females at 400 mg/kg/day would have this finding at the same time if it was due to simple hormonal variation.   Although all of the histological findings were of questionable toxicological significance, a health protective assumption was made that the increases at 400 mg/kg/day were treatment-related and adverse. The NOEL was 100 mg/kg/day based on clinical signs, reduced mean hemoglobin and hematocrit levels and histological changes in liver, lymph nodes and uterus at 400 mg/kg/day.   This study was acceptable to DPR toxicologists based on the FIFRA guidelines.

Dermal-Mouse

DEET (purity not reported) was applied to the flanks of 50 female Swiss mice at 0, 2, 10 or 20 mg/animal in acetone twice a week for 120 weeks (Stenbäck, 1976). There was no dose-related effect on tumor incidence. A NOEL could not be established for this study due to insufficient information. This study had many major deficiencies including no test article

Table 13. Effects in Dogs Receiving DEET in Capsules for 1 Year[a]

	Dose Level (mg/kg/day)			
	0	30	100	400
MALES				
Vomiting	1/4[+]	2/4	1/4	3/4
Salivation	0/4[++]	1/4	1/4	4/4*
Tremors	0/4[+]	0/4	0/4	2/4
Decreased defecation	2/4	3/4	4/4	4/4
Hemoglobin (g/dl), wk 52	16.5±1.1	15.9±1.8	15.6±0.9	14.6±1.1
Hematocrit (%), wk 52	47.1±4.0	45.7±6.1	44.0±2.5	40.8±4.0
Liver, mononuclear infiltration	0/4[+]	0/4	0/4	1/4
Mandibular lymph node, brown pigmentation	0/4[+]	1/4	2/4	3/4
FEMALES				
Vomiting	2/4	1/4	2/4	2/4
Salivation	2/4[++]	0/4	0/4	4/4
Tremors	0/4	0/4	2/4	1/4
Decreased defecation	1/4[+]	3/4	4/4	4/4
Hemoglobin (g/dl), wk 52	15.8±0.8	15.7±1.1	16.2±2.3	13.1±1.3
Hematocrit (%), wk 52	43.9±2.2	44.4±3.1	46.5±7.5	34.7±3.8*
Liver, mononuclear infiltration	0/4[++]	0/4	0/4	2/4
Uterus, hyperplasia	0/4[++]	0/4	1/4	3/4

[a]   Goldenthal, 1994

[+,++]   Significant trend based on the Cochran-Armitage trend test at $p < 0.05$ and 0.01, respectively (Gart *et al.*, 1986).

[*]   For quantal data, the incidence is significantly different from the control group based on the Fisher's exact test at $p < 0.05$. For continuous data, the mean is significantly different from the control group based on the Dunnett's test at $p < 0.05$.

analysis, clinical signs, body weights, food consumption, hematology, pathology for non-neoplastic lesions or individual data.

Dermal-Rabbit

DEET (purity not reported) was applied to the ears of 5 female New Zealand rabbits at 0, 2, 10 or 20 mg/animal in acetone twice a week for 95 weeks (Stenbäck, 1976). No tumors were observed in any treated animals. A NOEL could not be established for this study due to insufficient information. This study also had many major deficiencies including no test article

D. CHRONIC TOXICITY/ONCOGENICITY (cont.)

analysis, clinical signs, body weights, food consumption, hematology, pathology for non-neoplastic lesions or individual data.

E. GENOTOXICITY

Summary: All the genotoxicity studies for DEET were negative, except for a dominant lethal assay which had equivocal results. The studies conducted included 6 reverse mutation assays, four with *Salmonella typhimurium* and two with *Saccharomyces cerevisiae*, a dominant lethal assay, an *in vitro* cytogenetic assay using Chinese hamster ovary cells, and an unscheduled DNA synthesis (UDS) assay using rat primary hepatocytes. One of the reverse mutation assays with *S. typhimurium*, the cytogenetic assay, and the UDS assay met guidelines.

Gene Mutation

Negative results were reported for the reverse mutation assays that were available for DEET using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538. One of these studies was a published report in which *S. typhimurium* were exposed to DEET (purity not reported) at 0, 10, 33, 100, 333, 667, 1000, 2000 or 3333 μg/plate with and without metabolic activation (Zeiger *et al.*, 1992). There was insufficient information in this report to determine if the study met guidelines. Testing with metabolic activation included separate tests with both rat and hamster liver microsomal fractions. In an assay conducted by Brusick (1976), *S. typhimurium* strains were exposed to DEET (purity not reported) at 0, 0.01, 0.1, 1.0 or 5.0 μl/plate with and without metabolic activation. This study was unacceptable to DPR toxicologists since there was only one plate per concentration and there was no repeat assay. Another assay was conducted by the same laboratory; however, only a summary was available so it is uncertain at what concentrations it was tested or whether metabolic activation had been used (Macko and Weeks, 1980). Due to insufficient information, this assay was also unacceptable. San and Schadly (1989) conducted a fourth assay where *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to DEET (98.0% meta, 0.3% other isomers) at 0 (DMSO), 28, 83, 278, 833, 2778 or 8333 μg/plate without metabolic activation and 0 (DMSO), 2.8, 8.3, 28, 83, 278 or 833 μg/plate with metabolic activation. This study was acceptable to DPR toxicologists based on guidelines.

Two reverse mutation assays with *Saccharomyces cerevisiae* D4 were also negative. DEET (purity not reported) was tested at 0, 0.01, 0.1, 1.0, and 5.0 μg/plate with and without metabolic activation in one assay conducted by Brusick (1976). This study had several deficiencies including only one plate tested per concentration and no repeat assay. Since only a brief summary was available for the other test, it is uncertain what concentrations were tested (Macko and Weeks, 1980). Due to insufficient information, it was unclear if this test met guidelines.

Chromosomal Aberration

In a dominant lethal assay, 10 male ICR/Ha Swiss mice received a single dose of DEET (95% meta, remainder other isomers) at 600 mg/kg (Swentzel, 1978). Ten mice/group in the positive and concurrent control groups received 10 mg/kg of TEM and 5 mg/kg corn oil, respectively. The males were then cohoused sequentially with 3 untreated virgin female mice 5 days/week for 8 weeks. Females were sacrificed 13 days after the midweek of their cohabitation with a male. Although the fertility index was not significantly different from the

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E.   GENOTOXICITY (cont.)

concurrent controls, the total percentage of dams with less than 8 implantations over 8 weeks was greater in the males exposed to DEET than in the control animals (11.6% vs. 3.1%). This study had several deficiencies including only one dose level, too few pregnant females per group, and no individual data.

An *in vitro* cytogenetic assay was conducted for DEET (98.0% meta, 0.3% other isomers) using Chinese hamster ovary (CHO-K$_1$) cells with metabolic activation at 0.063, 0.125, 0.25 or 0.5 µl/ml and without metabolic activation at 0.125, 0.25, 0.50 or 1.0 µl/ml (Putman and Morris, 1989). There was no significant increase in chromosomal aberrations with DEET. DPR toxicologists found this study acceptable.

Other Genotoxic Effects

Rat primary hepatocytes were exposed to DEET (98.0% meta, 0.3% other isomers) at 0, 0.003, 0.01, 0.03, 0.1, 0.2 or 0.3 µl/ml in an unscheduled DNA synthesis assay (Curren, 1989). No increase in net nuclear grain counts were observed with either the initial or repeat assay. This study was acceptable to DPR toxicologists based on guidelines.

F.   REPRODUCTIVE TOXICITY

Summary: Four reproductive toxicity studies were conducted in rats with DEET administered by different routes; however, only one of these studies met guidelines. A slight increase (5-10%) in abnormal sperm were observed in males in two non-guideline studies; however, the toxicological significance of these findings is questionable since the differences were not statistically significant in either study. Urethral plugs and distended bladders were observed in males in another study, but it was unclear from the available data if the incidence was dose-related. There was no effect on sperm count or viability nor were there any microscopic lesions in the testes. Non-reproductive effects included increased hair loss, skin lesions, decreased rotorod performance, reduced body weights, elevated BUN levels (males), and renal lesions (males). The lowest reproductive NOEL was 146 mg/kg/day based on reduced neonatal pup weights (11-13%). The parental, non-reproductive NOEL was also 146 mg/kg/day based on48
 reduced body weights.

Inhalation-Rat

This study was done in conjunction with a subchronic inhalation study conducted by Macko and Bergman (1980) which has been previously described under the Subchronic Toxicity Section. The sperm were harvested from the epididymides after the animals were sacrificed at study termination. There was a consistently higher percentage of abnormal sperm in the treated animals. Although the differences in the percentage of abnormal spermheads were small (~5%), the investigators suggested that it was dose-related for several reasons. First, the difference was statistically significant at the highest dose level, 1500 mg/m^3, using the t-test. Second, the highest frequency of abnormal spermheads was observed at 1500 mg/m^3. Third, the unusually high frequencies of abnormal spermheads (> 2 SD above the mean) in one or two animals of the control and 250 mg/m^3 groups resulted in their means being higher and, consequently, the differences between the controls and the treatment groups were less pronounced.   Fourth, only 1 control animal exceeded the mean for any treatment group, but 7 of 10 high-dose animals exceeded the mean in the control group. A reanalysis of the raw data from this study found the ANOVA was not statistically significant, so that the significant t-test

F.   REPRODUCTIVE TOXICITY (cont.)

would not be meaningful (Sherman, 1980a). Since the increase was not statistically significant, the increase in abnormal spermheads was of questionable toxicological significance. Males at 1500 mg/m^3 also had higher testes weights (18-26%) at week 7, but not at week 13. However, the toxicological significance of this transient finding is also uncertain since there was no increase in histopathological lesions in the testes. Therefore, the NOEL for male reproductive effects in this study appear to be equal to or greater than 1500 mg/m^3 (360 mg/kg/day). This was an ancillary reproduction study and, consequently, did not meet guidelines.

<u>Diet-Rat</u>

Groups of 28 Charles River CD® rats/sex/group were administered DEET (95% meta isomers, 5% other isomers) in the feed at 0, 500, 2000 or 5000 ppm (M: 0, 36, 146 or 365 mg/kg/day; F: 0, 45, 179 or 449 mg/kg/day) for two generations (Schardein, 1989). The treatment period was 80-93 days for each generation prior to mating. An increased incidence of hair loss was seen in both generations of adult females at 5000 ppm. Significant reduction in the mean body weights (6%) were observed at 2000 ppm in F_0 males and at 5000 ppm in both sexes of F_0 adults (M: 7%; F: 6%) and F_1 adults (M: 8%; F: 12%). The body weight reductions in the F_0 males was not considered toxicologically significant because they were closer to 5% than the benchmark of 10% used in identifying an MTD. There was also a significant reduction in the mean body weights of the F_1 pups (M: 13%; F: 11%) and F_2 pups (M: 12%; F: 11%) at 5000 ppm from days 7-21 of lactation, but not at birth. The more pronounced body weight reduction in the F_1 adults compared to the F_0 adults suggests that the body weight reductions during lactation may have exacerbated the body weight reduction that occurred after weaning. Histopathological changes were observed in the kidneys of the F_1 adult males at all dose levels including hyaline droplets, chronic inflammation, the presence of regenerative tubules and renal cast formation (granular). It is unclear why these lesions were not observed in the F_0 adult males. There was no apparent treatment-related changes in food consumption or other reproductive parameters. The parental NOEL was 2000 ppm (M: 146 mg/kg/day; F: 179 mg/kg/day) based on the reduced body weights in F_1 adults (M: 8%; F: 12%). The reproductive NOEL was also 2000 ppm (M: 146 mg/kg/day; F: 179 mg/kg/day) based on the reduced neonatal pup weights (11-13%). This study was acceptable to DPR toxicologists based on guidelines.

<u>Subcutaneous-Rat</u>

Twenty male Sprague Dawley rats/dose were administered DEET (97-98% meta, 0.3% other isomers)by subcutaneous injection at 0, 0.30, 0.73, 1.15 or 1.80 ml/kg/day for 9 weeks (Wright *et al.*, 1992). Treated males were then allowed to mate with untreated females to evaluate for dominant lethal effects and male fertility. No males survived at 1.8 ml/kg/day. Most males developed skin lesions at one or several injection sites which the investigators attributed to scratching that was often observed after dosing. Disturbances in gait primarily in the hindlimbs was observed and was sometimes associated with cannibalization of the toes. A dose-related decrease in performance on the rotorod was observed by week 2. Urethral plugs made of coagulating gland and seminal vesicle secretions were observed in several treated males that died. The urinary bladder was often distended in these animals suggesting urinary blockage.   No incidence data were provided for the urethral plugs, so it is unknown if there was a dose-response relationship. BUN levels were elevated in blood collected from 4 moribund animals. The kidneys were not examined histopathologically, so it is uncertain if the elevated BUN levels were related to α_{2u}-globulin nephropathy or the urinary blockage. No effect on male fertility was seen based on the number of females impregnated, number, growth and survival of pups, and histological examination of the testes. Additionally, no evidence of induced dominant

lethal effects were found either. A NOEL could not be established for either the clinical signs or urethral plugs due to the limited information provided. This study had major deficiencies including the lack of individual data or summary tables for some effects and the study design did not follow the guidelines for reproductive toxicity studies.

Dermal-Rat

The effect of DEET on sperm morphology was also examined in a study in which DEET (98.3% meta, 0.2% other isomers) was applied topically to the shaved backs (unoccluded) of 80 male Sprague-Dawley rats/dose at 0, 100, 300 or 1000 mg/kg/day 5 days/week for 9 weeks (Brusick, 1980). This exposure period was selected to cover all stages of spermatogenesis. Twenty rats from each group were sacrificed at 36-37 days, 65-66 days, 95-96 days, and 125-126 days. There was no effect on body weight, food consumption or relative weights for testes. An increase in the relative liver and kidney weights was observed at 1000 mg/kg/day; however, neither tissue was examined microscopically. The increase in liver weights was considered adaptive in nature by DPR toxicologists based on pathological evidence of liver hypertrophy in other studies suggesting there was induction of microsomal enzymes (Johnson, 1987a&b). The increase kidney weights was probably due to the α_{2u}-globulin nephropathy that was observed in male rats (Goldenthal, 1989b & 1992). Only the testes were examined microscopically and no dose-related increase in lesions was found. An increase in the mean percent of abnormal sperm was seen at 100 (4.8%), 300 (6.9%) and 1000 (7.1%) mg/kg/day at the first interim sacrifice on days 36-37. These increases were not statistically significant and they were not observed at any other interim sacrifices or at the terminal sacrifice. The toxicological significance of this transient increase is uncertain for the following reasons: 1) the increase in abnormal sperm was less than 10%; 2) the increase was not statistically significant; 3) there was no effect on sperm count or viability; and 4) there were no microscopic lesions in the testes. Therefore, the NOEL for these limited male reproductive endpoints appears to be equal to or greater than 1,000 mg/kg, the highest dose level tested. This ancillary reproduction study did not meet guidelines.

G.   DEVELOPMENTAL TOXICITY

Summary: Six developmental toxicity studies were conducted in which DEET was administered to either rats or rabbits by the oral or dermal route. Only two of these studies met guidelines. Developmental effects included decreased implantations, increased resorptions, increased abortions, reduced fetal weights, increased skeletal variations, decreased neonatal weight gain, delayed neonatal development and increased neonatal death. However, many of these findings were only seen in studies of questionable quality. The skeletal variations were not considered adverse because the increase was not statistically significant when expressed on a litter basis and they were not associated with a reduction in fetal weight. Maternal effects included death, clinical signs, reduced body weight gain, reduced food consumption, and dermal irritation. The lowest developmental NOEL in an acceptable study was 250 mg/kg/day based on reduced fetal weights and increased preimplantation losses. The lowest systemic maternal NOEL in an acceptable study was also 250 mg/kg/day based on maternal deaths, clinical signs and reduced maternal weight gain and food consumption.

Gavage-Rat

DEET (purity not reported) was administered to 20 mated female SPF Wistar rats/dose by oral gavage in peanut oil at 0, 8, 20 or 80 µl/kg (0, 8.0, 19.9 or 79.7 mg/kg) on days 5 to 15

of gestation (Sterner, 1977). There were no maternal signs of toxicity other than a decreased weight gain (~30%) at 80 µl/kg. The mean food consumption was reduced by 22, 20 and 25% at 8, 20 and 80 µl/kg, respectively. The gestation index (percentage of pregnancies resulting in the birth of live litters) was decreased by 5, 10 and 15% at 8, 20 and 80 µl/kg, respectively. This decrease was probably due to the dose-related decrease in implantations and increase in resorptions, although the differences were not significant at any dose level. However, the decrease in implantations and increase in resorption were considered toxicologically significant at 80 µl/kg because of the dose-related trend and severity of the effect. There was no treatment-related increase in fetal malformations or variations. The maternal NOEL was 20 µl/kg (19.9 mg/kg) based on decreased maternal weight gain. The developmental NOEL was also 20 µl/kg based on the decreased implantations and increased resorptions. This study had major deficiencies including no justification for dose levels, no analysis of test article or dosing material, incomplete examination of fetuses for malformations, no individual data for fetal malformations or maternal clinical observations, and no gross necropsy data for dams.

Twenty-five pregnant female Charles River CD® rats/dose were administered DEET (98.0% meta, 0.3% other isomers) by gavage in corn oil at 0, 125, 250 or 750 mg/kg/day on gestation days 6 through 15 (Neeper-Bradley, 1990). Two dams at 750 mg/kg/day were sacrificed in a moribund condition on the second day of dosing. Their deaths appeared to be treatment-related. One dam at 125 mg/kg/day delivered early. A significant increase in clinical signs were observed in dams at 750 mg/kg/day, including hypoactivity (14/24), ataxia (11/24), decreased muscle tone (12/24), urine stains (13/24), foot splay (8/24), perinasal encrustation (10/24), and perioral wetness (17/24). The onset of the clinical signs was within the first day or two of dosing. There were significant reductions in the mean food consumption (12%) and maternal body weight gain (35%) at 750 mg/kg during the exposure period. However, the actual body weights were only 4% lower on day 15 in females at 750 mg/kg/day. Absolute and relative liver weights were significantly increased at 750 mg/kg/day. Although the liver was not examined microscopically, the increased liver weights were assumed to be adaptive in nature based on pathological evidence of liver hypertrophy in other studies suggesting there was induction of microsomal enzymes (Johnson, 1987a&b). There was an increase (137%) in percent preimplantation loss at 750 mg/kg/day. Although the increase was not statistically significant, it was considered toxicologically significant. The sex ratio (males:females) was increased slightly at 250 and 750 mg/kg/day; however, the toxicological significance of this finding is uncertain. The fetal body weights/litter were reduced (5%) at 750 mg/kg/day. No effect was seen on the number of corpora lutea or visceral and skeletal malformations. The developmental NOEL was 250 mg/kg/day based on the reduced fetal weight and increased preimplantation losses. The maternal NOEL was also 250 mg/kg/day based on the deaths, clinical signs, and decreased mean body weight gains (35%) and food consumption (12%). This study was acceptable to DPR toxicologists based on guidelines.

Gavage-Rabbit

Groups of 16 pregnant female New Zealand White rabbits/dose were administered DEET (98.69% meta, % other isomers not stated) by gavage at 0 (corn oil), 30, 100 or 325 mg/kg/day on gestation days 6 to 18 (Chun and Neeper-Bradley, 1991). One doe at 325 mg/kg/day died due to a dosing error and was removed from the study. No maternal clinical signs were seen. The mean maternal body weight gains and food consumption were reduced (69% and 30%, respectively) at 325 mg/kg/day during the exposure period. However, when the mean body weights were compared for day 18, only a 1% difference was seen primarily because the animals at 325 mg/kg/day were 2% heavier than the controls on day 6. There were no treatment-related effects on pregnancy rate, gestational length, and pre- or

G.   DEVELOPMENTAL TOXICITY (cont.)

postimplantation losses. There was an increase in some skeletal variations that were not dramatic, but when taken as a whole were suggestive of delayed skeletal development (Table 14). When expressed on a fetus basis, the incidence of a few variations (unossified or poorly ossified phalanges and extra rib) were significantly higher when compared to controls; however, the incidence of the skeletal variations was not significantly different from controls when expressed on a litter basis. There were also significant dose-related trends in the incidence of poorly ossified or unossified phalanges, unossified and split sternabrae and extra rib when expressed on either a fetus or litter basis. However, the toxicological significance of the increase in skeletal variations was uncertain because it was not significantly different from controls on a litter basis and it was not associated with a reduction in fetal weight. The developmental NOEL was equal to or greater than 325 mg/kg/day, based on the lack of any overt developmental toxicity. The maternal NOEL was 100 mg/kg/day based on the reduced body weight gain (69%) and food consumption (30%). The study was acceptable to DPR toxicologists based on guidelines.

Table 14. Incidence of Skeletal Variations in Rabbit Fetuses Exposed *In Utero* to DEET[a]

	Fetuses				Litters			
	0	30	100	325	0	30	100	325
	(mg/kg/day)				(mg/kg/day)			
Number Examined Skeletally	137	131	105	122	16	14	13	14
Phalanges (forelimbs), poorly ossified	13[+] (10%)	13 (10%)	4 (4%)	18 (15%)	5[+] (31%)	6 (43%)	3 (23%)	9 (64%)
Phalanges (forelimbs), unossified	7[+++] (5%)	10 (7%)	14* (13%)	27*** (22%)	4[+] (25%)	5 (36%)	5 (38%)	8 (57%)
Phalanges (hindlimbs), poorly ossified	0[+++] (0%)	0 (0%)	0 (0%)	6* (5%)	0[++] (0%)	0 (0%)	0 (0%)	3 (21%)
Sternabrae #5, unossified	4[+] (3%)	6 (5%)	4 (4%)	10 (8%)	3 (19%)	5 (36%)	3 (23%)	6 (43%)
Sternabrae #6, split	1[++] (1%)	0 (0%)	1 (1%)	4 (3%)	1[+] (6%)	0 (0%)	1 (8%)	3 (21%)
Extra rib #13 on thoracic arch #13, unilateral	1[++] (1%)	0 (0%)	2 (2%)	6* (5%)	1[+] (6%)	0 (0%)	2 (15%)	4 (29%)

[a]   Chun and Neeper-Bradley, 1991

*, ***   Significantly different from the control group based on the Fisher's exact test at p < 0.05 and 0.001, respectively.

+, ++, +++   Significant trend based on the Cochran-Armitage trend test at p < 0.05, 0.01, and 0.001, respectively.

Subcutaneous-Rat

In two replicate experiments, 35-37 Sprague-Dawley time-mated females/group were administered undiluted DEET (97-98% meta; 0.3% other isomers) by subcutaneous injection at 0 or 0.30 ml/kg/day on gestation days 6-15 (Wright *et al.*, 1992). Following the exposure period the groups were subdivided into two subgroups, one half of each group was

used for a teratology study and the other half for a postnatal viability study. A significant reduction in maternal body weight gain (25%) was observed at 0.3 ml/kg/day from gestation day 10 to 15. Beginning as early as gestation day 9 (day 3 of exposure), a gait disturbance was observed in the hindlimbs of treated females of both replicates.   There was no treatment-related differences in fetal weight, number of implants, live fetuses, fetal malformations or variations, and viability of pups. The maternal NOEL appears to be less than 0.30 ml/kg/day based on the gait disturbance. The developmental NOEL appears to be greater than or equal to 0.30 ml/kg/day. This study had major deficiencies including the lack of individual data, too few dose groups, and a non-standard protocol for evaluating developmental toxicity with respect to the guidelines.

Dermal-Rat

DEET (purity not reported) was applied to the sheared skin on the backs of 20 white (strain not specified) mated female rats/dose at 0 (alcohol), 100 and 1000 mg/kg/day on days 1 through 19 of gestation (Gleiberman *et al.*, 1975). On day 19 half of the dams at each dose level were sacrificed. The remaining dams were allowed to give birth and nurse the young for another 30 days. At 100 mg/kg/day, there was an increase in the percent preimplantation losses (22.8% vs. 13.0% for controls). At 1000 mg/kg/day, both the percent pre- and postimplantation losses were increased (26.3% and 9.3%, respectively) relative to controls (13.0% and 5.8%, respectively). In addition, a decrease in the mean neonatal weight at 60 days old (23%), delayed appearance of fur and eye opening (approximately 2 days later than controls), and an increase in the percent neonatal deaths (44.0% vs.15.7% for controls) were observed at 1000 mg/kg/day. The toxicological significance of the delayed fur appearance and eye opening is uncertain, although it may be related to the reduced neonatal weight gain. There was also an increase in percent neonatal deaths (27.4% vs.15.7% for controls) and delay in fur appearance and eye opening (approximately 1 day later) at 100 mg/kg/day, too. The developmental NOEL was less than 100 mg/kg based on the increased preimplantation losses. A maternal NOEL could not be established due to insufficient information. This study had other major deficiencies including no analysis of test article or dosing material, too few animals per group, only two dose levels tested, and no individual data.

Dermal-Rabbit

DEET (95% meta, 5% other isomers) was applied to the clipped dorsal skin of 20 mated female New Zealand White rabbits/dose at 0, 50, 100, 500 or 1000 mg/kg/day from gestation days 1 to 29 (Angerhofer and Weeks, 1980). The application site was covered with a screened foam pad which prevented ingestion of DEET. One female at 500 mg/kg/day and 6 at 1000 mg/kg/day starved to death due to trichobezors (hair ball or concretion in stomach) which the investigators suggested was due to licking near the application site. DPR toxicologists did not consider the deaths relevant to humans since licking is not a normal grooming behavior in humans. Dermal irritation was observed as early as day 7 at 100 mg/kg/day, but did not appear until day 14 at 50 mg/kg/day. No treatment-related systemic clinical signs were seen. A decrease in the mean daily food consumption was observed during the first two weeks of gestation at 500 (10%) and 1000 mg/kg/day (19%). Two does (one each at 500 and 1000 mg/kg/day) aborted their litters before termination of pregnancy. The number of resorptions were increased at 500 mg/kg/day. The only histopathological lesions were hyperkeratosis, parakeratosis, and acanthosis in skin at the application site. A significant increase in serum γ-glutamyl transpeptidase (γGT) were observed at 500 and 1000 mg/kg/day on day 7-30. The BUN levels were also elevated at 500 and 1000 mg/kg/day on day 30. The toxicological significance of the elevated γGT and BUN is uncertain since the liver and kidneys were not

G.   DEVELOPMENTAL TOXICITY (cont.)

examined microscopically in this study. Furthermore, none of the other liver enzymes measured in the serum were elevated. The maternal NOEL for local effects was less than 50 mg/kg/day based on dermal irritation. The maternal systemic NOEL was 100 mg/kg/day based on reduced food consumption. The developmental NOEL was 100 mg/kg/day based on the abortions and increased resorptions. This study had major deficiencies including no analysis of dosing material, insufficient number of animals at high dose, incomplete examination of fetuses for malformations, and no summary tables for maternal clinical signs or body weights or for fetal skeletal and visceral findings.

H.   NEUROTOXICITY

Summary: Three acute and 3 subchronic neurotoxicity studies were conducted where DEET was administered by the oral or inhalation route to rats. At approximately 500 mg/kg, mild neurological effects were observed including reduced endurance and balance, decreased activity, changes in heat sensitivity, tremors, and slower nerve conduction and repolarization. At 1,000 mg/kg, more severe neurological effects were seen including decreased reactivity and muscle tone, respiratory depression, decreased blood pressure, twitching, seizures, spongiform myelinopathy, and clear cytoplasmic clefts in the cerebellar roof nuclei. The lowest acute NOEL for neurotoxic effects was 200 mg/kg based on reduced heat sensitivity and motor activity, piloerection, and tremors. The lowest subchronic NOEL for neurotoxic effects was 180 mg/kg/day based on reduced endurance.   None of the studies met neurotoxicity guidelines; however, they were all conducted before the neurotoxicity guidelines were available and the deficiencies in most of these studies should not negate the effects.

Acute

Inhalation-Rat

The U.S. Army conducted an acute toxicity study designed to examine the behavioral effects at sublethal aerosol concentrations (Sherman, 1980b). In this study, 10 rats/sex/dose were exposed to DEET (approximately 95% meta (min.) and 0.5 other isomers (max.)) at 0, 2300, 2900 or 4100 mg/m^3 (0, 368, 464 or 656 mg/kg^3) for 4 hours. Endurance, passive and quick avoidance, balance, activity, tactile sensitivity, and auditory response were examined within one-hour after the exposure ended. A significant difference in response was observed with all the tests except auditory response at one or more dose levels. The NOEL appears to be less than 2300 mg/m^3 (368 mg/kg) based on reduced endurance and balance and increased heat sensitivity. When compared to the proposed U.S. EPA guidelines for neurotoxicity studies (1991), this study had several deficiencies including no individual data, no historical positive control data, very limited functional observational battery (FOB), and no individual pathology data. However, this study was conducted before these guidelines were available. Currently, DPR does not require this type of neurobehavioral tests under SB 950. U.S. EPA has a data call-in for these tests for selected chemicals under review for .

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[3]   Estimated assuming a respiratory rate of 0.16 m^3/kg/4 hrs for a rat (Zielhuis and van der Kreek, 1979).

H.   NEUROTOXICITY (cont.)

Gavage-Rat

Changes in the electrophysiology and neuropathology of rats administered DEET (98% meta isomer, % other isomers not reported) by oral gavage in arachis oil at 0.4 to 5 g/kg were examined (Verschoyle *et al.*, 1992). A rapid decrease in reactivity and muscle tone was observed at 2 g/kg and higher within 7 to 60 minutes after dosing. Rats given 2.5 g/kg and higher died within 24 hours with progressive respiratory depression. The CNS depression was interrupted by tremors, twitching and seizures. The twitching could be initiated by auditory or tactile stimuli. Marked slowing of the EEG and development of regular 2- to 5-Hz activity accompanied loss of muscle tone. Twitches were associated with spike discharges on the EEG. A decrease in blood pressure observed at 2 g/kg and higher paralleled the CNS depression. Auditory evoked responses in EEG at 2.5 g/kg and higher showed a large abnormal biphasic component at 24/32 msec. A spongiform myelinopathy (described as vacuolation of myelin sheaths in the cerebellar roof nuclei) and clear cytoplasmic clefts (primarily in cerebellar roof nuclei) were observed in rats receiving 1 g/kg and higher. All rats with lesions were either ataxic or prostrate, although some ataxic rats had no neuropathological lesions. All rats that had neuronal clefts exhibited semiprostration or prostration. There was insufficient information to establish a NOEL from this report. This study design did not follow the guidelines for neurotoxicity studies in rats.

