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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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JAN 24 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

**MEMORANDUM**

SUBJECT: DEET: Review of pharmacokinetics and metabolism studies on DEET

Caswell No. 346
MRID No. 419944-01
419944-02
419944-03

HED Project No. 1-2458
EPA ID No. 080301

TO: Jane Mitchell, PM Team (70)
Special Review and Re-registration Division (H7508C)

FROM: Whang Phang, Ph.D. 1/16/92
Pharmacologist
HFAS/Tox. Branch II/ HED (H7509C)

THROUGH: James Rowe, Ph.D. 1/16/92
Section Head, Section III
and
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Toxicology Branch II has been requested to review pharmacokinetics and metabolism studies on DEET. These studies were evaluated and the conclusions are as follows:

A. Selim, S. (1991) Pharmacokinetics and comparative absorption study of N,N-dimethyl-m-toluamide (DEET) in the rat. Study conducted by Biological Test Center, Study No. P01836. Submitted to EPA by DEET Joint Venture/Chemical Specialties Manufacturers Association. EPA MRID No. 419944-01

Conclusion: A series of experiments which consisted a preliminary and 6 definitive experiments was conducted to determine the absorption, distribution, elimination, and metabolism of DEET. In the preliminary experiment, groups of rats (4/sex) received a single dose (100 mg/kg bw) of

radiolabeled DEET by either oral (gavage) or dermal administration, and the blood radio- activity levels were measured at various intervals for 24 hours for determining the peak blood $^{14}C$-level. In the definitive experiments, groups of rats (5/sex/dose regimen) received DEET by single oral low dose (100 mg/kg), single oral high dose (500 mg/kg), repeated oral low dose (100 mg/kg), or single dermal low dose (100 mg/kg). Two groups (5 rats/sex/group), a single oral low dose and a single dermal low dose groups, were sacrificed at peak blood $^{14}C$ level to determine the radioacti-vity levels in various tissues. The results were as follows:

1). The peak blood level was reached in 0.5 hour after oral dosing in males while in females it took about 2 hours. In comparison, with dermal application, instead of a clear peak, a plateau which began approximately 1.5 hours after dosing in both male and female rats was found. This plateau persisted until the termination of the study (24 hours after dosing). These data indicated that when DEET was dermally applied on rats, a small amount of the test compound was continuously absorbed from the treatment site.

2). The fraction of the administered dose reaching the systemic circulation at peak $^{14}C$-blood level and the tissue residue levels following oral administration was substantially higher than that following dermal applications. At peak $^{14}C$-blood levels, approximately 17% and 5.3% of the dermally applied dose was absorbed by males and females, respectively; in comparison, approximately 53.3% and 65.25% of the orally administered dose was absorbed by males and females, respectively.

3). The major route of eliminated of DEET was via urine in both male and female rats. No marked difference was found in the total urinary or fecal radioactivity among the different dosing regimens or between male and female rats of different dosing regimens. However, there was a difference in the rate of urinary elimination of DEET among the different dose groups. For example, repeated oral dose groups or pretreatment groups showed the fastest rate of urinary excretion during the first 4 hours after dosing than any other dose groups; they were followed by single oral low-dose groups whose rate was faster than that of the single oral high-dose groups. The single dermal low-dose groups showed the slowest rate of urinary excretion; this finding might reflect the slow rate of dermal absorption. The increased rate of urinary elimination of radioactivity accompanied by slightly more total urinary radioactivity in repeated-dose groups relative to the other dose groups might reflect "an induction of liver microsomal enzyme system".

4). The liver, kidneys, lung, spleen, whole blood, and the

carcass contained higher radioactivity than any other tissues. The relatively higher radioactivity levels in the liver, kidneys, lung, and spleen could partially be due to higher perfusion rates since the radioactivity in whole blood was markedly greater than that in plasma. however, the total radioactivity found in all the tissues of various groups ranged from only 0.15% to 0.67% of the administered dose. Therefore, very little DEET was sequestered in the body.

5). The tissue radioactivity levels in animals sacrificed at the peak $^{14}C$-blood levels indicated a substantially higher amounts of tissue radioactivity in orally dosed animals than those of dermally treated animals. This finding also reflected a much slower rate of absorption by the skin than that by the G.I. tracts.

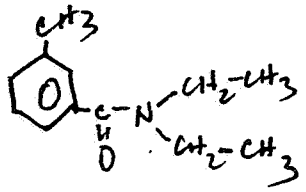
6). The means of the total radioactivity recovered in animals which received radiolabeled DEET by various regimens ranged from approximately 88% to 94% of the administered dose.

This study in combination with its addendum (MRID No. 41994-02) and the study with MRID No. 419944-03 meet the data requirements for a metabolism study (Guideline reference No. 85-1) and is classified as **acceptable.**

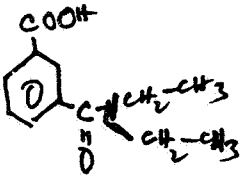
B. Selim, S. (1991) Addendum to Report Entitled "Pharmacokinetics and comparative absorption study of N,N-dimethyl-m-toluamide (DEET) in the rat". Study conducted by Biological Test Center, Study No.: Addendum P01836. Submitted to EPA by DEET Joint Venture/Chemical Specialties Manufacturers Association. EPA MRID No. 419944-02

Conclusion: In the metabolism phase of the study, a group of 5 rats/sex was administered radiolabeled DEET by gavage at a single dose of 500 mg/kg b.w. which was similar to the high dose in the ADE phase of the study. After dosing, the excreta of these animals were collected for isolation and identification of major metabolites. After identification of the major metabolites, a determination of the metabolite profile and quantitation of $^{14}C$ residues were conducted in urine samples obtained from the animals treated according to various dosing regimens used in the ADE phase of the study (MRID No. 419944-01).

