N,N,-Diethyl-\(m\)-Toluamide (DEET) Suppresses Humoral Immunological Function in B6C3F1 Mice

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N,N-diethyl-meta-toluamide (DEET) is a particularly effective broad-spectrum insect repellent used commonly in recreational, occupational and military environments. Due to its widespread use and suggested link to Gulf War Illness, this study examined the immunotoxicity of DEET. Adult female B6C3F1 mice were injected sc for 14 days with DEET at 0, 7.7, 15.5, 31, or 62 mg/kg/day. Due to differences in the dermal absorption of DEET between mice and humans, this study eliminated this confounding factor by utilizing sc injection and measured circulating blood levels of DEET to assess bioavailability from sc administration. Effects on lymphocyte proliferation, natural killer cell activity, thymus and spleen weight and cellularity, the antibody plaque-forming cell (PFC) response, and thymic and splenic CD4/CD8 lymphocyte subpopulations were assessed 24 h after the last dose. No effect was observed in lymphocyte proliferation, natural killer cell activity, thymic weight, splenic weight, thymic cellularity, or splenic cellularity. Significant decreases were observed in the percentage of splenic CD4/CD8- and CD4+/CD8- lymphocytes but only at the 62 mg DEET/kg/day treatment level and not in absolute numbers of these cell types. Additionally, significant decreases in the antibody PFC response were observed following treatment with 15.5, 31, or 62 mg DEET/kg/day. Pharmacokinetic (PK) data from the current study indicate 95% bioavailability of the administered dose. Therefore, it is likely that DEET exposure ranges applied in this study are comparable to currently reported occupational usage. Together, the evidence for immunospression and available PK data suggest a potential human health risk associated with DEET in the occupational or military environments assuming similar sensitivity between human and rodent responses.

Key Words: DEET; immune function; Gulf War Illness; PFC response; T-cell subpopulations.

N,N-diethyl-meta-toluamide, commonly known as DEET, is a personal use insect repellent. It was first registered in the United States for general public use in 1957, following its development in 1946 by the U.S. Army as protection for military personnel in insect infested areas (Schoenig et al., 1999). DEET is an aromatic amide that is used worldwide due to the unsurpassed protection it provides to a vast spectrum of biting insects including mosquitoes, sandflies, and deerflies (Carlson, 2006; Gilbert, 1966; Tawatsin et al., 2006). The U.S. EPA (1998a) has estimated that the average annual U.S. domestic usage is 4 million pounds of DEET. Approximately, 30% of the general U.S. population (≈90,000,000) use DEET-containing insect repellent products on an annual basis.

The U.S. Environmental Protection Agency (EPA) reports that DEET does not cause an unreasonable risk to humans or the environment (U.S. EPA, 1998a). Toxicity studies do not include general immunotoxicity assessments, but report on acute, chronic, carcinogenic, reproductive, developmental, neurotoxic, and mutagenic endpoints along with the kinetics of dermal absorption and metabolism. In general, effects were only observed at excessively high doses. For instance, in 90-day feeding studies with rats, kidney weights were increased at doses of 500 mg/kg and above and liver weights were increased at 100 mg/kg/day and above; whereas in studies with mice, liver weights were slightly increased at 300 mg/kg and significantly increased at 1000 and 3000 mg/kg (U.S. EPA, 1998a). In 90-day dermal exposure studies, DEET decreased body weights of male rats at 1000 mg/kg and increased kidney weights at 300 and 1000 mg/kg (U.S. EPA, 1998a). In two-generation reproduction studies renal tubular degeneration, granular cast formation, and inflammation were noted in male F2 rats at dietary doses of 500, 2000, and 5000 ppm. No effects on reproductive parameters were observed; however, decreases in body weight were noted in pups from the 2000 and 5000 ppm treatment groups (U.S. EPA, 1998a). Schoenig et al. (1993) report that DEET does not selectively target the nervous system following chronic (500, 2000, and 5000 ppm dietary concentrations) or acute exposures (50, 200, and 500 mg/kg by gavage).

The lack of serious effects in general toxicity studies and in human case studies suggests that DEET is relatively safe to the general population (Osmitz and Murphy, 1997; Sudakin and...
In a retrospective study reporting over 9000 accidental human overexposures to DEET, two-thirds of those exposed experienced no adverse effects or only temporary minor effects (Veltri et al., 1994). In a few reported human cases of DEET poisoning, toxicity included tremors, generalized seizures, coma, restlessness, mood changes, and ataxia (Gryboski et al., 1961; Roland et al., 1985; Zadikoff, 1979). Hence, Veltri et al. (1994) concluded that the risk of overt toxicity is low in comparison with its reported annual use and exposure estimates.

From August 1990 to April 1991, approximately 700,000 U.S. military service personnel were involved in the sea, air, and ground war in the Persian Gulf. Because their return, many of these service personnel have complained of a variety of nonspecific symptoms (Amato et al., 1997; Frost, 2000; Gouge et al., 1994; Haley and Kurt, 1997; Wittich, 1996) collectively labeled Gulf War Illness (GWI). Although no specific cause has been identified, it has been suggested that environmental exposures and stressors altered immune function contributing to GWI (Rook and Zumla, 1998; Soetekouw et al., 2000; Voidani and Thrasher, 2004). Several chemical exposures, including DEET, have been identified as potential causative agents due to the prevalence of use.

In light of these concerns, several recent reports examined the toxicity of DEET singly or in combination with other military-relevant chemicals (Abou-Donia et al., 1996a,b; Chaney et al., 2000; McCain et al., 1997; Peden-Adams et al., 2001). DEET in combination with pyridostigmine bromide resulted in increased mortality and neurotoxicity (Abou-Donia et al., 1996a,b; Chaney et al., 2000; McCain et al., 1997). DEET (500 mg/kg sc) singly caused increases in plasma butyrylcholinesterase, whereas DEET in secondary or tertiary mixtures with pyridostigmine or chlorpyrifos caused increased mortality, weight loss, and locomotor dysfunction (Abou-Donia et al., 1996a,b). Additionally, Chaney et al. (2000) demonstrated that DEET singly (200 mg/kg ip) does not alter cholinesterase activity in the diaphragm, heart, whole blood, or brain, whereas DEET combined with pyridostigmine (3 mg/kg orally) decreased brain cholinesterase. In addition, DEET increased DNA oxidative damage in rats exposed to a single dermal application (400 mg/kg; Abu-Qare and Abou-Donia, 2000).