Ten Crl:CD® VAF/Plus® rats/sex/dose were administered undiluted DEET (98.0% meta, 0.3% other isomers) at 50, 200 or 500 mg/kg by gavage (Schardein, 1990a). Controls were given white mineral oil by gavage. Animals were tested for neurotoxic effects at 1 hour, 24 hours, and 14 days after dosing. Mild salivation was observed at all dose levels immediately after dosing which the investigators suggested was a response to the taste of DEET rather than a systemic effect (Table 15). Excessive salivation was only reported (as drooling) in one child that developed toxic encephalopathy after topical application of DEET (Edwards and Johnson, 1987). Salivation was not observed in any animals after DEET was administered in the feed or applied dermally. Salivation was observed in dogs administered DEET in capsules at 30 mg/kg (Goldenthal, 1994 & 1995a), but not when administered in the feed up to 3000 ppm (92 mg/kg/day) (Goldenthal, 1995b). Increased salivation (reported as increased perioral wetness) was observed in a rat developmental toxicity study where DEET was administered by oral gavage in corn oil at 750 mg/kg/day (Neeper-Bradley, 1990). Consequently, the toxicological significance of the salivation, especially at the lower dose levels, is uncertain. It may be either the result of high peak blood levels as a result of the bolus dose when administered in capsules or by gavage or the result of local effects produced when DEET is administered orally at high concentrations. Several effects were seen at 500 mg/kg/day during the functional observational battery, including an increase in tremors at 24 hours, piloerection at 1 hour and 24 hours and vocalization on day 14 in males. Only an increase in piloerection was observed in females at 1 hour and 24 hours. In the thermal response test, reduced heat sensitivity (as measured by an increase in response time) was observed in both sexes at 500 mg/kg at 1 hour. However, the increase in response time was only significant when both sexes were combined. A decrease in rearing activity (as measured by vertical activity and time) were also observed in both sexes 1 hour after dosing at 500 mg/kg in the motor activity test. There was no consistent pattern of behavior at the 24-hr and 14-day observations or at the lower dose levels. A significant reduction (11%) in the food consumption of females was seen in week 2 when expressed on a g/animal/day basis. However, when expressed on a g/kg/day basis, the reduction (7%) was not significant. There was no effect on body weights, mortalities or gross pathological findings. The NOEL was 200 mg/kg based on the reduced heat sensitivity and motor activity, piloerection, and tremors. Based on the 1991 proposed guidelines for neurotoxicity studies, this study had minor deficiencies in the functional observational battery (FOB), no individual data for

H.   NEUROTOXICITY (cont.)

Table 15. Neurotoxic Effects in Rats After Acute Oral Exposure to DEET[a]

Effect	Dose Level (mg/kg)			
	0	50	200	500
MALES				
Clinical Signs				
Excessive Salivation	0/10[++]	5/10*	8/10***	7/10**
FOB				
Vocalization (14-day)	2/10[++]	1/10	1/10	6/10
Tremors, resting (24-hr)	0/10[++]	0/10	0/10	3/10
Piloerection (24-hr)	1/10[++]	2/10	2/10	6/10*
Heat Response Test (1-hr)				
Reaction Time (sec)	12.5±7.4	15.1±7.7	18.4±9.9	19.9±15.0*
Motor Activity Test (1-hr)				
Vertical Activity [b]	1072	1005	710	667*
Vertical Time (sec)	360	317	229	211*
FEMALES				
Clinical Signs				
Excessive Salivation	0/10[++]	5/10*	7/10**	8/10***
FOB				
Piloerection (24-hr)	0/10[+++]	0/10	2/10	6/10**
Heat Response Test (1-hr)				
Reaction Time (sec)	13.2±3.3	16.5±0.9	18.8±8.0	25.2±14.9*
Motor Activity Test (1-hr)				
Vertical Activity[b]	1136	1125	766	619*
Vertical Time (sec)	459	446	319	238*

[a]   Schardein, 1990a
[b]   Total number of beam interruptions that occurred in the vertical sensor during the 40 minute test period.
[++, +++]   Significant trend based on Cochran-Armitage trend test at $p < 0.01$ and 0.001, respectively (Gart *et al.*, 1986)
[*, **, ***]   For quantal data, the incidence is significantly different from the control group based on the Fisher's exact test at $p < 0.05$, 0.01, and 0.001, respectively. For continuous data, the difference is statistically significant ($p < 0.05$) by three-way repeated measures ANOVA where data from both sexes combined and post hoc Student-Newman-Keuls multiple range test.

FOB, incomplete historical positive control data, and no individual pathology data. However, this study was also conducted before these guidelines were available and these minor deficiencies should not negate the findings.

H.   NEUROTOXICITY (cont.)

Subchronic

Inhalation-Rat

In conjunction with a subchronic inhalation study described previously (Macko and Bergman, 1980), rats exposed at 0, 250, 750 or 1500 mg/m^3 (nominal; 0, 60, 180 or 360 mg/kg/day) were given several behavioral tests (balance, tactile sensitivity, and endurance tests) during weeks 2, 4, 6, 8, 11, and 13 of the study (Sherman, 1980c). Also, on week 13 they were given memory (quick avoidance), learning (passive avoidance) and short-term activity (tremors, locomotion) tests. The endurance of females was significantly increased at 250 mg/m^3, but significantly decreased at 1,500 mg/m^3. The females at 750 mg/m^3 performed consistently poorer on balance beam than at other dose levels, with fewer animals moving beyond the start point. Males at 750 and 1,500 mg/m^3 were significantly faster on the quick avoidance (memory) test than the controls. There was no obvious treatment-related effect on tactile sensitivity, learning, and short-term activity. The toxicological significance of the increased endurance at 250 mg/m^3 and the faster performance on the quick avoidance test at 750 and 1,500 mg/m^3 is uncertain. The reduction in performance at 1,500 mg/m^3 was considered an adverse effect. The NOEL for behavioral effects was 750 mg/m^3 (180 mg/kg) based on reduction in endurance in females. Based on the 1991 proposed guidelines for neurotoxicity studies, this study had several deficiencies including no individual data, no historical positive control data, very limited functional observational battery (FOB), and no individual pathology data. However, this study was conducted before these guidelines were available.

Gavage-Rat

Nerve conduction velocity and relative refractory period in the sciatic nerve was examined in 8 male Wistar rats/dose administered DEET (purity not reported) by gavage at 0, 0.6, 0.8, 1.0, 1.2, 1.4 or 1.6 g/kg/day for 28 days (Campbell, 1986). All animals at 1.6 g/kg/day died within the first few days of dosing. Approximately half of the animals at 1.4 g/kg/day died by the end of the experiment. No difference in nerve conduction velocity was found, but there was a significant change in the relative refractory period at all dose levels indicating both a slower conduction and slower repolarization of the nerve membrane after excitation. The average terminal body weight of the treated animals tended to be lower (~5-10%) than the controls, but the differences were not statistically significant at any dose level and did not exhibit a dose-relationship. The NOEL for these electrophysiological changes in the peripheral nerve was less than 0.6 g/kg. This study did not meet guidelines based on its limited scope.

Diet-Rat

Some of the F_2 weanling rats from a reproductive toxicity study for DEET (Schardein, 1989) were evaluated for neurotoxic effects following a 9 month exposure period at dietary concentrations of DEET (98.0% meta, 0.3% other isomers) at 0, 500, 2000 or 5000 ppm (M: 0, 19, 75 or 196 mg/kg/day; F: 0, 25, 99 or 275 mg/kg/day during weeks 41-48) (Schardein, 1990b). One rat/sex/litter from 20 litters were selected for each group. At the start of the neurotoxicity evaluation, the rats were approximately 40 weeks old. The neurotoxicity evaluations were conducted over an 8 week period. In addition to the usual functional observational battery and motor activity tests, the rats were further evaluated for learning and memory using M-maze, acoustic startle habituation, and passive avoidance tests. There were no clinical signs of toxicity; however, the mean body weights were significantly lower at 2000 (M: 8%; F: 11%), and 5000 ppm (M: 15%; F: 14%) between weeks 40 to 48 (Table 16). The

investigators attributed the differences in body weights at 500 and 2000 ppm to the randomization procedure since a significant reduction in body weights was only observed at 5000 ppm in the F_2 pups on day 21 of the reproductive toxicity study. However, there was a slight, although not significant, reduction in the mean body weights at 500 and 2000 ppm on day 21, suggesting some sort of delay in development that became more pronounced with continued exposure. A similar trend towards reduced body weights was also observed at 500 ppm and 2000 ppm in the F_1 offspring of the reproduction study. The only neurotoxic effect found was an increase in exploratory locomotor activity in both sexes at 5000 ppm which was of uncertain toxicological significance. No treatment-related increase in lesions in the central and peripheral nervous tissues were seen with neuropathological examination. The NOEL for this study was 500 ppm (M: 19 mg/kg/day; F: 25 mg/kg/day) based on the reduced body weights (M: 8%; F: 11%). Based on the 1991 proposed guidelines for neurotoxicity studies, this study had minor deficiencies in the functional observational battery (FOB), no individual data for FOB, incomplete historical positive control data, and incomplete individual pathology data. However, this study was also conducted before these guidelines were available and these minor deficiencies should not negate the findings. Furthermore, the animals in this study were exposed much longer than required by guidelines and they were evaluated for learning and memory deficiencies.

Table 16.   Postnatal Growth in Two Generations of Rats Exposed to DEET from Conception to Adulthood[a,b]

Body Weights	Dose Level (ppm)			
	0	500	2000	5000
MALES				
F_0 adults, week 16	513±52	497±46 (-4.1%)	478±37** (-6.9%)	478±34** (-6.9%)
F_1 pups, day 21	46.2±6.5	46.8±5.1 (+1.2%)	45.7±4.5 (-1.1%)	40.1±4.2** (-13.2%)
F_1 adults, week 16	506±46	494±46 (-2.4%)	490±54 (-3.2%)	464±32** (-8.3%)
F_2 pups, day 21	50.4±4.2	49.4±6.1 (-2.0%)	47.9±4.0 (-5.0%)	44.5±3.9** (-11.7%)
F_2 adults, week 48	711±7	666±91 (-6.3%)	655±69* (-7.9%)	602±50** (-15.3%)
FEMALES				
F_0 adults, week 11	249±29	257±25 (+3.2%)	255±31 (+2.4%)	234±15* (-6.0%)
F_1 pups, day 21	44.1±4.6	44.6±4.4 (+1.1%)	44.2±4.5 (+0.2%)	39.1±4.6** (-11.3%)
F_1 adults, week 16	271±26	272±22 (+0.4%)	261±19 (-3.7%)	239±22** (-11.8%)
F_2 pups, day 21	47.3±4.5	47.6±5.4 (+0.6%)	44.1±7.5 (-6.8%)	42.3±3.2** (-10.6%)
F_2 adults, week 48	377±57	362±48 (-4.0%)	337±53* (-10.6%)	326±62** (-13.5%)

[a]   Schardein, 1989
[b]   Schardein, 1990b
*, **   Significantly different from the control group by the Dunnett's test at $p < 0.05$ and 0.01, respectively.

III. RISK ASSESSMENT

A.   HAZARD IDENTIFICATION

Acute Toxicity

There are a small number of cases of seizures and/or toxic encephalopathy in children after topical application of DEET (Gryboski *et al.*, 1961; Zadikoff, 1979; Heick *et al.*, 1980, Edwards and Johnson, 1987; Oransky *et al.*, 1989; Roland *et al.*, 1985; Lipscomb *et al.*, 1992; U.S. EPA, 1998). In these cases, lower concentration (10-20%) DEET products had often been applied daily for several weeks to months, although in some cases seizures occurred after only a few applications of concentrated DEET (95-10%) products. There is one case report of a seizure in an adult male after only two topical applications of DEET of unknown concentration (U.S. EPA, 1998). Seizures were also observed in children and adults after ingestion of large amounts of concentrated DEET (47.5-95%) products (Tenebein, 1987). There were a few case reports of other adverse reactions in adults after dermal application of DEET, including manic psychosis, orthostatic hypotension, and bradycardia (Snyder *et al.*, 1986; Clem *et al.*, 1993). A number of symptoms were associated with heavy DEET use among park workers in the Everglades National Park, including skin rashes or blisters, chest pain or wheezing, depression, irritability, insomnia, difficulty starting and stopping urinary stream, muscle cramps, daytime sleepiness, memory problems, difficulty in concentrating, and absentmindedness (McConnell *et al.*, 1986). Although it was not possible to determine conclusively from any of these case reports that these adverse reactions were due to DEET, in cases where there was a history of excessive use there is a high probability that they are related.

A number of acute LD_{50}/LC_{50} studies were conducted for various DEET formulations; however, only a few studies were available for technical grade DEET (Table 17). DEET appears to be more toxic in young rats (11 days old) when compared to older rats (47-56 days old) based on their LD_{50} values (Verschoyle *et al.*, 1992). The clinical signs observed in these studies included prostration, unconsciousness, ataxia, convulsions, tremors, loss of balance, labored or slow breathing, rolling eyes, rear limbs extended, red urine, lethargy, bloody tears, nasal discharge, and ruffled hair. The gross pathological findings included mottled and/or congested lung, liver, kidneys, and spleen, petechial hemorrhages in the lung and intestines (oral exposure), and fluid-filled stomachs with pink or white pylorus (oral exposure). The LOELs for systemic effects in these studies ranged from approximately 600 mg/kg by the inhalation route to greater than 5,000 mg/kg by the dermal route.

Several acute neurotoxicity studies were available for DEET. Distinct neurological effects were observed in rats at 1 g/kg and higher in a study in which DEET was administered by oral gavage (Verschoyle *et al.*, 1992). There was a decrease in reactivity, muscle tone and blood pressure indicative of CNS depression at 2 g/kg and higher which was interrupted by episodes of tremors, twitching, and seizures. In a few rats implanted with electrodes, marked slowing of the EEG with prominent spike discharges was observed at 2.5 and 3 g/kg. Vacuolization of the myelin sheaths primarily in fibers of the cerebellar roof nuclei and clear cytoplasmic clefts in neurons diffusely distributed throughout the brain were observed in rats receiving 1 g/kg of DEET or higher. Although not all ataxic rats had neuropathological lesions, all prostrate rats had cytoplasmic clefts. Insufficient information was provided about the effects at the lower dosages to establish a NOEL in this study. The cardiovascular effects of DEET were investigated in another study in which rats were administered DEET intraperitoneally (Leach *et al.*, 1988). The NOEL for the cardiovascular effects was 56 mg/kg based on decreased heart rate and blood pressure at 113 mg/kg. It is unclear if the lower NOEL in this study was due to the route of administration. In a neurobehavioral study, reduced endurance

A.   HAZARD IDENTIFICATION (cont.)

Table 17.   Acute Effects of DEET and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL (mg/kg)	LOEL (mg/kg)	Ref.[a]
Intraperitoneal					
Rat	Single	Red. heart rate and blood pressure	56	113	1
Inhalation					
Rat[b]	Single, 4-hr	Death, ataxia, labored breathing, nasal discharge, red urine	------	592[c]	2
Rat[d]	Single, 4-hr	Reduced endurance and balance, increased heat sensitivity	------	368	3
Oral					
Rat[b]	Single, gavage	Lethargy, labored breathing	------	1250	4
Rat[b]	Single, gavage	Prostration, ataxia, tremors	------	1000	5
Rat[d]	Single, gavage	Reduced heat sensitivity and motor activity, piloerection, tremors	200	500	6
Rat[e]	11 Days, gavage	Fetal: Pre- and postimplantation losses	20	80	7
Rat[e]	10 Days, gavage	Maternal: Death, hypoactivity, ataxia, dec. muscle tone, foot splay (onset 1-2 days)	250	750	8*
		Fetal: Preimplantation losses, reduced pup weight	250	750	
Rabbit[e]	13 Days, gavage	Maternal: Dec. body weight gains and food consumption (onset 1-3 days)	100	325	9*
Subcutaneous					
Rat[e]	10 days	Maternal: Gait disturbance (onset day 3)	------	300	10
Dermal					
Rabbit[b]	Single, 24 hrs	Erythema, edema (no systemic effects)	------	2000	4
Rabbit[b]	Single, 24 hrs	Erythema	------	1800	5
Rat[b]	**Single, 24 hrs**	**None**	**5000**	------	**11***
Rat[e]	19 Days	Fetal: Preimplantation losses	------	100	12

a	References: 1. Leach *et al.*, 1988; 2. Macko and Bergman, 1980; 3. Sherman, 1980b; 4. Weil, 1973; 5. Macko and Weeks, 1980; 6. Schardein, 1990a; 7. Sterner, 1977; 8. Neeper-Bradley, 1990; 9. Chun and Neeper-Bradley, 1991; 10. Wright *et al.*, 1992; 11. Moore, 2000a; 12. Gleiberman *et al.*, 1975.
b	LD_{50}/LC_{50} study
c	Assuming a rat breathes 0.16 m^3/kg in 4 hours (Zielhuis and van der Kreek, 1979).
d	Neurotoxicity study
e	Developmental toxicity study: All fetal effects were considered acute effects; however, only maternal effects observed within the first few days of exposure were considered acute exposure.
f	This NOEL is equivalent to 25.8 mg/cm^2 based on the application of approximately 1 ml (1000 mg) to an application site that was 2 inches by 3 inches (38.7 cm^2).
*	Acceptable study based on FIFRA guidelines

and balance and increased heat sensitivity were observed in rats exposed to DEET at 2300 mg/m^3 (368 mg/kg) or higher for 4 hours (Sherman, 1980b). However, a NOEL was not established in this study. A NOEL of 200 mg/kg was established in another neurobehavioral study based on piloerection, tremors, reduced motor activity, and reduced heat sensitivity in rats administered DEET by oral gavage at 500 mg/kg (Schardein, 1990a).

The mechanism behind the neurotoxic effects associated with DEET is unclear. A sharp increase in serum ammonia levels was observed in mice administered DEET intraperitoneally at 0.5 g/kg which peaked about 3 hours after dosing (Heick *et al.*, 1988). The effects observed in mice were drowsiness followed by coma. However, the level of consciousness of the mice did not correlate with the ammonia levels. In an *in vitro* study with rat hepatocytes, DEET was found to increase the ammonia concentrations in reaction mixtures while it caused the urea levels to decrease (Brini and Tremblay, 1991). DEET also inhibited the production of glucose in these cells. These investigators speculated that DEET is affecting both the urea cycle and gluconeogenesis at a common site, such as energy charge, mitochondrial flux through pyruvate carboxylase or cytosolic availability of oxaloacetate and aspartate, based on the parallel changes in both. Fatal hyperammonia was observed in one case report of a 6-year-old girl who was a heterozygote for ornithine carbamoyl transferase deficiency (Heick *et al.*, 1980). This sex-linked genetic disorder is of variable severity in females, but is fatal in males during the neonatal period. This deficiency may be responsible for some of the cases of toxic encephalopathy in females, but it does not explain the cases in males. In most cases, it was not possible to determine if this deficiency may have been a contributing factor in the development of the toxic encephalopathy in girls since neither the activity of this enzyme or blood ammonia levels were reported. In one case involving an 8-year-old girl, the blood ammonia level was normal (Roland *et al.*, 1985). DEET did not inhibit ornithine carbamoyl transferase from human liver samples *in vitro* at concentrations ranging from 0.2 to 1.0 mmol/L (Rej *et al.*, 1990). More than one mechanism may be involved in the neurotoxic effects since DEET may have CNS excitatory properties similar to other *N,N*-dialkylamides, such as *N,N*-dimethyl acetamide or doxapram, which are capable of penetrating the blood-brain barrier (Snyder *et al.*, 1986).

Certain effects observed in developmental toxicity studies were also considered as acute effects. These include any maternal effects observed in the first few days of dosing and any fetal effects since, theoretically, they may have been caused by a single dose at a critical stage. Fetal effects (increased pre- and postimplantation losses and reduced pup weights) were observed in several rat developmental toxicity studies (Sterner, 1977; Neeper-Bradley, 1990; Gleiberman *et al.*, 1975). Neonatal effects including reduced neonatal weight gain, delayed fur appearance and eye opening, and increased neonatal death were observed in another developmental study in which half of the dams were allowed to give birth and nurse their pups for 30 days (Gleiberman *et al.*, 1975). There was a suggestion of delayed skeletal development in a rabbit study, although it was not clearly treatment-related (Chun and Neeper-Bradley, 1991). Acute maternal effects were observed in several of the developmental toxicity studies including death, hypoactivity, ataxia, decreased muscle tone, foot splay, gait disturbance, reduced body weight gain and food consumption (Neeper-Bradley, 1990; Chun and Neeper-Bradley, 1991; Wright *et al.*, 1975).

In selecting the critical NOEL, preference was given to the acute dermal toxicity studies based on the pharmacokinetic data for DEET. In studies of plasma levels after oral and dermal exposure, peak blood levels after oral (gavage) exposure were 18-fold higher than after dermal exposure (Selim, 1991a). Although the neurotoxicity studies provided the most thorough neurobehavioral evaluation, none of the available neurotoxicity studies for DEET exposed

animals by the dermal route. The uncertainty introduced by use of route-to-route extrapolation was considered greater than the uncertainty by use of dermal studies where only standard clinical observations were done. Only the acute dermal toxicity study conducted by Moore (2000a) established a NOEL for DEET by this route. No systemic effects were observed in this study at 5,000 mg/kg, the only dose level tested. This NOEL is the highest observed NOEL in any acute study, including other acute dermal toxicity studies. Most of the lower NOELs were in studies with inhalation or oral exposure which is not surprising based on the pharmacokinetics data for DEET. However, the LOELs observed in the other acute dermal toxicity studies were also lower than this NOEL. In two of these studies, the application site was covered with an occlusive wrapping during exposure which is not representative of actual use conditions and probably significantly increased absorption through increased hydration of the skin and decreased evaporation of DEET from the application site (Weil, 1973; Macko and Weeks, 1980). In a third study, an increase in preimplantation losses was observed at 100 mg/kg in pregnant rats exposed to DEET dermally for 19 days (Gleiberman *et al.*, 1975). However, it is unclear if the application site was covered, so some of the test material may have been ingested due to grooming rather than absorbed through the skin. The study had other major deficiencies, the most significant of these being the lack of analysis of the test compound for purity or dosing solution for concentration. Consequently, it is possible that impurities, degradation, errors in calculation or weighing, or nonhomogeneous mixing resulted in the animals receiving more or less test compound than reported.   Furthermore, a higher NOEL of 250 mg/kg was established for pre- and postimplantation losses in an oral rat developmental toxicity study which met FIFRA guidelines, including the analysis of test article and dosing solution (Neeper-Bradley, 1990). Use of the developmental toxicity studies also introduces some uncertainty regarding whether the effects are due to a single or repeated exposure. Consequently, the acute dermal toxicity study in rats conducted by Moore (2000a) was selected as the critical study for the evaluating acute exposure to DEET with a critical NOEL of 5000 mg/kg based on the lack of any systemic effects at this dose level. After correcting for dermal absorption (40%), the adjusted acute NOEL was 2000 mg/kg.

Evidence of dermal irritation (slight to moderate erythema and edema) was observed in several acute dermal toxicity studies at 1800 and 2000 mg/kg (Weil, 1973; Macko and Weeks, 1980).   A NOEL was not established for this endpoint with either study. A NOEL of 5000 mg/kg was established for dermal irritation in the acute dermal toxicity conducted by Moore (2000a). This NOEL is higher than the LOEL in the other two studies which is not surprising due to the use of semi-occlusive wrapping rather than occlusive wrapping. Since the semi-occlusive wrapping is more representative of actual use conditions than the occlusive wrapping, the study by Moore (2000a) was selected as the definitive study for evaluating the potential for DEET to cause dermal irritation from acute exposure. Since dermal irritation is a local effect, concentration on the skin was considered a more appropriate expression of dosage rather than on a body weight basis. Therefore, the critical NOEL of 5000 mg/kg was converted to 25.8 mg/cm^2 based on the application of approximately 1 ml (1000 mg) of technical grade DEET to the application site which was 2 inches by 3 inches (38.7 cm^2).

Subchronic Toxicity

Numerous subchronic studies were available for DEET including studies designed to address the species and sex-specificity of the renal lesions in male rats (Table 18). Included in Table 18 are also effects from neurotoxicity and reproductive toxicity studies. The renal lesions observed in male rats included cast formation (granular), multifocal inflammation in the renal cortex, regenerative tubules in the renal cortex, and hyaline droplets which were indicative of α_{2u}-globulin nephropathy. The presence of α_{2u}-globulin in the hyaline droplets was confirmed in

A.   HAZARD IDENTIFICATION (cont.)

one study using an immunocytochemical staining technique (Goldenthal, 1989b). Another study demonstrated that male NBR rats, which do not produce α_{2u}-globulin, did not develop the renal lesions (Goldenthal, 1992). Recent reviews of α_{2u}-globulin nephropathy have concluded that this effect is probably not relevant for humans since human males do not produce α_{2u}-globulin (Borghoff *et al.*, 1990; Flamm and Lehman-McKeeman, 1991; Hard *et al.*, 1993). Consequently, this effect was not used in selecting NOELs for evaluating human health effects of DEET.

Subchronic exposure to DEET caused death and a variety of clinical signs which were similar to those observed with acute exposure. In addition, other subchronic effects were seen including reduced body weights and food consumption, decreased nerve conduction and repolarization, abnormal sperm, changes in liver, kidney (dog), and testes weights, changes in clinical chemistry and hematological values, and miscellaneous microscopic lesions. Among the treatment-related microscopic findings were hepatocellular hypertrophy and fatty changes of the liver, cytoplasmic vacuolization of the kidney tubules (dog), testicular tubular degeneration, small epididymides with luminal cellular debris, erosion of the stomach mucosa, hypocellularity of the rib bone marrow, thymic atrophy and hemorrhage, and acanthosis and hyperkeratosis of the skin (dermal exposure only).

Increased liver weights were observed in many studies, but were rarely associated with any pathological lesions in the liver. In two studies, increased liver weights were associated with pathological findings in the liver including accentuated lobulation or round edges, diffuse multilobular mottling or foci, and hepatocellular hypertrophy which suggested there was induction of microsomal enzymes (Johnson, 1987a&b). These liver changes appear to be adaptive in nature and, therefore, were not considered adverse by DPR toxicologists. Changes in testicular weights were also seen in several studies; however, there was not a consistent direction in change or associated histopathological findings. Consequently, the changes in testicular weights alone were not considered toxicologically significant by DPR toxicologists. Increased kidney weights were often observed in male rats and were considered related to the α_{2u}-globulin nephropathy and not relevant to humans. An increase in kidney weights was observed in female rats in one study which was associated with common, naturally occurring renal lesions (hyaline casts and chronic inflammation) which were not considered treatment-related (Johnson, 1987c). A reduction in kidney weights was observed in dogs and was associated with cytoplasmic vacuolization of the kidney tubules. Since these findings were observed in dogs (both sexes) which do not develop α_{2u}-globulin nephropathy, DPR toxicologists considered them toxicologically significant (Goldenthal, 1995b).

The changes in clinical chemistry consisted of increased potassium, bilirubin, blood urea nitrogen, cholesterol, triglyceride and γ-glutamyl transpeptidase (γGT) levels, and decreased phosphorus, calcium, total protein, albumin, globulin, glucose, cholesterol and alanine aminotransferase levels. The toxicological significance of most of the clinical chemistry changes was uncertain since either they were only observed in one study and/or were not associated with any organ-specific histopathological lesions. Increased γGT levels are usually associated with cholestasis and precancerous changes in the liver (Hoffman et al, 1989). However, the liver and kidney (which is also high in γGT) were not examined microscopically in the one rabbit study where the γGT was elevated (Angerhofer and Weeks, 1980). The increased cholesterol and triglyceride levels were associated with fatty liver in another rabbit study (Haight *et al.*, 1980), and, therefore, were considered toxicologically significant. Increased BUN levels were also observed in several studies. In two studies it was observed in male rats and is probably due to impaired kidney function associated with α_{2u}-globulin

A. HAZARD IDENTIFICATION (cont.)

Table 18. Subchronic Effects of DEET and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL	LOEL	Ref.[a]
			(mg/kg)		
		Inhalation			
Rat	6 hrs/day, 5 days/wk, 13 wks	Disheveled and discolored fur	60[b]	180	1
		Reproductive: Abnormal sperm head	180	360	
Rat	6 hrs/day, 5 days/wk, 13 wks	Reduced endurance	180[b]	360	2
		Oral			
Rat[c]	11 days, gavage	Maternal: Reduced weight gain and food consumption	20	80	3
Rat[c]	10 days, gavage	Maternal: Reduced weight gain and food consumption	250	750	4*
Rabbit[c]	13 days, gavage	Maternal: Reduced weight gain and food consumption	100	325	5*
Rabbit	15 days, gavage	Fatty liver changes	132	264	6
Rat	28 days, gavage	Decreased nerve conduction and repolarization	------	600	7
Dog	8 weeks, capsule	Salivation, vomiting, relaxed nictitating membrane	100	200	8
Mouse	90 days, diet	Decreased defecation, reduced body weight	1000	3000	9
Hamster	90 days, diet	Reduced body weight and food consumption (M)	61	304	10*
Rat	90 days, diet	Reduced body weight and food consumption (F)	100	500	11
Rat[d]	2-gen., 80-93 days premating, diet	Parental: Reduced body weight	146	365	12*
		Reproductive: Reduced pup weights	146	365	
Rat	200 days, diet	Reduced body weight	333	652	13
		Dermal			
Rabbit[c]	29 days	Mat. Local: Dermal irritation	------	50	14
		Mat. Systemic: Reduced food consumption	100	500	
Rat[d]	5 days/wk, 9 wks	Abnormal sperm (at 36 days only)	------	100	15
Rat	5 days/wk, 13 wks	**Local: Dermal irritation**	(10)[e,f]	100	16*
		Systemic: Reduced body weight (M)	300	1000	
Micropig®	90 days	**Local: Dermal irritation**	(10)[e]	100	17*

a References: 1. Macko and Bergman, 1980; 2. Sherman, 1980c; 3. Sterner, 1977; 4. Neeper-Bradley, 1990; 5. Chun and Neeper-Bradley, 1991; 6. Haight *et al.*, 1980; 7. Campbell, 1986; 8. Goldenthal, 1995a; 9. Johnson, 1987a; 10. Goldenthal, 1989a; 11. Johnson, 1987b; 12. Schardein, 1989; 13. Ambrose *et al.*, 1959; 14. Angerhofer and Weeks, 1980; 15. Brusick, 1980; 16. Johnson, 1987c; 17. Goldenthal, 1991.
b Estimated assuming a respiratory rate of 0.24 m^3/kg/6 hrs for a rat (Zielhuis and van der Kreek, 1959).
c Developmental toxicity study: Only maternal effects observed after day 7 were included.
d Reproductive toxicity study
e Estimated by dividing the LOEL by an uncertainty factor of 10 (Dourson and Stara, 1983).
f This NOEL is equivalent to 61.5 μg/cm^2 assuming that a 0.2 kg rat has a surface area of 325 cm^2 (Harkness and Wagner, 1977) and that the application site was approximately 10% of the surface area according to FIFRA guidelines.
* Acceptable study based on FIFRA guidelines

A.   HAZARD IDENTIFICATION (cont.)

nephropathy. Elevated BUN levels were also observed in female rabbits; however, its toxicological significance is uncertain since there was no histological examination of the kidneys in this study (Angerhofer and Weeks, 1980). The reduction in total protein, albumin and globulin were seen in a 90-day feeding study in female rats and could be due to the reduction in food consumption that was observed in this study (Johnson, 1987b). In this same study, decreased lymphocytes and increased erythrocyte counts and hematocrits were also seen. The investigators suggested that some of these hematological changes, such as increased erythrocyte and hematocrits, could be due to the reduced water consumption in this study. Since these hematologic changes did not appear to correlate with any histological changes, their toxicological significance is uncertain.