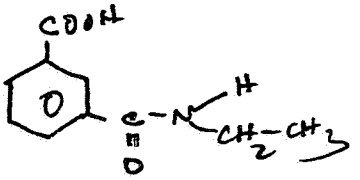
Two major metabolites were isolated and identified in the urine samples of all treated animals. The structures of DEET and its two major metabolites are indicated below:



DEET



Metabolite A



Metabolite B

Metabolite A, which accounted for approximately 50% of the administered radioactivity, was formed by oxidation of the methyl group on the aromatic ring. Metabolite B, which accounted for less than 18% of the administered dose, was formed by oxidation of the methyl group on the aromatic ring and N-dealkylation of an ethyl substituent on the amide moiety. The results indicated that DEET was quantitatively metabolized, and the presence of the parent compound was below the detection limit in the urine samples of the treated rats irrespective of sex or of dermal or oral routes of administration.

C. Lin, P. and Selim, S. (1991) Determination of expired $^{14}C$ volatiles following a single oral or dermal dose of N,N-diethyl-m-toluamide (DEET) in the rat. Study conducted by Biological Test Center, Study No. P01862. Submitted to EPA by DEET Joint Venture/Chemical Specialties Manufacturers Association. EPA MRID No. 419944-03.

Conclusion: Groups of rats (2/sex/dose regimen) received $^{14}C$-DEET by either oral (gavage) or dermal route at 100 mg/kg b.w.. The expired radioactivity was measured for 24 hours. The results indicated that less than 0.017% of the administered radioactivity was detected in both males and females of either orally or dermally dosed groups. There was no significant difference in the expired radioactive $CO_2$ and volatile metabolites between male and female rats and between oral and dermal dose groups.

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**DATA EVALUATION REPORT**

**Study Type:** Pharmacokinetics and comparative dermal absorption study of N,N-dimethyl-m-toluamide (DEET) in the rat

**Chemical:** DEET (N, N-diethyl-m-toluamide)

**Caswell No.** 346 **HED Proj. No.** 1-2458
**MRID No.** 419944-01 **EPA Case No.** 819244
**EPA ID No.** 080301

**Sponsor:** DEET Joint Venture/Chemical Specialties Manufacturers Association

**Testing Facility:** Biological Test Center (BTC)
P.O. Box 19791
2525 McGaw Ave.
Irvine, CA 92713-9791

**Citation:** Selim, S. (1991) Pharmacokinetics and comparative absorption study of N,N-dimethyl-m-toluamide (DEET) in the rat. Study conducted by Biological Test Center, Study No. P01836. Submitted to EPA by DEET Joint Venture/Chemical Specialties Manufacturers Association. EPA MRID No. 419944-01

**Conclusion:** A series of experiments which consisted of a preliminary and 6 definitive experiments was conducted to determine the absorption, distribution, elimination, and metabolism of DEET. In the preliminary experiment, groups of rats (4/sex) received a single dose (100 mg/kg bw) of radiolabeled DEET by either oral (gavage) or dermal administration, and the blood radio- activity levels were measured at various intervals for 24 hours for determining the peak blood $^{14}C$-level. In the definitive experiments, groups of rats (5/sex/dose regimen) received DEET by single oral low dose (100 mg/kg), single oral high dose (500 mg/kg), repeated oral low dose (100 mg/kg), or single dermal low dose (100 mg/kg). Two groups (5 rats/sex/group), a single oral low dose and a single dermal low dose groups, were sacrificed at peak blood $^{14}C$ level to determine the radioacti- vity levels in various tissues. The results were as follows:

1). The peak blcod level was reached in 0.5 hour after oral dosing in males while in females it took about 2 hours. In comparison, with dermal application, instead of a clear

peak, a plateau which began approximately 1.5 hours after dosing in both male and female rats was found. This plateau persisted until the termination of the study (24 hours after dosing). These data indicated that when DEET was dermally applied on rats, a small amount of the test compound was continuously absorbed from the treatment site.

2). The fraction of the administered dose reaching the systemic circulation at peak $^{14}C$-blood level and the tissue residue levels following oral administration was substantially higher than that following dermal applications. At peak $^{14}C$-blood levels, approximately 17% and 5.3% of the dermally applied dose was absorbed by males and females, respectively; in comparison, approximately 53.3% and 65.25% of the orally administered dose was absorbed by males and females, respectively.

3). The major route of elimination of DEET was via urine in both male and female rats. No marked difference was found in the total urinary or fecal radioactivity among the different dosing regimens or between male and female rats of different dosing regimens. However, there was a difference in the rate of urinary elimination of DEET among the different dose groups. For example, repeated oral dose groups or pretreatment groups showed the fastest rate of urinary excretion during the first 4 hours after dosing than any other dose groups; they were followed by single oral low-dose groups whose rate was faster than that of the single oral high-dose groups. The single dermal low-dose groups showed the slowest rate of urinary excretion; this finding might reflect the slow rate of dermal absorption. The increased rate of urinary elimination of radioactivity accompanied by slightly more total urinary radioactivity in repeated-dose groups relative to the other dose groups might reflect "an induction of the liver microsomal enzyme system".

4). The liver, kidneys, lung, spleen, whole blood, and the carcass contained higher radioactivity than any other tissues. The relatively higher radioactivity levels in the liver, kidneys, lung, and spleen could partially be due to higher perfusion rates since the radioactivity in whole blood was markedly greater than that in plasma. however, the total radioactivity found in all the tissues of various groups ranged from only 0.15% to 0.67% of the administered dose. Therefore, very little DEET was sequestered in the body.

5). The tissue radioactivity levels in animals sacrificed at the peak $^{14}C$-blood levels indicated substantially higher amounts of tissue radioactivity in orally dosed animals

than those of dermally treated animals. This finding also reflected a much slower rate of absorption by the skin than that by the G.I. tracts.

6). The means of the total radioactivity recovered in animals which received radiolabeled DEET by various regimens ranged from approximately 88% to 94% of the administered dose.

This study in combination with its addendum (MRID No. 41994-03) met the data requirements for a metabolism study (Guideline reference No. 85-1) and was classified as **acceptable.**

**Methods and Materials**

Test Article: The radiolabeled DEET was uniformly labeled with $^{14}C$ in the benzene ring. The specific activity was 22 μCi/mmole, and radiochemical purity was 97.4%. The nonradiolabeled technical grade DEET (Lot A-1-96) (purity 98.3%) was provided to the testing laboratory by Morflex Inc., and it was used for isotopic dilution in the study and as a standard for determining the purity of the radiolabeled compound. The purity of the compound was analyzed by HPLC.