Reliable estimates of exposure levels in soldiers do not exist. Abu-Qare and Abou-Donia (2000) have stated that a 400 mg DEET/kg dermal exposure level is a relevant exposure based on their reported personal communication with the U.S. Department of Defense. Other studies imply that 40 mg DEET/kg dermal exposure is a relevant exposure level for the soldiers (Abu-Qare and Abou-Donia, 2001; Abou-Donia et al., 2001). A high exposure level of DEET (500 mg/kg/day sc) in some studies (Abou-Donia et al., 1996a,b) is a dose of approximately 35,000 mg/kg/day assuming a 70 kg person and 100% absorption.

To compare these levels to the U.S. general population, the U.S. EPA estimates daily dermal exposure levels in adult males, adult females, children age 13–17, and children under age 12 to be 12.1, 9.68, 21.05, and 37.63 mg/kg/day, respectively (U.S. EPA, 1998a) when the compound is used during recreational activities. Dermal absorption studies have shown that following a single dermal application in rats, approximately 17 and 5.3% of the dermal dose is absorbed by males and females, respectively (U.S. EPA, 1998a). The major route of elimination is via urine (U.S. EPA, 1998a). Studies in various animal models report DEET absorption ranges of 7–72% depending on species, sex, dose, and site of applications. Additionally, human dermal absorption studies carried out with adult males demonstrated that in a 15% ethanol solution, approximately 20% of the applied dose was absorbed, whereas 12% was absorbed in an undiluted solution (U.S. EPA, 1998a). These percent absorption values were conservatively calculated to reflect differences in total recovery between the two treatment groups from the original study which reported a range of 3–8% with a mean of 5.6% for undiluted DEET and a range of 4–14% with a mean of 8.4% when DEET was diluted in ethanol (Selim et al., 1995). These reported absorption values in humans are consistent with the previous study by Feldmann and Maibach (1970) who found DEET absorption in human subjects to be 16.7% ± SD of 5.1%.

The goal of the current study was to determine if DEET, at dose concentrations greater than those expected in the general population, but within relevant exposure levels in the occupational or military environments, would alter Tier I or Tier II (Luster et al., 1988, 1992) immunotoxicological parameters. There are three reasons to assess the effects of DEET on immunity: (1) immune function alterations have been implicated in GWI, (2) DEET exposure has been implicated as a potential causative agent of GWI, and (3) initial studies from our laboratory identified that DEET modulates immune function. The current study evaluated dose-responsive alterations to determine what, if any, effects DEET would have on classical immune biomarkers and if possible, establish a no observable adverse effect level (NOAEL) and lowest observable adverse effect level (LOAEL).

**MATERIALS AND METHODS**

**Chemicals, antibodies, and supplies.** Unless otherwise specified all chemicals and mitogens were purchased from Sigma (St Louis, MO). DEET (Lot# 95H0968) was prepared in a vehicle of virgin olive oil. Sheep erythrocytes (SRBCs) in Alsever’s solution were obtained from BioWhitaker, Inc. (Walkersville, MD). Lyophilized guinea pig complement, nonessential amino acids (NEAA; 10mM 100×) and sodium pyruvate (100mM) were obtained from Gibco Laboratories (Grand Island, NY). RPMI-1640 (with l-glutamine and sodium bicarbonate), phosphate-buffered saline (PBS; with and without Ca²⁺ and Mg²⁺), and penicillin/streptomycin (5000 IU/ml, 5000 µg/ml) were purchased from Cellogro (Mediatech; Herndon, VA). The radio-isotopes, sodium chromate and tritiated thymidine, were purchased from ICN (Costa Mesa, CA). The fluorescent antibodies, rat IgG 2a (isotype control), fluorescein isothiocyanate conjugated rat-anti-mouse CD4 (monoclonal), and phycoerythrin (PE) conjugated rat-anti-mouse CD8 (monoclonal) were purchased from
Caltag (Burlingame, CA). Luma plates, Unifilters, and Microscint 20 were purchased from Packard (Meriden, CT). YAC-1 cells were purchased from ATCC (Manassas, VA). Tissue culture plates and other disposables were purchased from Fisher Scientific. Metaphone (methoxyflurane) was purchased from Schering-Plough (Kenilworth, NJ).

**Animal care and dosing: 14-day exposure studies.** Mice were housed in plastic shoebox cages and received food and water *ad libitum*. Prior to starting a particular experiment, 7- to 8-week-old female B6C3F1 mice were placed randomly three to a cage in a treatment room. They acclimated to the conditions of the treatment room (12-h light/dark cycle, 22 ± 2°C, 60-65% relative humidity) for 1 week before dosing began. Four doses of DEET (7.7, 15.5, 31, and 62 mg DEET/kg/day) and an olive oil control (0 mg DEET/kg/day) were used. Mice were dosed daily for 14 days via sc injection with either DEET or the olive oil control. There were six animals per treatment group. All procedures were approved by the Institutional Animal Care and Use Committee and conducted in an accredited facility by Association for Assessment and Accreditation of Laboratory Animal Care.

**Animal care and dosing: pharmacokinetic studies.** Mice were housed in plastic shoebox cages and received food and water *ad libitum*. Prior to starting a particular experiment, 7- to 8-week-old female B6C3F1 mice were placed randomly three to a cage in a treatment room. They acclimated to the conditions of the treatment room (12-h light/dark cycle, 22 ± 2°C, 60-65% relative humidity) for 1 week before dosing began. In the first experiment 3 mice per time point (1, 2, 4, 6, 8, 12, and 24 h) were injected once sc with either 15.5 or 31 mg DEET/kg and 0.8–1 ml of whole blood was collected via cardiac puncture following anesthesia with Metaphone. After blood collection, mice were euthanized via CO₂ asphyxiation. Tissue were harvested, anaerobically washed with PBS, and stored at −80°C. Plasma samples were stored at −80°C until analysis. Mice were euthanized by CO₂ asphyxiation. The serum concentrations for the individual time points were imported from Microsoft Excel into WinNonLin Professional 5.2 (Pharsight Corp., Pharsight.com) after conversion to molar units. The parameters for the sc data were calculated assuming extra-vascular input and a noncompartmental model with uniform weighting. The linear trapezoidal method was used for the calculation of area under the curve (AUC). The terminal elimination rate constant was calculated from the slope of the data points in the terminal elimination phase (log

**Natural killer cell activity.** Natural killer (NK)-cell activity was assessed as a modification of Duke et al. (1985). Briefly, YAC-1 cells were used as the target and were labeled with 51Cr. Spleens were processed, counted and diluted to 1 × 10⁶ cells/ml in Complete RPMI-1640 (RPMI-1640, 10% fetal calf serum and 1% pen/strep). The ratios of spleen cells to YAC-1 cells used was 100:1, 50:1, and 25:1 and these were plated in triplicate for each sample in a 96-well microtiter plate. Additionally, five wells of a negative control (spontaneous release) and a positive control (maximum release) were prepared. Plates were incubated for 4 h at 37°C and 5% CO₂. After the incubation, plates were centrifuged, 50 μl of supernatant was transferred to a LumaPlate, and plates dried overnight. 51Cr counts were assessed using a Packard Top Count-NXT scintillation counter. Results are reported as mean lytic units/10⁶ cells (Bryant et al., 1992).