One of the more common effects observed in animals with subchronic exposure to DEET was body weight reductions. The reductions in body weights were often associated with reductions in food consumption (Sterner, 1977; Johnson, 1978b; Goldenthal, 1989a&b, 1994, 1995a,b&c; Neeper-Bradley, 1990; Chun and Neeper-Bradley, 1991). The reduced food consumption may be the result of decreased palatability of the diet from DEET. However, reductions in body weights and/or food consumption were also observed in studies where DEET was administered by oral gavage or by the dermal route indicating that palatability may not be the only factor responsible (Sterner, 1977; Angerhofer and Weeks, 1980; Johnson, 1987c; Neeper-Bradley, 1990; Chun and Neeper-Bradley, 1991). Furthermore, reduced body weights were sometimes observed without a significant reduction in food consumption (Ambrose *et al.*, 1959; Johnson, 1987a&c; Goldenthal, 1989a; Schardein 1989&1990b). Although reduced food consumption may be partially responsible for the body weight reductions, another mechanism that might be involved is inefficiency in the metabolism of food if DEET inhibits a common site in both the urea cycle and gluconeogenesis pathways as Brini and Tremblay (1991) have proposed. The body weight reductions at the LOELs were generally modest (~10%); however, their toxicological significance becomes more important since few other signs of overt toxicity were seen at the higher doses until lethal doses (750 to 2000 mg/kg/day) were given (Neeper-Bradley, 1990; Goldenthal, 1989a; Johnson, 1987b). Clinical signs (salivation, vomiting, relaxed nictitating membrane) were seen in dogs at doses that were not lethal (200 mg/kg/day), but these signs may have been due to transient increases in blood levels from administering DEET in a bolus. Due to its prevalence and sensitivity, reduced body weights was selected as the endpoint of concern for evaluating seasonal exposure to DEET in humans. Since the main route of exposure to DEET is the dermal route, an acceptable 90-day dermal toxicity study in rats was selected as the definitive study (Johnson, 1987c). The critical NOEL was 300 mg/kg/day based on the reduced mean body weights (9%) in males. After correcting for dermal absorption (40%), the adjusted NOEL was 120 mg/kg/day.

The lowest LOEL for local effects was 50 mg/kg/day for dermal irritation in a rabbit dermal developmental toxicity study (Angerhofer and Weeks, 1980). However, this study had major deficiencies including no analysis of dosing material, insufficient number of animals at high dose, incomplete examination of fetuses for malformations, and no summary tables for clinical signs or body weights for dams or for skeletal and visceral findings in fetuses. A LOEL of 100 mg/kg/day was observed for dermal irritation in two other acceptable subchronic studies, one in rats and the other in micropigs® (Johnson, 1987c; Goldenthal, 1991). A NOEL was not established in either of these studies; therefore, a NOEL for local subchronic effects was estimated to be 10 mg/kg/day by using the default of dividing the LOEL by an uncertainty factor of 10 (Dourson and Stara, 1983). The NOEL for dermal irritation was equivalent to 61.5 $\mu g/cm^2$ assuming that a 0.2 kg rat has a surface area of 325 cm^2 (Harkness and Wagner, 1977) and that the application site is approximately 10% of the surface area based on FIFRA guidelines.

A.   HAZARD IDENTIFICATION (cont.)

Chronic Toxicity

The chronic toxicity of DEET was evaluated in three species by the oral route (Table 19). The effects observed in the chronic studies included clinical signs (vomiting, salivation, tremors, ataxia, convulsions, and decreased defecation), reduced body weights and food consumption, increased liver weights, reduced hemoglobin levels and hematocrits, increased platelet counts, reduced cholesterol levels, increased alkaline phosphatase activity and potassium levels, brown pigment in the mandibular lymph node, uterine hyperplasia, and mononuclear infiltration of the liver. As discussed under the Subchronic Toxicity Section, the increased liver weights were assumed to be due to microsomal enzyme induction and, therefore, were considered adaptive in nature. The reductions in the hemoglobin and hematocrit were suggestive of anemia and were considered adverse particularly in view of the hypocellularity of the bone marrow that was observed in dogs in a subchronic toxicity study (Goldenthal, 1995b). The toxicological significance of the increased platelet counts in dogs is uncertain. The reduced cholesterol levels and increased potassium levels in dogs were also of uncertain toxicological significance. The increased alkaline phosphatase in male dogs was considered toxicologically significant since mononuclear infiltration of the liver was observed in this study (Goldenthal, 1994). The increased cholesterol levels in rats was also considered toxicologically significant based on its association with fatty liver in rabbits (Haight *et al.*, 1980). All three chronic studies met FIFRA guidelines.

A NOEL of 100 mg/kg/day was observed in two species, rats and dogs. Although the NOELs were identical in these two studies, the toxicological effects were somewhat different. In rats, the effects observed at the LOEL, 400 mg/kg/day, were reduced mean body weights (13%) and food consumption (7.5%) and increased mean cholesterol levels (43%) in females (Goldenthal, 1995). In dogs, clinical signs, reduced mean hemoglobin (M: 11%; F: 15%) and hematocrit levels (M: 12%; F: 16%), mononuclear infiltration of the liver (M: 1/4; F: 2/4), brown pigmentation of the lymph node (M: 3/4) and hyperplasia of the uterus (F: 3/4) were seen at 400 mg/kg/day (Goldenthal, 1994). The effects in the dogs appear to be more severe than those in the rat, but the different responses is probably due to bolus dosing rather than differences in species sensitivity. Bolus dosing results in higher peak blood levels which are probably responsible for the clinical signs (vomiting, salivation, tremors, ataxia, and convulsions) that are more typical of acute exposure. The other effects in dogs were of questionable toxicological significance because the differences at the highest dose level were usually not statistically significant when compared to controls probably because of the small number of animals. Consequently, the rat study was selected as the definitive study for evaluating the risk for adverse health effects in humans from chronic exposure to DEET. The critical NOEL was 100 mg/kg/day based on the reduced body weights and food consumption, and increased cholesterol levels in rats.

Oncogenicity/Genotoxicity

The oncogenic potential of DEET was evaluated in three species by either the oral or dermal route. No dose-related increase in tumors was observed in any of the studies; however, both of the dermal studies in mice and rabbits had major deficiencies including no test article analysis, clinical signs, body weights, food consumption, hematology, pathology for non-neoplastic lesions or individual data (Stenbäck, 1976). The oral mouse and rat oncogenicity studies for DEET did meet FIFRA guidelines (Goldenthal, 1990; Goldenthal, 1995c). An increase in renal tumors in male rats has been observed with a number of chemicals that cause α_{2u}-globulin nephropathy (Borghoff *et al.*, 1990; Flamm and Lehman-McKeeman, 1991; Hard *et*

A.   HAZARD IDENTIFICATION (cont.)

Table 19. Chronic Effects of DEET and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL	LOEL	Ref.[a]
			(mg/kg/day)		
Mouse	78 weeks, diet	Hyperplastic liver nodules, increased liver weights	500	1000	1*
Rat[c]	2 years, diet	**Reduced body weights and food consumption, increased cholesterol levels (females)**	100	400	2*
Dog[c]	1 year, capsule	Clinical signs, reduced hemoglobin and hematocrits, histological changes in the liver, lymph nodes and uterus	100	400	3*

a   References: 1. Goldenthal, 1990; 2. Goldenthal, 1995c; 3. Goldenthal, 1994.
*   Acceptable study based on FIFRA guidelines.

al., 1993). The increase in renal tumors was attributed to an increase in cell death and subsequent regeneration. Since the dose levels for the male rats in the chronic study were low enough to not produce any evidence of α_{2u}-globulin nephropathy, it is not surprising that an increase in renal tumors was not observed in the rat chronic toxicity study for DEET. However, even if renal tumors had been observed in male rats after chronic exposure to DEET, this oncogenic effect is probably not relevant to humans since they do not produce a protein similar to α_{2u}-globulin in high concentration in the kidney.

All the genotoxicity studies for DEET were negative, except for a dominant lethal assay which had equivocal results. The studies conducted included 6 reverse mutation assays, four with *Salmonella typhimurium* (Zeiger *et al.*, 1992; Brusick, 1976; Macko and Weeks, 1980; San and Schadly, 1989) and two with *Saccharomyces cerevisiae* (Brusick, 1976; Macko and Weeks, 1980), a dominant lethal assay (Swentzel, 1978), an *in vitro* cytogenetic assay using Chinese hamster ovary cells (Putman and Morris, 1989), and an unscheduled DNA synthesis (UDS) assay using rat primary hepatocytes (Curren, 1989). In the dominant lethal assay, the fertility index was not significantly different from the concurrent controls; however, the percentage of dams with less than 8 implantations was greater in the treated males than controls. One of the reverse mutation assays with *S. typhimurium*, the cytogenetic assay, and the UDS assay met FIFRA guidelines.

B.   EXPOSURE ASSESSMENT

Non-Occupational

Exposure estimates for the general public were based on several surveys conducted by the registrants (Sanborn, 1999). From a usage survey of 542 individuals, these investigators determined the amount applied per application. The amount applied to skin only or dermal dose was determined by weighing the container before and after application. The average dermal dose from a single application was determined for the following population subgroups: male adults, female adults, male adolescents (12-17 years old), female adolescents (12-17

A.   HAZARD IDENTIFICATION (cont.)

years old), male children (<12 years old), and female children (<12 years old) (Table 20). The average dermal dose was then divided by the average surface area of the arms, hands, legs and head from U.S. EPA's Exposure Factors Handbook (U.S. EPA, 1996) to estimate the daily dermal concentration (DDC) of DEET in the skin for these population subgroups. The average DDCs ranged from 69 $\mu g/cm^2$ for adult females to 192 $\mu g/cm^2$ for female children less than 12 years old. Since the dermal dose represents the average dose applied after a single application, a high-end estimate was calculated for each exposure scenario. An analysis of the usage data indicated that the annual aggregate mean and 95th percentile for all age groups (skin only) was 0.038 and 0.12 g/day, respectively. Based on this analysis, the upper end estimates assumed DEET was applied 3 times as frequently as the average. The high DDCs ranged from 207 $\mu g/cm^2$ for female adults to 576 $\mu g/cm^2$ for female children. From a mail survey of 8,000 households, it was determined that people used DEET an average of 7.5 times during the heavy use months of June and July. The Seasonal Average Dermal Concentration (SADC) was estimated by assuming that DEET was used 7.5 times during the peak-use months of June and July (i.e., SADC = DDC x 7.5 ÷ 61 days). The average SADCs ranged from 8 $\mu g/cm^2$ for female adults to 24 $\mu g/cm^2$ for female children. The high SADCs ranged from 24 $\mu g/cm^2$ for female adults to 72 $\mu g/cm^2$ for female children.

The absorbed daily dosages (ADDs) for various population subgroups was estimated by multiplying the average dermal dose by the dermal absorption of DEET in humans (8.4%) and then dividing by the average body weight for these population subgroups from U.S. EPA's Exposure Factors Handbook (U.S. EPA, 1996). The average ADDs ranged from 840 $\mu g/kg/day$ for female adults to 3,610 $\mu g/kg/day$ for female children less than 12 years old. The high ADDs ranged from 2,520 $\mu g/kg/day$ for female adults to 10,830 $\mu g/kg/day$ for female children. The seasonal average daily dosage (SADD) was then estimated by assuming DEET was used 7.5 times during the peak-use months of June and July (i.e., SADD = ADD x 7.5 ÷ 61 days). The average SADDs ranged from 103 $\mu g/kg/day$ for female adults to 443 $\mu g/kg/day$ for female children less than 12 years old. The high SADDs ranged from 309 $\mu g/kg/day$ for adult females to 1,329 for female children. Based on a syndicated market survey, it was determined that 53-60% of DEET products are sold during June and July. The average yearly DEET use was estimated by multiplying the average DEET use in June and July (ADD x 7.5) and dividing by the percentage of yearly product sold in the peak months of June and July. The annual average daily dosage (AADD) was then determined by dividing the average yearly DEET use by 365 days. The average AADDs ranged from 57 $\mu g/kg/day$ for female adults to 130 $\mu g/kg/day$ for female children. The high AADDs ranged from 111 $\mu g/kg/day$ for female adults to 390 $\mu g/kg/day$ for female children.

Two types of inhalation exposure could occur with the use of DEET products. It could occur during application with the use of aerosol products or after application with any of the products due to evaporation. There was no attempt in the usage survey to determine how much of the product was actually inhaled versus absorbed through the skin. DPR considered estimating exposure using inhalation exposure data from personal care products (e.g., hair spray, deodorant) as surrogate data. However, because the parts of the body sprayed with personal care products were different from DEET products, these data were not used. Estimation of inhalation exposure from evaporation was also not attempted since the amount volatilized per unit time would decrease with time.

B.   EXPOSURE ASSESSMENT (cont.)

Table 20. Estimated Exposure Dosages for DEET in Various Population Subgroups

	Male Adult	Female Adult	Male (13-17 yrs)	Female (13-17 yrs)	Male (≤12 yrs)	Female (≤ 12 yrs)
Dermal Dose (g/day)	0.95	0.65	1.07	1.07	0.94	0.94
Surface Area[a] (m^2)	1.14	0.94	0.95	0.92	0.50	0.49
DDC[b] (µg/cm^2)						
Average[c]	83	69	113	116	188	192
High[d]	249	207	339	348	564	576
SADC[e] (µg/cm^2)						
Average	10	8	14	14	23	24
High	30	24	42	42	69	72
Body Weight (kg)	78.1	65.4	61.1	55.0	22.8	21.9
ADD[f] (µg/kg)						
Average	1,020	840	1,470	1,630	3,460	3,610
High	3,060	2,520	4,410	4,890	10,380	10,830
SADD[g] (µg/kg/day)						
Average	126	103	181	201	426	443
High	378	309	543	603	1,278	1,329
AADD[h] (µg/kg/day)						
Average	57	37	51	56	124	130
High	171	111	153	168	372	390

[a] Average surface area of the head, arms, hands, and legs (U.S. EPA, 1996).
[b] DDC = Daily Dermal Concentration = dermal dose ÷ surface area.
[c] Mean exposure for a single application.
[d] Upper end estimate assuming DEET was applied 3 times more frequently than the average person.
[e] SADC = Seasonal Average Dermal Concentration, assuming 7.5 applications per 61 day season (June and July)
[f] ADD = Absorbed Daily Dosage, assuming a dermal absorption of 8.4%
[g] SADD = Seasonal Average Daily Dosage, assuming 7.5 applications per 61 day season (June and July)
[h] AADD = Annual Average Daily Dosage, assuming 53-60% of annual use in June and July

Occupational

In their 1980 Reregistration Eligibility Document for DEET, U.S. EPA estimated the total annual exposure for the military personnel to be 43 g, assuming 1 ml of a 75% formulation was applied 60 times per year (U.S. EPA, 1980). This is equivalent to 0.72 g/day. They also estimated a research biologist in the Florida Everglades was exposed to 4.25 g/day or 60.7 mg/kg/day based on a report that he used 2 1-oz. bottles of 28.74% DEET per week on skin.

B. EXPOSURE ASSESSMENT (cont.)

Applications were made 4 days per week from May to October. This is similar to exposure estimates for park workers in the Everglades National Park by McConnell *et al.* (1985) which ranged from 0.2 g/day (median) to 11.5 g/day (95th percentile) from April to October. As expected, these estimates indicate that the average or median daily occupational exposure can be similar to that estimated for the general public, but it can also be significantly higher. The most apparent difference between non-occupational and occupational exposure is the frequency of application which may be daily over several months in a climate such as Florida. The population of biting insects in California is not likely to be as great as Florida due to a more temperate climate; therefore, the frequency of application is likely to be less. There are no data available for estimating the frequency of occupational use in California. Therefore, occupational exposure was estimated using the survey and usage data for the general public with different assumptions about the frequency of applications. Since adult males had the highest exposure per application for adults, initial exposure estimates for workers were based on the adult male exposure. The acute exposure for workers was assumed to be the same as the high-end exposure for adult males (i.e., ADC = 21 $\mu g/cm^2$ and ADD = 3,060 $\mu g/kg/day$). For seasonal exposure, the assumption was made that workers used DEET once a day for 5 days per week during the peak months of June and July. The resultant SADC for workers was 59 $\mu g/cm^2$. The SADD was 729 $\mu g/kg/day$ for workers. (Note: The SADD for workers is equivalent to an external dose of approximately 0.6 g/day which is above the median seasonal exposure of 0.2 g/day reported for park workers in the Everglades.) As with the general public, the assumption was made that 55% of the use occurred in June and July. The resultant AADD for workers was 222 $\mu g/kg/day$.

C. RISK CHARACTERIZATION

<u>Non-Occupational</u>

<u>Acute Toxicity</u>

The risk for non-oncogenic human health effects is expressed as a margin of exposure (MOE). The MOE is the ratio of the NOEL from experimental animal studies to the human exposure dosage.

$$Margin \ of \ Exposure \ = \ \frac{NOEL}{Exposure \ Dosage}$$

The MOEs for local effects were calculated using the estimated acute NOEL for dermal irritation (25.8 mg/cm^2) and the estimated average and high DDCs in the skin from Table 20. The MOEs for acute dermal irritation using the average DDCs ranged from for 130 female children less than 12 years old to 370 for female adults (Table 21). The MOEs for acute dermal irritation using the high DDCs ranged from 45 for female children to 120 for female adults. The MOEs for acute systemic effects were calculated using the acute NOEL of 2000 mg/kg and the estimated average and high ADDs from Table 20. The acute MOEs based on the average ADDs ranged from 550 for female children less than 12 years old to 2400 for female adults. The acute MOEs based on the high ADDs ranged from 180 to 790.

Table 21.   Margins of Exposure for DEET in Various Population Subgroups[a]

	Male Adult	Female Adult	Male (13-17 yrs)	Female (13-17 yrs)	Male (≤12 yrs)	Female (≤ 12 yrs)
Local Effects						
Acute[b]						
Average	310	370	230	220	140	130
High	100	120	76	74	46	45
Subchronic[c]						
Average	6	8	4	4	3	3
High	2	3	1	1	<1	<1
Systemic Effects						
Acute[d]						
Average	2000	2400	1400	1200	580	550
High	650	790	450	410	190	180
Subchronic[e]						
Average	950	1,200	660	600	280	270
High	320	390	220	200	94	90
Chronic						
Average	1,800	2,700	2,000	1,800	800	770
High	580	900	650	600	270	260

[a]   Margin of Exposure = NOEL / Exposure Dosage. See Table 20 for exposure dosages for various population subgroups. Values rounded to two significant figures.
[b]   The estimated acute NOEL for dermal irritation was 25.8 mg/cm^2 (rabbits).
[c]   The estimated subchronic NOEL for dermal irritation was 61.5 µg/cm^2 (rats).
[d]   The acute NOEL for systemic effects was 2000 mg/kg (rats - piloerection, tremors, reduced heat sensitivity and reduced motility).
[e]   The adjusted subchronic NOEL was 120 mg/kg/day (rats - reduced body weights).
[f]   The chronic NOEL was 100 mg/kg/day (rats - reduced body weights and food consumption, increased cholesterol levels).

Subchronic Toxicity

The subchronic MOEs for dermal irritation were calculated using the subchronic NOEL for dermal irritation (61.5 µg/cm^2) and the estimated average and high SADCs from Table 20. The subchronic MOEs for dermal irritation ranged from 3 for female children to 8 for female adults using the average SADCs. Using the high SADCs, the subchronic MOEs for dermal irritation ranged from less than 1 to 3.

The subchronic MOEs for systemic effects were calculated using the adjusted NOEL of 120 mg/kg/day for reduced body weight and the average and high SADDs from Table 20. The subchronic MOEs for systemic effects ranged 270 for female children to 1,200 for female adults

based on the average SADDs. Using the high SADDs, the seasonal MOEs ranged from 90 to 390.

Chronic Toxicity

The chronic MOEs for systemic effects were calculated using the NOEL of 100 mg/kg (reduced body weights and food consumption, increased cholesterol levels in female rats) and the average and high AADDs from Table 20. The chronic MOEs ranged from 770 for females children less than 12 years old to 2,700 for adult females based on the average AADDs. Using the high AADDs, the chronic MOEs ranged from 260 to 900.

Occupational

The MOEs for acute occupational exposure to DEET was assumed to be the same as the MOEs for high-end exposure for adult males in the general population (i.e., MOE for dermal irritation is 100 and MOE for systemic effects is 650). The MOEs with seasonal occupational exposure to DEET were 1 for dermal irritation and 165 for systemic effects. The MOE for systemic effects with chronic occupational exposure to DEET was 450.

IV. RISK APPRAISAL

Introduction

Risk assessment is the process used to evaluate the potential for human exposure and the likelihood that the adverse effects observed in toxicity studies with laboratory animals will occur in humans under the specific exposure conditions. Every risk assessment has inherent limitations on the application of existing data to estimate the potential risk to human health. Therefore, certain assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment, and exposure assessment processes. These, in turn, result in uncertainty in the risk characterization which integrates all the information from the previous three processes. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, the degree or magnitude of the uncertainty can vary depending on the availability and quality of the data, and the types of exposure scenarios being assessed. Specific areas of uncertainty associated with this risk assessment for DEET are delineated in the following discussion.

Hazard Identification

An acute dermal neurotoxicity study would have been the most ideal study for establishing an NOEL for DEET to address uncertainties related to route of exposure and potential neurotoxic effects. Since one was not available, the dermal LD_{50} studies for the technical grade DEET were evaluated for possible identification of an acute NOEL. Possible treatment-related effects were seen in one study at 1800 mg/kg, but a NOEL was not established (Macko and Weeks, 1980). This study was not useful because the application site was covered with an occlusive covering and it was unclear which signs were seen at 1800 mg/kg.   In another study, dermal irritation, but no clinical signs were observed at 2,000 mg/kg (Weil, 1973). However, this study was also of questionable value since the application site was covered with an occlusive covering and it was unclear at what dose level pathological lesions were observed in this study. A third study could not be used because there was insufficient information provided in the report to determine if signs were observed at any dose level tested (Ambrose *et al.*, 1959). A NOEL was established for systemic and local effects in a recent study in which rats were exposed to a limit dose of 5,000 mg/kg. The application site was covered with a semi-occlusive wrapping which could represent a worse-case scenario since humans usually apply DEET to exposed skin. Assuming this study represents a worse-case exposure scenario, the acute NOEL for systemic and local effects could be higher. On the other hand, only five animals per sex were tested in this study and some of the more subtle neurobehavioral effects seen in the neurotoxicity studies, such as reduced heat sensitivity and motor activity, may have been missed. Consequently, the acute NOEL may be lower than assumed.

A NOEL of 200 mg/kg was established in an acute oral neurotoxicity study for DEET based on reduced heat sensitivity, reduced motility, piloerection and tremors (Schardein, 1990a). This study wasn't used because route-to-route extrapolation would have been needed to evaluate dermal exposure to DEET. In the pharmacokinetic study conducted by Selim (1991a), the peak blood levels were much higher after oral administration than with dermal administration. If the oral NOEL from the neurotoxicity study was adjusted for differences in the body burden (i.e., area under plasma concentration curve or AUCs) during the first 24 hours after oral and dermal administration of DEET, the acute dermal NOEL would be 4.5 times higher. However, the urinary and fecal excretion after dermal exposure suggests that body burden may have been greatest 48 to 72 hours after exposure. Therefore, following the plasma concentration for only 24 hours probably significantly underestimated the total body burden with

dermal exposure. On the other hand, if the neurological effects were associated with the peak blood levels, then the oral NOEL could be multiplied by 18 to come up with an equivalent dermal NOEL of 3,600 mg/kg. Interestingly, this NOEL is similar to the NOEL established in the acute dermal toxicity study. The use of the peak plasma levels from the Selim (1991a) study is problematic for two reasons. The dermal peak blood levels may have been underestimated because the blood levels were monitored for only 24 hours and urinary data suggests the peak levels may have occurred at 48 to 72 hours with dermal exposure. Then again, the peak blood levels with dermal exposure may have been overestimated since the exposure site was covered with an occlusive glass enclosure.

The acute dermal NOEL and MOEs may also be lower than estimated based on possible acute effects (increased preimplantation losses) in a dermal developmental toxicity studies (Gleiberman *et al.,* 1975). This study was not selected as the definitive study for evaluating acute exposure to DEET because of uncertainty as to whether these effects were from a single or repeated exposure. In addition, there were deficiencies with this study, the most significant being the lack of analysis of the test compound for purity or dosing solution for concentration. Consequently, it is possible that impurities, degradation, errors in calculation or weighing, or nonhomogeneous mixing resulted in the animals receiving more or less test compound than reported.   Furthermore, a higher NOEL of 250 mg/kg was established for preimplantation losses in an oral rat developmental toxicity study which met FIFRA guidelines, including the analysis of test article and dosing solution (Neeper-Bradley, 1990). However, if this study had been used, the acute MOE for adult women would be 50-fold lower than estimated.

Concentration at the application site is an important factor in development of dermal irritation (Mathias, 1983). Therefore, it was considered more appropriate to express the NOEL in terms of amount applied per body surface area (i.e., mg/cm^2), rather than amount applied per kg body weight. The exact size of the application site was unknown in most of the dermal toxicity studies. Therefore, certain assumptions were made regarding the size of the application site and the surface area of the animals exposed. The use of these assumptions was considered superior to leaving the dosage in mg/kg. However, if the acute MOEs for dermal irritation had been calculated using the NOEL in mg/kg and the ADDs without the adjustment for dermal absorption, they would be slightly higher than estimated. If the subchronic MOEs for dermal irritation had been calculated using the NOEL in mg/kg and the SADDs without the correction for dermal absorption, they would be almost identical. Dermal penetration is critical in order for dermal irritation to occur (Mathias, 1983). It may have be more appropriate to have adjusted the NOELs and exposure dosages for dermal absorption. These adjustments were not made because it is unclear if all the factors that contribute to species differences in dermal absorption also contribute to species differences in dermal irritation. However, if these values had been adjusted for dermal absorption, the MOEs would be approximately 4 times higher than estimated (i.e., dermal absorption in animals ÷ dermal absorption in humans = 40% ÷ 10% = 4).

The critical NOEL for evaluating seasonal exposure to DEET was 300 mg/kg/day based on a statistically significant reduction in body weights (9%) in males rats that were exposed at 1000 mg/kg/day by the dermal route for 90 days (Johnson, 1987c). This modest body weight reduction was considered toxicologically significant because it could not be attributed to decreased food consumption due to the palatability of the diet since DEET was administered by the dermal route. Body weight reduction was one of the most common effects seen in the subchronic studies for DEET. In addition, these modest reductions in body weights were often

the only effects seen before lethal doses were administered. In the 90-day dermal study with micropigs®, no effects were seen at 1000 mg/kg/day, the highest dose tested. This study was not selected because there was no data on absorption of DEET through the skin of micropigs®. If the dermal absorption was similar to humans (~10%), the adjusted NOEL for this study (~100 mg/kg/day) would be very similar to the adjusted NOEL for the 90-day dermal study in rats (120 mg/kg/day).

The critical NOEL for evaluating chronic exposure to DEET was 100 mg/kg/day based on reduced body weights and food consumption and increased cholesterol levels in rats fed DEET at 400 mg/kg/day for 2 years (Goldenthal, 1995). An identical NOEL was observed in dogs given DEET in capsules at 400 mg/kg/day for 1 year based on vomiting, salivation, tremors, decreased defecation, reduced body weights and food consumption, reduced hemoglobin and hematocrit levels, mononuclear infiltration of the liver, brown pigmentation of the lymph node and hyperplasia of the uterus (Goldenthal, 1994). The effects in the dogs appear to be more severe than those in the rat, but the different responses are probably due to bolus dosing rather than differences in species sensitivity. Bolus dosing results in higher peak blood levels which are probably responsible for the clinical signs (vomiting, salivation, tremors, ataxia, and convulsions) that are more typical of acute exposure. The other effects in dogs were of questionable toxicological significance because the differences at the highest dose level were usually not statistically significant when compared to controls probably because of the small number of animals. However, if the dog study had been used for the definitive chronic study, it would have had no impact on the estimated chronic MOEs since the NOELs were the same. Regardless of which chronic study was used, the NOELs derived from these oral studies are probably much higher than would have been observed with dermal exposure based on the pharmacokinetics study by Selim (1991a).

Exposure Assessment

The dermal absorption for humans was estimated from the Selim (1992) study, assuming only the radioactivity excreted in the urine was absorbed. However, some radioactivity was not accounted for in the material balance of this experiment. It is uncertain if this radioactivity was not recovered from the containers, skin or if it was absorbed and not yet excreted. If it was bound to the skin, but available for absorption or if it had penetrated the skin, but not been excreted, then the actual dermal absorption would be higher than estimated. Assuming that all the unrecovered radioactivity was absorbed, the dermal absorption would be 12% and 20% for the undiluted DEET and the 15% DEET in ethanol, respectively. If the higher of these dermal absorption values had been used in the calculation of the exposure dosages, the dosages would be approximately 2.5 times higher than estimated. A similar increase in the exposure estimates would have also occurred if the dermal absorption value for humans was estimated from the Feldman and Maibach (1970) study where the dermal absorption was estimated to be 16.7% after correction for unrecovered material with intravenous administration. However, this study used acetone as the vehicle which may have enhanced dermal absorption and it had a low material balance (52%). Dermal absorption studies in monkeys suggest the dermal absorption varies significantly (14% to 68%) based on the site of application (Moody *et al.*, 1989). If there are similar variations in the dermal absorption of DEET in humans, the dermal absorption may be greater than estimated since the site of application in the human studies (forearm) had the lowest dermal absorption of the various sites tested in monkeys.

In calculating the SADCs to evaluate dermal irritation, the exposure is amortized over a two month period (i.e., assumed small exposures every day for two months). However, it is

IV. RISK APPRAISAL (cont.)

unclear if the dermal irritation response to small daily dosages is equivalent to high dosages once a week for two months. There may be sufficient time for recovery between the weekly dosages for the skin to recover, so that these weekly dosages are more like separate acute exposures rather than small daily exposures.

Data from the usage survey indicate that children less than 12 years old receive as much DEET per application as adult male on average regardless of the difference in their body weight or surface area. Since this seems illogical, exposure estimates for adult females, juveniles (ages 12-17), and children (less than 12 years old) were also estimated by adjusting the adult male dermal dosage of approximately 1,000 mg/application based on the average body surface area for these population subgroups from the U.S. EPA's Exposure Factors Handbook (U.S. EPA, 1996). The revised exposure dosages are summarized in Table 22. A comparison of the exposure dosages based on the alternative method in Table 22 with those estimated from the survey data in Table 20 indicate that if the same concentration of DEET were applied per surface area of skin in adults and children, the ADDs and SADDs for children would be at least 50% lower and the AADDs would be at least 30% lower. Consequently, the acute, subchronic and chronic MOEs would all be greater than 100 for children using this alternative method for calculating the exposure dosages. Although the alternative method appears more logical, it is only theoretical and the original method is based on the usage survey in which the amount used was determined by weighing the container before and after application.

Table 22. Alternative Method for Estimating Exposure Dosages for DEET in Various Population Subgroups Based on Body Surface Area

	Adult Males	Adult Females	Juveniles (12-17 yrs)	Children (<12 yrs)
Surface Area[a] (m^2)	1.94	1.69	1.58	0.86
Dermal Dose (mg/day)	1000	871	814	443
Body Weight (kg)	78.1	65.4	58.1	22.4
ADD[d] (µg/kg)	1,076	1,119	1,177	1,661
SADD[e] (µg/kg/day)	132	138	145	204
AADD[f] (µg/kg/day)	51	53	56	78

[a] Total body surface area
[b] Mean exposure for a single application
[c] Upper end estimate assuming 3 applications of DEET per day
[d] ADD = Absorbed Daily Dosage, assuming a dermal absorption of 8.4%
[e] SADD = Seasonal Average Daily Dosage, assuming 7.5 applications per 61 day season (June and July)
[f] AADD = Annual Average Daily Dosage, assuming 56.5% of annual use in June and July

There was no estimate of inhalation exposure from use of aerosol products since there was no attempt in the usage survey to determine how much of the product that was actually inhaled versus absorbed through the skin. Consequently, the exposure estimates are probably higher than estimated for individuals using aerosol products. However, U.S. EPA noted in their recent Reregistration Eligibility Document (RED) for DEET that while inhalation and oral exposure were not included in their exposure estimate, "this omission is not expected to

significantly underestimate exposure as the dermal exposure is so much greater than inhalation or oral" (U.S. EPA, 1998).