Test Animals: Seven week old Charles River Sprague-Dawley rats were obtained from Charles River Breeding Lab., Portage, Michigan.

**Study Design**

A series of experiments was conducted to determine the absorption, distribution, elimination, and metabolism of DEET.

**1. Preliminary $^{14}C$ blood level experiments**

a. Oral administration
Four male and 4 female rats received radiolabeled DEET (≈100 mg/kg bw) by gavage following an 18 hour fasting. The compound was administered as a constant volume of 2 ml/kg bw in corn oil. Blood samples were collected from the tail vein of the treated rats at 1/4, 1/2, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours after dosing. After 24 hour collection of the blood samples, the treated animals were sacrificed, and this part of the experiment was terminated.

b. Dermal application
The back of 4 male and 4 female rats were shaven the day before the treatment. A "glass contoured rectangular enclosure (2.5 cm x 5 cm; max. height 1.3 cm) was glued on the middle of each animal's back with a cyanoacrylate-based glue.

Silicone medical adhesive , Type A, was applied as a seal around the outside of each enclosure" (MRID No. 419944-01). After 18 hours of fasting, each animal received 20 μL of $^{14}C$-DEET at 100 mg/kg bw with a micropipette. "The actual amount of the test material applied to each animal was determined by weighing the dosing pipette before and after delivery of the test material. After dosing, the rectangular cell was covered with a glass slide to prevent disturbance of the application site and mechanical loss of the test material" (MRID No. 419944-01). Blood sample collections and the rest of experiment were similar to that described in the oral dose experiment.

**2. Definitive experiments**

The test groups and the number of animals/group were as follows:

| Group No. | No. of Rats | Dosing* | |
|---|---|---|---|
| Group 1 | 5 rats/sex | Single oral low-dose | (100 mg/kg) |
| Group 2 | 5 rats/sex | Single oral high-dose | (500 mg/kg) |
| Group 3[a] | 5 rats/sex | Repeated oral low-dose | (100 mg/kg) |
| Group 4[b] | 5 rats/sex | Single oral low-dose | (100 mg/kg) |
| Group 5 | 5 rats/sex | Single dermal low-dose | (100 mg/kg) |
| Group 6[b] | 5 rats/sex | Single dermal low-dose | (100 mg/kg) |

*: by gavage.

a: The animals in this group received cold DEET (100 mg/kg bw) for 13 days prior to a single oral low-dose of radiolabeled DEET (≈100 mg/kg).

b: These 2 groups of animals were sacrificed at the time of peak $^{14}C$-blood level for determining the $^{14}C$ levels in various tissues.

Following treatment, all rats were maintained in individual metabolism cages. Four hours after treatment, food and drinking water were provided ad libitum. Urine and feces samples were collected from rats in groups 1, 2, 3, and 5 at intervals of 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours. Seven days after termination of treatments, the animals in Groups 1, 2, 3, & 5 were sacrificed, at least 5 ml of blood was collected from the thorax, and the selected tissues and organs, such as gastrointestinal (GI) tract, GI contents, bone, brain, spinal cord, sciatic nerve, fat, gonads, heart, kidneys, liver, lungs, blood, muscle, spleen, and carcass, were removed, weighed, and analyzed for $^{14}C$

levels. The cages were rinsed with methanol, and the rinse was analyzed for radioactivity with a Beckman liquid scintillation spectrophotometer.

For the dermal groups, the skin treatment sites and the enclosures were removed, rinsed with methanol, and processed for $^{14}C$-activity determination. The report did not clearly state how long the compound remained on the treatment site. However, based upon the results for Group 5 animals, the compound remained on the treatment site under the skin enclosure for 7 day. For Group 6 animals, the dermal application went on for 3.5 hours.

A quality assurance statement was signed and included in the report.

**RESULTS**

I. $^{14}C$ blood level experiments (Preliminary experiments)

a. Oral administration (gavage)

Four rats/sex received DEET at ≈ 100 mg/kg by gavage, and the amounts of radioactivity received by the test animals were 20.1 and 15.6 μCi for males and females, respectively. No signs of toxicity were seen in treated animals. In males, the peak $^{14}C$-blood levels was found at 0.5 hours after dosing (Table 1 and Figure 1). Subsequently the blood radioactivity level dropped precipitously, and at 5 hours after dosing the level reached a plateau which persisted until the end of the study (Figure 1). In females, the peak $^{14}C$-blood level occurred at 2 hours, after which the level dropped rapidly and reached a plateau at approximately 5 hours. The plateau lasted until the end of the experiment.

b. Dermal application

The mean applied doses were 103.2 and 111.6 mg/kg for male and female rats, respectively. The total amounts of the administered radioactivity were 21 and 17 μCi for males and females, respectively. In both males and females, the blood radioactivity level reached a plateau 1 hour after dermal application, and a clear peak of $^{14}C$-blood level could not be determined (Table 2 and Figure 1). However, the 3.5 hour was selected as the peak radioactivity level following dermal administration. The plateau was maintained until the termination of the study.

II. Definitive experiments

1. Single oral low dose (Group 1)

In male rats, the mean administered dose and radioactivity level were 103.9 mg/kg and 19.3 μCi, respectively. A mean of 86.72% of the administered radioactivity was eliminated via urine while 3.58% was detected in the feces (Table 3). During the first 12 hours following dosing, approximately 75% of the administered radioactivity was eliminated in the urine whereas 0.01% was eliminated in the feces. A total (mean) of 90.3% of the administered radioactivity was eliminated in urine and feces. The radioactivity in all the tissues examined was 0.67% of the administered $^{14}C$, 96% of which was the radioactivity in the carcass (or 0.65% of the administered $^{14}C$) (Table 4). The mean radioactivity level in the brain was 0% in male rats. The mean total recovery of $^{14}C$ approximately 91% of the administered radioactivity.