**Antibody plaque-forming cell assay.** The number of plaque-forming cells (PFCs) was determined using the Cunningham modification of the Jerne plaque assay (Cunningham and Szenberg, 1968; Jerne and Nordin, 1963). Four days prior to euthanasia, mice were administered 0.1 ml of a 20% sheep red blood cell (SRBC) suspension in PBS via ip injection. All SRBCs for the experiments were drawn from a single, donor rabbit. Mice spleens were processed, cell densities counted and suspensions diluted to 2.0 × 10⁶ cells/ml in Supplemented RPMI-1640 (as previously described). Aliquots of the cell suspension were added to Eppendorf tubes containing Supplemented RPMI-1640 and SRBCs. Fifty microliters of reconstituted lysophosphatidylcholine complement (diluted 1:2, vol/vol) was then added on a tube by tube basis. Aliquots of the solution were placed in Cunningham chamber slides, sealed, and slides were incubated at 37°C and 5% CO₂ for 1 h. Slides were counted using an Olympus microscope (BH2) at 4× power. Results are reported as PFCs/million cells.

**Spleenic and thymic CD4/CD8 subpopulations.** For each sample and cell type 100 μl of cell suspension (1 × 10⁵ cells/ml = 1 × 10⁶ cells/well) was added to a single well of a 96-well plate. Additionally, appropriate controls were included (i.e., isotype, cells only, label only, etc.). Plates were centrifuged at 390 g for 3 min, the supernatant was aspirated, the plate vortexed to resuspend the pellet, and 100 μl of flow buffer (0.1% sodium azide and 0.1% bovine serum albumin [BSA] in PBS without Ca²⁺ or Mg²⁺, pH 7.4) was added to each well. Antibodies (Ab) were diluted in flow buffer to give 1 μg Ab/10⁶ cells. Ten μl of both CD4 and CD8 fluorescent labels were added to each sample well and the plates were incubated in the dark at 6°C for 30 min. Plates were then centrifuged, aspired, vortexed, 150 μl of lysis buffer (150mM ammonium chloride and 12mM sodium bicarbonate in PBS without Ca²⁺ or Mg²⁺, pH 7.0) was added and plates were incubated for 5 min at 37°C. Plates were then centrifuged, aspirated, vortexed, washed twice with 15 μl of FACS buffer, and then 200 μl of a 0.1% paraformaldehyde (in PBS without Ca²⁺ or Mg²⁺) was added to each well. Plates were incubated for 5 min in the dark on ice then were centrifuged and washed twice with flow buffer before finally adding 200 μl of cold analysis buffer (0.1% BSA and 0.1% sodium azide in PBS with Ca²⁺ and Mg²⁺, pH 7.8). Flow cytometric analysis was performed using a Becton Dickinson flow cytometer (FACSCalibur; San Jose, CA). Results are reported as both percent (%) of 1 × 10⁶ cells and as absolute cell numbers.

**Analysis of DEET in whole blood and PK assessment.** Mice were injected sc or iv and blood was collected via cardiac puncture, following anesthesia with Metaphene, at intervals from 2 min to 7 h as described above. Mice were then euthanized with CO₂ asphyxiation. The serum concentrations for the individual time points were imported from Microsoft Excel into WinNonLin Professional 5.2 (Pharsight Corp., Pharsight.com) after conversion to molar units. The parameters for the sc data were calculated assuming extra-vascular input and a noncompartmental model with uniform weighting. The linear trapezoidal method was used for the calculation of area under the curve (AUC). The terminal elimination rate constant was calculated from the slope of the data points in the terminal elimination phase (log

**Lymphocyte proliferation.** Spleens were processed and diluted to 2.0 × 10⁶ cells/ml in Supplemented RPMI-1640 (RPMI-1640, 10% fetal bovine serum, 1% NEAAs, 10mMHEPES, 1% pen/strep, and 10μg/2-mercaptoethanol, pH 7.4). One hundred microliter aliquots of the resulting cell suspensions were dispensed into 96-well plates (2.0 × 10⁵ cells per well) containing triplicate wells of 100 μl of either concanavalin A (Con A, type IV-S), phytohemagglutinin, lipopolysaccharide (0111:B4), or RPMI-1640. All mitogen concentrations used were 5 μg mitogen/ml of culture. Plates were incubated for 48 h at 37°C and 5% CO₂. After 48 h, 100 μl of a 0.5 μCl/ml stock of tritiated thymidine in RPMI-1640 was added to each well. Cells were harvested onto Unifilter plates 12 h later using a Packard Filtermate 96-well plate harvester and the plates were allowed to dry. Once dry, 25 μl of Microscint 20 was added to each well and the samples were analyzed using a Packard Top Count-NXT scintillation counter. Results are reported as stimulation indices (SI = counts per minute (CPM) stimulated/CPM control).
RESULTS

Body Mass, Organ Mass, and Splenic and Thymic Cellularity

There were no signs of overt toxicity in any of the animals as indicated by weight loss, lack of activity, lack of grooming, or cloudy eyes. In fact, mice in all groups appeared to gain weight equally over the course of the 14-day study, therefore, no differences were observed in final body mass or body mass change between the DEET treated and control animals (Supplementary Data 2). Secondary immune organ mass (spleen and thymus) and liver mass also exhibited no change as compared with control groups (Supplementary Data 2). Cellularity of the spleen and thymus were not altered following treatment with DEET at any of the dose levels assessed (Supplementary Data 3).

Lymphocyte Proliferation, NK-Cell Function, and PFC Assessments

In addition to organ mass and cellularity, three other Tier I immunotoxicological endpoints were assessed. These were proliferative responses, NK-cell activity, and the PFC cell response. The ability of lymphocytes to proliferate is important to both parts of adaptive immunity (e.g., cell mediated and humoral immunity). To be able to generate sufficient numbers of effector lymphocytes to fight an infection the activated lymphocyte must be able to undergo clonal expansion; thereby, requiring proliferation of the activated lymphocyte. Exposure to DEET caused no effect on either T- or B-cell mitogen induced proliferation (p = 0.1341 and p = 0.4886, respectively; Fig. 1). Suggesting that neither lymphocyte type would have difficulty undergoing clonal expansion.

