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

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SEP 27 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Assays of Blood Levels of Deet and Fenvalerate in Dogs and Correlation with Appearance of Symptoms

TO: Mr. George LaRocca, PM 15
Registration Division (H7505C)

FROM: Byron T. Backus, Toxicologist Byron T. Backus 9/18/89
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HED (H7509C)

THROUGH: K. Clark Swentzel K. Clark Swentzel 9/19/89
Acting Section Head, Review Section II
Fungicide/Herbicide/Antimicrobial Toxicology Branch
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and

Marcia van Gemert, Branch Chief Marcia van Gemert 9/20/89
Fungicide/Herbicide/Antimicrobial Toxicology Branch
HED (H7509C)

EPA Record No. 236656, 235657

Project No. 9-0546

EPA Reg. Nos. 2596-114, 2596-115

Tox. Chem. 77A, 346

Action Requested:

Review a study titled: "Domestic Animal Safety: In Vivo Toxicology Estimation of the Safety Factor of an Aerosol Product in Dogs by Assay of Blood Levels of the Insecticides."

Comments and Recommendations:

1. The Agency did not request this study, and had no opportunity to examine or comment on the protocol before its initiation.

2. Symptoms (including ataxia, tremors and emesis) were associated with IV administration of cumulative amounts of 3 mg/kg Fenvalerate or 100 mg/kg Deet. However, these cumulative

dosages were each divided into five equal doses, and symptoms occurred (in at least some dogs) following a single injection (which would have contained either 0.6 mg/kg Fenvalerate or 20 mg/kg Deet).

3. Intravenous administration of the combination of Deet and Fenvalerate (at 30 and 3 mg/kg respectively) involved a 10:1 ratio of these two actives, but the Blockade formulation is approximately 10% Deet and 0.1% Fenvalerate (a 100:1 ratio). The 3 mg/kg IV dosage of Fenvalerate alone had been demonstrated to cause symptoms, while findings with only 30 mg/kg IV administration of Deet (given as five 6 mg/kg at 10 minute intervals) were equivocal. It is noteworthy then that the symptoms occurring following this combination of Deet and Fenvalerate (all dogs showing tremors, with symptoms up to 4 hours after the last injection) were substantially greater than those observed from a cumulative dosage of 3 mg/kg Fenvalerate alone (ataxia in a single dog up to 1 hour after the fifth injection, emesis in another dog, and equivocal findings in the other two). This appears to contradict the statement made in the report (p. 8) that "IV injections of a combination of the two ingredients did not reveal any enhancement of reactions..."

4. The highest level of Deet observed in the blood of any one dog following dermal exposure to Blockade was 3.267 ppm in one animal (#907) two hours after application of a single 4.5 g/kg treatment. Presumably, this correlates with the rate of absorption of Deet through the skin. The dermal exposures to Blockade in this study involved single applications at 1.5 and 4.5 g/kg and multiple (4X) applications of 1.5 and 4.5 g/kg (cumulative dosages of 6 and 18 g/kg). There were no evident symptoms of toxicity in any of the dogs as a result of dermal exposure to Blockade at these levels. No Fenvalerate was detected in the blood of any of the dogs which were dermally exposed to Blockade.

5. Overall, while the study findings are interesting, and the results (primarily the lack of symptoms in the dogs which were dermally exposed to Blockade) do suggest that there is probably a safety factor associated with single dermal exposure of dogs to Blockade at 4.5 g/kg and to four 4.5 g/kg exposures (cumulative dosage of 18 g/kg), it is not readily apparent as to how (or even whether) a margin of safety could be calculated by comparison of the IV administration and dermal application findings. The study is classified as core supplementary data.

6. A copy of the attached DER should be provided to the registrant.

Reviewed by: Byron T. Backus Byron T. Backus 9/16/89
Section 2, HFASB (H7509C)

Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 9/20/89
Section 2, HFASB (H7509C)

DATA EVALUATION REPORT I

STUDY TYPE: Toxicity of Blockade by the intravenous route in dogs TOX CHEM NO. 77A, 346

MRID NO: ACC. NO: 409279-01

TEST MATERIALS: Fenvalerate, Deet, Fenvalerate + Deet

SYNONYMS: Blockade

STUDY NUMBER(S): Hartz Test No. 1022

SPONSOR: Hartz Mountain Corporation
Harrison, NJ 07029

TESTING FACILITY: [redacted]

Note: the name of the testing facility has been labeled as confidential information by the registrant

TITLE OF REPORT: Domestic animal safety: In vivo toxicology estimation of the safety factor of an aerosol product in dogs by assay of blood levels of the insecticides.