In female rats, The mean administered dose and radioactivity level were 104.5 mg/kg and 12.3 μCi, respectively. For 7 days of monitoring, the total means of the radioactivity eliminated in the urine and feces were 86.7% and 3.5% of the administered radioactivity, respectively (Table 5). During the first 12 hours after dosing, 75% of the administered radioactivity was eliminated. The mean total radioactivity in the tissues examined was 0.24%; as in the males, the majority of the radioactivity was found in the carcass. The mean radioactivity level in the brain was 0% in female rats (Table 6). The mean total $^{14}C$ recovery was 88.4% of the administered radioactivity.

2. Single oral high dose (Group 2)

Males: The mean dose and radioactivity level received by this groups of male rats were 532.8 mg/kg and 20.4 μCi, respectively. The amounts of radioactivity eliminated in the urine and feces, expressed as % of the administered dose, at various time intervals were summarized in Table 7. The total means of 86.70% and 5.92% of the administered radioactivity were found in the urine and feces, respectively. Majority of the radioactivity in the urine was found during the first 36 hours after dosing (Table 7). In feces, the radioactivity level was below the detection limit during the first 12 hours, and most of it was found between 12 to 72 hours. A total of 92.62% of administered radioactivity was found in the urine and feces during the duration of the study (7 days).

A total of only 0.23% of the dosed radioactivity was found in the tissues and carcass, and the majority of the 0.23% was detected in the carcass (Table 8). The radioactivity level in the brain was below the detection limit. The total mean of the recovered radioactivity was 92.86% of the dose

(Table 25, page 39).

Females: This group of female rats received 523.4 mg/kg (mean) with the mean radioactivity level of 13.2 μCi. The means of the amount of the radioactivity eliminated in the urine and feces were 88.81% and 4.90% of the administered dose, respectively (Table 9). In urine most of the radioactivity was eliminated during the first 36 hours following dosing. In feces, the radioactivity level was below the detection limit during the first 12 hours after dosing, and most of the radioactivity was detected between 12 and 72 hours after dosing. A mean of 93.7% of the dosed radioactivity was found in urine and feces.

In the tissues and carcass a total of 0.39% of the dosed radioactivity was detected. Most of it was found in the carcass, and radio activity in the brain was below the detection limit (Table 8). The total mean radioactivity recovery was 94.10% for this group of females (Table 25).

3. Repeated oral low-dose (Group 3)

Males: The male rats in this group received 104.4 mg/kg (mean) with a mean radioactivity level of 18.9 μCi. With repeated oral dosing, 58.13% of the administered radioactivity was eliminated in the urine during the first 4 hours after dosing, and by 12 hours following dosing, approximately 85% of the administered radioactivity was detected in the urine (Table 10). A total of 90.59% of the dosed radioactivity was found in the urine. In the feces, a total 3.12% of the administered dose was found, and the majority of this fecal elimination occurred between 12 and 24 hours after dosing. The radioactivity was not detected in the feces during the first 8 hours after dosing (Table 10). A total of 93.71% of the administered radioactivity was eliminated in urine and feces of the male rats.

A total of 0.15% of the dosed radioactivity was found in the tissues and carcass of male rats, and most of it was contributed by that of the carcass (0.13%). A detectable level of radioactivity was not found in the brain (Table 11). For this group of male rats, the mean total radioactivity recovery was 93.86% of the administered dose (Table 25).

Females: The mean dose received by this group of females was 103.1 mg/kg with a mean radioactivity level of 11.8 μCi. Like males, 45.18% of the dosed radioactivity found in the urine was eliminated during the first 4 hours after dosing, and by 12 hours 82.22% of the dose $^{14}C$ was detected in the urine (Table 12). The total radioactivity elimi-

nated in the urine during the duration of the study was 90.09% of the dose. In feces, only 3.36% of the administered radioactivity was detected, and most of it was eliminated during the interval of 12 to 24 hours after dosing. The total radioactivity eliminated in the urine and feces was 93.45% of the administered dose.

In the tissues and carcass examined, only 0.42% of the administered dose was found. A detectable level of radioactivity was not found in the brain or the reproductive organs. Total mean recovery was 93.71% of administered radioactivity for this group of female rats (Table 25).

4. Single oral low-dose/animals sacrificed at peak 14C-blood levels (Group 4)

Male rats: The mean dose received by this group of rats was 104.1 mg/kg with a mean radioactivity level of 20.5 μCi. When the animals were sacrificed at peak $^{14}C$-blood level (0.5 hour after dosing), a total of 46.70% of the administered radioactivity remained in the gastrointestinal (GI) tract (Table 13). Most of this radioactivity was contributed by that of the stomach (30.32%) and small intestinal contents (10.03%). Based on these results, approximately 53.3% of the administered radioactivity was absorbed from the GI tracts 0.5 hour after dosing. The tissue radioactivity levels were examined, and the results were reported in units of ppm (Table 14). At peak blood level, DEET appeared to be distributed into every tissues examined (Table 14). If the plasma radioactivity level (48.7 ppm) was used as a basis of comparison and excluding that in the GI tracts, other organs with more radioactivity than that of plasma were liver (90.4 ppm), kidney (270.2 ppm), and fat (51.9 ppm). However, the brain (16.4 ppm) had less than half of that of the plasma.

Females: The mean dose of DEET received by this group of females was 105.5 mg/kg with a mean radioactivity level of 13.9 μCi. When these female rats were sacrificed at the peak $^{14}C$ level, a total of 34.7% of the radioactivity remained in the GI tracts (Table 13). As in males, much of the radioactivity was contributed by the radioactivities in the stomach (22.3%) and intestinal intestinal (4.4%) and the stomach itself (5.3%). Based upon these data, approximately, 65.3% of the administered radioactivity was absorbed from the GI tracts at 2 hours after dosing. As in males, at peak blood level radioactivity was found in all tissues examined (Table 14). Using the radioactivity level in the plasma (29.2 ppm) as a basis of comparison and excluding those in the GI tracts, the tissues which contained more radioactivity than that in the plasma were lungs (37.2 ppm), liver (58.5 ppm), kidneys (158.0 ppm),

and sciatic nerve (65.1 ppm). The radioactivity level in the brain (14.2 ppm) was less than that in the plasma.