<u>Risk Characterization</u>

Generally, an MOE of at least 100 is considered sufficiently protective of human health when the NOEL for an adverse systemic effect is derived from an animal study. The MOE of 100 allows for humans being 10 times more sensitive than the most sensitive animal species and for the most sensitive humans being 10 times more sensitive than the least sensitive humans. The MOEs for acute systemic effects were greater than 100 for all population exposure groups based on either average or high-end exposure estimates. The MOEs for subchronic systemic effects was greater than 100 for all population subgroups based on average exposure estimates and for adults and juveniles based on high-end exposure estimates. Only the subchronic MOEs for children based on high-end exposure estimates were less than 100. The MOEs for chronic systemic effects were greater than 100 for all population subgroups based on either the average or high-end exposure estimates. The MOEs for occupational exposure were greater than 100 for acute, subchronic and chronic exposure.

The critical NOEL for subchronic systemic effects was based on a mild effect (9% body weight reduction in males relative to controls), suggesting that the subchronic MOEs of 90 and 94 for male and female children, respectively, may be adequately protective. However, the case reports involving toxic encephalopathy in children exposed to DEET suggests that the MOEs for children may not be adequate. Several of these cases involved daily application of low concentration formulations (10-20% DEET) daily for several weeks to months. An argument could be made that seizures are common among children and that the number of case reports involving DEET is relatively small given the large percentage of the population that uses DEET; however, seizures or other symptoms in children may be underreported if parents or doctors do not suspect they are related to exposure to DEET.

For dermal irritation, an MOE of 10 is probably adequately protective. There are species differences in the thickness of the stratum corneum or number of cell layers that affect barrier function (Monteiro-Riviere, 1996). In general, the domestic pig and non-human primate are the best animal models for evaluating skin permeability in humans. If species differences in dermal absorption of DEET are any indication of species differences in sensitivity to dermal irritation, then humans appear to be 4 times less sensitive than rabbits or rats. Therefore, a 10-fold uncertainty factor for interspecies variation may not be necessary with dermal irritation. The acute MOEs for dermal irritation were greater than 10 for all populations subgroups using either average or high-end exposure estimates. On the other hand, the subchronic MOEs for dermal irritation were less than 10 for all population subgroups using either average or high-end exposure estimates. The MOEs for dermal irritation from occupational exposure were greater than 10 for acute exposure, but less than 10 for seasonal exposure. This would suggest that dermal irritation is a major problem with repeated exposure. Cases of dermal irritation from DEET may be underreported since this is not a very severe effect and some people may not seek medical attention when they develop it. Nevertheless, more case reports of dermal irritation would be expected given the large number of users. Therefore, the risk for dermal irritation with repeated use may be overestimated. Possible explanations for the overestimation of risk for dermal irritation are: 1) the NOELs should have been adjusted for species differences in dermal absorption; 2) the subchronic NOEL for dermal irritation is higher than estimated by dividing the LOEL by 10; 3) the assumptions regarding the size of the application site were incorrect and 4) the variation in sensitivity within the human population is less than 10-fold. On

the other hand, there are several reports of dermal irritation in humans after the use of high concentration formulations (50-75% DEET) or heavy use, suggesting that the risk for dermal irritation is not negligible (Rabinovich, 1966; Lamberg and Mulrennan, 1969; Reuveni and Yagupsky, 1982; McConnell *et al.*, 1986). In addition, Steinberg et al. (1975) reported that DEET was more irritating than salicylic acid in people exposed for 21 consecutive days when the application site was occluded. The relevance of this finding with respect to the normal use of DEET is unclear since people don't normally occlude their skin after treatment with DEET.

<u>Issues Related to the Food Quality Protection Act</u>

The Food Quality Protection Act of 1996 mandated U.S. EPA to "upgrade its risk assessment process as part of the tolerance setting procedures" (U.S. EPA, 1997a and b). The improvements to risk assessment were based on the recommendations from the 1993 National Academy of Sciences report, "Pesticides in the Diets of Infants and Children" (NAS, 1993). The Act required an explicit finding that tolerances are safe for children. U.S. EPA was required to use an extra 10-fold safety factor to take into account potential pre- and post-natal developmental toxicity and the completeness of the data unless U.S. EPA determined, based on reliable data, that a different margin would be safe. In addition, U.S. EPA must consider available information on: 1) aggregate exposure from all non-occupational sources; 2) effects of cumulative exposure to the pesticide and other substances with common mechanisms of toxicity; 3) the effects of *in utero* exposure; and 4) the potential for endocrine disrupting effects. Since DEET has no food uses and, therefore, no food tolerances, issues related to the Food Quality Protection Act do not necessarily apply. However, these issues will be discussed as much as they pertain to DEET.

Pre- and Postnatal Sensitivity

Six developmental toxicity studies were conducted in which DEET was administered to either rats or rabbits by the oral or dermal route. Only two of these studies met FIFRA guidelines. Developmental effects included decreased implantations, increased resorptions, increased abortions, reduced fetal weights, increased skeletal variations, decreased neonatal weight gain, delayed neonatal development and increased neonatal death. The skeletal variations were not considered adverse because the increase was not statistically significant when expressed on a litter basis and they were not associated with a reduction in fetal weight. Maternal effects included death, clinical signs, reduced body weight gain, reduced food consumption, and dermal irritation. In an acceptable rat developmental toxicity study, the developmental NOEL was 250 mg/kg/day based on the reduced fetal weight and increased preimplantation losses (Neeper-Bradley, 1990). The maternal NOEL was also 250 mg/kg/day based on the deaths, clinical signs, and decreased mean body weight gains (35%) and food consumption (12%). In an acceptable rabbit developmental toxicity study, the developmental NOEL was equal to or greater than 325 mg/kg/day, based on the lack of any overt developmental toxicity (Chun and Neeper-Bradley, 1991). The maternal NOEL was 100 mg/kg/day based on the reduced body weight gain (69%) and food consumption (30%). The lowest reported developmental NOEL was 20 mg/kg/day based on decreased implantations and increased resorptions (Sterner, 1977). The maternal NOEL for this study was also 20 mg/kg/day based on reduced maternal weight gain. These data suggest there is no increased prenatal sensitivity to DEET.

Four reproductive toxicity studies were conducted in rats with DEET administered by different routes; however, only one of these studies met FIFRA guidelines. Abnormal sperm

were observed in males in two studies. Urethral plugs of the coagulating gland, seminal vesicle secretions and distended bladders were observed in males in another study. Non-reproductive effects included increased hair loss, skin lesions, decreased rotorod performance, reduced body weights, elevated BUN levels (males), and renal lesions (males). In the study that met FIFRA guidelines, the parental NOEL was 2000 ppm (M:146 mg/kg/day; F: 179 mg/kg/day) based the reduced body weights in F_1 adults (M: 8%; F: 12%) (Schardein, 1989). The reproductive NOEL was also 2000 ppm based on the reduced neonatal pup weights (11-13%). This study suggests there is no increased postnatal sensitivity to DEET.

There was evidence of increased postnatal sensitivity to DEET in two neurotoxicity studies. In a subchronic neurotoxicity study, reduced mean body weights (relative to controls) were observed in 48-week old rats at 2000 ppm (M: 8%; F: 11%) and 5000 ppm (M: 15%; F: 14%) (Schardein, 1990b). The NOEL for this endpoint was considered 500 ppm (M: 19 mg/kg/day; F: 25 mg/kg/day) even though slight reductions in mean body weights (M: 7%; F: 4%) were observed at this dose level. Given that the only other effect observed when the dose level was increased 10-fold (5000 ppm) was increased locomotor activity, the toxicological significance of the reduced body weights at 500 ppm is questionable. The investigators suggested that the reduced body weights at 500 and 2000 ppm were an artifact from the randomization procedure since there was not a significant reduction in the body weights in the reproductive toxicity study from which these rats came, except at 5000 ppm. However, examination of the pup weights from both F_1 and F_2 generations at day 21 of the reproductive toxicity study showed a trend towards lower body weights at 2000 ppm in both sexes (see Table 16). A slight body weight reduction (6%) was also seen in F_0 adult males of the reproductive toxicity study at 2000 ppm. The lower NOEL for body weight reduction in the 48-week old rats could be due either to the longer exposure period and/or increased postnatal sensitivity. The length of exposure does not appear to be the only factor since the reduction in body weights in the 48-week old rats was more pronounced than that observed in the 2-year rat study (F: 13% at 400 mg/kg/day) despite being conducted by the same laboratory with the same strain of rats. This suggests that the exposure during critical growth periods *in utero* and prior to weaning may have exacerbated the body weight reduction seen with long-term exposure.

In a non-standard neurotoxicity study, the LD_{50} values for 11-day-old and 47-56-day-old rat were compared (Verschoyle *et al.*, 1992). The LD_{50} values for females increased from 667 mg/kg at 11 days to 3429 mg/kg at 47-56 days while for males they increased from 891 mg/kg at 11 days to 3564 mg/kg at 47-56 days, suggesting an increase in postnatal sensitivity. This difference in sensitivity could be due to the immature development of the liver, kidneys or brain (Snodgrass, 1992; NRC, 1995; Bearer, 1995).

A small number of case reports associated exposure to DEET in children with the occurrence of seizures. It is unclear if these cases represent an increased sensitivity to DEET toxicity in children, but in some of these cases, excessive exposure appears to have been a contributing factor. Daily topical application over several weeks to several months occurred in several cases before seizures developed (Gryboski *et al.*, 1961: Zadikoff, 1979; Heick *et al.*, 1980; Edwards and Johnson, 1987). However, seizures occurred in some cases after only a few topical applications (Roland *et al.*, 1985; Lipscomb *et al.*, 1992).   In at least two of these cases, 95-100% DEET products had been used. A contributing factor in at least one girl was being a heterozygote for ornithine carbamoyl transferase (OCT) deficiency. This deficiency is a sex-linked condition that is fatal in males during the neonatal period, but of variable severity in females which are usually heterozygotes. It is possible this deficiency contributed to the seizures in some of the other girls, but it is unlikely to have been a factor in the seizures in

boys. Some seizures may have occurred in children after DEET exposure by coincidence. In U.S. EPA's RED document for DEET, they noted that the estimated incidence for afebrile seizures in children (0-19 years) is 15,000 to 20,000 per year while an estimated 17 million children are exposed to DEET in a year (U.S. EPA. 1998). The possibility that the seizures were coincidental is strongest when they occurred after only a few applications. However, it seems unlikely that all cases of seizures after DEET exposure are coincidental, especially where there is a clear history of excessive use. Even if all these case reports of seizures were due to DEET, the incidence is still relatively low considering that approximately 17 million children use DEET each year.

Children may also be at greater risk for adverse effects from DEET because of their greater surface area to body weight ratio so that they get a higher exposure than adults on a mg/kg basis. However, the usage study conducted by Boomsma and Parthasarathy (1990) suggests that children receive more than adults even on a mg/cm^2 basis. In other words, parents may be applying DEET more liberally to their children than themselves. Other factors that may increase children's exposure are greater hand to mouth behavior and possibly greater permeability of skin in children relative to adults. Children below the age of 8 years old are generally considered to be more susceptible to skin irritation (Weltfriend *et al.*, 1996).

Endocrine Effects

Possible endocrine-related effects were observed in several animal studies after exposure to DEET. Abnormal sperm were observed in male rats in two studies (Macko and Bergman, 1980; Brusick, 1980). Increases in testicular weights were seen in several rat studies (Ambrose *et al.*, 1959; Macko and Bergman, 1980; Johnson, 1987b); however, reductions in testicular weights were seen in hamsters and dogs (Goldenthal, 1989a; Goldenthal, 1995a). In addition, hamsters had testicular tubular degeneration and small epididymides with luminal cellular debris (Goldenthal, 1989a). Urethral plugs made of coagulating gland and seminal vesicle secretions was observed in rats in another study (Wright *et al.*, 1992). Although most of the potential endocrine-related effects were seen in males, one potential endocrine-related effect was seen in female dogs, uterine hyperplasia (Goldenthal, 1994). There was insufficient information available to determine the exact mechanism behind any of these possible endocrine-related effects.

Cumulative Toxicity

There are no other registered pesticides that are known to have a common mechanism of toxicity with DEET. However, there is evidence that indicates DEET may act synergistically with other chemicals. The enhanced toxicity from combined exposure to DEET and fenvalerate appears to have been a factor in the deaths of some cats treated with a product that contained both of these chemicals as active ingredients (Dorman *et al.*, 1990; Mount *et al.*, 1991). The synergistic toxicity of several chemicals used by military personnel (DEET, permethrin, pyridostigmine bromide, chlorpyrifos) in the Persian Gulf War have been suspected in the "Gulf War syndrome" (McCain, 1995; Abou-Donia and Wilmarth 1996; Abou-Donia *et al.*, 1996; Chaney *et al.*, 1998 a&b). Possible explanations for the enhanced toxicity of these combined exposures include facilitation by DEET of the transport of chemicals across skin (fenvalerate) or the blood brain barrier (pyridostigmine bromide), inhibition by cholinesterase inhibitors (e.g., pyridostigmine bromide and chlorpyrifos) of non-specific esterases involved in the detoxification of DEET and overloading of metabolic pathways. This evidence suggests that gardeners and agricultural workers who use DEET while working with insecticides may be at greater risk for

toxicity from either DEET or the insecticide. However, there is insufficient information at this time to quantitate the risks.

Aggregate Exposure

The only registered use for DEET is as an insect repellent on pets (cats, dogs and horses) and humans. Therefore, there is no anticipated aggregate exposure for DEET from dietary, residential and/or occupational exposure.

<u>U.S. EPA's Reregistration Eligibility Document for DEET</u>

U.S. EPA issued a Reregistration Eligibility Document (RED) for DEET in September, 1998. Based on the low acute toxicity and lack of evidence of oncogenicity in the available toxicological data, U.S. EPA concluded that the normal use of DEET did not present a human health concern and therefore, did not do a quantitative risk assessment for DEET. However, because of its direct application to skin and clothing and its association with seizure incidents, U.S. EPA required improved label warnings and product restrictions. The new labeling requirements include directions on the method of application with special instructions for aerosol and pump sprays, special precautions for children, and elimination of any direct or indirect claims of child safety. U.S. EPA is also considering canceling registration of products that contain both DEET and sunscreen, but has deferred that decision until it has solicited views from various governmental agencies and other groups. Their concern about these products is that the combination of these products could result in more frequent application of DEET than is needed for pesticidal efficacy.

V. CONCLUSIONS

The risks for potential adverse human health effects in the general population with recreational exposure to DEET were evaluated. The MOEs for acute systemic effects in the general population were greater than 100 for all population exposure groups based on either average or high-end acute exposure estimates. The MOEs for acute occupational exposure were also greater than 100. The MOEs for subchronic systemic effects was greater than 100 for all population subgroups based on average exposure estimates and for adults and juveniles based on high-end exposure estimates. Only the subchronic MOEs for children based on high-end exposure estimates were less than 100. The small number of case reports of seizures in children exposed to DEET suggests that the MOEs for children may not be adequate with heavy use. The MOEs for chronic systemic effects were greater than 100 for all population subgroups based on either the average or high-end exposure estimates. The subchronic and chronic MOEs for workers were also greater than 100.

For dermal irritation, an MOE greater than 10 may be sufficiently health protective since humans are probably less sensitive than laboratory animals to dermal irritation. The MOEs for acute dermal irritation were greater than 10 for all population subgroups, including children and workers, when based on either the average or high-end exposure estimates. On the other hand, the MOEs for subchronic dermal irritation were less than 10 for all population subgroups using either the average or high-end exposure estimates. The subchronic MOEs for dermal irritation in workers was also less than 10. Risks for dermal irritation with repeated exposure may have been overestimated since the subchronic NOEL for this endpoint was estimated and humans are probably less sensitive than the animals. However, several literature reports of dermal irritation with high concentration formulations or heavy use suggest that the risk for dermal irritation is not negligible.

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Human Exposure Assessment for DEET

By

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HS-1740

January 20, 1999

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EXECUTIVE SUMMARY

DEET Human Exposure Assessment

January 20, 1999

Worker Health and Safety Branch
Department of Pesticide Regulation
California Environmental Protection Agency

Purpose

The Department of Pesticide Regulation (DPR) prepared an exposure assessment for DEET (*meta*-methyl *N,N*-diethyl toluamide) because of observations of adverse neurological outcomes in laboratory animal studies and reports from the published literature of neurological symptoms in children treated with this repellent for protection from nuisance or disease-transmitting arthropods. Comparison of the exposure data with the information derived from animal toxicology studies allows the estimation of risk and/or oncogenic potential during use of products containing DEET.

BACKGROUND

Humans apply DEET to their skin and clothing to repel nuisance and disease-transmitting arthropods. Extensive data exist on the use patterns of DEET, human absorption, and metabolism after skin application. In the time period between 1982-1995, 11 illnesses or injuries were reported to DPR for products containing this active ingredient.

METHODS
The document summarizes the exposure information on DEET from the peer-reviewed published literature and from companies that sell products containing this active ingredient. The data utilized for the development of the exposure assessment included a national survey of use characteristics of DEET (formulation type, time of year, amount used for each application), and human pharmacokinetics and metabolism after dermal treatment. From this information, we derived estimates of daily, annual and lifetime exposure.

FINDINGS AND CONCLUSIONS
For a single application of DEET, daily absorbed dosages were 1076 and 1661 μg/kg in adult females and children 12 years or younger, respectively. The Annual Average Daily Dosage (AADD) for DEET ranged from ~37-130 μg/kg/day for the different age groups. When the exposure information is compared to the animal toxicology data, DPR can estimate risks associated with the use of this arthropod repellent.

VOLUME 2

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
**WORKER HEALTH AND SAFETY BRANCH**

HUMAN EXPOSURE ASSESSMENT

DEET

January 20,1999

INTRODUCTION

DEET, *N,N* diethyl *meta*-toluamide, (CAS No. 134-62-3, formula $C_{12}H_{17}NO$, MW 191.27) is a repellent applied to humans for protection from biting arthropods, primarily mosquitoes, fleas and ticks. Some of these species are vectors for diseases, such as, Rocky Mountain Spotted Fever, Lyme's Disease, malaria, encephalitis and human granulocytic ehrlichiosis. The structure of DEET is shown below:

$$\overset{\displaystyle O}{\overset{\|}{C}}N(C_2H_5)_2$$

(benzene ring with CH_3 at meta position)
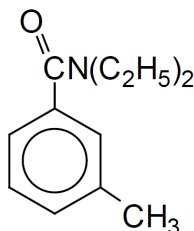
Some physical-chemical properties of DEET are listed below [a]:

Boiling Point (°C,1.3 mbar)	111
Vapor Pressure (Pa, 25 °C)	0.22
K_{ow}	2.0

[a]Tomlin, 1994

EPA STATUS

The US Environmental Protection Agency (US EPA) has DEET under review because of concerns about adverse health effects after use. As a part of this process, they are considering additional label language to reduce the frequency of illness associated with use of this repellent.

USAGE

Since DEET is not applied in production agriculture, its use is not required to be reported in California under the full Pesticide Use Report process. However, California sales data are available and provide some indication of use in California. Table 1 contains a compilation of the pounds of DEET products and DEET sold in 1991-1995.

Table 1. Pounds of DEET Products and DEET Sold in California: 1991-1995[a]

	Pounds Sold	
Year	DEET Products	DEET
1991	588,101	119,389
1992	708,797	145,735
1993	1,771,522	363,354
1994	810,780	129,478
1995	590,472	104,082
Mean	895,534	172,408
SD	445,459	96,430

Mill Tax Assessment Database[a]

Sanborn, WHS, 1999

These data demonstrate approximately a three-fold variability in the amount of product sold from year to year. Using the ratio of pounds sold for DEET and DEET products, the average percent of active ingredient in DEET products is 19.3% (range 16-20%) for the 5 years evaluated. This value is similar to the estimate of ~24.5% percent active ingredient that was based on a national survey (see Table 10).

FORMULATIONS

Accessible via the department web site, the Department of Pesticide Regulation (DPR) label database lists 164 currently registered products that contain DEET as the active ingredient. DEET is formulated as an aerosol, gel, cream, spray and ready-to-use liquid, to name a few.

LABEL PRECAUTIONS

The following label precautions are listed on a product containing 100% DEET. The product has the signal word **CAUTION.** This was selected as an example of a label because it provides the maximum likelihood for potential effects that may result from exposure to this repellent.

DO NOT APPLY TO EYES AND MOUTH. MAY CAUSE EYE INJURY. DO NOT SPRAY DIRECTLY ON FACE. DO NOT APPLY TO HANDS OF YOUNG CHILDREN. DO NOT APPLY TO SEVERELY SUNBURNED OR OTHERWISE DAMAGED SKIN.

ILLNESSES

The Pesticide Illness Surveillance Program data indicates that there were 11 reports of illness related to DEET exposure from 1982 through 1995 (WH&S, 1999). The types of illness reported were systemic (2), eye (6) and skin (3). Two days of lost work time were reported for 9 of the 11 cases; in two reports, lost work time is unknown. No days of hospitalization are reported for any of the exposures. Only one non-occupational incident was reported; an individual experienced and allergic reaction after spraying an entire can of DEET in his van. There were 10 occupational incidents: three persons sprayed DEET directly onto their eyes; two were exposed when handling defective containers; and two suffered possible allergic reactions; and three had DEET transfer from skin to eyes or mouth via sweat or rubbing.

TOXICITY

Acute toxicity by the dermal route is likely to be low, since the acute oral toxicity (LD_{50}) for male rats is reported as ~2,000 mg/kg (Tomlin, 1994). The acute dermal LD_{50} values in the rat, mouse and rabbit, are 5000 mg/kg, 3170 μl/kg and 3180 μl/kg, respectively (RTECS, 1997). The latter two values are reported in unconventional units of volume. Since the density of DEET is 0.996 g/l, these microliter values are numerically equivalent to milligrams.

DERMAL ABSORPTION

The dermal absorption of DEET has been investigated in rats, hairless dogs, monkeys and humans. The following discussion summarizes these studies.

Rats (single application)
Male Sprague-Dawley rats (6-8) were dosed (44 μg/4.2 cm^2 in 100 μl acetone) mid-dorsally with ^{14}C-ring-labeled DEET (specific activity 4.35 mCi/mmole, >98% pure), Moody *et al.*, 1989. The treated area was washed at 24 h with 50% Radiac$^®$ soap. The amount absorbed in 24 h was determined to be 36 $\pm$ 8% by analysis of the excreta. The urinary $t_{1/2}$ after dermal application was observed to be 20 h.

Monkeys (single application)
Rhesus monkeys (6-8) were dosed similarly to the rat, except several anatomical sites were treated to determine the effect on absorption (Moody *et al.*, 1989). The dermal absorption values after application to various sites are shown in Table 2. These ranged from 14% for the middorsal forearm to 68% for the ventral forepaw. The elimination

half-life followed the magnitude of dermal absorption with 4 h for the middorsal forearm to 8 h for the ventral forepaw.

Table 2. Influence of Anatomical Site on the Dermal Absorption of ^{14}C-DEET in Monkeys

Anatomical Site	Absorption (%)	Urinary $T_{1/2}$ (h)
Middorsal Forearm	14 ± 50	4
Forehead	33 ± 11	6
Dorsal Forepaw	27 ± 30	7
Ventral Forepaw	68 ± 90	8

Moody *et al*., 1989

Rat/Monkey (multiple applications)
In addition to the single administration studies, Moody *et al.* (1989) evaluated multiple dermal applications of DEET made to the forearm of monkeys and the dorsum of rats (Table 3). These experiments were conducted to determine whether there was a difference in dermal absorption between multiple and single applications. This is particularly relevant for humans as this repellent is generally applied several times daily to provide continued protection against biting arthropods. Multiple skin applications are required for continued efficacy because loss occurs from volatilization and dermal absorption.

Table 3. Influence of Multiple Applications of DEET on Dermal Absorption and Urinary Half-Life ($t_{1/2}$) in Rats and Monkeys

Animal	No. Applications	Time Interval (hr)	Absorption (%)	Urinary $t_{1/2}$ (h)
Rat	1	-	36 ± 8	20
Rat	3	2	31 ± 5	16
Monkey	1	-	14 ± 5	4
Monkey	3	0.5	12 ± 1	4

Moody *et al*., 1989

These data indicate that multiple applications of DEET do not markedly affect either the magnitude of the absorbed dose or the urinary half-life. With respect to the time interval between applications, the study in rats appears to be most relevant to human use of this repellent. It is improbable that a human would apply DEET three times in 0.5 h, as attained in the monkey study. While humans may apply DEET twice during a 2-hour period, this is still likely an excessive use, even in an area where the biting arthropod pressure is very high. It is more likely that humans may apply this repellent several times in a 16-hour period, with 2-4 h elapsing between applications. Despite improbability of humans using DEET twice during a 2-hour period, these data clearly demonstrate that multiple DEET dermal applications over relatively short time frames do not significantly impact the dermal absorption or the elimination half-life of this repellent.

Hairless Dog (single application)
Reifenrath *et al*., 1981 investigated dermal absorption of undiluted DEET in the hairless dog. Two dose rates were used, 340 and 4 µg/cm^2. Three dogs were used per dose with a study duration of 96 h. Using an intravenous (iv) dose, the dermal absorption estimate was corrected for incomplete urinary excretion. Dermal absorption values for the high and low doses were $9.4 \pm 3.6\%$ and $12.8 \pm 4.6\%$, respectively. Considering variability, the latter value is comparable to that obtained by Feldmann and Maibach, 1970, where humans were treated at the same dose rate. The high treatment rate is also similar to the values employed in the human studies by Selim, 1991a, 1991b, and 1992, and Selim *et al.,* 1995, where the doses were about 1.5-fold higher than this dog study.

Humans
Feldmann and Maibach, 1970, investigated the dermal absorption of DEET in humans. Subject's forearms (4) were treated with ^{14}C-labeled DEET in acetone (position of label, specific activity and radiopurity unspecified) at a dose of 4 µg/cm^2. The site of application was not covered or washed for 24 h. Urine samples were collected 120 h post-application for analysis of radioactivity. Since the study did not indicate the amount of radioactivity removed by washing at twenty-four hours, it is not possible to determine the material balance. Data from an iv administration of DEET were used to estimate incomplete renal excretion after dermal absorption. The iv administration data indicated that 52.3% radioactivity was recovered in the urine with a $t_{1/2}$ of 4 h. For dermally administered DEET, 16.7% (SD = 5.1%) was absorbed over the duration the study. By contemporary criteria this study has some deficiencies (estimate of material balance, metabolite characterization, solvent relevance to a commercial formulation). However, when the ~17% dermal absorption is compared with later investigations in other animals, including non-human primates (Moody *et al*., 1989), this value compares favorably.

The loss of DEET's repellent efficacy over time results from skin absorption/adsorption and volatilization from the site of application (Moody *et al*., 1989, Spencer *et al*., 1979). Evaporative loss is important for repellency of biting arthropods. Spencer *et al*., 1979 investigated, in some detail, the loss of DEET from both *in vitro* and *in vivo* skin and found 30-45 min. post application, the evaporation rate was 4.0 ± 2.9 and 3.5 ± 1.6 µg/cm^2/h, respectively. These data can be used to estimate material balance for the Moody *et al. in vivo* studies: 9.6% evaporated; 27.1% associated with a skin wipe; and 11.9% associated with skin stripping for a total recovery of 48.3%. The dose rate of 25 µg/cm^2 in these *in vitro* experiments by Spencer *et al.,* 1979 lies in the range (10-70 µg/cm^2) that is effective for the repellency of biting arthropods (Gabel *et al*., 1976).

In the multiple dermal absorption studies reported by Moody *et al*., 1989, one male subject was treated on the back, chest, and forearm with 15g of a commercial insect repellent formulation containing 95% DEET (Muskol$^®$, dose rate = 3,450 µg/cm²) or 14.25 g DEET (dose rate = 3,277.5 µg/cm²). This dose rate is 47-325 fold greater than those found to be effective for arthropod repellency (Gabel *et al*., 1976). The male human subject showered four hours after treatment. Urine samples were collected for

48 h post administration. Table 4 contains the data for the time dependent elimination of DEET and the primary metabolite, *N*-ethyl *m*-toluamide (NEMT).

Table 4: Urinary titers of DEET and NEMT after treatment of a single human male with 15g Muskol®

| Time (hrs)	DEET (µg/ml)	NEMT (µg/ml)
0	9 ± 8	4 ±< 1
4	84 ± 62	11 ± 97
8	78 ± 38	129 ± 11
12	32 ± 28	28 ± <1
24	17 ± <1	20 ± 1
48	5 ± <1	35 ± 16

Moody *et al*., 1989

The units (µg/ml) do not allow estimation of a cumulative excreted dose because the urine volumes at each time period were not provided. Therefore, it is not possible to estimate either the total amount of DEET or NEMT at each sampling period. Further, it is not possible to estimate the percent absorbed in this study. However, it is possible to estimate a urinary half-life for elimination of DEET, but not for the metabolite, NEMT. A value for the half-life for NEMT cannot be estimated because the urinary metabolites reach a plateau at 48 h with no apparent decrease in their titer during the duration of the study. The half-life for elimination of DEET from this single subject was 10.7 h. This is less than the value for rats (16-20 h) and greater than the values for the monkey (4-8 h). The plot of these urinary data is shown below

Figure 1

Urinary Elimination of DEET
N = 1

Y = -0.028X + 1.99 R = -0.97
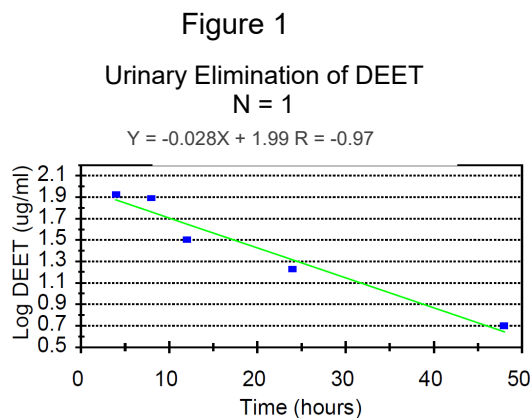
In the study by Moody *et al*., 1989 NEMT (Figure 2) was isolated after treatment of the urine samples with acid or *β*-glucuronidase. The structure was determined by gas chromatography coupled with mass spectrometry (GC/MS). Since this metabolite does not contain a hydroxyl or amine moiety, it is not obvious why treatment was required to isolate this metabolite. An explanation for the need for either treatment is that the intermediate from oxidative metabolism was immediately conjugated as a glucuronide. Support for excretion of a metabolite of DEET as a glucuronide may be found in the earlier work of Wu *et al*., 1979.