Natural killer cell activity is important in nonspecific, or innate, immunity because these cells provide an early defense against certain intracellular infections. NK cells are important in early responses to both bacterial (e.g., Listeria monocytogenes) and viral infections. Natural killer cell activity was increased at the 15.5 and 62 mg DEET/kg/day treatment levels but this increase did not reach statistical significance compared with control (p = 0.08) (Fig. 2).

The antibody PFC response to the T-cell dependent antigen SRBC, requires antigen presenting cells, T-helper cells, and B-cells in the production of SRBC-specific IgM. Treatment with 15.5, 31, or 62 mg DEET/kg/day resulted in suppression (p < 0.0001) of the antibody plaque-forming cell response (57, 53, and 29% of control, respectively; Fig. 3). The calculated ED50 for this response was 27 mg/kg/day sc (R² = 0.92, y = −0.61x + 5.50).

Splenic and Thymic CD4+/CD8− Subpopulations

Assessment of T-cell subpopulations, a Tier II endpoint, was also determined for both splenic and thymic T-cells. Although subcutaneous treatment with DEET decreased the percentages of splenic CD4+/CD8− cells (p = 0.0024) and caused increases in the percentages of CD4−/CD8− cells (p = 0.011) following

Statistics. Data were tested for normality (Shapiro-Wilk W-test) and homogeneity (Bartlett’s test for unequal variances) and, if needed, appropriate transformations were made. A one-way ANOVA was used to determine differences among doses for each endpoint using JMP 4.0.2 (SAS Institute Inc., Cary, NC) in which the standard error used a pooled estimate of error variance. When significant differences were detected by the F-test (p < 0.05), Dunnett’s t-test was used to compare treatment groups to the control group. Unless otherwise stated, all immune experiments were repeated three times.

The PFC dose-response was assessed by regression analysis. The log transformation of the endpoint (PFC/million cells) was plotted against the log dose. The log dose needed to achieve 50% suppression of the immune response was determined from the regression equation and the inverse log calculated to determine the calculated ED50 (mg/kg/day).
treatment with 62 mg/kg/day, this alteration was not large enough to impact absolute numbers of these cell types or splenic CD4+:CD8+ ratios (Table 1). Thymic subpopulations were not altered by treatment with DEET as indicated by both percent and absolute numbers of cells (Table 2). However, treatment with 7.7 and 15.5 mg DEET/kg/day decreased thymic CD4+:CD8+ ratios but treatment with 31 or 62 mg/kg/day did not (p = 0.035; Table 2).

**DEET Absorption and Bioavailability following sc or iv Injection**

Preliminary studies assessing absorption from the sc exposure indicated the peak of absorption was most likely prior to the first time point measured (1 h; Supplementary Data 1). The concentration values shown in Supplementary Data 1 are apparent T\(_{\text{max}}\) values as determination of the true T\(_{\text{max}}\) requires sufficient sampling to allow model fitting. Because data points prior to 1 h in this initial experiment were not obtained, modeling was not attempted. For the 31.1 mg/kg exposure, the serum concentration at 1 h was statistically indistinguishable from that at 2 h. This suggests that during this interval the kinetics are temporarily near steady state, with the absorption rate constant being rate limiting and close to the value of the elimination rate constant. Thus, the serum concentration values are in a plateau between 1 and 2 h after the high dose and that plateau value must be very close to the real T\(_{\text{max}}\) as it is kinetically impossible for T\(_{\text{max}}\) to be greater than the plateau value if there is only one absorption rate constant.

Studies utilizing both sc and iv exposure for comparison, therefore, focused on sample collection at earlier time points. The terminal elimination phase of DEET from mouse serum after iv dosing is shown in Figure 4A. The regression analysis used the last 10 data points to determine lamda _z_ (λ\(_{z}\)) and produced a correlation coefficient (R\(^2\)) of 0.68. The terminal elimination phase for DEET was similar after sc dosing (two data points are obscured by the predicted curve) (Fig. 4B). In this case, the regression analysis used the last four data points and achieved a correlation coefficient of 0.84. Only four points were used because of an unusually low value at 120 min; eliminating this point allowed the analysis to use nine data points (R\(^2\) = 0.9, terminal half-life = 56 min, data not shown).

Lamda _z_ for iv and sc dosing were comparable (0.0057 and 0.0081, respectively; Table 3) demonstrating a long terminal elimination half-life (122 and 85 min for iv and sc dosing, respectively, range between 85 and 420 min). As would be expected, the apparent T\(_{\text{max}}\) occurred later with sc injection (20 min) than with iv injection (6 min). The 6-min lag time after the iv injection is possibly due to partial extravasation of the dose into tissues around the injection site.

The volume of distribution appears exceptionally large in this analysis because it is calculated as V = Dose/(λ\(_{z}\) × AUC) within the noncompartmental model. This analysis does not reflect the earlier phases of distribution and elimination. Here lamda _z_ is quite small (slow terminal elimination) leading to a large apparent volume. Total clearance is rapid, approximately 20 l/min/kg in both dose settings. The mean residence time in plasma was approximately 30 min. For all these parameters good correlation between the observed and predicted values was noted. The AUC\(_{\infty}\) after iv dosing was 3984 min x nM (4.0 min x μM). After sc dosing it was

<table>
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<tr>
<th>DEET (mg/kg/day)</th>
<th>%CD4+</th>
<th>%CD8+</th>
<th>%DN</th>
<th>%DP</th>
<th>Ratio CD4/CD8</th>
<th>Absolute CD4+ (cells x 10(^6))</th>
<th>Absolute CD8+ (cells x 10(^6))</th>
<th>Absolute DN (cells x 10(^6))</th>
<th>Absolute DP (cells x 10(^6))</th>
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<tbody>
<tr>
<td>0</td>
<td>20.3 ± 0.6</td>
<td>11.1 ± 0.3</td>
<td>68.3 ± 0.8</td>
<td>0.24 ± 0.03</td>
<td>1.8 ± 0.07</td>
<td>21.5 ± 1.3</td>
<td>11.7 ± 0.35</td>
<td>72.4 ± 3.2</td>
<td>0.26 ± 0.04</td>
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<td>7.7</td>
<td>21.4 ± 0.3</td>
<td>10.1 ± 0.4</td>
<td>67.5 ± 0.5</td>
<td>0.26 ± 0.02</td>
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<td>62</td>
<td>18.1 ± 0.7*</td>
<td>9.9 ± 0.6</td>
<td>71.6 ± 1.1*</td>
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<td>11.1 ± 0.81</td>
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Note. DP = CD4+/CD8-, DN = CD4-+CD8-. *Significantly different from respective control, p ≤ 0.05. Absolute values were determined by multiplying the percent gated cells by the total number nucleated cells counted in the spleen. Data are presented as mean ± SEM. Sample size for all groups is 6. Absolute cell numbers are reported as (mean ± SEM) x 10\(^6\). Data from a single experiment are shown, as results were representative of multiple experiments.
lives (data not shown), a rapid distribution phase (error (Supplementary Data 5). This analysis yielded three half-
model (Supplementary Data 4) with relatively small residual
from the sc injection employed in this study was 95% (Fig. 4).