5. Single dermal low-dose (Group 5)

Males: This group of male rats received a mean value of 98.4 mg/kg with a mean radioactivity level of 15.0 μCi. With dermal application, less than 1% of the administered dose was eliminated in the urine during the first 4 hours following dosing (Table 15). A majority of the radioactivity in the urine (58.7%) was eliminated during the interval from 12 to 72 hour after dosing. A total of 77.86% of the administered radioactivity was eliminated in the urine. In the feces, only 3.98% of the administered radioactivity was detected, and most of the radioactivity was eliminated after 12 hours following administration (Table 15). A mean of 81.84% of the administered radioactivity was eliminated in the urine and feces.

A total of 0.21% of the administered radioactivity was found in all tissues examined, and the carcass contained most of the radioactivity (0.19%). The mean radioactivity levels in the brain, kidneys, and blood were 0.0% (Table 16). A total of 6.46% of the administered dose was found in the skin-treatment site, skin rinse, and the enclosure rinse (Table 17). Based upon the data, the total mean radioactivity level for male rats was 88.51% of the dose (Table 25).

Female rats: This group of female rats received DEET at 98.5 mg/kg (mean) with a mean radioactivity level of 10.3 μCi. During the first 12 hours of dosing, only 10.2% of the dosed radioactivity was eliminated in the urine of female rats, and by 72 hours following dosing, 69.25% was eliminated (Table 18). A total of 73.95% of the dose was detected in the urine. In the feces, a total 7.12% of the administered radioactivity was found (Table 18). During the first 12 hours following dosing, no radioactivity was detected in the feces. Most of the radioactivity in the feces was detected in the interval of 36 and 144 hours. A total mean of 81.07% of the administered radioactivity was found in urine and feces.

In the tissues examined, a total of 0.28% of the administered dose was found, and 0.25% was contributed by that in the carcass (Table 16). No radioactivity was found in the brain, kidneys, and blood. A total mean of 6.76% of the administered dose was found in skin, skin rinse, and enclosure rinse in female rats (Table 17). A total recovery of 88.10% of the dose was found in found in this group of female rats (Table 25).

6. Single dermal low-dose/animals sacrificed at "peak" 14C blood levels (Group 6)

Male rats: This group of male rats received a mean of 99.6 mg/kg with a mean radioactivity level of 15.2 μCi. With dermal application, 1 hour after dosing the blood radioactivity level reached a plateau which persisted for several days, and a clear peak blood radioactivity level with dermal application was not present. However, the time of 3.5 hours after dosing was selected as the "peak time" which was well into the plateau region. The animals were sacrificed at this time, and approximately 83.37% of the applied radioactivity was found in treatment site and skin rinse (Table 19). At the time of the estimated peak blood level, approximately 17% of the applied radioactivity was absorbed from the skin. Tissue analyses showed that plasma contained approximately 2.1 ppm of the radioactivity. Using the plasma radioactivity level as a comparative basis, the tissues which contained higher levels of radioactivity than plasma were liver (6.1 ppm), kidneys (16.6 ppm), stomach (3.1 ppm), small intestine (8.8 ppm), large intestines (2.16 ppm), fat (4.1 ppm), sciatic nerve (4.0 ppm), prostate (8.4 ppm), and seminal vesicles (2.6 ppm). The brain contained only 0.58 ppm which was approximately 1/4 that of the plasma level.

Females: The females received a mean of 100.7 mg/kg with 10.4 μCi of DEET by dermal application. With peak blood level sacrifice (3.5 hours), mean radioactivity levels of 2.10 and 92.63% of the applied dose were found in the dosed skin and the skin rinse, respectively. Based upon these data, approximately 5.3% of the applied dose was absorbed from the skin. The data on the tissue radioactivity levels showed that plasma had approximately 1.1 ppm (Table 20). When using the plasma level as basis for comparison, tissues, which had higher radioactivity, were liver (2.0 ppm), kidneys (7.0 ppm), stomach (1.14 ppm), small intestine (3.7 ppm), large intestine (3.9 ppm), fat (5.0 ppm), bone (1.5 ppm), sciatic nerve (1.4 ppm), uterus (2.5 ppm), ovaries (1.2 ppm), and carcass (1.2 ppm). The brain had approximately 0.27 ppm which was less than 1/3 that of the plasma level.

**DISCUSSION**

The data of the preliminary $^{14}C$-blood level experiments with rats, which received a single oral or dermal dose of DEET (100 mg/kg), indicated that with oral administration the peak $^{14}C$-blood level occurred in 0.5 hour after dosing in males and in approximately 2 hours in females. With dermal application in males and females, a clear peak was not seen; instead a plateau occurred which began

approximately 1.5 hours and persisted until the termination of the study (24 hours after dosing) (Figure 1). These data indicated that when DEET was dermally applied on rats, a small amount of the test compound was continuously absorbed from the treatment site.

In addition, the above data (Figure 1) and the results obtained from the animals, which received either single oral or dermal dose and were sacrificed at peak $^{14}C$-blood levels, indicated, at peak $^{14}C$-blood level, the fraction of the administered dose reaching the systemic circulation and the tissue residue levels following oral administration was substantially higher than that following dermal applications (Table 23). This conclusion is further supported by the following finding: With dermal application, 83.4% and 94.7% of the administered dose in males and females, respectively, remained in the treated skin and the skin rinses of animals sacrificed at peak $^{14}C$-blood level (Table 19). In comparison, approximately 46.7% and 34.75% of the administered dose remained in the G.I. tracts of orally dosed males and females, respectively (Table 13). In other words, from the time of dosing to the time of peak $^{14}C$-blood levels, approximately 17% and 5.3% of the administered dose was absorbed by the dermally treated males and females, respectively, while approximately 53.3% and 65.25% of the administered dose was absorbed by orally treated males and females, respectively.

The major route of elimination of DEET was via urine in both male and female rats. No marked difference was found in the total urinary or fecal radioactivity levels among the different dosing regimens or between male and female rats of different dosing groups as indicated in Tables 21 and 22. However, there was a difference in the rate of urinary elimination of DEET among the different dose groups. For example, repeated oral dose groups or pretreatment groups showed the fastest rate of urinary excretion during the first 4 hours after dosing than any other dose groups; they were followed by single oral low-dose groups whose rate was faster than that of the single oral high-dose groups. The single dermal low-dose groups showed the slowest rate of urinary excretion (Table 21); this finding might reflect the slow rate of dermal absorption. The increased rate of urinary elimination of radioactivity accompanied by slightly more total urinary radioactivity in repeated-dose groups relative to the other dose groups might reflect "an induction of the liver microsomal enzyme system".