Figure 2
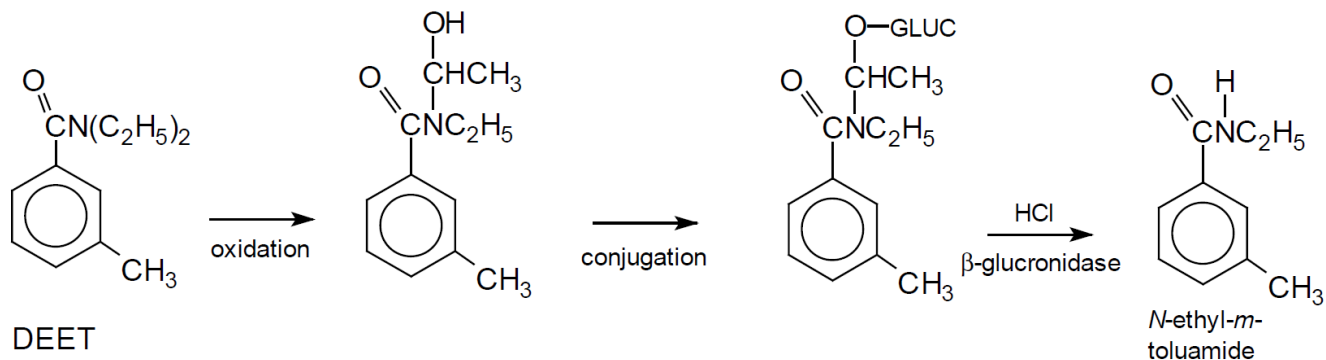
Wu *et al.*, 1979, focused on spectroscopic identification of the various metabolites in urine present after dosing. An individual treated himself topically with 10.5 g of a 75% formulation (7.88 g DEET). Small amounts of the intermediate hydroxylated species following acid hydrolysis were observed spectroscopically; it resulted in formation of NEMT. This is only an inference for the presence of the glucuronide in urine, as the authors did not isolate the glucuronide for mass spectral analysis. Interestingly, the authors found small amounts of methyl-oxidized products, namely the alcohol and carboxylic acid. The latter was transformed into the methyl ester for characterization. In addition, Wu *et al.,* 1979, found unmetabolized DEET in the urine. This constituted 10-14% of the applied dose (133 mg/kg) in the first hour and ~2% at the fourth hour. In blood, at 8 h the concentration of DEET was determined to be 0.3% (300 µg/100 ml blood). At 18 h after application detectable DEET was still in the urine. At the end of the study, the carboxylic acid, before methylation was determined to be the major human metabolite in the urine. In the most recent human dermal absorption study (to be discussed later in this document) the carboxylic acid metabolite was observed via use of high-pressure liquid chromatography (HPLC) (Selim, 1992 and Selim *et al.*, 1995).

Recently, the DEET Steering Committee, evaluated the dermal absorption in humans (Selim, and 1992 and Selim *et al.*, 1995). The forearms of twelve healthy volunteers (six/formulation) were treated with 12-15 mg ^{14}C-ring-labeled DEET (98.9% radiopurity, 97.87% chemical purity, specific activity 22 mCi/mmole) either undiluted or as a 15% (w/v) solution in ethanol. The dosing area was 24 cm^2 (6X4 cm). Those treated with the 15% material received a dose of ~500 µg/cm^2. For subjects administered undiluted DEET, the dose was slightly higher, 620 µg/cm^2. The treated area was covered with an aluminum dome that contained air holes for circulation, but prevented physical contact with the ^{14}C-DEET. At 8 h post-treatment, the cover was removed and the treated area washed with *iso*-propanol moistened cotton swabs. Tape strippings of the treated area were conducted at 1, 23 and 45 h after the cover and protective wrappings were removed. Blood samples from the treated and untreated arms were taken at 0, 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96 and 120 h post application. Urine samples were collected at 0-4, 4-8, 8-12, 12 to 120 h and then 120-128 h. The subjects were not allowed to bathe until the last tape stripping was completed 45 h after the protective

appliance was removed. At the end of the eight-hour exposure period, the location of administered doses for the two treatments were marked on the skin.

Table 5: Distribution of ^{14}C-DEET equivalents after application to human volunteers: Effect of formulation

	Percent of Applied Dose	
Region	Undiluted	15% in Ethanol
Swabs	60.80	50.89
Pipettes	5.33	2.46
Skin rinse	1.14	1.05
Gauze	0.28	0.32
Aluminum dome	21.08	25.54
Tape Stripping	0.08	0.07
Feces	0.02	0.08
Urine	5.61	8.33
Total	94.34	88.74

Sanborn, WH&S, 1999 after Selim, 1992 and Selim *et al.*, 1995

While this study did not utilize an iv dose to correct for incomplete renal elimination, sufficient data exists for a material balance calculation and estimation of dermal absorption.

The following table compares the dermal absorption values from Feldmann and Maibach, 1970 and Selim, 1992 and Selim *et al.*, 1995.

Table 6: Comparison of Dermal Absorption of DEET in Humans

Study	Vehicle	Dose Rate (μg/cm^2)	Absorption (%)
Feldmann & Maibach	Acetone	4	16.7 (5.1)[a/]
Selim	None	620	5.6 (2.8)
Selim	Ethanol	500	8.4 (3.6)

a/ Arithmetic standard deviation

Sanborn, WH&S, 1999

Despite the 125-fold greater dose rates (500 and 600 μg/cm^2) in the studies by Selim, 1992, and Selim *et al.*, 1995 vs. those by Feldmann and Maibach, 1970, (4 μg/cm^2) only a two-fold difference exists in the dermal absorption when a solvent was utilized (16.7% vs. 8.4%).   In the studies by Selim, 1992 and Selim *et al.*, 1995 where the dose rates were comparable (620 vs. 500 μg/cm^2), the difference in dermal absorption is likely related to use of the ethanol as a dosing vehicle. Considering the variability in the Selim data (cv ~43%), the dermal penetration values for the studies with solvent are likely the same. Statistical treatment of these data with a 2-tailed t-test for independent samples indicates that they are not different at $p \leq 0.05$ and are only different at $p = 0.24$. This assumes equal variances. The Selim study used dose rates about 10-fold higher than are reported by Gabel *et al.*, 1976 to provide protection from nuisance

arthropods (10-70 ug/cm^2). These higher dose rates may be related to the specific activity of the radiolabeled DEET.

METABOLISM

Humans

In contrast to the report by Moody *et al.*, 1989, following dermal administration, Selim, 1992 and Selim *et al.*, 1995 found two urinary metabolites of DEET; these are depicted below:
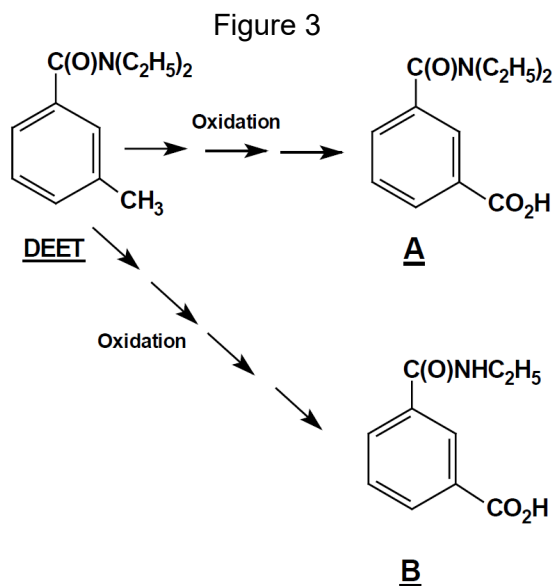
Figure 3

Table 7 presents the mean percentages of these two metabolites in the urine, as well as, the percentage of these metabolites as a function of the applied dose.

Table 7: Urinary Metabolites in Humans after Dermal Dosing of DEET in Two Formulations

DEET Dosing Regime	Metabolite	% Metabolite in Urine	% Metabolite Applied Dose
15% in Ethanol	A	32.6	2.7
15% in Ethanol	B	16.0[a/]	1.1
Undiluted	A	34.6	2.0
Undiluted	B	11.0[a/]	0.7

[a/] Likely underestimated because of incomplete resolution on HPLC

Sanborn, WH&S, 1997 after Selim, 1992 and Selim *et al.*, 1995

These metabolites were not identified spectroscopically (mass spectrometry), but were characterized qualitatively by comparison of relative retention times on HPLC to those initially observed in a rat metabolism study after an oral dose. The difficulty of resolving metabolite B from another urinary metabolite interferes with precise quantitation of this degradation product. The small fraction of the applied dose as identifiable metabolites

may make exposure assessment from urinary biomonitoring of humans exposed to DEET somewhat difficult unless small inter- and intra-individual variation exists for these metabolites. If small variation is observed, then it might be possible to use these metabolites as biomarkers of DEET exposure.

Comparison of these metabolism data with data from Moody *et al.*, 1989, provides a different picture of the human metabolites. None of the degradation products in Selim's work were found in Moody's study and vice versa. The apparently facile benzylic oxidation of the methyl moiety in DEET observed in the investigations by Selim is not unexpected.

EXPOSURE ASSESSMENT

Non-occupational
Using several types of surveys, researchers estimated exposure of humans to DEET in non-occupational settings (Boomsma and Parthasarathy, 1990). These surveys consisted of a mail survey, usage survey and a syndicated market share survey. In the syndicated market survey, data was gathered on products sold, timing of sales, etc.

The mail survey was sent to 8,000 households, nationally balanced to the US Census data on age, household income, region, population and household size. Questionnaires asked people about insect repellent use in the last year. If anyone in the household responded positively, the household was targeted for further study and asked to keep a daily diary of repellent use during June and July. In the diary, information was recorded on date of use, who used it, brand name and number of applications. June and July were selected because sales data show most repellents are sold in these months; this time period accounted for 53-60% of annual use.

In the usage survey, 542 individuals were observed applying DEET products. Observers recorded areas treated, whether applied to clothes, skin or both, whether label directions were followed and the amount applied (product weighed before and after use). The use survey was conducted in Green Bay, Wisconsin, Tampa, Florida and Portland, Oregon, representing high, medium and low exposure, respectively. Even though this was not California-specific data, the information from this national survey will be used to estimate exposure of Californians to DEET.

Type of Products. Table 8 lists DEET products sold in 1989 and 1990 and the results of the 1990 use survey for these same products. Data from the market survey and use survey show good concordance.

Frequency of Use. Table 9 contains data on insect repellent use from the initial mail survey. Obviously, households targeted for further study reported higher use of DEET; about 4 out of 5 respondents indicated that they had used an insect repellent sometime in the previous year. For the whole survey population, about 3 out of 10 reported use of an insect repellent during the previous year. Neither of these surveys total 100% because of non-respondents.

Table 8: Market Share of DEET Products Sold in 1989 and 1990 and 1990 Usage Study

	Market Share (%)[a]		
Formulation Type	1989	1990	Use Survey (%)[b]
Aerosol	75.5	71.9	75
Pump Sprays	15.6	15.0	16
Lotions/Creams	1.3	1.4	6
Liquids	4.8	6.0	1
Roll On/Stick	0.8	0.7	1
Towellettes	2.0	0.2	1
Other	-	4.8	-

[a] Table 6, Boomsma and Parthasarathy, 1990
[a] Table 41, Boomsma and Parthasarathy, 1990

Boomsma and Parthasarathy, 1990

Table 9: Frequency of DEET Use in Mail Survey; Use of Any Insect Repellent in Previous Year

Household	Sample Size	Yes (%)	No (%)
Initial Survey Population[a]	12,224	37	62
Targeted Survey Population[b] (later kept diary of use)	5,536	82	17

[a] Table 7, Boomsma and Parthasarathy, 1990
[b] Table 8, Boomsma and Parthasarathy, 1990

Boomsma and Parthasarathy, 1990

Data from the mail survey indicated that during June and July of 1990, people used DEET products an average of 7.5 times (N=1,571).

Usage Amounts. Table 10 shows the distribution of the amount of DEET product and amount of DEET applied during a one-time application to skin and clothing. As stated before, these data were obtained by weighing the product container before and after use, ensuring an accurate determination of the amount of DEET used.

Table 10: Amount of DEET Product and DEET Applied to <u>Skin and Clothing</u> from a single application

DEET Product[a]			DEET[b]	
Amount of Product Applied (g)	Percent of Population		Amount of DEET Applied(g)	Percent of Population
0.0-2.99	40		0.00-0.99	56
3.0-5.99	32		1.00-1.99	27
6.00-8.99	15		2.00-2.99	10
9.00-11.99	5		3.00-3.99	5
12.00-14.99	5		4.00-4.99	1
$\geq$15.00	3		5.00-5.99	1
			$\geq$6.00	1
Mean (g) = 4.9[c]			Mean (g) = 1.2[c]	

[a] Table 46, Boomsma and Parthasarathy, 1990; Sample size = 542
[b] Table 51, Boomsma and Parthasarathy, 1990; Sample size = 542
[c] Mean: midpoint applied range x percent population use, summed/100; therefore it is a weighted mean

<div align="right">After Boomsma and Parthasarathy, 1990</div>

The mean amount used allows estimation of the approximate percent DEET in the products applied for arthropod repellency. These data indicate that DEET products used contain an average of 24.5% DEET (1.2/4.9 X100).

<u>Estimation of Daily Potential Exposure and Absorbed Daily Dosage (ADD)</u>. The following exposure and dosage estimates use data from application to skin only. Table 11 provides information on the variability of the DEET exposure data. The exposure values in this table represent the overall mean for the population, including all formulations, age groups and both sexes, mean + 2 standard deviations (SD) and the 90th and 95th percentiles. (No justification was provided in the reference exposure document for the calculation of the upper end values.) The small variability in these data is somewhat unexpected. In contrast, exposure studies conducted in production agriculture generally exhibit large ranges, often more than 10-fold. Production agriculture exposure data are therefore often log-normally distributed, requiring geometric statistical treatment to calculate central tendencies. In contrast, for these aggregate population data, the difference between the mean value and the 95th percentile is less than 4-fold. Apparently the self-application of DEET or application to others does not have the variability routinely observed in other exposure studies.

Table 11: DEET Exposure to the Skin - Data Variability (All age groups)

	Aggregate Mean Exposure[a]			
Exposure	(g/day)	g/day + 2 SD[b]	g/day (90th)[c]	g/day (95th)[d]
Skin Only	0.038	0.042	0.081	0.12

[a] From Table 4, Boomsma and Parthasarathy; Total overall average (composite all age groups)
[b] SD-Standard Deviation
[c] 90th percentile
[d] 95th percentile

<div align="right">Sanborn, WH&S, 1999, after Boomsma and Parthasarathy, 1990</div>

To understand the data in Tables 12 and 13, annual average daily dosages (AADD) and seasonal average daily dosages (SADD values), it is necessary to provide some information on how the data in these tables were derived. From the usage survey, researchers determined the amount applied per application. From the mail survey, users reported the number of applications during the heavy-use months (June and July). To obtain the total amount used during the heavy use months, the amount per application was multiplied by the number of applications. To derive the average yearly and daily amount used the following two equations were used:

Average Yearly DEET Use (g) = $\dfrac{\text{Average DEET usage in June/July}^{1/}}{\text{\% Yearly Product Sold in June/July}}$

[1] Grams/application x # application

Average Daily DEET Use (g) = $\dfrac{\text{Average Yearly DEET Use}}{\text{365 days}}$

Data in Table 11 reflect an overall mean for the general population (male, female, and all age groups). In addition, it may be important to derive values for different age groups (Table 12). While the amount of DEET applied in each age group is relatively constant (less than two-fold difference), the body weights of the different ages differ more than 10-fold from infants/children to adults. Mean weights were selected from the US EPA Exposure Factors Handbook (US EPA, 1996). When there is a range of ages, *i.e.*, for young children <12, the weight at the midpoint of the age (*i.e.* 1-12 y) is utilized. The exposure data in the table below only indicates about a 3.6-fold variation. Table 12 summarizes these age-related exposures.

Table 12: DEET Exposure by Age and Gender - Annual Average Daily Dosage (Skin Only)

Gender: Age Group:	Male Adult	Female Adult	Male (13-17)	Female (13-17)	Male (<12)	Female (<12)
Dermal Exposure (g/d)[a]	0.053	0.029	0.037	0.037	0.034	0.034
Body Wt (kg)	78.1	65.4	61.1[c]	55[c]	22.8[c]	21.9[c]
AADD (µg/kg/d)[b]	57.0	37.1	50.7	56.4	124.5	129.6

[a] From Table 4, Boomsma and Parthasarathy, 1990, Annual Averaged Daily Dermal Exposure
[b] Annual Average Daily Dosage: Dermal absorption = 8.4% (Selim, 1992); sample calculation for
     male 0.053 g/day/78.1 x 0.084 x 10^6 µg/g = 57 µg/kg/day
[c] Body weight-midpoint of the age range, Draft US EPA Exposure Factors Handbook, 1996

Sanborn, WH&S, 1999 after Boomsma and Parthasarathy, 1990

If there are age-related concerns regarding the use of DEET as a repellent, then these exposure data can be used with the toxicology information to provide either estimates of risk or margins of exposure (MOE). The greater AADD values of children less than 12 yr. as compared to adults are the result of lower body weight and <u>data</u> from the usage survey where approximately the same amount was applied to this age group despite the smaller surface area.

An aspect of the exposure estimates in Table 12 is unreasonable from a use perspective. The adult female daily dermal exposure is less than either of the younger age groups. In terms of body weight, the adult female is not much different than the 13-17 year old male as indicated in Table 12. A modified exposure assessment will be derived later in the document that takes into account both surface area and body weight of the different age groups.

Seasonal Average Daily Dosage
Data from a single exposure can be amortized over time to obtain a Seasonal Average Daily Dosage. From the mail survey, we know people used DEET an average of 7.5 times per season. Table 13 contains the seasonal dosage by gender and age.

Table 13: Seasonal Average Daily Dosage (SADD) by Age from 7.5 Applications (Skin Only)

Gender:	Male	Female	Male	Female	Male	Female
Age Group:	Adult	Adult	(13-17)	(13-17)	(<12)	(<12)
Exposure (g/d)[a]	0.95	0.65	1.07	1.07	0.94	0.94
Body Wt (kg)	78.1	65.4	61.1[c]	55[c]	22.8[c]	21.9[c]
SADD (µg/kg/d)[b]	125.6	102.6	180.9	200.9	425.8	443.3

[a] From Table 50, Boomsma and Parthasarathy, 1990
[b] Seasonal Average Daily Dosage: Dermal absorption = 8.4% (Selim, 1992; Selim *et al*., 1995),
   Sample calculation for male: (0.95 g/day x 7.5 applications/season / 61 days/season) / 78.1 kg x
   0.084×10^6 µg/g = 125.6 µg/kg/day
[c] Body weight-midpoint of the age range, Exposure Factors Handbook, US EPA, 1996

<div align="right">Sanborn, WH&S, 1999 after Boomsma and Parthasarathy, 1990</div>

Absorbed Daily Dosages from a Single Application. The estimates of ADD values from a single application are compiled in Table 14.

Table 14: Exposure by Age from a Single Application (Skin Only)

Gender:	Male	Female	Male	Female	Male	Female
Age Group:	Adult	Adult	(13-17)	(13-17)	(<12)	(<12)
Exposure (g/d)[a]	0.95	0.65	1.07	1.07	0.94	0.94
Body Wt (kg)	78.1	65.4	61.1[c]	55[c]	22.8[c]	21.9[c]
ADD (µg/kg/d)[b]	1,020	840	1,470	1,630	3,460	3,610

[a] From Table 50, Boomsma and Parthasarathy, 1990
[b] Absorbed Daily Dosage: Dermal absorption = 8.4% (Selim, 1992; Selim *et al*., 1995), Sample
   calculation for male: 0.95 g/day/ 78.1 kg x 0.084×10^6 µg/g = 1,020 µg/kg/day
[c] Body weight-midpoint of the age range, Exposure Factors Handbook, US EPA, 1996

<div align="right">Sanborn, WH&S, 1999 after Boomsma and Parthasarathy, 1990</div>

Comparison of the SADD data in Table 13 with AADD values in Table 12 indicates that seasonal exposure is significantly greater. This not unexpected given the reduction in exposure that occurs when exposure is amortized over a use season or over an entire year.

Alternative Non Occupational Exposure Assessment

As indicated earlier, the exposure estimates in Tables 12-14 are not logical, even though they are based on a usage survey of considerable size. It is unrealistic to think that a child less than 12 years old will receive approximately the same dermal dose as older age groups when the surface area is considerably less. Table 15 contains an alternative dermal exposure assessment for users of products containing DEET. Two assumptions were used to develop the data in this table: 1) the adult male applies 1,000 mg each application and 2) the treated area for the male is 1.94 m^2.

The surface area (1.94 m^2) is the 50 percentile in the US EPA Exposure Factors Handbook (US EPA, 1996). The dermal doses for the other age groups were derived from the adult male dose in mg/cm^2 and the body surface area of each age group. To obtain the ADD values a dermal penetration value of 8.4% and the appropriate body weights were utilized.

Table 15. DEET Dermal exposure and absorbed daily dosage (ADD): Single application
Basis: Surface Area and Body Weight for Three Age Groups

	Adult Male	Adult Female	Child 12-17	Child < 12
SA (m^2)[a] 50 percentile	1.94	1.69	1.58[b]	0.86[b]
Dermal Dose (mg/per/applic.)	1000	871[c]	814[c]	443[c]
BW (kg)	78.1	65.4	58.1[d]	22.4[d]
Dermal Dose[e] (mg/kg)	12.8[e]	13.3	14.0	19.8
ADD[f] (µg/kg/d)	1076	1119	1177	1661

[a] Exposure Factors Handbook, US EPA, 1996
[b] Mean of male and female surface areas at midpoint of age range
[c] Sample Calculation, Dermal Dose: (1000 mg male dose) x SA female/SA male = 871
[d] Mean of male and female body weights 50th percentile; Exposure Factors Handbook, US EPA, 1996
[e] Sample calculation: Potential Exposure (male) = Dermal Dose (1000 mg)/BW (78.1kg) = 12.8 mg/kg
[f] Absorbed Daily Dosage = Dermal dose (mg/kg) x 8.4% (Selim, 1992; Selim *et al*., 1995)

Sanborn, WHS, 1999

When the ADDs in Tables 14 and 15 are compared, substantial differences exist for the youngest age group. The nearly 2-fold difference in ADDs for the child <12 y is related to the difference in the method of estimating the dermal dose. Both of these exposure scenarios (Tables 14,15) assume only <u>one</u> application per day. The differences between the estimated ADD values in Table 12 compared to 13, 14 or 15 relate to the absence of amortization of single applications over time.

In addition to the ADDs calculated in Table 15, seasonal average daily dosages (SADD) and annual average daily dosage estimates (AADD) may be required for comparison to some toxicology endpoints. Table 16 contains these estimates.

Table 16: DEET Absorbed Dosages - Daily, Seasonal and Yearly (µg/kg/day)

Value	Adult Male	Adult Female	Child 12-17	Child < 12
ADD[a/]	1076	1119	1177	1661
SADD[b/]	132	138	145	204
AADD[c/]	50.8	52.8	55.6	78.5

a/ Values from Table 15
b/ Seasonal Average Daily Dosage = ADD x 7.5 days/61 days, June, July.
c/ Annual Average Daily Dosage = ADD x 7.5 days/365/0.435 to account for use months other than of June/July

<div align="right">Sanborn, WHS, 1999</div>

These data differ from those reported in Tables 12 and 13 because they were generated with a consideration that the dose applied reflects the surface area of the human. The most pronounced differences occur for the children <12. Using surface area to define the dose was the basis of Table 15. The SADD and AADD values in Table 16 were derived from the ADD values in Table 15.

Occupational Exposure
Exposure estimates are available for workers in mosquito control programs who used this repellent (Robbins and Cherniack, 1986). The data in Table 17, abstracted from this paper, also includes information from a US EPA document that estimated upper end exposures for the general population as well as workers in mosquito control programs in the Florida everglades. Everglade biologist exposures indicated below as mosquito control workers, have been included as a point of reference even though this level of use in California is not likely to occur because of the more temperate climate that results in lower populations of biting arthropods.

Table 17: Comparison of Occupational Exposures to DEET

		Dermal Exposure	
Group	Conc. %	Daily(g/day)	Seasonal (g)[a/]
Military Person	75	-	43
Mosquito Control	28.7	4.25	442
Mosquito Control (Upper 5%)	15-75	>2kg/7 mo	>1710

[a/] Use: May to October

<div align="right">Sanborn, WH&S, 1999, after Robbins and Cherniack, 1989</div>

Clearly, the insect pressure present in the Florida Everglades occurs infrequently if at all in California. Nevertheless, it is important to be cognizant of the level of dermal exposure that may occur in other regions of the country. Using these dermal exposure values in Table 16 would result also in much higher ADDs as compared to those in Table 15. These data while informative for comparative purposes, do not possess the sample size and documentation to make them useful for the exposure assessment process for use of DEET in California.

EXPOSURE: INHALATION

The preceding estimates of exposure focused on the dermal route of exposure. The biological activity of DEET depends in part on its volatility, which is related to its relatively high vapor pressure (0.22 Pa).   Evidence for evaporation after application may be found in a study that reported about 27% of a dose at 25 μg/cm^2 volatilized in 45 minutes after *in vitro* treatment of skin sections (Spencer *et al*., 1979). Estimates of exposure to DEET via the inhalation route cannot be based on active ingredient-specific data because none exist. Two types of inhalation exposures may occur, one short term (during application) and the other with a longer duration after application (evaporation from clothing/skin). The latter inhalation exposure component will be difficult to match with a toxicology endpoint because the amount volatilizing per unit time decreases resulting in a decreased exposure by inhalation. Further with respect to direct human inhalation exposure from aerosol products, DEET labels specifically state for these products that they should not be sprayed directly on the face or neck. Rather, the labels indicate that DEET should be sprayed first on the hands for subsequent application to other parts of the body (neck, ankles, *etc*.,).

For the estimation of inhalation exposure to products that contain DEET, consideration was given to the utilization of inhalation exposures (CFC propellant) from personal care products (hair spray, body spray, antiperspirant) as a surrogate (Hartop and Adams, 1989). These products, unlike DEET are directly sprayed either on the body or hair. Because these aerosol personal care products are directly sprayed on the skin or hair rather than as prescribed for DEET products, no estimates for inhalation exposure for this insect repellant have been derived using these data as a surrogate.

EXPOSURE APPRAISAL

The information used to estimate exposure of humans to DEET is substantial. There are data on dermal absorption in humans, metabolite characterization after controlled human exposure, human use data with respect to types of products, frequency of use, the site of application (skin or skin/clothing) and the amount applied for a single application. These data were derived from several surveys that have a relatively large sample size. The usage survey suggests that small children receive the same absolute dermal dose as an adult. Since this is not very reasonable because of the smaller surface area of small children as compared to an adult, an alternative exposure assessment based on surface area has been derived which should be used in the estimates of risk from DEET exposure.

From the data in Table 8, we can see that approximately 87-92% of the DEET used is formulated as products that can be sprayed on the clothing or skin. These contain label language that directs against applications to the face and neck. Thus, the relevance of the dermal penetration values determined in laboratory animals and humans could be questioned. In laboratory animal dermal absorption studies, DEET was applied with a pipette rather than by spray to ensure accurate dosing and acceptable material

balance. While direct application will result in more reaching the site of application, it may reduce the inhalation component of exposure. This is especially the case if devices are used to trap DEET evaporating from surface of the treated animals or humans. This would be of minor concern if biological monitoring were used to provide exposure information for dermal and/or inhalation routes. Given the relatively high vapor pressure of DEET, inhalation exposure is likely from both during spray application and evaporation from skin and clothing after application. However, because only small percentages of two metabolites were observed in the studies by Selim, 1992 and Selim *et al.,* 1995, it is not feasible at this time to determine whether biological monitoring can be used successfully to estimate exposure of DEET users. If the urinary metabolites, regardless of the percentage of applied dose, are found to be constant and linearly related to the excretion of radiolabel, and there is little intra- and/or interpersonal variation, then it may be possible to use biomonitoring to refine further exposure estimates.

There is no indication, from human or animal studies, that the dermal absorption or elimination after single or multiple doses will differ. For example, in laboratory studies in rats and monkeys, the rate of dermal penetration and subsequent elimination of DEET and its metabolites after multiple applications were similar to a single application (Moody *et al.,* 1989). Therefore, when humans apply DEET several times in a day (which may occur where there is high arthropod pressure) the kinetics of penetration and elimination likely would not differ from those after a single dermal application.

Two issues have not been specifically addressed in this document, exposure via inhalation and upper-end exposure values for comparison to acute toxicology endpoints. Exposure to DEET via inhalation is likely to occur because most products sold are aerosol products that were reported from the use data to be sprayed on the clothing or the skin. Data to empirically base an inhalation exposure estimate do not exist. Therefore any estimate of exposure via inhalation would not be active ingredient-specific, empirically based and therefore not technically defensible. While other pesticides maybe sprayed from aerosol containers, the spray is directed **away** from the applicator and not **on** the user. Because data do not exist for estimation of inhalation exposure for products such as DEET that are sprayed on humans, an estimate for this route will not be derived even though it is unlikely to be zero. Earlier discussion indicated that personal care product exposure information was not appropriate for estimate of inhalation exposure. The best way to estimate exposure from products applied as sprays would be via biomonitoring which is not possible until more information exists about the usefulness of the urinary metabolite profile as a biomarker of DEET exposure.

With respect to high-end exposure estimates, the data in Table 11 can be used. Therefore, high-end exposure estimates from the dermal dose values can be estimated from the ratio of 95th percentile to the mean. The ratio of the mean exposure to 95th percentile is just slightly greater than 3. The high-end exposure estimate may be compared with acute toxicology data to estimate an acute risk while using DEET as an arthropod repellent.

The database for exposure to DEET contains sufficient exposure information to assess the risks associated with the use of this repellent. In particular, the number of human studies with DEET reduces the necessity of using animal data (such as dermal absorption) to derive an absorbed dosage for the assessment of risk.

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Office of Environmental Health Hazard Assessment

MEMORANDUM

Winston H. Hickox
Agency Secretary

Gray Davis
Governor

TO: Joyce Gee, Ph.D., Acting Chief
Medical Toxicology Branch
Department of Pesticide Regulation
830 K St.
Sacramento, California 95614-35

FROM: Anna M. Fan, Ph.D., Chief
Pesticide and Environmental Toxicology Section

DATE: October 5, 1999

SUBJECT: COMMENTS ON THE DEPARTMENT OF PESTICIDE REGULATION'S
DRAFT RISK CHARACTERIZATION DOCUMENT FOR THE ACTIVE
INGREDIENT N, N-DIETHYL-M-TOLUAMIDE (DEET)

We have completed our review of the draft risk characterization document (RCD) for the active ingredient N,N-diethyl-m-toluamide (DEET) prepared by the Department of Pesticide Regulation (DPR). DEET is used as an all-purpose insect repellant applied either directly to the skin, or to clothing, bedding and tents of users. In California, over 160 products are registered which contain DEET as the active ingredient, and in 1995, 104,082 pounds of the active ingredient were sold. The principal users are the general public and outdoor workers such as park and forestry personnel. It is estimated that 38 percent of the general public in the United States (U.S.) uses insect repellents containing DEET.

The draft RCD package submitted to the Office of Environmental Health Hazard Assessment (OEHHA) consisted of the draft RCD (June 28, 1999) prepared by the Medical Toxicology Branch. Additional information obtained independently included a summary of toxicology data for DEET (last revised on June 6, 1999) prepared by the Medical Toxicology Branch and a DEET use survey submitted to DPR by a registrant. OEHHA staff also conducted a brief review of the published literature on DEET.