3786 min × nM (3.8 min × μM). Both values are close to the
value predicted by the model, and less than 2% of the total
calculated value was extrapolated. Therefore, bioavailability
value predicted by the model, and less than 2% of the total

Data from the sc dosing experiment fits a three compartment
model (Supplementary Data 4) with relatively small residual
error (Supplementary Data 5). This analysis yielded three half-
lives (data not shown), a rapid distribution phase (t_{1/2} ~ 7 min),
a rapid elimination phase (t_{1/2} ~ 7 min), and a slow terminal
elimination (t_{1/2} ~ 90 min). This last phase is consistent with
the terminal elimination calculated by the noncompartmental

<table>
<thead>
<tr>
<th>DEET  (mg/kg/day)</th>
<th>% CD4+</th>
<th>%CD8+</th>
<th>%DN</th>
<th>%DP</th>
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<td>Absolute CD8+ (cells × 10^6)</td>
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<tr>
<td>31</td>
<td>11.4 ± 0.28</td>
<td>4.1 ± 0.23</td>
<td>4.9 ± 0.28</td>
<td>79.6 ± 0.64</td>
</tr>
<tr>
<td>62</td>
<td>11.4 ± 0.70</td>
<td>3.8 ± 0.30</td>
<td>4.8 ± 0.52</td>
<td>79.9 ± 1.34</td>
</tr>
</tbody>
</table>

Note. DP = CD4+/CD8+, DN = CD4−/CD8−. *Significantly different from respective control (p ≤ 0.05). Absolute values were determined by multiplying
the percent gated cells by the total number nucleated cells counted in the spleen. Data are presented as mean ± SEM. Sample size for all groups is 6. Absolute
values are reported as (mean ± SEM) × 10^6. Data from a single experiment are shown, as results were representative of multiple experiments.

Table 2

**DISCUSSION**

DEET exposure has been suggested as a causative agent in
GWI via mechanisms of immune modulation (Rooke and
Zumla, 1998; Soetekouw et al., 2000; Vojdani and Thrasher,
2004). This is the first study designed to establish a NOAEL
and LOAEL for DEET based on immunotoxicity testing.
Therefore, this study assessed the effects of DEET to determine
if immunological alterations in a surrogate species (the mouse)
could occur at levels of exposure that might be relevant for the
recreational, occupational or military environments.

The anti-SRBC antibody (IgM) PFC response is considered
highly predictive of immunotoxicity with a concordance of
78% for prediction of immunotoxicity and 73% to decreased
host resistance (Luster et al., 1992, 1993; Selgrade, 1999). The
U.S. EPA specifies this test in combination with NK-cell
activity and alterations in immunophenotypes as necessary and
adequate to assess immunotoxicity in vivo (U.S. EPA
Immunotoxicity Test Guidelines, OPTTS 870.7800; U.S.
EPA, 1998b). Changes in IgM antibody responses and CD4/78 lymphocytic subpopulations are parameters both affected in
this study and together are considered highly predictive of
immunotoxicity with a concordance of 91% (Luster et al.,

Doses of DEET starting at 15.5 mg/kg/day increased the
suppression of IgM humoral immunity as a function of
increasing dose when measured by the PFC response. At this
time, the mechanism of suppression is not known and could
affect human health risk assessments depending on the
sensitivity of humans as compared with mice. However, the
reduction in the antibody response was not due to reduced
TABLE 3
DEET Absorption and Bioavailability in Female B6C3F1 Mice following iv or sc Exposure to 15.5 mg/kg DEET in an Olive Oil Carrier

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>iv dosing</th>
<th>sc dosing</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^2$</td>
<td>0.68</td>
<td>0.84</td>
</tr>
<tr>
<td>Correlation XY</td>
<td>-0.83</td>
<td>-0.92</td>
</tr>
<tr>
<td>Number of points for $\lambda_z$</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>$\lambda_z$ (1/min)</td>
<td>0.0057</td>
<td>0.0081</td>
</tr>
<tr>
<td>Half-life lower limit (min)</td>
<td>75</td>
<td>150</td>
</tr>
<tr>
<td>Half-life upper limit (min)</td>
<td>420</td>
<td>360</td>
</tr>
<tr>
<td>Half-life (min)</td>
<td>122</td>
<td>85</td>
</tr>
<tr>
<td>$T_{max}$ (min)</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>$C_{max}$ (nmol/l)</td>
<td>933</td>
<td>165</td>
</tr>
<tr>
<td>AUC$_{FI\text{N}}$ observed (min $\times$ nmol/l)</td>
<td>3984</td>
<td>3786</td>
</tr>
<tr>
<td>AUC$_{FI\text{N}}$ predicted (min $\times$ nmol/l)</td>
<td>3965</td>
<td>3781</td>
</tr>
<tr>
<td>% AUC extrapolated</td>
<td>1.54</td>
<td>0.78</td>
</tr>
<tr>
<td>$V_z$ observed (l/kg)</td>
<td>3569</td>
<td>2636</td>
</tr>
<tr>
<td>$V_z$ predicted (l/kg)</td>
<td>3586</td>
<td>2640</td>
</tr>
<tr>
<td>Cl observed (l/min/kg)</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>Cl predicted (l/min/kg)</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>AUMC$_{FI\text{N}}$ observed (min $\times$ min $\times$ nmol/l)</td>
<td>125,455</td>
<td>132,065</td>
</tr>
<tr>
<td>Mean residence time observed (min)</td>
<td>31</td>
<td>35</td>
</tr>
<tr>
<td>Mean residence time observed (min)</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td>$F$ (%)</td>
<td>—</td>
<td>95</td>
</tr>
</tbody>
</table>

Note. AUMC = area under the moment curve, IFN = infinity, $V_z$ = volume of distribution, Cl = clearance, $F$ = bioavailability

numbers of B-cells as it has been shown that 15.5 and 31 mg DEET/kg/day did not significantly decrease splenic B220+ cell numbers (Peden-Adams et al., 2001). Alteration of the CD4:CD8 ratio was observed at 7.7 and 15.5 mg DEET/kg/day, but this response did not vary with dose. Therefore, the LOAEL was conservatively established using the PFC data and not based on alterations observed in the CD4:CD8 ratio. These data indicate the NOAEL is 7.7 mg DEET/kg/day.