The data on the tissue radioactivity levels of animals in various treatment groups showed that liver, kidneys, lung, spleen, whole blood, and the carcass contained higher radioactivity level than any other tissues (Table 24). The relatively higher radioactivity levels in the lung, liver, spleen, and kidneys could partially be due to higher perfusion rates since the radioactivity in whole blood was markedly greater than that in plasma. The total radioactivity found in all the tissues of various groups ranged from 0.15% to 0.67% of the administered dose (Table 25), and essentially very little DEET was sequestered in the body.

The tissue radioactivity levels in animals which were sacrificed at peak $^{14}C$-blood level showed substantially higher amounts in orally dosed animals than that of dermally treated animals (Table 23). This finding also reflected a much slower rate of absorption by the skin than that by the G.I. tract.

The means of the total radioactivity recovered in animals which received radiolabeled DEET by various regimens ranged from approximately 88% to 94% of the administered dose (Table 25).

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009065

**Reviewer:** Whang Phang, Ph.D. 1/15/92
HFAS/Tox. Branch II (H7509C)

**Secondary Reviewer:** Alberto Protzel, Ph.D. 1/15/92
HFAS/Tox. Branch II (H7509C)

**DATA EVALUATION REPORT**

**Study Type:** The metabolism phase of the pharmacokinetics and comparative dermal absorption study of N,N-dimethyl-m-toluamide (DEET) in the rat

**Caswell No.** 346
**MRID No.** 419944-02
**EPA ID No.** 080301

**HED Proj. No.** 1-2458
**EPA Case No.** 819244

**Chemical:** DEET (N, N-diethyl-m-toluamide)

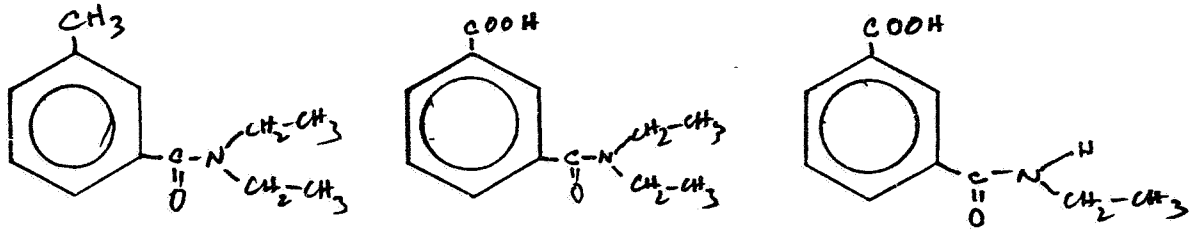
**Sponsor:** DEET Joint Venture/Chemical Specialties Manufacturers Association

**Testing Facility:** Biological Test Center (BTC)
P.O. Box 19791
2525 McGaw Ave.
Irvine, CA 92713-9791

**Citation:** Selim, S. (1991) Addendum to Report Entitled "Pharmacokinetics and comparative absorption study of N,N-dimethyl-m-toluamide (DEET) in the rat". Study conducted by Biological Test Center, Study No.: Addendum P01836. Submitted to EPA by DEET Joint Venture/Chemical Specialties Manufacturers Association. EPA MRID No. 419944-02

**Conclusion:** In the metabolism phase of the study, a group of 5 rats/sex was administered radiolabeled DEET by gavage at a single dose of 500 mg/kg b.w. which was similar to the high dose in the ADE phase of the study. After dosing, the excreta of these animals were collected for isolation and identification of major metabolites. After identification of the major metabolites, a determination of the metabolite profile and quantitation of $^{14}C$ residues were conducted in urine samples obtained from the animals treated according to various dosing regimens used in the ADE phase of the study (MRID No. 419944-01).

Two major metabolites were isolated and identified in the urine samples of all treated animals. The structures of DEET and its two major metabolites are indicated below:



DEET Metabolite A Metabolite B

Metabolite A, which accounted for approximately 50% of the administered radioactivity, was formed by oxidation of the methyl group on the aromatic ring. Metabolite B, which accounted for less than 18% of the administered dose, was formed by oxidation of the methyl group on the aromatic ring and N-dealkylation of an ethyl substituent on the amide moiety. The results indicated that DEET was quantitatively metabolized, and the presence of the parent compound was below the detection limit in the urine samples of the treated rats irrespective of sex or of dermal or oral routes of administration.

**Methods and Materials**

Test material: Radiolabeled DEET (uniformly labeled with $^{14}C$ on the benzene ring) with specific radioactivity of 22 μCi/mmole and chemical purity of 98.9% was used.

Animals: Seven to 8 weeks old Charles River CD rats were obtained from Charles River Lab., Portage, Michigan. These animals were acclimated to the conditions in the testing laboratory for approximately 3 weeks prior to the initiation of the study.

Study Design: The study evaluated the metabolism phase of the comparative pharmacokinetics study with DEET. The objectives of this portion of the study were to isolate, purify, and identify major metabolites in the urine of rats dosed orally with $^{14}C$-DEET and to identify and quantitate the radioactive residues in the urine samples of animals from the absorption, distribution, and elimination (ADE) phase of the study in which animals received the test chemical according to different dosing regimens [see the Data Evaluation Report (DER) on the study with MRID No. 419944-01].

Identifying major metabolites: Five male and 5 female rats received radiolabeled DEET (500 mg/kg b.w.) by gavage. To ensure the availability of a sufficient quantity of DEET metabolites, the test animals received 2 doses of radiolabeled DEET; one dose at the initiation of the study, and a

second dose 5 days later. The dosing solutions were prepared by adding measured amounts of non-radiolabeled and 14C-DEET into the corn oil. The values for the final specific activity of the dosing solutions were 256,059 dpm/mg of DEET and 276,093 dpm/mg of DEET for the first and second dosing solutions, respectively. The animals received 2 ml dosing solution/kg b.w. (500 mg/kg b.w.). The actual dose was "measured by weight as a differential between the weight of syringe before and after dosing".