AUTHOR(S): Klaassen, C.

REPORT ISSUED: 11/17/88

CLASSIFICATION: Core supplementary data

COMMENTS AND CONCLUSIONS:

1. It is emphasized that the Agency did not request this study, and had no opportunity to examine or comment on the protocol before its initiation.

2. Symptoms (which included ataxia, tremors, emesis) were associated with IV administration of cumulative amounts of 3 mg/kg Fenvalerate or 100 mg/kg Deet. However, these cumulative amounts were divided into 5 equal doses, and symptoms occurred (in at least some dogs) following a single injection (which would have been 0.6 mg/kg Fenvalerate or 20 mg/kg Deet).

3. Intravenous administration of the combination of Deet (at 30 mg/kg) and Fenvalerate (at 3 mg/kg) involved a 10:1 ratio of these two actives, but the Blockade formulation is approximately 10% Deet and 0.1% Fenvalerate (a 100:1 ratio). The 3 mg/kg IV dosage of Fenvalerate alone had been demonstrated to cause symptoms, while findings with only 30 mg/kg IV administration of Deet (given as five equal doses at 10 minute intervals) were equivocal. It is noteworthy then that the symptoms observed then with this combination of Deet and Fenvalerate (all dogs showing tremors, with symptoms up to 4 hours after the last injection) were substantially greater than those observed from the 3 mg/kg Fenvalerate alone (ataxia in a single dog up to 1 hour after the last injection, emesis in another dog, and equivocal findings in the remaining two). This appears to contradict the statement made in the report (p. 8) that "IV injections of a combination of the two ingredients did not reveal any enhancement of reactions..."

4. The highest level of Deet observed in the blood of any one dog following dermal exposure to Blockade was 3.267 ppm in animal 907 two hours after application of a single 4.5 g/kg treatment. Presumably, this correlates with the rate of absorption of Deet through the skin. The dermal exposures to Blockade in this study involved single applications at 1.5 and 4.5 g/kg, and multiple (4x) applications of 1.5 and 4.5 g/kg (cumulative dosages of 6 and 18 g/kg). There were no evident symptoms of toxicity in any of the dogs as a result of dermal exposure to Blockade at these levels. No Fenvalerate was detected in the blood of any of the dogs which were dermally exposed to Blockade.

5. Overall, while the study findings are interesting, and the results do suggest that there is probably a safety factor associated with single dermal exposure of dogs to Blockade at 4.5 g/kg, and to four 4.5 g/kg exposures (cumulative dosage of 18 g/kg), it is not readily apparent as to how (or even whether) a margin of safety could be calculated from the results. The study is classified as core supplementary data.

A. MATERIALS:

1. Test compounds: Fenvalerate (sample no. 8521: 0.604%), DEET (sample no. 8483: 19.09%; 8520: 6.097%) and a mixture of the two compounds (sample 8508: average 0.602% Fenvalerate, 5.96% DEET). The remainder of each of these samples consisted of propylene glycol.

   Blockade formulation (lot #MR 10727; analytical results are reported giving 0.095% Fenvalerate and 10.16% DEET).

2. Animals used: Thirty-six mongrel dogs, of both sexes, ranging in age from 1 to 8 years of age, and in weight from 17 to 67 lbs (mean of 34.33 with a standard deviation of 9.53 lbs at initiation of the study).

B. STUDY DESIGN:

The study was divided into two phases. Following a range-finding study in which it was determined that IV injection of 3 mg/kg Fenvalerate or 100 mg/kg DEET (findings at 50 mg/kg were equivocal) were the lowest levels at which symptoms (ataxia, tremors) occurred, these substances, either singly or in combination, were intravenously injected into dogs and samples of blood were subsequently taken from these animals for analyses of these compounds.