DEET is generally cleared from plasma within 24 h in both humans and mice. The PKs of DEET following sc administration at the LOAEL indicated a peak plasma level at 20 min and a half-life of 85 min in B6C3F1 female mice. In humans exposed to radiolabeled DEET on the forearm for 8 h, peak plasma levels were detected at 2 h and quantifiable levels were observed for only 4-h postexposure. The estimated half-life associated with the plasma means from Selim et al. (1995) are approximately 123 min for undiluted DEET and 82 min for DEET in 15% ethanol, as compared with 85 min in the current sc mouse model. In the current experiments, the half-life after an iv injection was 122 min.
also similar to those estimated from Selim et al. (1995). Therefore, the effects on humoral immunity observed in mice were not likely from accumulating doses of DEET, but rather following daily exposure to DEET that was generally cleared prior to the next dose.

Dermal exposure to DEET in humans results in absorption into the blood yielding systemic exposure without incurring first pass metabolism in the liver. In the liver, DEET is biotransformed into six metabolites that are primarily eliminated via urine (Schoenig and Osimitz, 2001; Selim et al., 1995). Oral dosing would require a first pass through the liver and would modify exposure to DEET and the spectrum of its metabolites. Thus, only sc or iv dosing can effectively mimic dermal dosing.

The PKs of these three routes vary in time to peak plasma levels, but the immunological effects observed in this study are not likely to be time to peak concentration ($T_{\text{max}}$) dependent. In some instances, $T_{\text{max}}$ can be used to monitor certain effects which are peak concentration ($C_{\text{max}}$) dependant such as central nervous system (CNS) effects (i.e., tremors, convulsions) and cardiovascular effects (i.e., changes in blood pressure and electrocardiogram) (Dixit, 2007). Effect dependence on peak plasma concentration ($C_{\text{max}}$) as noted by $T_{\text{max}}$ (time to $C_{\text{max}}$) has been observed with CNS effects in rats exposed to DEET (Schoenig and Osimitz, 2001). Nevertheless, it is unlikely that the timing of the peak concentration ($T_{\text{max}}$) influences humoral immune effects in this study. In the current study, the PK data demonstrate that detectable DEET concentrations in the plasma were generally cleared within 24 h (i.e., half-life 85 min). In the study by Selim et al. (1995) plasma DEET concentrations were undetectable after 4 h following removal of DEET from the skin. For most target organ toxicities a substantial presence is required for toxicity including immune function. For example, TCDD a potent immunotoxicant does not alter CD4/8 lymphocytic subpopulations in the spleen following acute exposure (Kerkvleit and Brauner, 1990). The PFC-response measures numbers of spleen cells producing IgM, and this response must be measured 4–5 days after SRBC injection.
Therefore, it is highly unlikely that the timing of the plasma peak associated with DEET via sc injection would influence this endpoint when measured 24 h after the last injection.

Assessment of human health risk from laboratory studies is fraught with problems including extrapolations between species, dose routes, and exposure time frames. Inherent in the process is exposure assessment, toxicity assessment, and risk characterization that utilizes information from the two previous framework components (e.g., exposure and toxicity assessment). This information is well reviewed elsewhere (Faustman and Omenn, 2001; National Research Council, 1994a,b, 1996; Selgrade, 1999; U.S. EPA, 1994, 2003). The traditional paradigm, when little PK or mechanistic data is available, might err on the side of caution and use 10-fold uncertainty factors (UFs) for extrapolations including exposure time, animals to humans, human variability and database limitations to arrive at reference concentrations or reference doses (RfD) and rely on the NOAEL (Faustman and Omenn, 2001). Margins of exposure (MOE) also rely on the NOAEL to bridge to estimated human exposure (Faustman and Omenn, 2001). In this paradigm, incorporation of mode of action or dosimetry of the active toxicant form is not included, often because it is not known. The best dose metric for a given toxicity is the concentration of the compound at the target tissue but the use of blood concentrations is an acceptable dose metric in place of actual organ concentrations. For example, spleen concentrations of DEET in humans are not likely concentrations that would be available for risk assessment, but blood concentrations following dermal application are available. Utilization of blood levels assumes the relationship and the human (Clewell et al., 2002). In the exposure assessment, information on human exposure is compiled from various sources. This information is then coupled with animal toxicity studies as presented here to characterize risk. Depending on the available data various approaches can be employed to assess risk including the traditional approaches using NOAEL and UF to achieve RfD and MOE. If PK or physiologically based pharmacokinetic (PBPK) data are available \( C_{\text{max}} \) and \( \text{AUC} \) can also be used as dose metrics to compare data between animal models and humans. Nevertheless, a full PBPK model is not always required to support a PK risk assessment (Clewell et al., 2002). To determine if DEET exposure during Gulf War deployment may have posed a risk related to suppressed immune function (Rook and Zumla, 1998; Soetekouw et al., 2000; Voidiani and Thrasher, 2004), the remainder of the discussion will address these points.

There is a wealth of data evaluating dermal absorption of DEET in various in vivo and in vitro models. Exhaustive reviews of these data are not required here as this has been well reviewed elsewhere (Qiu et al., 1998; Robbins and Cherniack, 1986; Schoenig and Osmint, 2001; Stinecipher and Shah, 1997). However, some mention is required as these data are often misconstrued and correct values are required to establish relevant, rather than excessive, exposure levels for this study.