Urine or feces samples were collected at intervals of 0-24, 24-48, and 48-72 hours after dosing. Daily urine or fecal samples were pooled by sex to obtained sufficient quantity of $^{14}C$-residues for isolation and identification of metabolites. All the samples collected following the second dosing were frozen as backup samples.

Identifying and quantitating the metabolites in the urine samples from the ADE phase of the kinetics study: Urine samples from animals of single oral-low-dose, single oral-high-dose, repeated oral-low-dose, and single dermal-low-dose groups were pooled according dose regimen and sex and analyzed.

The methods for sample preparation, radioassay, analytical procedures, and the statistical methods are excerpted from the report and are presented in Appendix A. A schematic presentation of the metabolite isolation procedure is also excerpted from the report and is presented in Appendix B.

**Results**

a. No clinical signs of toxicity were seen in animals which received two oral doses of DEET (500 mg/kg b.w.) at 5 days apart. The means of the total radioactivity eliminated in the urine and feces were excerpted from the report and presented in Table 1.

Table 1+. Means of radioactivity (as % of dose) in urine and feces of DEET treated rats (500 mg/kg)

| Time (hours) | Urine | | Feces | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| 0-24 | 77.87 ±6.23 | 63.46 ±8.27 | 3.18 ±1.22 | 2.18 ±0.98 |
| 24-48 | 10.77 ±6.92 | 18.26 ±3.70 | 1.49 ±0.39 | 3.06 ±1.27 |
| 48-72 | 0.54 ±0.26 | 1.89 ±1.26 | 0.28 ±0.05 | 0.59 ±0.32 |
| Total | 89.18 ±3.42 | 83.60 ±3.67 | 4.95 ±1.19 | 5.83 ±1.79 |

+: Data excerpted from the report (MRID No. 419944-02)

The data presented in Table 1 were consistent with those of the ADE phase of the study. Approximately 89% and 84% of the administered radioactivity were found in the urine samples of males and females, respectively, while approximately 5% and 6% were seen in the feces of male and female rats, respectively.

b. Isolation, purification, and identification of metabolite metabolites: Using HPLC, metabolite profiles of the pooled raw urine samples and of the post-XAD methanol fraction were obtained. A diagram of the scheme for metabolite isolation is presented in Appendix B. A representative elution profile is excerpted from the report and is presented in Figure 1. Two major peaks of radioactivity were found in the post-XAD fraction of pooled urine samples. The tallest peak, with a retention time (RT) of approximately 17.5-20.3 minutes, was designated as Metabolite A, and the second peak, with a RT of 9.8-13.7, was designated as Metabolite B.

HPLC fractions corresponding to each of these two peaks were collected, fractionated further, and analyzed by mass spectrometry. The HPLC and mass spectral results are presented in Figures 2, 3, 4, & 5. Based upon the mass spectral analyses, Metabolite A was identified as m-[(N,N-diethylamino)carbonyl]-benzoic acid, and Metabolite B as m-[(ethylamino)carbonyl]-benzoic acid. The molecular weights of DEET, Metabolite A, and Metabolite B and their structures are presented in Table 2.

Since the radioactivity in the feces samples was less than 10% of the administered radioactivity, no further analyses of these samples were conducted.

c. Analysis of urine samples from the ADE phase of the study: The urine samples from rats which received a single oral low-dose, repeat oral low-dose, a single oral high-dose, and a single dermal low-dose in the ADE phase of the study were analyzed for the parent compound and major metabolites. A representative chromatogram of the urine samples from a single oral high-dose group and another from a single dermal low-dose group are presented in Figure 6. The HPLC chromatographic profile of the 2 analyses shown in Figure 6 were similar to each other and to that of the single oral high-dose group of the metabolism phase of the study (Figure 1). Two major peaks were apparent in these chromatograms, and they eluted with similar RT values in urine samples from the various treatment groups. However, HPLC results from the single oral low-dose and repeated oral low-dose groups in the ADE phase of the study were not available for examination.

The amount of radioactivity in several peaks was measured to to determine the fraction of the dose corresponding to each peak. The results are presented in Table 3. These results indicated that the majority of the radioactivity was concentrated in peaks designated as Metabolite A and Metabolite B. Approximately 46% to 59% of the administered dose was associated with Metabolite A in the various dosing regimens. Metabolite B accounted for 10 to 17% of the administered dose in all dose groups except in the males of low-dose oral and dermal regimens in which Metabolite B accounted for approximately 3% of the administered dose. Two polar zones with multiple metabolites and two unknown metabolite zones were also found. The two unknown metabolite zones (Unknown 1 & 2) were not analyzed further because each zone accounted for less than 10% of the administered dose except in the samples of the repeat oral low dose males which had approximately 10.05% of the administered dose. No parent compound was found in the urine.

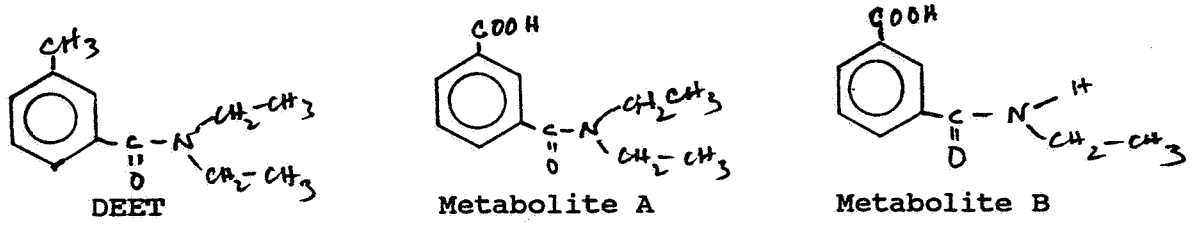
Based upon these results, a metabolic pathway for DEET was proposed by the author (Figure 7). According to the proposed pathway, the methyl group on the aromatic ring of DEET can be oxidized to a carboxylic acid moiety leading to Metabolic A. On the other hand, one of the ethyl substituents on the amide moiety may undergo N-dealkylation in combination with oxidation of the ring methyl group and leading to the formation of Metabolite B. In addition, N-dealkylation of Metabolite A can also lead to the formation of Metabolite B.