We obtained the document entitled "Human Exposure Assessment for DEET" (January 20, 1999) from the Worker Health and Safety Branch of DPR, which is apparently a final document. OEHHA had not previously reviewed the exposure assessment for DEET. We

understand that the exposure assessment is prepared separately from the RCD by the Worker Health and Safety Branch. However, it is not possible for us to review RCDs without the exposure assessments, and any related documentation. Therefore, it would be helpful if draft exposure assessments were provided prior to (preferable) or together with the submission of the RCD package. Having to obtain the exposure assessment independently might delay our review of the RCD and submission of our comments to DPR.

The draft RCD is well written The document includes a summary of the extensive toxicological database for DEET. In the risk identification section, the toxicological data are adequately evaluated for relevance to the hazard DEET might pose to exposed humans. This was a difficult undertaking since DEET rarely produced any consistent toxicity across different exposure time frames and animal species. The draft RCD states that seizures have been reported to occur in individuals following exposure to DEET. Experimental animals exposed to DEET might also exhibit seizures and other neurological effects. Other toxicities reported are not as well defined and vary among species and exposure period. Dermal irritation was also reported. Margins of exposure (MOEs) for DEET were determined to be at least 100 for all subpopulations under acute, subchronic or chronic exposures, except for high-end acute exposures to children under 12, for which the margin of exposure was estimated to be ten.

The U.S. Environmental Protection Agency (U.S. EPA) has recently recommended reregistration of DEET [Reregistration of the Insect Repellent DEET (fact sheet) April 28, 1998]. However, this decision apparently was based on a generalized assessment of the toxicity database for DEET rather than a risk characterization based on toxicity endpoints [Federal Insecticide, Fungicide, and Rodenticide Scientific Advisory Panel Meeting, June 1997]. U.S. EPA no longer allows child safety claims on DEET product labels.

Based on our review of the draft RCD for DEET, we feel that the document needs some revision before finalization. In general, the assumptions and conclusions stated in the draft RCD require additional scientific support and analysis, as well as additional detailed discussion regarding exposure assessment in order to provide an acceptable characterization of the risks posed by the use of DEET in California. Our major technical concerns are listed below:

1) There is an inadequate discussion of exposure estimates in the draft RCD.
2) The contribution of inhalation exposure to the overall risk is not assessed in the draft RCD. It is not clear whether this was the result of a limited database, an oversight, or a scientifically based omission. Inhalation is likely to contribute significantly to the overall exposure to DEET in aerosol products.
3) The potential hazards to children, especially those under 12 years old, are not adequately addressed in the draft RCD.

4) The upper bounds of exposure and the degree of DEET absorption appear to be underestimated.
5) Occupational risks are not estimated, despite the stated purpose of the draft RCD to do so.
6) The inclusion of reference exposure levels (RELs) in the RCD would be appropriate in order to compare health-based exposure levels with measured or estimated levels of exposure.

Assuming that the document was revised to address these concerns, the MOEs would be less, indicating an increased hazard potential. We are concerned about the potential for excessive exposures, particularly for products containing high levels of the active ingredient DEET. We are also concerned that the hazards from using 100 percent DEET containing products were not evaluated for the general population and particularly for children under 12.

Thank you for the opportunity to comment on the draft RCD for DEET. If you have any questions about our comments, please contact Dr. Michael J. DiBartolomeis or me at (510) 622-3170.

cc: Joan E. Denton, Ph.D., Director, OEHHA
    Val Siebal, Chief Deputy Director, OEHHA
    George V. Alexeeff, Ph.D., DABT, Deputy Director for Scientific Affairs, OEHHA
    Michael J. DiBartolomeis, Ph.D., PETS/OEHHA
    Chuck Andrews, Chief, WH&S/DPR

Attachment

Comments on the Draft Risk Characterization Document
for N,N-diethyl-m-toluamide

General Comments

The draft risk characterization document (RCD) for N,N-diethyl-m-toluamide (DEET) is well written. The document includes a summary of the rather extensive toxicological database for DEET. In the risk identification section, the toxicological data are adequately evaluated for relevance to the hazard DEET might pose to exposed humans. This was a difficult undertaking since DEET rarely produced any consistent toxicity across different exposure time frames and animal species. Based on the data, it can be concluded that DEET is as hazardous from acute exposure as from subchronic or chronic exposures based on the actual and derived no-observed-adverse-effect levels (NOAELs).

The exposure assessment portion of the draft RCD is too brief to allow a comprehensive review. Our evaluation included review of the document entitled "Human Exposure Assessment for DEET" (Sanborn, 1999), which was not provided with the draft RCD package. Even with this document prepared by the Worker Health and Safety Branch, it was difficult to follow the exposure assumptions and check calculations. We recommend providing more detail in the RCD on the exposure assessment, and that the Sanborn (1999) document be revised. (More comments follow on the separate exposure assessment document.)

DEET is a relatively low-toxicity pesticide and adverse reactions in humans are uncommon. However, DEET is unique among pesticides in being applied repeatedly, often at high concentrations, directly to human skin. It is, therefore, important to acknowledge in the RCD its potential for overuse, and the resulting possibility of toxic effects, particularly to children. This is addressed in the body of the RCD document, but we recommend that a discussion emphasizing this concern also be provided in the summary.

Dermal absorption appears to have been underestimated in the draft RCD. While whole-body (exposed surface) dermal absorption cannot be accurately determined from the available data, we suggest that 17 percent absorption is a more scientifically defensible mean absorption estimate than the value used (8.4 percent). Twice the estimated mean, or 34 percent dermal absorption, is probably a reasonable upper limit estimate for children. Conditions of the cited human study (Selim, 1991b) clearly underestimate potential dermal absorption under field conditions. A detailed explanation for this comment is provided under Specific Comments.

In addition, the range and population distribution of possible doses with repeated dermal applications is inadequately addressed. Despite the low toxicity of DEET, severe systemic toxic effects have resulted from dermal applications as noted in the "Illness Reports." The conditions or reasons for these overdoses should be more extensively discussed. These were generally repeated-dose applications in which DEET was applied several times, possibly over several days, to a significant portion of the body surface of a child. (See: Fradin, 1998; Qiu et al., 1998;

Brown and Hebert, 1997; Garrettson, 1997; Osimitz and Murphy, 1997; Osimitz and Grothaus, 1995; Veltri, 1994; Robbins and Cherniak, 1986.) The risk of an adverse reaction in this likely "worst-case" scenario should have been included in the hazard estimates and margins of exposure (MOE) calculations.

Major concerns posed in the draft RCD include special sensitivity in humans and potential synergistic toxic interactions of DEET with other commonly used chemicals. Special sensitivity in humans was described in the section "Illness Reports" but not discussed in the "Hazard Identification or Risk Appraisal" sections in any detail that could be developed into a specific recommendation for mitigation (e.g., additional warnings on the label). Similarly, the synergistic interactions of DEET were addressed in the "Toxicity" section with a substantial number of studies illustrating that the toxicities of other chemicals (often pesticides) can be potentiated by DEET. However, no mention of this was made again. Both of these effects suggest that DEET can be more hazardous under some conditions of normal use than would be estimated from the standard toxicity studies. We recommend that the impact of these conditions be factored into the human health risk assessment. If this is not possible based on the available scientific data, then some discussion of this limitation is needed in the technical summary and risk appraisal sections.

Many products containing DEET are sprays, applied by the individual to their clothing or skin. Therefore, it is not clear why inhalation exposure is not addressed in the draft RCD. There is likely to be an inhalation exposure component. If the exposure cannot be estimated, it should be addressed as a limitation and uncertainty in the technical summary and risk appraisal sections.

The inclusion of reference exposure levels (RELs) would be appropriate in order to compare health-based exposure levels with measured or estimated levels of exposure.

Specific Comments

The comments that follow are grouped according to the headings used in the draft RCD. Please note that although a comment may appear under a specific heading, its impact may not be limited to that specific section; it may have relevance to other sections of the draft RCD.

Summary

Page iii states that there is "no evidence of increased sensitivity in infants and children to DEET from available developmental and reproductive toxicity studies." This may be true for studies conducted in experimental animals. However, there is evidence of several cases of young children with acute toxicity resulting from a few topical applications of DEET-containing products as mentioned in the "Illness Reports" section. This may indicate increased children's sensitivity to DEET. Child cases of convulsions and death upon exposure to DEET mentioned in the Illness Reports section should be addressed and recognized in the Summary.

On page iv, it is written that "a review by the Hazard Evaluation Section of OEHHA is acknowledged." This is an obsolete name; it should be changed to "Pesticide and Environmental Toxicology Section." The relevance of the reference to the Adverse Effects Advisory Panel is also unclear.

I. Introduction

Page 2, I. D. Usage. It is stated "It was estimated that approximately 30% of the population used DEET-containing repellents in the last year." Please specify the year referred to.

Page 2, I. E. Illness Reports. This section identifies cases of human intoxication with DEET-containing products. Notably, cases of toxic encephalopathy and seizures and some deaths were reported. A significant number of the intoxications were of children under the age of 12. Although copious or excessive applications of DEET were attributed to be the cause of some intoxications, in one case, a five-year-old boy suffered a seizure after only two applications of DEET (95 percent concentration) in one day. In another case, a 61-year-old woman suffered a severe, short-duration illness after a single application of DEET. In neither case can the reaction be conclusively attributed to the DEET application, although no other potential precipitating events were identified. We recommend that more discussion on the exposure conditions that may lead to such adverse effects be included in this section and in the "Risk Characterization" section.

II. Toxicology Profile

A. Pharmacokinetics: Dermal Absorption. It is clear from this narrative that DEET is rapidly absorbed through skin, although there is uncertainty about relative amount of absorption in different skin areas and under different conditions.

Page 9, first paragraph. The human study of Selim (1991b) is noted as providing the human dermal absorption estimate of 8.4 percent, but no detailed evaluation of the study is provided. The data are also given in Selim et al. (1995), which is the source of details for these comments. For this study, ^{14}C-DEET was applied to the forearms of adult males from a 100 percent product or a 15 percent solution in ethanol. The application site was covered with a non-occlusive dome, and after eight hours the area was washed with isopropanol. Later the epidermis was sampled repeatedly by tape-stripping to assess surface skin reservoir of DEET. Urine and feces were assayed for ^{14}C. Most of the radioactivity was recovered in the isopropanol washes (a mean of 62 percent for undiluted DEET and 52 percent for the 15 percent DEET). Another 21 percent and 25 percent, respectively, was recovered from the dome, and 5 percent and 2.5 percent, respectively, from the application devices. No correction was applied for incomplete elimination of absorbed dose, although recovery losses (6 percent for undiluted DEET and 11 percent for 15 percent DEET) are greater than the estimated urine plus fecal elimination of 5.6 percent and 8.4 percent for the respective preparations. The earlier study of Feldman and Maibach (1970) demonstrated a 52 percent elimination recovery after dermal administration of DEET, compared to recovery after intravenous administration.

We believe that the 8.4 percent value from the Selim study (1995) significantly underestimates the potential for dermal absorption of DEET for the following reasons:

1) The isopropanol wash at eight hours may have more efficiently removed DEET than a standard soap and water wash; in addition, some application periods for outdoor uses may last much longer than eight hours. Therefore, the initial absorption period in normal use can be significantly prolonged compared to the test conditions, resulting in enhanced absorption.

2) Skin sites vary in permeability. In a monkey study, dermal penetration of DEET on the forearm was least among the four sites studied (14 percent on forearm, 33 percent on forehead, 68 percent on the ventral forepaw, and 27 percent on the dorsal forepaw). Variations among human sites have been shown to be similar.

3) Skin permeability is generally greater under hot, humid conditions (where mosquitoes are likely to be contacted), whereas the study was conducted in a medical clinic under controlled environmental conditions.

4) Lack of a correction for percentage of dose collected in urine likely underestimates the absorption by half. This corresponds to the corrected absorption value of 16.7 percent for DEET calculated by Feldman and Maibach (1970) (applied to the forearm, under laboratory conditions).

Considering all of the factors involved, we believe that the Feldman and Maibach (1970) value represents a moderate to low absorption estimate. This value appears more defensible than the estimate from the Selim (1995) study, although the former probably underestimates the potential for dermal absorption of DEET by children in a hot, humid environment. We propose the use of a default "mean" dermal absorption value of 17 percent (rounded) and an upper-limit value of twice that amount, or 34 percent. Alternatively, calculations based on the range of absorptions reported by Robbins and Cherniak (1986) (9 percent to 56 percent, with a mean of 17 percent) could be appropriate. Acute, seasonal, and chronic exposures should be calculated for the upper-limit absorption estimates as well as the mean values because exposure conditions are likely to be consistent with repeated uses for any given individual.

No discussion on inhalation absorption was included in the draft RCD. It is not clear whether that is because there is no information, it was an oversight, or the analysis was omitted for scientific reasons. Inhalation of DEET through aerosols is likely to be a significant source of exposure. The limited discussion on the potential for DEET inhalation exposure in Sanborn (1999) provides a good starting point for a discussion of DEET inhalation in the RCD. We recommend that the exposure assessment (Sanborn 1999) and the RCD be revised to include more information, and if appropriate, more analysis of this potential source of exposure.

B. Acute Toxicity

The numerous toxicity studies conducted on DEET reveal relatively high lowest-observed-adverse-effect-levels (LOAELs), about 1,000 mg/kg by the oral route. Toxicities include prostration, ataxia, and tremors. The LOAEL for systemic effects after dermal exposure was

1,800 mg/kg based on lethargy, extended rear limbs, and hematoma. This was comparable to the LOAEL for acute dermal irritation (about 2,000 mg/kg).

Synergistic interactions of DEET with other chemicals are addressed in a separate section (pages 19 to 23) and are a cause for concern. It appears that interactions may occur among some of the active ingredients in DEET products formulated with other chemicals, as well as with separate products used concurrently with DEET. However, the potential toxicological consequences of such interactions are not addressed in the draft RCD. We recommend providing a relevant discussion in this section as well as in the "Risk Characterization" section.

C. Subchronic Toxicity

The notable findings for subchronic experimental exposures to DEET are male rat nephropathy, decreased kidney weight, increased liver weight, and decreased body weight. The rat nephropathy was judged to be associated with α_{2u}-globulin accumulation, and thus not relevant human health, since humans do not produce α_{2u}-globulin.

Decreased kidney weight in dogs was judged to be an adverse effect. The increased liver weights were observed in a number of studies without concurrent evidence of histopathological changes, and were assumed to be "an adaptive response rather than an adverse effect" (page 24, first paragraph). Body weight decrease was observed after multiple routes of administration, and was considered to be an adverse effect. The lowest LOAEL by the oral route was 200 mg/kg-day for dogs and 300 mg/kg-day for rats by the dermal route. For both studies, the no-observed-adverse-effect-level (NOAEL) was 100 mg/kg-day. The LOAEL for dermal irritation was 100 mg/kg day. These results mirror the range of sensitivity to DEET that was exhibited in the chronic studies.

The computation of the high dose level for the Goldenthal subchronic diet dog study (1995) is problematic. In this study, the high dose group of 6,000 ppm rejected ingestion of the DEET diet, which was subsequently reduced several times in an attempt to accommodate this rejection. However, the reduction in concentration did not solve the problem. The result was that the highest dose group exhibited the most toxicity, but the average daily dose over the eight-week study is less than the next highest dose. It is likely that the toxicity exhibited in this high-dose group is the result of the initial ingestion of the high dose rather than the average dose received during the exposure period. Thus, it does not appear appropriate to list a "LOEL" (lowest-observed-effect-level) of 12 mg/kg-day and "NOEL" (no-observed-effect-level) of 92 mg/kg-day for the same study in this section (page 29) and in the "Hazard Identification" section (Table 18, page 55). We recommend that the RCD not include any conclusion about the dose-response of DEET from the results derived from the highest dose group in this study.

D. Chronic Toxicity

The sensitivity for adverse effects from chronic oral or dermal exposures to DEET in experimental animals was similar to that seen with subchronic exposures. No evidence of DEET-related increases in tumor formation was found. An increase in hepatic hyperplastic nodules and bile duct hyperplasia observed in mice treated with 1,000 mg/kg-day was judged to

be treatment- related and to be a potential preneoplastic event. Hyperplastic liver nodules are a rather common pathology in older mice. It would be helpful if a discussion of any evidence that this increased nodule incidence is within the range of incidence in historical control populations was included in the RCD.

It is not clear why rat renal nephropathy for tumor formation is discussed in the "Summary" for this section when it is not addressed in the individual study summary which is found later in this section.

From both chronic rat and dog oral studies a NOAEL of 100 mg/kg can be identified. A transient increase (observed at interim sacrifice periods of 6, 12, and 18 months but not at study termination) in female rat cholesterol levels was noted at the 400 mg/kg dose and attributed to DEET exposure. From the chronic dog study, a LOAEL was identified based on changes to hemoglobin levels, hematocrit, liver, lymph nodes, and uterus at 400 mg/kg.

F and G. Reproductive and Developmental Toxicity

From the several reproductive studies conducted on DEET, the most reproducible effect was an increased incidence of abnormal sperm; the "NOEL" for this effect was judged to be less than 100 mg/kg-day. It is not clear if the increase in sperm abnormalities was in number or in type or both. Slight increases in anomalies and changes in fetal and maternal body weights appeared within the same dose range as the effects noted in other studies.

III. Risk Assessment

A. Hazard Identification

For the acute local (dermal irritation) risk assessment, we agree with the selection of the most appropriate study and endpoint for the LOAEL/NOAEL determination in the draft RCD. However, for the acute systemic risk assessment, we do not agree with the selection of the most appropriate study and endpoint for the LOAEL/NOAEL determination in the draft RCD. We realize that there are several factors used when selecting appropriate toxicity endpoints. However, the criteria used in the draft RCD for selecting the appropriate studies were not clearly delineated. The appropriate studies for determining the NOAEL/LOAELs would be those in which DEET was administered dermally, which is the predominant exposure route. The draft RCD used oral studies for risk determination. However, the rationale or explanation for selecting oral studies is not clear or adequately presented in the draft RCD. Furthermore, it appears that the draft RCD places its emphasis on studies that meet Federal Insecticide, Fungicide, and Rodenticide (FIFRA) requirements, while excluding from risk assessment those that did not.

We recommend that the selection of the critical study for risk determination for acute systemic effects be reconsidered. The draft RCD concludes that the single dose rat gavage study (Schardein, 1989) is more suitable for acute risk determination than the developmental, reproductive, or dermal toxicity studies listed under acute effects (Table 17). The criterion appears to be whether or not a study met FIFRA requirements. For acute systemic effects, an oral rat study was selected in which the "NOEL" was 900 mg/kg (adjusted for pharmacokinetic

considerations from a "NOEL" of 200 mg/kg). Among the dermal studies, the results from one study indicates an increase in preimplantation loss (this is a systemic effect which is interpreted as a potential acute effect apparently following internal DPR policy) at a LOAEL of 100 mg/kg. This rat dermal developmental toxicity study (Gleiberman et al., 1975) was not used because, according to the draft RCD, it did not meet FIFRA requirements. The estimated NOAEL from this study would be 10 mg/kg after applying an uncertainty factor of 10 to the LOAEL of 100 mg/kg-day (without route adjustment factor). Similarly, from another oral developmental study in rats (Sterner, 1977), a NOAEL of 90 mg/kg (adjusted from a NOAEL of 20 mg/kg) could be used. The problem with the selection criterion used in the draft RCD is that it excludes a body of data that indicate the toxic effect occurs at lower doses. The exclusive use of the data from the Schardein (1989) study is not the best scientific or public health approach for this risk assessment. Therefore, we recommend that the revised RCD reassess the body of evidence and reconsider the use of the higher adjusted NOAEL of 900 mg/kg when lower NOAELs have been identified. We also recommend that the selection criteria for the critical studies and toxicological endpoints be clearly delineated in the RCD. If the higher dose oral study is to be used, there should be some scientific explanation in the RCD for not using the lower dose oral or dermal studies.

The oral/dermal dose-route correction that has been used (a factor of 4.5) is specific for rats. The relative areas under the plasma concentration curve after oral and dermal exposures are a function of both absorption and metabolism rates. No evidence has been presented to suggest that the ratio of these two rates is the same in humans as it is in rats. We expect that it would not be. Further examination and discussion of these issues would be needed before this general assumption should be accepted for this risk assessment.

The subchronic studies reveal a wider range of systemic effects, but the comparable LOAELs were not much lower than those observed in the acute studies. Effects were mostly of minor toxicological importance, such as changes in clinical chemistry, slight histopathological changes in the kidney, and signs of increased liver metabolic activity. Body weight reduction was common, which was at least partly attributable to decreased food intake. The critical NOAEL for subchronic exposure (which was used for the seasonal exposure risk assessment) was based on a dermal study by Johnson (1997) with a NOAEL of 300 mg/kg-day, and when adjusted for 40 percent dermal absorption, resulted in an absorbed dose of 120 mg/kg for systemic effects. We agree that dermal studies should be used whenever possible and support using this determination to arrive at a subchronic NOAEL.

The estimated NOAEL of 10 mg/kg for dermal irritation from the subchronic exposure study is the same as from the acute exposure study. Both were estimated from a LOAEL 100 mg/kg, divided by 10 to derive a NOAEL from a LOAEL (see page 57). We agree with this determination.

The NOAEL determination for chronic exposure was similar to that of the subchronic exposure. Unadjusted NOAELs for several studies were about 100 mg/kg-day. Thus, there was little evidence for increased severity upon prolonged dosage with DEET. Although we may agree that DEET may not severely impact major organ systems upon prolonged exposure, we do feel that

there may be limitations inherent in the chronic experimental studies that do not address major neurological effects seen upon human exposure to DEET.

Since there was no evidence of carcinogenicity, and genotoxicity studies were negative except for one dominant lethal assay with equivocal results (page 59, second paragraph), no carcinogenic assessment was conducted. We agree with this determination.

More consistency in presenting information in Tables 17, 18, and 19 would be helpful. It is difficult to compare the dermal doses when one is presented in mg/cm^2 and another on a body weight basis. When computing the MOEs later it is clear that applied oral and dermal doses are converted to absorbed doses (as described in the narrative on page 54). It would be helpful if these summary tables included the adjusted absorbed doses so appropriate comparisons among dermal and oral studies, for acute, subchronic, and chronic studies can be made.

Page 55 and "Reference" section. There is an improper reference to Dourson and Strata; it is Dourson and Stara.

B. Exposure Assessment

The draft RCD refers to the exposure assessment entitled "Human Exposure Assessment for DEET" (Sanborn, 1999), which was not submitted for our review and which we obtained from the Worker Health and Safety Branch with a significant delay. The exposure assessment is a critical part of any RCD, which in the past has been attached to the RCD as an appendix. In general, we recommend that the draft RCD be revised to provide more details on the assessment of exposure in the main document. Attaching the exposure assessment document would also enhance the RCD and make it easier to follow.

We are concerned that the separate exposure assessment document (Sanborn, 1999) was finalized without OEHHA review. We submit the following comments on the draft RCD as well as the separate exposure assessment document with the expectation that the exposure assessment will be revised, as appropriate, based on our comments.

The mean and upper limit values for the daily exposure estimate for DEET is derived from a public survey funded by the registrant (Boomsma and Parthasarathy, 1990). The upper end estimates for acute dermal doses were assumed to be three times the average amount applied during a single application. The estimates of seasonal exposure are based on average exposures, applied only "7.5" times. Although this is a mathematical average, there is no practical means to apply a partial dose of DEET. Therefore, we recommend that reference to frequency of applications be presented in whole numbers (e.g., an average of seven or an average of eight applications). In addition, this seasonal exposure estimate does not include the range of exposures among normal users, and does not address the observed toxicity problem caused by repeated heavy usage of DEET. The user surveys conducted by Boomsma and Parthasarathy (1990) provide information on the distribution of use patterns, which should be included in the exposure assessment and considered in the RCD.

Slow absorption through the dermal pathway may contribute to cumulative toxicity. Based on information on the distribution of cumulative doses over a few days or a use season, particularly for children, an estimate of the potential for adverse effects could be produced. We recommend that the exposure assessment and the draft RCD be revised to include an appropriately detailed discussion of the patterns of use that have resulted in toxic effects.

Pages 59 to 60, Non-occupational exposures. The exposure estimates in Sanborn (1999), derived from the registrant-sponsored studies described in Boomsma and Parthasarathy (1990) appear to contain inconsistencies. Average daily exposures are listed as 0.038 grams/day in Table 11 and 0.65 to 1.07 grams/day in Table 13, with no explanation of the difference. Reference to the original tables of Boomsma and Parthasarathy (1990) did not clarify the problem. However, the higher values (~ 1 gram/day) appear to be representative of moderately high daily dermal exposures, by comparison with the estimated occupational dermal exposure of 4.25 grams/day for workers in the Florida Everglades that was provided in Table 17 of Sanborn (1999). Although it is acceptable to apply these non-occupational dermal exposure values to the draft RCD dose estimates in Table 20, page 60, we recommend that the conditions that result in these exposure estimates be better defined in the documents.

For the calculation of Seasonal Applied Daily Dose (SADD), using only the average seasonal usage of "7.5" applications does not address the issue of potential over-exposures, and the resulting toxicity. We recommend including more information and discussion of the distribution of uses under different conditions.

We agree in general that conditions similar to those in the Everglades would be infrequent in California, as stated on page 18 of the exposure assessment document (Sanborn, 1999). However, some estimate of the range and distribution of exposures to DEET, which are relevant to California, should be derived. Use of three applications in one day (for the acute exposure estimate) as the only measure of high-end exposures is clearly inadequate for the assessment of possible (and likely) applications. High-end dose estimates should be provided for Annual Applied Daily Dose (AADD) as well as for SADD. These dose estimates should consider the variability in absorption (as discussed above), the variability in application rates (amount per application), and the variability in number of applications per season and per year. This will presumably result in some MOEs much less than 100, particularly for children. The relevance of these high-end exposure estimates to the reported toxic effects should be discussed, and the conclusions of the risk characterization for children should be carried over to the "Summary" section.

The first paragraph of the draft RCD Summary (page ii) states that exposures of park and forestry workers are to be considered. However, we could not find any discussion in the draft RCD of the potential for health risks to adults who by occupation would be more likely to use DEET (e.g., park and forestry workers). Exposures in some classes of outdoor workers exceed the 7.5 applications per season used for the seasonal exposure estimate. A complete exposure and risk assessment would include consideration of the expected high-end exposures in this group.

Human Exposure Assessment (Sanborn, 1999)

This document contains essential elements pertinent to the draft RCD. We have read the human exposure assessment as well as documents relating to exposure assessment published by Selim (1991a,b; 1992) and Boomsma and Parthasarathy (1990) and have the following comments.

We have observed previously that upper-bound exposure estimates need to be included for all time and exposure scenarios. The minimal consideration of variability in uses of DEET, including the use of high-concentration products, fails to address the potential for over-exposure to DEET. In consideration of the available data, it should be noted that the study of Boomsma and Parthasarathy (1990) does not specifically state whether participants counted re-applications in a day as individual applications or as one collective application. However, this publication does provide some data (Table 50) on which to base estimates of high-end exposures. It appears, for example, that about 8 percent of adult men and 5 percent of children of the ages 12 and under applied more DEET to their skin in a single application (greater than 3 grams) than assumed for the high-end acute exposure estim ates in the draft RCD (page 60, Table 20). The estimate of total seasonal use included in the draft RCD is only about twice these single high-end exposures, with no estimate of variability or distribution of uses. The draft RCD therefore does not address the use patterns that have resulted in the known toxic events.

We recommend that estimates of high-end seasonal exposures be included for both children and occupational use patterns. These estimates might have to be based on exposure scenario assumptions if no further data are available. Such scenarios might include a summer vacation in a mosquito area for children, based on average vacation length. An occupational scenario might include one month of exposure at the rates of DEET usage observed in the Florida Everglades (Sanborn, 1999, page 18). The resulting MOEs will be much lower than presently stated in the draft RCD.

C. Risk Characterization

The derived MOEs for various populations were presented. The lowest MOE for acute systemic effects was 83, for children less than 12 years old. The lowest MOE for local effects (dermal irritation) was 14 for children under a high use acute exposure. If our recommendations for NOAEL selections were adopted, the MOEs would be lower.

It is not clear how the acute dermal irritation MOE was calculated from the NOAEL, which was provided only in terms of mg/cm^2 Inclusion of the calculation and assumptions used would be helpful.

IV. Risk Appraisal

The uncertainties and limitations associated with this risk assessment are not adequately addressed in this section, particularly with respect to exposure-derived uncertainties. There is no separate section addressing scientific uncertainties as has been included in recent documents. If our recommendation for more discussion of upper-end exposures is followed (particularly

regarding occupational exposures), the uncertainties in these estimates should be addressed in this section.

The following comment relates to the statement on page 64 that "Data from the usage survey indicate that children less than 12 years old receive as much DEET per application as [an] adult male on average regardless of the difference in their body weight or surface area. Since this seems illogical, exposure estimates for adult females, juveniles (ages 12-17), and children (less than 12 years old) were also estimated by adjusting the adult male dermal dosage...." In the Boomsma and Parthasarathy study (1990), it was clear that juveniles under 12 were more likely to be sprayed with DEET by a parent. It was reported that parents were prone to over-apply DEET to their children. The data are from real applications and should not be dismissed or discounted. Therefore, we recommend that this alternate exposure estimation be dropped from the draft RCD and from the separate exposure assessment document.

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Department of Pesticide Regulation

dpr

Paul E. Helliker
Director

MEMORANDUM

Gray Davis
Governor

Winston H. Hickox
*Secretary, California
Environmental
Protection Agency*

TO: Chuck Andrews, Chief
                   Worker Health and Safety Branch

FROM: James R. Sanborn, Staff Toxicologist **[original signed by J. Sanborn]**
                   Worker Health and Safety Branch
                   (916) 445-4262

DATE: March 14, 2000

SUBJECT: RESPONSE TO OEHHA's COMMENTS ON DEET EXPOSURE
                   ASSESSMENT

Page/Paragraph

1/4 Underestimation of dermal absorption

Dermal absorption appears to have been underestimated in the draft RCD. While whole-body (exposed surface) dermal absorption cannot be accurately determined from the available data, we suggest that 17 percent absorption is a more scientifically defensible mean absorption estimate than the value used (8.4 percent). Twice the estimated mean, or 34 percent dermal absorption, is probably a reasonable upper limit estimate for children. Conditions of the cited human study (Selim, 1991b) clearly underestimate potential dermal absorption under field conditions. A detailed explanation for this comment is provided under Specific Comments.

WHS Response: The exposure assessment author disagrees with OEHHA that the exposure assessment used a value for dermal absorption that was too low. First, the exposure time of the Selim study (8 hours) appears to be more relevant to the use characteristics of this repellent than the 24 hour exposure time of the Feldmann and Maibach 1970 study. Secondly, the reviewers did not take into account the high vapor pressure (0.22 Pa) of this repellent, which is critical to its activity as a repellent. This will significantly reduce any dermal absorption value measured under carefully controlled laboratory conditions. Thirdly, the primary selection criterion for use of the contemporary study by Selim (1991b) over that of Feldmann and Maibach 1970, was the amount and quality of the experimental detail. It is preferable to have a well-conducted contemporary study with extensive description experimental design, as the source of the dermal absorption value for use in the exposure assessment. Finally, neither these studies were conducted indoors, mirror the conditions that exist for individuals that use DEET in an outdoor environment. DEET will volatilize more rapidly in an outdoor environment thus in less resulting in less absorption than would occur in any indoor study.

As for the use of a high-end dermal absorption value (suggested above, 34 percent) the exposure assessor is unaware of any precedence either in a regulatory context or in the open, peer-reviewed literature on exposure assessment. OEHHA's recommendation to use two times the mean of 17 percent does not take into account the variability of the dermal absorption data for DEET. If it is necessary use a high-end dermal absorption value for exposure assessment, it is much more technically defensible to use the complete data set to estimate a high-end value (in this case dermal absorption).