Literature on DEET absorption is often misrepresented by studies that quote dermal penetration as opposed to actual absorption. Penetration has been reported in various human and animal models at ranges of 7–56% (Reifenrath and Robinson, 1982; Reifenrath et al., 1991; Schmidt et al., 1959; Smith et al., 1963; Spencer et al., 1979). Spencer et al. (1979) suggested that the \( ^{14} \text{C}-\text{DEET} \) that penetrated human skin was most likely only partially absorbed. Absorption studies in various animal models report DEET absorption ranges of 7–72% depending on species, sex, dose, and site of application. Reports of in vitro absorption values ranging from 14–95% in human skin samples and in animal models are often used to imply DEET absorption; however, no dermal in vivo human absorption rates have been reported above 20%. Published literature citing human dermal absorption at ranges of 50% or greater must then be carefully considered. For example, suggestions of 50% absorption referencing Spencer et al. (1979) miss-quote the original paper. Spencer et al. (1979) reported that Smith et al. (1963) estimated that because 50% of the dose evaporated, 50% of the applied DEET penetrated the skin. Data from Spencer et al. (1979) indicates that in vitro studies with human skin resulted in 9.7% evaporation and penetration of 50.8% (residual in skin). Moreover, they clearly delineate the difference between penetration and total absorption and suggest that due to dermal retention, lower absorption (less than 50% of the applied dose) would be expected.

Two published studies report in vivo dermal absorption in humans (Feldmann and Maibach, 1970; Selim et al., 1995). Feldmann and Maibach (1970) reported the average total absorption of DEET (% of applied dose) over 5 days was 16.7%. Selim et al. (1995) attempted an actual mass-balance and concluded that the average absorption was 5.6% for the neat compound and 8.4% for DEET in 15 percent ethanol, but the means were not statistically different. For further purposes of discussion the low (5.6%), average (10%), and high (16.7%) dermal absorption values will be used for comparison between studies to ascertain estimates of human exposure relevance.

According to U.S. EPA estimates, dermal exposure for adult males is 12.1 mg DEET/kg/day (U.S. EPA, 1998a). Using low, medium and high dermal absorption values, blood levels are estimated to range from 0.7 to 2.0 mg/kg/day (Table 4). Reliable estimates of exposure levels in soldiers and other occupational exposures do not exist. The only published data that evaluates exposure of humans under occupational conditions are from a 1986 National Institute for Occupational Safety and Health (NIOSH) self survey of employees in the Everglades National Park (McConnell et al., 1986). The mean weekly estimate of dermal DEET application established was 14.6 g/week (30 mg DEET/kg/day assuming a 70 kg person). However, 5% of the employees applied 57.5 g/week (145 mg DEET/kg/day) or more to the skin and 1% applied 392 g/week (801 mg DEET/kg/day). Based on the influence of low, average, or high dermal
absorption rates, the estimated blood absorption range is 1.7–134 mg/kg/day for the NIOSH survey (Table 4). This overlaps with that of deployed soldiers with reported exposure of either 40 or 400 mg DEET/kg/day (Abu-Qare and Abou-Donia, 2000, 2001; Abou-Donia et al., 2001).

Because 95% of the sc dose given in the current study was absorbed, the doses administered approximate human dermal applications ranging from 44 to 1052 mg/kg/day based on low, average or high human dermal exposure estimates (Table 5). Specifically, the NOAEL (7.7 mg DEET/kg/day sc) in this study translates to a human dermal exposure of 130, 73, or 44 mg DEET/kg/day based on 95% bioavailability giving an absorbed dose of 7.3 mg DEET/kg/day and the low (5.6%), average (10%), and high (16.7%) human dermal absorption estimates, respectively (Fig. 5B). Utilization of the blood concentration in the mouse allows better estimation of the human dermal exposure that would be required to achieve that same blood level based on the varied estimates of DEET dermal absorption percentages (Fig. 5). Subsequently, the LOAEL (15.5 mg DEET/kg/day sc) translates to a human dermal exposure of 263, 147, or 88 mg DEET/kg/day based on low, average, or high human dermal absorption estimates, respectively (Table 5). The dermal exposure estimates for the LOAEL overlap with reported human exposure estimates in the occupational and military environments (Abu-Qare and Abou-Donia, 2000, 2001; Abou-Donia et al., 2001; McConnell et al., 1986). With the application of an UF of 10 for extrapolation of data between species (rodent to human), the NOAEL established in this study would clearly include the reported occupational and military exposure estimates and would overlap levels of estimated DEET usage in the general population (Table 6).

Based on estimated absorbed plasma levels, the MOE is calculated to be 6.1 based on the U.S. EPA estimate of 12.1 mg DEET/kg/day dermal exposure (10% absorption) for the adult male in the general population and the NOAEL estimate of 7.3 mg DEET/kg/day absorbed blood levels (Table 4). This value is less if using the exposure estimates for those working in the Everglades or deployed by the military (Table 4). The MOE is the ratio of the NOAEL in the animal study to exposure levels estimated in the human population. MOE values less than 100 may serve as an alert for further testing (Faustman and Omenn, 2001). Although we have used absorbed DEET levels based on

### Table 4

<table>
<thead>
<tr>
<th>Reported estimated dermal exposure levels (mg/kg/day)</th>
<th>Low ( (5.6%) )</th>
<th>Average ( (10%) )</th>
<th>High ( (16.7%) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult male (general population)</td>
<td>12.1</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Adult female (general population)</td>
<td>9.68</td>
<td>0.5</td>
<td>0.97</td>
</tr>
<tr>
<td>Child 13–17 (general population)</td>
<td>21.05</td>
<td>1.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Child under 12 (general population)</td>
<td>37.63</td>
<td>2.1</td>
<td>3.8</td>
</tr>
<tr>
<td>Occupational exposure</td>
<td>30</td>
<td>1.7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>136</td>
<td>7.6</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>801</td>
<td>45</td>
<td>80</td>
</tr>
<tr>
<td>Deployed soldiers</td>
<td>40</td>
<td>2.2</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>22.4</td>
<td>40</td>
</tr>
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<td></td>
<td></td>
<td>67</td>
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<td>0.33</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

\( ^a \) (Feldmann and Maibach, 1970; Selim et al., 1995).

\( ^b \) This was calculated as 5.6, 10, or 16.7% of the reported estimated dermal exposure levels and represents, based on these varied dermal absorption rates, the amount that would be absorbed into the blood from the applied dermal exposure reported in the literature.

\( ^c \) MOE = mouse blood NOAEL/human blood exposure. This was calculated by determining the mouse blood exposure level based on 95% bioavailability (7.3 mg/kg/day absorbed) of sc injected NOAEL dose (7.7 mg/kg/day) observed in the current study and calculated human blood exposure levels from the reported dermal estimates.

\( ^d \) Mean percent dermal absorption of applied dermal exposure from Selim et al. (1995).

\( ^e \) Mean percent dermal absorption of applied undiluted DEET from Feldmann and Maibach (1970).

\( ^f \) Mean percent dermal absorption of applied DEET from Feldmann and Maibach (1970).

\( ^g \) (U.S. EPA, 1998a).

\( ^h \) (McConnell et al., 1986).