**Discussion**

In the metabolism phase of the study, a group of 5 rat/sex was administered radiolabeled DEET by gavage at a single dose of 500 mg/kg b.w. which was similar to the high dose in the ADE phase of

the study. The excreta of these animals were collected for isolation and identification of major metabolites. After identification of the major metabolites, the determination of the metabolic profile and quantitation of $^{14}C$ residues were conducted in the urine samples obtained from the animals of the various dosing regimens in the ADE phase of the study (MRID No. 419944-01).

Two major metabolites (A & B) were isolated and identified in the urine samples of all treated animals. The structures of DEET and its two major metabolites are indicated below:



DEET Metabolite A Metabolite B

Metabolite A, which accounted for approximately 50% of the administered radioactivity, was formed by oxidation of the methyl group on the aromatic ring while the rest of the molecule remained intact. Metabolite B, which accounted for less than 18% of the administered dose, was formed by oxidation of the methyl group on the aromatic ring and N-dealkylation of one of the ethyl substituents on the amide moiety. The results indicated that DEET was quantitatively metabolized, and the presence of the parent compound was below the detection limit in the urine samples of the treated rats irrespective of sex or of dermal or oral routes of administration.

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009065

**Reviewer:** Whang Phang, Ph.D. 1/15/92
HFAS/Tox. Branch II (H7509C)

**Secondary Reviewer:** Alberto Protzel, Ph.D. 1/15/92
HFAS/Tox. Branch II (H7509C)

**DATA EVALUATION REPORT**

**Study Type:** Determination of expired $^{14}C$ volatiles following a single oral or dermal dose of N,N-diethyl-m-toluamide (DEET) in the rats

**Chemical:** DEET (N,N-diethyl-m-toluamide)

**Caswell No.** 346 **HED Proj. No.** 1-2458
**MRID No.** 419944-03 **EPA Case No.** 819244
**EPA ID No.** 080301

**Sponsor:** DEET Joint Venture/Chemical Specialties Manufacturers Association

**Testing Facility:** Biological Test Center (BTC)
P.O. Box 19791
2525 McGaw Ave.
Irvine, CA 92713-9791

**Citation:** Lin, P. and Selim, S. (1991) Determination of expired $^{14}C$ volatiles following a single oral or dermal dose of N,N-diethyl-m-toluamide (DEET) in the rat. Study conducted by Biological Test Center, Study No. P01862. Submitted to EPA by DEET Joint Venture/Chemical Specialties Manufacturers Association. EPA MRID No. 419944-03.

**Conclusion:** Groups of rats (2/sex/dose regimen) received $^{14}C$-DEET by either oral (gavage) or dermal route at 100 mg/kg b.w.. The expired radioactivity was measured for 24 hours. The results indicated that less than 0.017% of the administered radioactivity was detected in both males and females of either orally or dermally dosed groups. There was no significant difference in the expired radioactive $CO_2$ and volatile metabolites between male and female rats and between oral and dermal dose groups.

**Methods and Materials:**

Test Article: The radiolabeled DEET was uniformly labeled with $^{14}C$ in the benzene ring. The specific activity was 22 μCi/mmole, and radiochemical purity was 97.4%. The nonradiolabeled technical grade DEET (Lot A-1-96) (purity 98.3%) was provided to the testing laboratory by Morflex Inc., and it was used for isotopic dilution in the study and as reference standard. The purity of the compound was analyzed by HPLC.

Test Animals: Ten week old Charles River Sprague-Dawley rats were obtained from Charles River Breeding Lab., Portage, Michigan.

**Study Design**

Groups of rats (2/sex/dose regimen) received $^{14}C$ DEET by either gavage or dermal application at a dose of 100 mg/kg b.w. For dermal application, the test material was applied on a shaven area, and the application site was covered with a contoured rectangular enclosure (2.5 cm x 5 cm x approximately 1.3 cm). enclosure was glued on to the shaved area with a cyanoacrylate-based glue.

Immediately after dosing, the animals were transferred to individual Roth glass metabolism cages. Expired $CO_2$ and volatile metabolites were collected in a reservoir (150 ml) containing 2:1 mixture of ethanolamine and cellosolve. Samples were collected at 2, 4, 8, and 24 hours. Aliquots (2 ml) of all trapping solutions were counted directly in liquid scintillation cocktail with a Beckman LS 3801 liquid scintillation spectrometer.

**Results and Discussion**

The amount of radioactivity received by both dermal and oral dose groups (2/sex) ranged from approximately 11 to 16 $\mu$Ci per rat. The average radioactivity expired by rats in various dose regimens at different times is excerpted from the report and presented in Table 1. In 24 hours, the average cumulative radioactivity expired by male and females in the dermally dosed group was 0.014 and 0.016% of the administered radioactivity, respectively, and that by the orally dosed males and females was 0.013% and 0.016%, respectively.

The results indicated that no significant difference existed in the amount of radioactivity expired between dermally and orally dosed animals and between males and females. In addition, only a minimal amount of radioactivity (<0.017% of the administered dose) was expired within 24 hours by rats which were dosed either dermally or orally.

Table 1+. Average cumulative percent of dosed $^{14}C$ recovered in the expired air of orally and dermally dosed rats

| Time Intervals (hours) | Males Average cum.* % dose expired | Females Average cum. % dose expired |
|---|---|---|
| | Dermally dosed group (100 mg/kg b.w.) | |
| 2 | 0.002 | 0.002 |
| 4 | 0.005 | 0.006 |
| 6 | 0.008 | 0.009 |
| 8 | 0.011 | 0.012 |
| 24 | 0.014 | 0.016 |
| | Orally dosed group (100 mg/kg b.w.) | |
| 2 | 0.003 | 0.004 |
| 4 | 0.006 | 0.006 |
| 6 | 0.008 | 0.009 |
| 8 | 0.010 | 0.012 |
| 24 | 0.013 | 0.016 |

+: Data excerpted from the report (MRID No. 419944-03)
*: Cumulative percent of dose between time 0 and the indicated time.