1/5 Inadequate discussion of multiple exposures and illnesses

In addition, the range and population distribution of possible doses with repeated dermal applications is inadequately addressed. Despite the low toxicity of DEET, severe systemic toxic effects have resulted from dermal applications as noted in the "Illness Reports." The conditions or reasons for these overdoses should be more extensively discussed. These were generally repeated-dose applications in which DEET was applied several times, possibly over several days, to a significant portion of the body surface of a child. (See: Fradin, 1998; Qiu et al., 1998; Brown and Hebert, 1997; Garrettson, 1997; Osimitz and Murphy, 1997; Osimitz and Grothaus, 1995; Veltri, 1994; Robbins and Cherniak, 1986.) The risk of an adverse reaction in this likely "worst-case" scenario should have been included in the hazard estimates and margins of exposure (MOE) calculations.

WHS Response: OEHHA provided several references to support their view, that a need exists to address scenarios of overuse/over exposure that may lead to adverse health outcomes especially in children. In these articles, the precise dose delivered to the individual is not always known nor is the timeframe well documented. Because of the absence of dose and/or timing information, it is not possible to develop defensible MOEs for these cases.

Generally, DPR does not estimate exposures from small sample sizes (as described in several of these articles) for the purpose of an MOE derivation. Rather, they originate from animal toxicology studies with large number of animals or human studies with sufficient replicates where exposures have been measured or estimated with some certainty. Finally, it is difficult to summarize the information in these articles into "worst-case" exposure estimates and perhaps MOE values.

2/2 Inhalation exposure
Many products containing DEET are sprays, applied by the individual to their clothing or skin. Therefore, it is not clear why inhalation exposure is not addressed in the draft RCD. There is

likely to be an inhalation exposure component. If the exposure cannot be estimated, it should be addressed as a limitation and uncertainty in the technical summary and risk appraisal sections.

WHS Response: DPR agrees that inhalation of DEET during use of aerosol products could be a route of exposure. However, DEET-specific data or even appropriate surrogate data do not exist either in the published literature or in documents submitted by the registrants of DEET to address inhalation exposure during and after application. Further DEET aerosol product labels admonished against direct application of DEET to skin or clothing surfaces proximal to the **breathing zone. Rather, the labels suggest that** spray formulations should be applied to the hands, which then are used to apply DEET to other regions of the body.

Interestingly, two other regulatory agencies, Health Canada and the U.S. EPA have not addressed the issue of inhalation exposure as they recognized the difficulty of assessment without DEET-specific data. Finally, in animal toxicology studies treated dermally, it is likely that both an inhalation and oral route of exposure exist. Th latter because of the cleaning and preening laboratory animals do as apart of normal behavior.

Specific Comments

4/1 II Toxicology Profile: Dermal absorption
4/ following: We believe that the 8.4 percent value from the Selim study (1995) significantly underestimates the potential for dermal absorption of DEET for the following reasons:

1) The isopropanol wash at eight hours may have more efficiently removed DEET than a standard soap and water wash; in addition, some application periods for outdoor uses may last much longer than eight hours. Therefore, the initial absorption period in normal use can be significantly prolonged compared to the test conditions, resulting in enhanced absorption. Given the very small amounts of DEET that have been applied and the amount of wash solution applied, one could speculate that both IPA or soap and water would be equally effective in washing off residues of this repellent.

WHS Response: The reviewers suggest, without a reference to support their supposition, that IPA may be more efficacious that aqueous soap and water in removing DEET from the skin. Without data to support the concern of the reviewers, it is inappropriate to speculate whether soap or IPA would be more effective in removing the residues. Further, given the water solubility of DEET, it is likely that surfactant-water mixtures could be as effective as IPA in the removal of surface residues that were about 15 mg at the highest dose.

2) Skin sites vary in permeability. In a monkey study, dermal penetration of DEET on the forearm was least among the four sites studied (14 percent on forearm, 33 percent on forehead, 68 percent on the ventral forepaw, and 27 percent on the dorsal forepaw). Variations among human sites have been shown to be similar.

WHS Response: The author of exposure assessment is aware of the differential absorption rates of different regions of the human or primate body. However, the application of this knowledge about differential absorption rates on various anatomical regions to refine the exposure assessment is unclear.

3. Skin permeability is generally greater under hot, humid conditions (where mosquitoes are likely to be contacted), whereas the study was conducted in a medical clinic under controlled environmental conditions.

WHS Response: The author is aware that several environmental variables affect skin permeability. However, to conduct a study outdoors rather then indoors to generate data for use in an exposure assessment would not allow the control of as many of the experimental variables as possible. Control of the experimental variables during the dermal absorption study reduces the potential for extreme variability in the study. While humidity certainly is a known factor known to affect the dermal absorption of pesticides through skin. However, designing an experiment to generate data useful to the regulatory process that takes account of this variable is no straightforward. Further, to address the concerns of OEHHA about the role of humidity in dermal absorption of DEET would require multiple studies with differing humidity. Research experiments of this type are not generally conducted for use in a regulatory context.

4. Lack of a correction for percentage of dose collected in urine likely underestimates the absorption by half. This corresponds to the corrected absorption value of 16.7 percent for DEET calculated by Feldmann and Maibach (1970) (applied to the forearm, under laboratory conditions).

WHS Response: Table 5 in the exposure assessment document indicates that the material balance for the undiluted and ethanol-diluted studies had ^{14}C-material balances of 94.34% and 88.74%, respectively. Examination of the urinary excretion curve for ^{14}C metabolites shown

below indicates that elimination is rapid and occurs within 24 hours regardless whether it is formulated in ethanol or administered neat.

DEET Absorption in Humans
Neat and 15% in Ethanol
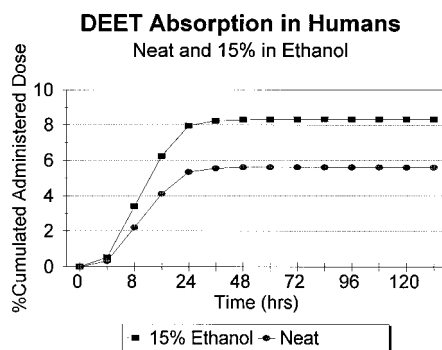
Given the excellent material balances observed in the studies and plateauing of the urinary excretion curves at 24 hours, it is difficult to justify a higher value for the dermal absorption. For comparison other regulatory agencies, Health Canada used a dermal value of 7.5 percent. The U.S. EPA used a value of ~20 percent which they obtained by adding the unaccounted radioactivity in the ethanol-diluted study to the total amount of recovered radioactivity. Given this high vapor pressure of DEET, the U.S. EPA dermal absorption value does not appear to be justifiable as no literature or regulatory precedence exists suggesting that the unaccounted radioactivity should be added to the to the urine, feces, carcass to arrive at a dermal absorption value. Conference call discussions with U.S. EPA have not clarified their position on this issue.

4/1 Considering all of the factors involved, we believe that the Feldman and Maibach (1970) value represents a moderate to low absorption estimate. This value appears more defensible than the estimate from the Selim (1995) study, although the former probably underestimates the potential for dermal absorption of DEET by children in a hot, humid environment. We propose the use of a default "mean" dermal absorption value of 17 percent (rounded) and an upper-limit value of twice that amount, or 34 percent. Alternatively, calculations based on the range of absorptions reported by Robbins and Cherniak (1986) (9 percent to 56 percent, with a mean of 17 percent) could be appropriate. Acute, seasonal, and chronic exposures should be calculated for the upper-limit absorption estimates as well as the mean values because exposure conditions are likely to be consistent with repeated uses for any given individual.

WHS Response: The use of upper end dermal absorption values during the assessment of absorbed dose is not the practice currently used by the Department of Pesticide Regulation. Further it is not the general procedure of two other regulatory agencies, Health Canada or the

U.S. EPA. Finally, the certainty of upper end dermal absorption values are less certain than those at the central tendency.

4/3 No discussion on inhalation absorption was included in the draft RCD. It is not clear whether that is because there is no information, it was an oversight, or the analysis was omitted for scientific reasons. Inhalation of DEET through aerosols is likely to be a significant source of exposure. The limited discussion on the potential for DEET inhalation exposure in Sanborn, (1999) provides a good starting point for a discussion of DEET inhalation in the RCD. We recommend that the exposure assessment (Sanborn, 1999) and the RCD be revised to include more information, and if appropriate, more analysis of this potential source of exposure.

WHS Response: This was addressed earlier in the general comment section; no data exist to estimate inhalation exposure of DEET either during or after application.

B. Exposure Assessment

8/3 The draft RCD refers to the exposure assessment entitled, "Human Exposure Assessment for DEET" (Sanborn, 1999), which was not submitted for our review and which we obtained from the Worker Health and Safety Branch with a significant delay. The exposure assessment is a critical part of any RCD, which in the past has been attached to the RCD as an appendix. In general, we recommend that the draft RCD be revised to provide more details on the assessment of exposure in the main document. Attaching the exposure assessment document would also enhance the RCD and make it easier to follow.

We are concerned that the separate exposure assessment document (Sanborn, 1999) was finalized without OEHHA review. We submit the following comments on the draft RCD as well as the separate exposure assessment document with the expectation that the exposure assessment will be revised, as appropriate, based on our comments.

WHS Response: No response is required.

8/6 The mean and upper limit values for the daily exposure estimate for DEET is derived from a public survey funded by the registrant (Boomsma and Parthasarathy, 1990). The upper end estimates for acute dermal doses were assumed to be three times the average amount applied during a single application. The estimates of seasonal exposure are based on average exposures, applied only "7.5" times. Although this is a mathematical average, there is no practical means to apply a partial dose of DEET. Therefore, we recommend that reference to frequency of applications be presented in whole numbers (e.g., an average of seven or an average of eight applications). In addition, this seasonal exposure estimate does not include the range of

exposures among normal users, and does not address the observed toxicity problem caused by repeated heavy usage of DEET. The user surveys conducted by Boomsma and Parthasarathy (1990) provide information on the distribution of use patterns, which should be included in the exposure assessment and considered in the RCD.

WHS Response: The author re-examined the relevant tables (17-24, 50) in Boomsma and Parthasarathy 1990 to see whether data existed to address reviewer's concern about that "upper end" exposure scenarios. Because they are summary tables, the data are not useful. Without the individual data values that were used to construct these tables, it is not possible to develop the types of upper end exposure scenarios suggested by reviewers.

9/1 Slow absorption through the dermal pathway may contribute to cumulative toxicity. Based on information on the distribution of cumulative doses over a few days or a use season, particularly for children, an estimate of the potential for adverse effects could be produced. We recommend that the exposure assessment and the draft RCD be revised to include an appropriately detailed discussion of the patterns of use that have resulted in toxic effects.

WHS Response: This concern is not supported by the user survey data where an average of 7.5 applications occurred in the heavy use season over a period of several months. The average frequency of use over a 60-day use season results in an application every ~7-8 days. Further, from the previous graph on elimination in the urine, it appears that the $t_{1/2}$ is less than 24 hours. Assuming the half-life is about 12 hours after dermal application, then 16 half-lives will have elapsed between applications 7-8 days apart. It appears then that a small body burden would exist at the time of another application. After 16 half-lives, 0.0015 percent would remain as an absorbed dose. It appears then that a small body burden exists at the time of another application. Clearly, at the next dose, the small amount remaining from the previous application would be inconsequential.

With respect to the inclusion of detailed discussion of patterns of use that have resulted in toxic effects, normal procedure in exposure assessments is to use the information in our Pesticide Illness Surveillance Program (PISP) which focuses primarily illnesses in agricultural settings. Literature data may be used to supplement this information. Generally, it has been our experience that literature information may not provide the detailed information on dose and timing that is essential to quantify patterns of pesticide use that result in adverse outcomes. Illness reports for the PISP have a standardized format that allows much better characterization of the relationship between exposure and illness. Literature reports of illnesses after pesticide exposure do not follow a standardized format.

9/2 Pages 59 to 60, Non-occupational exposures. The exposure estimates in Sanborn (1999), derived from the registrant-sponsored studies described in Boomsma and Parthasarathy (1990) appear to contain inconsistencies. Average daily exposures are listed as 0.038 grams/day in Table 11 and 0.65 to 1.07 grams/day in Table 13, with no explanation of the difference. Reference to the original tables of Boomsma and Parthasarathy (1990) did not clarify the problem. However, the higher values (~ 1 gram/day) appear to be representative of moderately high daily dermal exposures, by comparison with the estimated occupational dermal exposure of 4.25 grams/day for workers in the Florida Everglades that was provided in Table 17 of Sanborn (1999). Although it is acceptable to apply these non-occupational dermal exposure values to the draft RCD dose estimates in Table 20, page 60, we recommend that the conditions that result in these exposure estimates be better defined in the documents.

WHS Response: Estimates of occupational exposure were not the focus of the WHS exposure assessment for several reasons. First, California does not have the climate of Florida or the rest of the Gulf Coast where humidity and high temperatures combine to serve as an ideal environment for mosquitoes and therefore extensive mosquito control is required. It is expected that workers in these areas would use large amounts of DEET during the control programs. While mosquito abatement occurs in California, state-specific exposure assessment data to base an exposure assessment do not exist. Secondly, the data cited in the exposure assessment were summary data without sufficient background information to justify making a detailed exposure assessment. Without more detailed information about the source of the Everglades military occupational exposure data, it is inappropriate to make definitive, quantitative statements about occupational exposures in California.

9/3 For the calculation of Seasonal Applied Daily Dose (SADD), using only the average seasonal usage of "7.5" applications does not address the issue of potential over-exposures, and the resulting toxicity. We recommend including more information and discussion of the distribution of uses under different conditions.

WHS Response: Sufficient information does not exist to address the concern in the above paragraph. Additionally, previous discussion characterized the rationale as for not addressing over exposures.

9/4 We agree in general that conditions similar to those in the Everglades would be infrequent in California, as stated on page 18 of the exposure assessment document (Sanborn, 1999). However, some estimate of the range and distribution of exposures to DEET, which are relevant to California, should be derived. Use of three applications in one day (for the acute exposure estimate) as the only measure of high-end exposures is clearly inadequate for the assessment of possible (and likely) applications. High-end dose estimates should be provided for Annual Applied Daily Dose (AADD) as well as for SADD. These dose estimates should consider the

variability in absorption (as discussed above), the variability in application rates (amount per application), and the variability in number of applications per season and per year. This will presumably result in some MOEs much less than 100, particularly for children. The relevance of these high-end exposure estimates to the reported toxic effects should be discussed, and the conclusions of the risk characterization for children should be carried over to the "Summary" section.

WHS Response: Estimates of high-end seasonal and annual exposures do not appear to be justifiable given the type of data in the use survey. Further, it seems unlikely and improbable that every time an application occurs that it would be at the high end thus resulting in exposures that might cause an adverse health reaction. If the survey data were in a format that could be examined by a probabilistic analysis, it might be possible to examine some of the concerns raised in the above paragraph. However, the data existed in a summary display, which does not allow the type of analysis that the reviewers deem necessary to ensure the safety of children exposed to excessive amounts of DEET.

10/2 We have observed previously that upper-bound exposure estimates need to be included for all time and exposure scenarios. The minimal consideration of variability in uses of DEET, including the use of high-concentration products, fails to address the potential for over-exposure to DEET. In consideration of the available data, it should be noted that the study of Boomsma and Parthasarathy (1990) does not specifically state whether participants counted re-applications in a day as individual applications or as one collective application. However, this publication does provide some data (Table 50) on which to base estimates of high-end exposures. It appears, for example, that about 8 percent of adult men and 5 percent of children of the ages 12 and under applied more DEET to their skin in a single application (greater than 3 grams) than assumed for the high-end acute exposure estimates in the draft RCD (page 60, Table 20). The estimate of total seasonal use included in the draft RCD is only about twice these single high-end exposures, with no estimate of variability or distribution of uses. The draft RCD therefore does not address the use patterns that have resulted in the known toxic events.

WHS Response: Earlier commentary addressed the summary format of the data (Tables 17-20, 50) which does not allow a quantitative estimation of the quantitative frequency of multiple applications. Further, the criticism that "**the study does not specifically state whether participants counted re-applications in a day as individual applications or as one collective application**" may be valid but the exposure assessor did not review the use survey prior to implementation. It is unjustified to critique a survey, because it does not report the data in a format to address a concern of the reviewers. It is important to point out that the DEET-use survey provided more information on a consumer product than exists for any other similar product.

With respect to the estimation of high-end exposures that may lead to adverse health outcomes, as iterated several times previously, insufficient, quantitative information exists in the user survey to make definitive and technically supportable estimates of exposure. Further, literature reports of adverse health outcomes from use of this repellent do not provide the type and form of data that allows discussion of the application amounts and timing that result in illness.

10/3 We recommend that estimates of high-end seasonal exposures be included for both children and occupational use patterns. These estimates might have to be based on exposure scenario assumptions if no further data are available. Such scenarios might include a summer vacation in a mosquito area for children, based on average vacation length. An occupational scenario might include one month of exposure at the rates of DEET usage observed in the Florida Everglades (Sanborn, 1999, page 18). The resulting MOEs will be much lower than presently stated in the draft RCD.

WHS Response: The exposure assessor disagrees with the statement that:

> **"These estimates (high end) might have to be based on exposure scenario assumptions if no further data are available as the normal course of exposure assessment"**

Use of unsubstantiated or untested assumptions in an exposure assessment may promote the concept that the estimated high-end exposures may have some reality. Throughout this exposure assessment, every attempt had been made to base exposure estimates on empirical data rather than on assumptions.

Further, the certainty of high-end exposure estimates even if based on some data (which for DEET does not exist) is always subject to conjecture and criticism. Because they are at one end of the exposure distribution, the confidence limits are always much larger than at the point of central tendency. It is therefore inappropriate, without supporting data, to develop high-end exposure estimates to address DEET-related illness episodes reported in the literature.

10/ 6 IV. Risk Appraisal

The uncertainties and limitations associated with this risk assessment are not adequately addressed in this section, particularly with respect to exposure-derived uncertainties. There is no separate section addressing scientific uncertainties as has been included in recent documents. If our recommendation for more discussion of upper-end exposures is followed (particularly regarding occupational exposures), the uncertainties in these estimates should be addressed in this section.

The following comment relates to the statement on page 64 that, "Data from the usage survey indicate that children less than 12 years old receive as much DEET per application as [an] adult male on average regardless of the difference in their body weight or surface area. Since this seems illogical, exposure estimates for adult females, juveniles (ages 12-17), and children (less than 12 years old) were also estimated by adjusting the adult male dermal dosage…." In the Boomsma and Parthasarathy study (1990), it was clear that juveniles under 12 were more likely to be sprayed with DEET by a parent. It was reported that parents were prone to over-apply DEET to their children. The data are from real applications and should not be dismissed or discounted. Therefore, we recommend that this alternate exposure estimation be dropped from the draft RCD and from the separate exposure assessment document.

WHS Response: OEHHA suggests that the exposure assessment based on surface area should be removed from the RCD without providing some justification. The author disagrees with this proposal as the techniques of exposure assessment continue to evolve and no single method used today provides definitive and representative estimates of exposure. Therefore, when alternative possibilities for exposure assessment exist then it is appropriate for the assessor to develop them.

Department of Pesticide Regulation

Paul E. Helliker
Director

Gray Davis
Governor

Winston H. Hickox
Secretary, California
Environmental
Protection Agency

M E M O R A N D U M

TO: Joyce Gee
              Acting Supervising Toxicologist
              Medical Toxicology Branch

VIA: Keith Pfeifer
              Senior Toxicologist
              Medical Toxicology Branch

FROM: Carolyn Lewis
              Associate Toxicologist
              Medical Toxicology Branch

DATE: March 2, 2000

SUBJECT: RESPONSE TO OEHHA'S COMMENTS ON THE RISK
              CHARACTERIZATION DOCUMENT FOR DEET

General Comments

Many of the comments under this section pertain to the exposure assessment. The discussion of the exposure assessment in the RCD was elaborated, including the addition of high-end exposure estimates for seasonal and chronic exposure and occupational exposure estimates. However, the decision regarding the human dermal absorption is the professional judgement of the toxicologist responsible for the exposure assessment. The Medical Toxicology Branch will leave any response to that comment to the Worker Health and Safety Branch.

A discussion of the synergistic potential of DEET was added to the Summary and Risk Appraisal section, although it was not possible to quantitate the risks from synergistic interaction of multiple chemicals with different mechanisms.

An explanation for why inhalation exposure was not addressed in the exposure assessment had been included in both the exposure assessment document and in the Risk Appraisal section of the RCD. The explanation why it was not done was moved up to the Exposure Assessment section, but the impact on the risk estimates was left in the Risk Appraisal section. A discussion of this was added to the Summary.

DPR has only calculated RELs or RfCs in our toxic air contaminant documents. Its usefulness with toxic air contaminants is understandable since an REL can be used for enforcement purposes. Since DEET is self-administered and exact exposure levels are unknown, it is unclear how this value will be of use.

Specific Comments

Summary

Page iii: The reviewer must not have read the sentence following the statement "no evidence of increased sensitivity in infants and children to DEET from available developmental and reproductive toxicity studies" because it stated "However, evidence from two neurotoxicity studies suggest a possible increase in postnatal sensitivity, including reduced body weights and increased mortalities in 11 day old pups versus 47-56 day old pups." This second sentence was rewritten to "However, evidence of increased sensitivity in infants and children was found in two neurotoxicity studies including….". The increased incidence of seizures and death in children was added as a lead into this paragraph; however, it will be pointed out that in a number of cases excessive exposure may have contributed to the development of these effects.

Page iv: The error regarding the name of the section in OEHHA reviewing the document was corrected.

I. Introduction

Page 2, I.D. Usage: The statement refers to the year prior to when the survey was sent out. This appears to be 1989. The document was revised to be more specific.

Page 2, I.E. Illness Reports: Exposure conditions in case reports are not reliable because they depend on the recall of the patient or family. Recall is subject to bias especially when associated with an adverse event. There already was some discussion of the frequency to which DEET was applied in these case reports. Further discussion of possible exposure conditions that might have contributed to the adverse events would be speculative and was not considered appropriate here. However, a discussion of this issue was added in the Pre- and Postnatal Sensitivity section under Issues Related to the Food Quality Protection Act in to the Risk Appraisal. In U.S. EPA's RED document for DEET, they note that the estimated incidence of afebrile seizures in children (0-19 years) is 15,000 to 20,000 per year while an estimated 17 million children are exposed to DEET in a year. Therefore, some seizures may occur by coincidence after exposure to DEET. This may well be the case with the five-year-old boy who had a seizure after only two DEET applications (only one of which was 95% DEET). It seems inappropriate to speculate on what exposure conditions contributed to the event when it is not clearly due to DEET exposure. The same is true for the cardiovascular problems developed in the 61-year-old after copious amounts of sunscreen and DEET were applied.

II. Toxicology Profile

A. Pharmacokinetics

The Selim (1991b) and Feldman and Maibach (1970) studies are described in detail in the exposure assessment document for DEET. In this discussion, several deficiencies in the Feldman and Maibach study were mentioned, including the estimate of material balance, solvent relevance and metabolite characterization. Since the selection of the study for human dermal absorption is the decision of the toxicologist responsible for the exposure assessment, no further detail was provided in the Risk Characterization Document. However, a reference to the exposure assessment document was added after the statement that the human dermal absorption value is based on the Selim (1991b) study. It should be noted that the dermal absorption value DPR selected was slightly higher than the dermal absorption value selected by Health Canada (7.5%) in their draft risk assessment for DEET that DPR recently reviewed. On other hand, U.S. EPA estimated a slightly higher dermal absorption for DEET of 12% for the undiluted DEET and 20% for 15% DEET in ethanol. They used the same study that DPR used to estimate dermal absorption, but apparently assumed all of the unrecovered radioactivity was absorbed.

Inhalation absorption was not discussed because there was no estimate of inhalation exposure in the exposure assessment document. The reason why inhalation exposure was not estimated was explained in the Risk Appraisal section. It was moved up to the Exposure Assessment section and elaborated slightly to include more of the discussion from the exposure assessment document. The decision to not include an inhalation exposure assessment is the professional judgement of the toxicologist responsible for the exposure assessment. It should be noted that neither Health Canada nor U.S. EPA included inhalation exposure in their risk assessments for DEET. U.S. EPA noted in their Reregistration Eligibility Document (RED) that while inhalation and oral exposure were not included in their exposure estimate, "this omission is not expected to significantly underestimate exposure as the dermal exposure is so much greater than inhalation or oral."

B. Acute Toxicity

Regulators are still struggling with how to quantitate the risk for multiple chemicals with common mechanisms of action such as organophosphates. Therefore, attempting to quantitate the risk for multiple chemicals with different mechanisms of action seems very problematic and questionable at this point. However, a discussion of the possible synergistic actions with other chemicals used simultaneously was added to the risk appraisal. This section is considered the more appropriate place for this discussion than the Risk Characterization since it can not be quantitated and is speculation.

C. Subchronic Toxicity

The discussion of the dose response was removed from the discussion of the 8-week feeding study in dogs in both the Toxicology Profile and the Hazard Identification.

D. Chronic Toxicity

No historical control data were available on the incidence of preneoplastic lesions in CD-1® mice for this laboratory.

A discussion of the lack of renal tumors in the male rats was added to the individual study summary for the chronic rat study.

F. Reproductive and Developmental Toxicity

As indicated by the use of the terms "frequency" and "percentage", the number of abnormal sperm increased. There was no discussion of different types of abnormal sperm in either study.

III. Risk Assessment

A. Hazard Identification

DPR did not exclude studies that do not meet FIFRA guidelines in its risk assessment for DEET. They were presented in the Toxicology Profile and Hazard Identification sections. As indicated by the lack of asterisks next to studies in Tables 17 and 18 in the Hazard Identification section many of the acute and subchronic studies for DEET do not meet FIFRA guidelines including the one selected as the definitive study for evaluating the acute toxicity of DEET. This study was selected because it had the most thorough evaluation of the neurobehavioral effects from DEET. No dermal neurotoxicity studies were available for DEET, probably because it was difficult to produce neurological effects with a single dermal exposure. In the draft risk assessment that Health Canada recently prepared for DEET, they selected the same oral neurotoxicity study that DPR did to evaluate the acute toxicity of DEET.

As described in the Hazard Identification section, the two rat studies with pre- and post-implantation losses at 80 and 100 mg/kg were not selected because of major deficiencies with these studies including no analysis of test article or dosing material, too few animals per group, incomplete examination of fetuses for malformations, no gross necropsy of dams and no individual data. The most important of these deficiencies was the lack of analysis of the test article for purity or the dosing material for concentration, so there is no confirmation that the

animals received what the investigators reported they did. Impurities, degradation, errors in calculations and weighing can result in the animals receiving more or less than intended. Furthermore, higher NOELs for pre- and post-implantation losses were found in a rat developmental toxicity study, which included the analysis of the test article and dosing material. Although route of exposure is an important consideration in selecting the definitive study for the critical NOEL, study quality is more important in the professional judgment of the primary author. A discussion of why these studies were not selected was elaborated with an explanation of why study quality was considered more important than route of administration.

The adjustment of the oral NOEL by the ratio of the area under the plasma concentration curves with oral and dermal exposure was not intended to account for differences between humans and rats in the absorption and metabolism of DEET. It is merely an adjustment for the differences in absorption and metabolism with oral and dermal exposure in rats. The difference in dermal absorption between rats and humans is taken into consideration when the exposure dosage is converted to an internal dosage by multiplying the amount applied by 8.4%. Differences in metabolism between rats and humans should be taken into consideration in by the default uncertainty factor of 10 for interspecies variation that is applied when determining an acceptable MOE.

Reevaluation of the pharmacokinetics data on which this adjustment factor was based raised several concerns that resulted in the adjustment factor being eliminated. The main reason was the period for which the blood levels were followed was too short and probably underestimated both the total body burden and the peak blood levels with dermal exposure. Closer examination of the urinary and fecal excretion data indicates that with dermal exposure the peak excretion was not until 48 to 72 hours after dosing. There is a strong possibility that blood levels follow a similar pattern. Second, the application site was covered with a glass enclosure following exposure, which appears to have significantly increased the dermal absorption. Although the blood levels would probably be lower with a non-occlusive or semi-occlusive wrapping, the occlusive covering introduces more uncertainty in the route-to-route extrapolation. Since a NOEL from a well-conducted acute dermal toxicity on the technical material would eliminate the need for this route-to-route extrapolation, it did not seem reasonable to use a novel approach such as this when there was so much uncertainty in the pharmacokinetic data on which it was based. A discussion of the limitations of the blood level data has been added to the Risk Appraisal section and why it was not used.

The use of different units for the NOEL/LOEL for acute and subchronic dermal irritation was obviously confusing. Because dermal irritation is a local effect, it seemed more appropriate to express the NOEL in terms of concentration on the skin, rather then in terms of body weight, whenever possible. Initially, an acute dermal toxicity study was not used because the size of the application site was unknown. In response to the OEHHA comment, this issue was reexamined and a new method for converting the dosage in the dermal toxicity studies was developed. The

assumption was made that the application site was 10% of surface area of the animal as recommended by the FIFRA guidelines and that the animal had a certain surface area for a given body weight, depending on the species. While the exact size of the application site would be more accurate, the use of these assumptions was considered superior to expressing the NOEL for this endpoint in mg/kg. This approach allowed for a more consistent treatment of this endpoint between acute and subchronic exposure. Consequently, the dermal irritation study was no longer used for the definitive study for acute dermal irritation, but instead the dermal toxicity study with the lowest LOEL used to estimate the acute NOEL for dermal irritation. To avoid confusion with units used for other NOELs/LOELs, the NOELs/LOELs for dermal irritation were initially expressed in mg/kg with a footnote showing the conversion to mg/cm^2. The study used for the subchronic NOEL for dermal irritation did not change, except the NOEL was converted to mg/cm^2, assuming the application site was 10% of the surface area.

It is unclear as to the intent of the OEHHA comment about the limitations of the chronic studies in addressing neurological effects. It should be noted that the exposure period in one of the subchronic neurotoxicity studies was approximately 48 weeks (not counting *in utero* exposure) which approaches the length of chronic studies. Furthermore, the exposure of the rats *in utero* enabled the evaluation of DEET on neurological development.

The misspelling of "Stara" was corrected.

B. Exposure Assessment

In the future, the exposure assessment document will be included with the RCD for review. The exposure assessment document is no more finalized than the RCD; however, it is the responsibility of the toxicologist who prepared the exposure assessment to revise that document, as he considers appropriate. The dermal absorption will not be revised in the RCD unless the exposure assessment document is revised; however, discussion of the uncertainty in the dermal absorption was added to the Risk Appraisal section.

The patterns of use that were associated with illness reports have already been discussed under the Illness Reports section of the Introduction. The author does not consider the Exposure Assessment section the appropriate section for any further discussion of factors that may have lead to the development of seizures in some children since this is speculation. However, this discussion was added to the Pre- and Postnatal Sensitivity discussion under Issues Related to the Food Quality Protection Act in the Risk Appraisal section.

The difference in the values in Tables 11 and 13 of the exposure assessment document are because the dosages in Table 11 were averaged for all age groups over an entire year and the values in Table 13 are the averages by age and sex from a single application. The discussion in

the RCD does mention the dosages in Table 20 are based on the average dermal dose from a single application. This was elaborated to indicate it was to the skin only. The discussion of the variability was elaborated to indicate that the mean is the annual aggregate mean for all age groups.