\( ^i \) Assuming a 70 kg adult male.

\( ^j \) (Abu-Qare and Abou-Donia, 2001; Abou-Donia et al., 2001).

\( ^k \) (Abu-Qare and Abou-Donia, 2000).
estimates of human dermal exposure levels (Table 6) could be used to support human exposure levels (mg/kg/day) required to attain mouse blood exposure levels\(^a\)

<table>
<thead>
<tr>
<th>DEET treatment (mg/kg/day)</th>
<th>Estimated mouse blood exposure level (mg DEET/kg/day)</th>
<th>Low(^c) (5.6%)</th>
<th>Average(^d) (10%)</th>
<th>High(^e) (16.7%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.7</td>
<td>7.3</td>
<td>130</td>
<td>73</td>
<td>44</td>
</tr>
<tr>
<td>15.5</td>
<td>14.7</td>
<td>263</td>
<td>147</td>
<td>88</td>
</tr>
<tr>
<td>31</td>
<td>29.5</td>
<td>527</td>
<td>295</td>
<td>177</td>
</tr>
<tr>
<td>62</td>
<td>58.9</td>
<td>1052</td>
<td>589</td>
<td>353</td>
</tr>
</tbody>
</table>

\(^a\)Based on low (5.6%), average (10%), or high (16.7%) human dermal percent absorption values reported in the literature (Feldmann and Maibach, 1970; Selim et al., 1995).

\(^b\)Based on 95% bioavailability from the subcutaneous injection into the blood.

\(^c\)Mean percent dermal absorption of applied undiluted DEET from Selim et al. (1995).

\(^d\)Average of the three reported mean for in vivo dermal absorption percentages for DEET of 5.6, 8.4, and 16.7% from the literature (Feldmann and Maibach, 1970; Selim et al., 1995).

\(^e\)Mean percent dermal absorption of applied DEET from Feldmann and Maibach (1970).

95% bioavailability from the sc injection as a basis of comparison to reported human exposure levels (Fig. 5), there are several additional aspects involved in characterization of risk not considered in this manuscript such as mechanism of effect, differences in species susceptibility/sensitivity related to the mechanism, and additional factors of uncertainty (Faustman and Omenn, 2001). Utilization of compound specific data and identification of the mechanism of action related to suppressed IgM production by DEET could greatly influence the RfD and risk assessment for this compound; however, as the mechanism of action is not known modifying factors were not used and the UF defaults of 10-fold were applied (Table 6). Additional data on the mechanism along with utilization of a parallelogram approach (Selgrade et al., 1995) would decrease uncertainty and allow for modification of the UF used in the assessment of risk related to DEET-induced IgM suppression.

With available PK data presented here and reported in the literature for humans it is possible to compare data from human studies with the current mouse model using \(C_{\text{max}}\) (peak plasma concentration) as an estimate of exposure. In an unpublished study funded by the DEET Joint Venture and reviewed by Schoenig and Osimitz (2001) adult males (57 mg/kg/day) and females (43 mg/kg/day) were exposed to undiluted DEET on the skin for 8 h either for a single exposure or a repeated exposure (4 consecutive days) with mean peak plasma levels \((C_{\text{max}})\) ranging from 0.25 to 0.62 \(\mu\)g/ml. Estimated \(C_{\text{max}}\) from means presented in Selim et al. (1995) are 0.074 and 0.047 \(\mu\)g/ml for diluted and undiluted DEET, respectively. In the current mouse model, \(C_{\text{max}}\) for the LOAEL was 0.031 \(\mu\)g/ml which is lower than the human estimates noted above. If the suppression of SRBC-specific IgM noted in the current B6C3F1 model is peak plasma level dependent (\(C_{\text{max}}\)) then humans exposed to DEET could be at risk for immunotoxic effects. However, \(C_{\text{max}}\) is often best used for indicating the likelihood of toxicity when associated with endpoints that are short-term or rapidly reversible such as acute neurotoxicity effects (Clewell et al., 2002). In these instances the effect results from the current concentration in the tissue or blood. As suppression of IgM production was measured 24 h after the last dose and \(C_{\text{max}}\) occurred within 20 min of the injection, it is not likely that the effects observed are \(C_{\text{max}}\) related.

Long-term effects of those not \(C_{\text{max}}\) dependant may be cumulative depending on the concentration, frequency and duration of exposure. In these cases, AUC may be appropriate for comparison of animal data to humans (Clewell et al., 2002; Schoenig and Osimitz, 2001). AUC determinations have been used previously to establish predictive relationships between immune parameters and chemical-induced stress (Prueitt et al., 2000). AUC equates to total exposure. Using the plasma means reported in Selim et al. (1995) an AUC was estimated for comparison and was 63,316 nmol × min/l for undiluted DEET and 32,276 nmol × min/l for DEET diluted in 15% ethanol. The DEET used by Selim et al. (1995) was radiolabeled and the method of detection was scintillation counting; therefore,
the measured parameters represent total radioactivity from DEET and its metabolites. These values greatly overestimate the actual exposure to DEET. In the DJV study, human volunteers were exposed to DEET on the skin continuously for 8 h for 1 or 4 consecutive days. This resulted in AUCs ranging from 659 to 1603 nmol × min/L. The calculated AUC in the current study for a single sc injection at the LOAEL was 3786 nmol × min/L. This is between two to six times greater than that achieved by an 8-h application in humans for 1 or 4 consecutive days (Schoenig and Osimite, 2001).

In summary, the T-dependent IgM response was dose-responsively suppressed by DEET in B6C3F1 mice. Using the mouse as a surrogate species for human risk assessment in immunotoxicology is appropriate as alterations in murine immune function have been linked to human health risk (Selgrade, 2007). When comparing the DEET doses used in this rodent study, the half-life is similar to that reported in humans, the calculated reference dose with UFs overlaps with current estimates of occupational or military exposure, the MOE warrants further investigation, and the AUC is within a 10-fold difference of that reported for humans. These data, therefore, suggest that DEET may affect immune function at exposure levels reported in occupational and military settings. This is important as altered immune function and increased disease susceptibility has been implicated as a causative effect in reported symptoms of Gulf War Veterans. At this time, it is unclear if DEET is a potential health hazard for the general population without more information delineating the mode and/or mechanism of action and further examination of the duration of exposure. Based on the widespread usage of DEET and estimated levels of exposure in the general population, further investigation is necessary for accurate risk characterization and to assess the status of immune function in adults, as well as consider vulnerable populations such as children. Therefore, determination of the mode and/or mechanism of action for DEET are essential to accurate risk characterization.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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