It is unclear how more information can be provided in the discussion of the SADD on the distribution of uses under different conditions when these data are not available. Generally, DPR does not include upper end estimates for seasonal and chronic exposure assessments. However, upper end estimates for seasonal and chronic exposure were added, using the same assumption used with acute exposure (i.e., upper end exposure is 3 times the average exposure) since this assumption was based on the variation in the annual average use reported by Boomsma and Parthasarathy (1990). These high-end exposure estimates were carried over to the Summary.

The omission of the park and forestry workers exposure was an oversight. Since the exposure assessment document did not calculate ADDs, SADDs and AADDs from the limited data available on occupational, MOEs were not originally calculated. However, a discussion was added on the limited data from the exposure assessment document and the study of Everglades National Park workers conducted by McConnell et al. (1986). In addition, estimates for occupational exposure were added to the document. The acute occupational exposure was assumed to be the same as the high end estimate for the general population. The seasonal exposure was estimated by assuming that workers used DEET once a day for 5 days per week during the peak months of June and July. The chronic exposure was estimated from the seasonal exposure assuming that 55% of the DEET use was during June and July.

C. Risk Characterization

The acute NOEL for systemic effects and the NOELs for dermal irritation were changed as previously discussed under the response to comments on the Hazard Identification. As explained previously, the acute NOEL for systemic effects was no longer adjusted for differences in body burden with oral and dermal exposure. In addition, the NOEL from a dermal toxicity study was used for dermal irritation instead of the dermal irritation study. The NOELs from the acute and subchronic dermal toxicity studies were converted to mg/cm^2 by making certain assumptions about the size of the application site. Because of these changes, the acute and seasonal MOEs are lower than previously calculated. The acute NOEL was slightly lower than estimated before (3.54 vs. 8 mg/cm^2), so the acute MOEs were slightly lower. The conversion of the subchronic NOEL from mg/kg to mg/cm^2, resulted in a dramatic reduction in the MOEs calculated. However, most of that reduction is because the absorbed exposure dosages were incorrectly used to calculate the MOEs. This resulted in the MOEs being 12-fold higher than they would have been if they were calculated using the external exposure dosages.

MOEs for high end seasonal and chronic use have been added to this section.

D. Risk Appraisal

This section has been elaborated to include a discussion of what the MOEs would be if the lower NOELs for pre- and postimplantations losses were used for adult women. In addition, a discussion of the uncertainties in the calculation of the NOELs and MOEs for dermal irritation was included. Uncertainties in the estimation of dermal absorption in humans were also added. Finally, the patterns of use and possible increased sensitivity that may have been responsible for the illnesses in children were added to the Pre- and Postnatal Sensitivity discussion under Issues Related to the Food Quality Protection Act.

Despite the objection of OEHHA to the presentation of the alternative method for estimating exposure in the risk appraisal, this will be left in. Although actual use suggests more DEET is applied to children on a mg/cm^2 basis, it is reasonable to assume a similar concentration is applied to children and adults. Since these estimates were only theoretical, they were only presented in the risk appraisal and no MOEs were calculated. The purpose of the Risk Appraisal section is to present information that may indicate possible uncertainties and whether the risks were likely underestimated or overestimated.

CHEMICAL SPECIALTIES MANUFACTURERS ASSOCIATION

Product Ingredient
Review Program

May 5, 2000   Via Federal Express

Gary Patterson, Ph.D.
Director, Medical Toxicology Branch
Department of Pesticide Regulation
California EPA
830 K Street
Sacramento, CA 95814

Re: DEET Characterization Document

Dear Dr. Patterson:

On behalf of the DEET Joint Venture (DJV), membership list attached, additional commentary
on the revised draft of the DEET Risk Characterization Document ("Document") are provided
herein. The DJV appreciates the opportunity to provide this additional commentary.

While the principal focus of these comments is on the risk assessment aspects of the Document,
the DJV still has concerns that many key sections of this document are dominated by the
discussion of findings from older studies and/or studies that were conducted by routes of
administration other than the dermal route of exposure to which humans are exposed. In
addition, there are numerous cases throughout the document where study findings are discussed
without any mention as to the route of administration. These situations are particularly prevalent
in sections of the document where data are summarized, i.e. Document Summary (p. ii);
Subchronic Toxicity (p. 23); Chronic Toxicity/Oncogenicity (p. 33); Reproductive Toxicity (p.
38); Developmental Toxicity (p. 41); Pre- and Postnatal Sensitivity (p. 69); and Endocrine
Effects (p. 71).

Since a much larger array of toxic effects can be produced by the oral, inhalation, subcutaneous
or intraperitoneal routes of administration, this presentation of data gives a misleading
impression concerning DEET's potential to produce effects in humans by the route to which
humans are exposed. For example, there are only two or three sentences in the entire Summary
at the front of the Document dedicated to the findings, or lack thereof, observed in studies
conducted by the dermal route of administration, while the remainder of paragraphs two through
six describe findings by the oral, inhalation, intraperitoneal and subcutaneous routes of
administration.

A simple solution to this problem would be to separate clearly the findings observed by the dermal versus the non-dermal routes of exposure in each section of the document where data are summarized, and clearly state the route of exposure when discussing the results of any study in all sections of the report. For example, the following type of paragraph could be added as the second paragraph to the Summary in the front of the Document in order to provide the needed balance to the other five paragraphs in which observations from studies conducted by the less relevant routes of exposure are discussed.

"DEET is of relatively low toxicity to laboratory animals, especially when administered by the dermal route of exposure to which humans are exposed. For example, no deaths or clinical signs of toxicity were observed in rats administered a single dose of DEET dermally at a dose level of 5000 mg/kg. Further, in 90-day subchronic dermal toxicity studies conducted in rats and micropigs, no clear evidence of systemic toxicity was observed at dose levels up to 1000 mg/kg/day.[1] While DEET is relatively nontoxic by the dermal route of exposure, systemic toxicity, including neurotoxicity, can be produced when large enough doses are administered by the oral, inhalation, subcutaneous or intraperitoneal routes of exposure. However, because these more invasive routes of exposure do not represent significant routes of exposure in humans, the findings in studies conducted by these routes were not interpreted as having a high likelihood of occurring in humans under normal conditions of human use. On the other hand, the fact that DEET has been evaluated at maximum tolerated doses by the more rigorous oral route of administration in a number of key studies, provides good assurance that DEET is not a potential human teratogen, carcinogen, or reproductive toxin."

In addition, to the above discussion on a specific concern, other comments are provided directly in the margins of the enclosed copy of the Document.

Commentary on Risk Assessment for Acute Exposure

When the original draft of this document was reviewed by the DJV, the approach taken by the authors to define the acute NOEL was to adjust the acute NOEL of 200 mg/kg from the acute oral neurotoxicity study for the differences in pharmacokinetics between oral and dermal exposure. This provides for an adjusted acute NOEL of 900 mg/kg (200 mg/kg x 4.5). When this adjusted NOEL was used to calculate the margins of exposure (MOEs) for acute exposure, only two MO Es in Table 21 were less than 100. These were the MO Es for high acute exposure to children ≤ 12 years of 87 and 83. However, since the initial review by the DJV, the authors have decided not to adjust the acute NOEL of 200 mg/kg from the acute oral neurotoxicity study for differences in pharmacokinetics. This decision reduced all of the MO Es for acute exposure by a factor of 4.5 and has resulted in MO Es below 100 for all high acute exposure scenarios and the average acute exposure scenario for children ≤ 12 years. It also has resulted in the need for the authors to add a disclaimer to the Document that the risks for acute systemic effects probably are greatly overestimated.

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[1] The DJV realizes that CDPR currently considers the NOEL for the rat 90-day dermal toxicity study to be 300 mg/kg/day. However, further evidence supporting a 1000 mg/kg/day NOEL for this study is provided below.

The initial approach that was taken by the authors was considered novel and not necessarily the approach that the DJV would have taken. However, because it was reasonable from a scientific perspective and produced MOEs that, for the most part, were >100, no commentary was provided on it in the earlier set of comments. However, now that the decision has been made to use the unadjusted NOEL from the acute oral neurotoxicity study to calculate MOEs for acute risk, the DJV would like to provide input as to the approach or approaches that it feels should be considered to define the MOEs for acute exposure.

One approach is very simple since a NOEL from a well-conducted acute dermal toxicity on the technical material recently has been submitted to CDPR. In this full guideline GLP study, undiluted technical grade DEET was administered to rats at a dose level of 5000 mg/kg. No clinical signs or deaths were observed. Also supporting this approach is the fact that the NOEL in two full guideline GLP subchronic dermal toxicity studies (rats and micropigs) is 1000 mg/kg/day (See footnote, p. 3).

Another approach supports the position that the unadjusted NOEL from the acute oral neurotoxicity study should not be used to define the MOEs for acute exposure, but allows the data from this study to be used for quantitative risk assessment. This is an approach to risk assessment advocated by the DJV through the use of DEET blood level studies.

After neurotoxic effects were observed in two studies involving oral dose administration, the DJV realized that additional information would be needed to assist in the risk assessment process. The additional information developed were data that defined the profile of the actual systemic exposure to DEET under the experimental conditions in which the neurotoxic effects were observed and the actual systemic exposure humans received at the 95th percentile of human exposure. Another blood level study was conducted under conditions which simulated the exposure the rats received in a 90-day dermal toxicity study. The purpose of this study was to provide data that would aid in risk assessment for subchronic exposure. The reports for these blood studies have been submitted to California EPA. In addition, the results of these studies are described in a document entitled "Response to California EPA Notice to Pesticide Registrants Regarding a Risk Assessment on the Active Ingredient N,N-Diethyl-m-toluamide (DEET)."
This document was submitted to CDPR in March of 1998. For your convenience, another copy of this document is enclosed for your review.

In the DEET Risk Characterization Document, these studies are described in the section on Pharmacokinetics, but the data from these studies are not really considered in the risk assessment section. Because of the relevance and usefulness of these data to the risk assessment process, it would be unfortunate if they were not seriously considered for this purpose.

As noted in the enclosed document, the blood profiles in both dogs and rats following oral bolus administration of DEET were much different than those in humans under use conditions. For example, in the human blood level study that was conducted at the 95th percentile of human use, DEET did not appear in the blood of humans until one to two hours after dermal application, after which time the DEET plasma levels gradually increased until the material was washed off

eight hours after application. The DEET plasma profiles and peak plasma levels were similar in males and females and did not increase after repeated dosing. The overall mean peak plasma level was 0.45 µg/ml. The overall mean area under the DEET plasma concentration versus time curve (AUC) was 3.51 µg • hr/ml.

In contrast, in dogs administered an oral bolus dose of 75 mg/kg, peak plasma levels were observed within 30 minutes after dosing and plasma levels were back below the limit of quantitation within three to four hours after dose administration. Consistent with humans, 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 14.7 µg/ml. The overall mean AUC was 12.59 µg • hr/ml. Since the effects that were observed in the dogs occurred shortly after dosing, they appear to be more closely associated with the time frame in which peak plasma levels would have occurred rather than being associated with AUC. A comparison of the overall mean peak plasma levels between dogs and humans showed a 33-fold difference, i.e. 14.7 µg/ml versus 0.45 µg/ml.

In order to put this 33-fold difference in peak blood level into perspective, two things need to be considered. First, since these blood level data provide a direct comparison of the true systemic exposure, a 10-fold difference in plasma levels generally is considered to be at least equivalent to a 100-fold safety factor or MOE based on whole body exposure on a mg/kg basis.[2] The second thing that needs to be considered is that these blood level comparisons are based upon data developed in humans at the 95th of human use and in dogs at the NOEL for the clinical signs that were observed. Therefore, the observed 33-fold difference in overall mean peak blood levels is comparable to a 330-fold safety factor or MOE developed on the basis of whole body exposure.

As was the case with dogs, the blood profiles and peak blood levels observed in rats administered a single oral bolus dose at the NOEL observed in the rat acute neurotoxicity study, i.e. 200 mg/kg, were much different than those observed in humans under use conditions. In this case, 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 four-hour time period in which the plasma levels were measured. Average peak plasma levels were 9.58 µg/ml in male rats and 13.61 µg/ml in female rats. These values represent 21-to 30-fold increases over the peak plasma levels observed in the human study. Since AUC values could not be determined in this rat study because DEET plasma levels did not return to baseline over the four-hour period in which they were measured, a second rat study was conducted in order to characterize more fully the time-course of elimination of DEET from the plasma following an oral bolus dose. In the second study, the same dose level (200 mg/kg) was administered; however, the DEET plasma profile was evaluated for 48 rather than four hours. In this study, peak plasma levels occurred 30 minutes after dosing and a comparison of peak plasma levels between rats and humans shows

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[2] While these type of comparative toxickinetic data rarely are available for pesticides, they are routinely used for human risk assessment of pharmaceutical products by the U.S. Food and Drug Administration. The 10-fold difference in blood levels is used as the "gold standard" in the same way as the value of 100 is used to define an MOE that represents a safe level of exposure on a mg/kg b. w. /day basis.

that the levels in male and female rats were 7.06 to 15.8 µg/ml, respectively. As was the case in dogs, the effects that were observed in the rats occurred shortly after dosing. Therefore, they appear to be closely associated with the time frame during which peak plasma levels would have been expected to occur in the rat acute neurotoxicity study rather than AUC. A comparison of peak plasma levels between the rats and humans in this study show 16- to 34-fold differences. These data clearly demonstrate that it is not appropriate to perform quantitative risk assessment by comparing the NOEL from a study in which DEET was administered orally as a bolus dose to potential human exposure on a total body weight basis. The data from these studies also provide an alternate way of performing quantitative risk assessment on DEET for the endpoint of neurotoxicity that was observed in the dog and rat oral toxicity studies.

Commentary on Risk Assessment for Subchronic Exposure

When the original draft of the DEET Risk Characterization Document was reviewed, only average exposure scenarios were presented in Table 21 for Subchronic Exposure. Since this initial review, high exposure scenarios have been added resulting in MOEs for children ≤ 12 years of age of 94 and 90.

The endpoint used for subchronic risk assessment is a body weight effect observed at the high-dose level (1000 mg/kg/day) in a rat 90-day dermal toxicity study (Johnson 1987c). The NOEL for this effect was determined to be 300 mg/kg/day. For risk assessment purposes, this value was reduced to 120 mg/kg/day based upon the assumption that only 40% of the applied DEET was absorbed dermally.

While an inspection of the mean body weight data for male rats in this 90-day dermal toxicity study appears to support the conclusion that the differences in mean body weight between the control and high-dose level is a treatment-related effect, there are a number of items that suggest either that the effect was secondary to other effects and/or it is less of an effect than is considered currently. The two causes to which the body weight effects in the high-dose group males may be secondary are skin irritation resulting from the inherent skin irritating properties of technical grade DEET, and kidney lesions resulting from accumulation off α_{2u}-globulin in renal tubule cells.

Humans do not experience significant skin irritation when using DEET and the type of renal lesions observed in this study are unique to certain strains of male rats. Therefore, if the differences in body weight observed in this study were attributable to these effects, the differences in body weight would not be an appropriate endpoint for human risk assessment. In this case, the NOEL in this study for toxicologically meaningful endpoints would be 1000 mg/kg/day. This NOEL also is supported by a 1000 mg/kg/day NOEL in a full guideline 90-day dermal toxicity study in the micropig, a species of laboratory animal whose renal system is similar to that of man.

While the authors of the Document acknowledge that the body weight effects in the high-dose male rats may be due to skin irritation, the authors are reluctant to definitively attribute these body weight effects to skin irritation because the high-dose female rats experienced the same

level of skin irritation without showing comparable body weight effects. The authors also place a higher level of toxicological significance on the body weight effects in the high-dose males because they are close to the 10% standard for defining an MTD. While these appear to be reasonable positions based upon a comparison of terminal body weights, a closer look at the mean body weight data presents a different picture for both males and females.

In the case of the high-dose male rats, the difference in mean body weight from controls at terminal sacrifice was 534 versus 484 g or 9.4%. However, all mean body weight values in this study actually decreased between study weeks 12 and 13, probably because the rats were weighed after being fasted for blood studies. Therefore, the mean data for study week 12 are more representative of the true difference in mean body weight between the two groups. At study week 12, the mean difference was 541 versus 501 or 7.4%. In addition, on the day prior to the start of dose administration, the mean body weights of the control and high-dose male rats were 290 versus 283, respectively, Therefore, the mean value for the high-dose group males already was 2.4% lower than controls before treatment began. When this 2.4% is subtracted from the 7.4% difference noted at study week 12, the adjusted difference is only 5.0%.

The same exercise was performed on the mean body weight values for female rats. In this case the differences in mean body weight at week 12 are 320 versus 311 or 2.8%. However, the mean body weight value for the high-dose female rats on the day prior to the initiation of dosing was 205 versus 201 for the controls. This represents a 2.0% difference and, if added to the 2.8% difference observed at study week 12, the adjusted difference in body weight for the high-dose females is almost the same as the adjusted values for the males, i.e. 4.8%. This analysis lead to an evaluation of the relative mean body weight gains for male and female rats over the first 12 weeks of the study. Somewhat surprisingly, these gains are almost identical for the male and female rats, i.e. 13 and 12 percent of the respective controls.

The analysis demonstrates two things. First, the difference between the mean body weight values for the control and high-dose male rats is not as great as that reflected by the terminal body weights. Second, an almost identical body weight effect occurred in the female rats. It also makes more sense of the data since the degree of skin irritation observed in both the male and female rats in the high-dose group would be expected to be reflected in some sort of an effect on body weight gain.

The results of another study cited in the Document also suggests that the body weight effects in the high-dose group males are overestimated based on terminal body weights in this 90-day study. In this other study (Brusick 1980), Sprague Dawley rats received dermal doses of undiluted DEET at dose levels of 100, 300 and 1000 mg/kg/day for a period of nine weeks. In this study, no body weight effects were observed in males or females in any group.

With regard to the possible relationship between the body weight effects in the high-dose males and renal lesions related to α_{2u}-globulin nephropathy, the authors of the Document do not feel that it is appropriate to assume that the body weight effects are secondary to renal lesions on the basis that renal lesions also were observed in the low- and mid-dose males without producing comparable body weight effects. This conclusion also appears reasonable because the

differences in severity and incidence of the renal lesions was not markedly different across the different treatment groups. However, while not markedly different, the incidence and severity was greater in the high-dose group for all α_{2u}-globulin related lesions, i.e. granular casts, inflammation, regeneration and hyaline droplets. Also, the kidney weights were significantly increased (P<0.05) only in the high-dose group and the incidence of macroscopic changes also was noticeably higher in the high-dose group. In addition, while not statistically significantly different (P > 0.05), the mean body weights of the male rats in the low- and mid-dose groups at study week 12 also were lower than the control, in a clearly dose related fashion, i.e. 541, 522 (-3.5%), 516 (-4.6%) and 501 (-7.4%), respectively. Therefore, as would be expected, the renal lesions caused a dose related decrease in body weights in the male rats in all treatment groups. The reason that the body weight depressions were the greatest in the high-dose animals is because the renal lesions were of a somewhat greater incidence and severity and the skin irritation also was of greater severity.

Another piece of data developed by the DJV also strongly supports the position that the differences in mean male body weight are not a direct effect of DEET exposure. These data are in the form of a blood level study that was conducted in male and female rats under conditions that simulated the conditions of this 90-day dermal toxicity study. (This study is discussed in detail under the title of "Blood Level Studies to Support the Use of the Rat and Micropig 90-Day Dermal Toxicity Studies for Quantitative Risk Assessment" beginning on page 6 in the enclosed document). In this blood level study, male and female rats were administered undiluted DEET dermally at a dose level of 1000 mg/kg/day. DEET plasma levels were profiled following the first and fifth consecutive daily applications. In this study, it was shown that DEET plasma levels were four to five times higher in female rats. Therefore, if dermally applied DEET had the potential to cause a direct effect on body weight, one would expect to see a more pronounced effect in the female rats.

In conclusion, a closer look at the body weight and pathology data from the definitive rat 90-day dermal toxicity study along with data from a full guideline 90-day dermal toxicity study in micropigs, a second subchronic dermal toxicity study in rats and a blood level study that simulated the exposure the rats received in the definitive 90-day dermal toxicity study, provide very strong evidence that the body weight differences observed in the definitive rat 90-day dermal toxicity study are both overestimated based on terminal body weight values and secondary to skin irritation and renal lesions. Based upon this evidence, it is the position of the DJV that the body weight effects observed in the rat 90-day dermal toxicity study should not be used as an endpoint for human risk assessment and that the NOEL for all other relevant findings in this key study is 1000 mg/kg/day.

If 1000 rather than 300 mg/kg/day is used as the no-effect level for subchronic toxicity, the MOEs developed by comparing potential human exposure to NOELs developed in animal toxicology studies on a total body weight basis for even the high exposure scenario are greater than 100. However, the data from the human blood level study and the rat blood level study described above provide an even stronger alternate way of looking at subchronic risk assessment. There are two important findings from these studies. One is that the DEET plasma profile in the rat dermal blood level study was qualitatively very similar to that obtained in the human dermal

blood level study. This supports the position that the data from the 90-day dermal toxicity studies are the most appropriate data to use for human risk assessment. The other important finding is that marked quantitative differences in DEET plasma levels were observed between humans and rats in both peak plasma levels and AUC. What is particularly impressive are the 27- and 72-fold differences between humans and male and female rats, respectively, based upon AUC data, which in the case of subchronic dermal exposure appears to be the most appropriate metric for comparison. As discussed earlier, these differences in blood levels are equivalent to MOEs of 270 to 720 developed on a mg/kg b.w./day basis.

Commentary on Conclusion Section of Document

One item the reader would expect to find in the Conclusion section of this Document is the authors' clearly stated conclusion as to whether or not DEET is a safe chemical for use as a personal insect repellent. Currently, there only is a discussion of MOEs, many of which are less than 100.

Perhaps when the commentary provided herein are considered there will be adjustments made such that there will not be any MOEs below 100, which in turn will allow the authors to feel comfortable making a clear statement regarding DEET's safety as a personal insect repellent

Conclusions

The DJV has been in the process of developing a state of the art safety data base on DEET for the past 15 years in order to address the regulatory requirements of California Senate Bill 950 (SB-950) and EPA Re-registration. To date, about seven million dollars have been spent on this effort. This new data base was deemed necessary because most of the studies that were conducted on DEET prior to 1985 did not meet today's toxicology testing standards. Because of the lower standards under which much of this earlier work was conducted, the validity of some of the findings is questionable. Findings that were not reproduced in the subsequent state of the art studies are particularly suspect. Therefore, the DJV strongly encourages a close examination of the source and validity of the data that are provided in the Document so as to avoid giving these earlier data undue credibility and prolonging their existence and further dissemination. For example, if some of the older studies discussed in the Document had been submitted to CDPR to satisfy SB-950 requirements, they probably would have been rejected outright.

The DJV always has taken the most scientifically valid approach to developing its data base on DEET and has developed far more data than required by regulation. For example, in cases where a maximum tolerated dose (MTD) could not be achieved by the dermal route of administration, the DJV always conducted the subject study by the more rigorous oral route of administration. This due diligence approach has led to findings that would not have been observed if the studies were conducted by the dermal route of administration. In addition, many of these findings have little or no relevance to human health. Therefore, blood level studies have been conducted to support this position and to aid in the overall risk assessment process. It now is the hope of the DJV that the data from these studies are interpreted in the most appropriate scientific manner and that all of the data that were developed are used in the risk assessment process.

The DJV appreciates the opportunity to provide additional commentary on the DEET Risk Characterization Document and hopes that you and your colleagues find this commentary to be useful. As always, we are available to respond to any questions or requests for additional information. We also would welcome the opportunity to meet with you and your staff after you have a chance to review our commentary to discuss any major points of disagreement. Questions or requests for information of a scientific nature should be directed to the Toxicology Consultant for the DJV, Dr. Gerald Schoenig at (804) 977-5957. Other requests should go to Ms. Susan Little, Executive Director, PIR Program, at (202) 872-8110.

Sincerely,

Stephen S. Kellner
Sr. Vice President
For the DEET Joint Venture

Enclosures

cc: Ms. Anne Pritchard, Cal EPA, letter only

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VIA: Keith Pfeifer
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FROM: Carolyn M. Lewis
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DATE: March 2, 2000

SUBJECT: RESPONSE TO REGISTRANT'S COMMENTS TO THE RISK
CHARACTERIZATION DOCUMENT FOR DEET

The following comments are in response to the comments from the Chemical Specialties
Manufacturers Association on the Risk Characterization Document for DEET

SUMMARY

The route of exposure and/or dose level was added to the second and third paragraphs wherever
it was considered appropriate. In addition, the lack of effects or mild effects in the dermal
studies was added. Some of the effects seen in studies of more questionable validity were
removed from the summary since they were only considered supplemental data. The statements
in the third and fourth paragraphs about the similarity in effects with acute, subchronic and
chronic exposure were removed since this was misleading.

No statement about the overall adequacy of the toxicology database or the quality of the
exposure data was added because generally statements to this effect are not included in the risk
assessment documents. Although all the data gaps for DEET have been fulfilled according the
requirements of SB950, the acute dermal toxicity studies for DEET are inadequate for
establishing an acute NOEL for DEET. Furthermore, while the exposure data for DEET go a
long way towards addressing exposure to DEET, some questions remain. For example, does the
amount of DEET applied vary with the type and concentration of the formulations? What is the
inhalation exposure to DEET with sprays? Finally, a discussion of the alternative exposure
scenario was not included in the summary since this is speculative and in the opinion of the
author did not warrant any further discussion.

I.B.   CHEMICAL IDENTIFICATION

The purpose of discussing the toxicity of DEET is to address its mechanism of toxicity, which is usually discussed in this section. However, this discussion was shortened since it is discussed in more detail in the Hazard Identification section.

I.E.   ILLNESS REPORTS

Case reports by nature are anecdotal and that is why we summarize these reports under illness reports rather than under toxicity studies. A discussion of these cases was added to the Hazard Identification and Risk Appraisal. It was mentioned in these discussions that it was not possible to determine conclusively that DEET was responsible for these adverse reactions, but in cases where there was a history of excessive use there is a high probability that they are related. Revisions were made to this section to make it clearer whether conclusions were those of the author of the referenced article or the author of the RCD.

Specific Comments

The last sentence of the second paragraph was moved to the discussion in the risk appraisal since this is speculative.

The sixth sentence in third paragraph was revised from "brief topical applications" to "a few topical applications".

In the fourth paragraph, large quantities of concentrated DEET products were ingested and, therefore, it is doubtful that alcohol was the sole source of the fatal outcome in these cases. The first sentence was revised to indicate the large quantities of concentrated DEET products were ingested with the concentration of the DEET products in parentheses.

The last sentence of the fifth paragraph was revised to indicate that it was unclear if DEET played any role in the development of these malformations since chloroquine was also used.

II.A.   PHARMACOKINETICS

All the suggested changes to this section were incorporated into the document.

II.C.   SUBCHRONIC TOXICITY

All the suggested changes to this section of the document were incorporated.

II.F.   REPRODUCTIVE TOXICITY

Summary:

The sentence regarding the abnormal sperm was revised to indicate that the toxicological significance of these findings was questionable since the differences were not statistically significant in either study. Consequently, the lowest reproductive NOEL was changed to 146 mg/kg/day.

Dermal- Rat

The transient increase in abnormal sperm was reevaluated. It was concluded its toxicological significance is questionable because 1) the increase in abnormal sperm was less than 10%; 2) the increase was not statistically significant; 3) there was no effect on sperm count or viability; and 4) there were no microscopic lesions in the testes. Therefore, the NOEL for this study was changed to 1,000 mg/kg/day.

II.G.   DEVELOPMENTAL TOXICITY

The suggested changes to this section were incorporated.

II.H.   NEUROTOXICITY

Summary:

The approximate dose levels at which effects were seen was added. Although the overall quality of the registrant studies were slightly better than the Army studies, it is the opinion of the author that the differences are not worth mentioning in the summary, especially since this was not done in other sections except to indicate whether or not studies met FIFRA guidelines.

Acute
Gavage-Rat

The statement about the deficiencies in the study was not changed except that the description of the FOB was changed from inadequate to incomplete.

Subchronic
Diet-Rat

The statement about the deficiencies in the study was not changed except that the description of the FOB was changed from inadequate to incomplete. In addition, a sentence was added

indicating that the study was much longer than required by guidelines and the animals were tested for learning and memory deficiencies.

IV.   RISK APPRAISAL

Hazard Identification

The statement about the deficiencies with the acute oral neurotoxicity study was modified to indicate these deficiencies were minor. In addition, some discussion of the acute dermal toxicity studies was added to indicate why none of these was used for the acute NOEL.

Although originally the acute NOEL was adjusted for differences in the pharmacokinetics with dermal and oral absorption, reevaluation of the pharmacokinetics data raised several concerns that resulted in the adjustment factor being eliminated. The main reason was the period for which the blood levels were followed is too short and probably underestimated both the total body burden and the peak blood levels with dermal exposure. Closer examination of the urinary and fecal excretion data indicates that with dermal exposure the peak excretion was not until 48 to 72 hours after dosing. There is a strong possibility that blood levels follow a similar pattern. Second, the application site was covered with a glass enclosure following exposure, which appears to have significantly increased the dermal absorption. Although the blood levels are probably lower with a non-occlusive or semi-occlusive wrapping, the occlusive covering introduces more uncertainty in the route-to-route extrapolation. Since a NOEL from a well-conducted acute dermal toxicity on the technical material would eliminate the need for this route-to-route extrapolation, it did not seem reasonable to use a novel approach such as this when there was so much uncertainty in the pharmacokinetic data on which it was based. A discussion of the limitations of the blood level data had been added to the Risk Appraisal section and why it was not used.

Exposure Assessment

While the application of more DEET to children on a surface area basis seems illogical, it is supported by registrant's usage study. It is possible adults overapply DEET to their children thinking they are being more health protective. Since exposure estimates using the alternative method are only theoretical and there are usage data to indicate otherwise, there is little justification for using theoretical estimates. Consequently, these theoretical estimates were not given any more weight than a discussion in the Risk Appraisal section.

Pre- and Postnatal Sensitivity

The author is aware of the rationalization the registrant provided for not considering the reduction in body weights in the animals for the subchronic neurotoxicity study to be treatment-

related. The body weight data from both generations in the reproductive toxicity study were examined and it was determined that a similar pattern was seen in those pups, although the differences were not statistically significant. Why body weight reductions were not seen in the 2-year rat study at similar dose levels as the subchronic neurotoxicity may have been due to differences in exposure during critical growth periods during development. In addition, the author considers the lower LD_{50} values in young versus old pups to be a valid indication of increased postnatal sensitivity. While it probably should not be the only data considered in determining if there is any increased pre- and postnatal sensitivity, it is appropriate to use these data in a weight of evidence approach. Further consideration of the potential risk to children was added to this section because children are at greater risk due to greater exposure. The few case reports of seizures in children after exposure to DEET also suggest there may be increased risk for adverse effects in this population subgroup. However, it is uncertain if the increased risk comes from increased sensitivity or increased exposure, assuming these seizures are related to DEET exposure.

V.   CONCLUSIONS

It is the opinion of the author that it is not appropriate to mention the MOEs when adjusted for surface area in the conclusions since this is theoretical and not supported by the registrant's own usage study. However, the conclusions were revised to indicate that the risks for acute systemic effects were probably greatly overestimated by using an oral NOEL. A reference to the difference in the peak blood levels with oral and dermal exposure was added.