In the second phase of this study, Blockade formulation was sprayed on dogs at either a 1X or 4X use level, and blood samples were taken from these dogs for analyses.

1. Administration by IV:

The following dosage levels were given by IV injection: Placebo (propylene glycol alone), Fenvalerate at 3 mg/kg; DEET at 30 mg/kg; DEET at 100 mg/kg; and a combination of Fenvalerate at 3 mg/kg and DEET at 30 mg/kg administered in propylene glycol. The total dosage volume per animal (including those receiving propylene glycol alone) was 0.5 ml/kg, divided into 5 equal injections of 0.1 ml/kg given at 10-minute intervals into the cephalic or jugular vein.

Blood samples (each 4.5 mls) were drawn from the cephalic or jugular vein (but not the vein used for the series of IV injections) at the following times: before injection, 1 minute after the first injection, and at 1, 5, 10, 15, 30 and 45 minutes and 1, 2, 3, 4, 5, 6, 12, 24, 48 and 72 hours after the last injection. Blood samples were immediately frozen and shipped (still frozen) to Hartz Laboratories for analyses.

2. Dermal application of Blockade:

The following dose levels of Blockade were sprayed on the dogs:

a) a one time "normal" application of 1.5 g/kg.

b) four "normal" applications (1.5 g/kg) applied over a 3-hour period (total of 6 g/kg).

c) a one time 3X application (4.5 g/kg)

d) four 3X applications, each 4.5 g/kg, applied over a 3-hour period.

Each test group consisted of two male and two female dogs.

3. GLP practices statement and quality assurance:

On page 3 of the report there is a dated Good Laboratory Practices Compliance Statement, which is signed by the two directors and by a representative of the study sponsor.

There is a signed and dated Quality Assurance Unit Statement on page 212 from the laboratory which conducted the analytical work.

C. METHODS AND RESULTS:

1. Observations:

Placebo: no reactions were observed in the dogs, and no Fenvalerate or DEET was detected in their blood samples.

Fenvalerate (3 mg/kg): symptoms (which in two subjects included fasciculations/tremors) were reported for all 4 subjects. In one subject a possible symptom ("anxiety") was still being observed 3 hours after the final injection.

The following analytical results from blood are reported for fenvalerate (3 mg/kg):

Time after injection	range of values (ppm)	average of four replicates (ppm)
1 min after 1st injection	2.09-2.82	2.53
1 min after 5th "	2.07-4.31	2.89
5 min after 5th "	0.79-1.43	1.12
10 min after 5th "	0.53-0.86	0.75
15 min after 5th "	0.48-0.66	0.58
30 min after 5th "	0.23-0.36	0.30
45 min after 5th "	0.11-0.20	0.17
1 hr after 5th "	0.10-0.14	0.11
2 hr after 5th "	0.05-0.06	0.06
3 hr after 5th "	0.03-0.05	0.04
4 hr after 5th "	0.02-0.03	0.03
5 hr after 5th "	N.D.-0.03	0.02
6 hr after 5th "	N.D.-0.02	N.D.
12 hr after 5th "	N.D.	N.D.

There were no symptoms in 2/4 dogs injected with Deet at 30 mg/kg; in the other two findings were equivocal, consisting of anxiety during the period the animals were being injected.

The following analytical results from blood are reported for Deet (30 mg/kg):

Time after injection	range of values (ppm)	average of four replicates (ppm)
1 min after 1st injection	5.02-14.98	8.90
1 min after 5th "	13.27-21.23	17.94
5 min after 5th "	12.24-20.64	16.28
10 min after 5th "	10.91-16.61	12.85
15 min after 5th "	8.24-14.78	10.77
30 min after 5th "	5.91-11.13	7.61
45 min after 5th "	4.30-9.80	6.06
1 hr after 5th "	3.11-7.48	4.46
2 hr after 5th "	0.93-2.72	1.49
3 hr after 5th "	N.D.-1.66	0.48
4 hr after 5th "	N.D.-0.66	N.D.
5 hr after 5th "	N.D.-0.33	N.D.
6 hr after 5th "	N.D.	N.D.

At 100 mg/kg Deet all dogs showed symptoms (including ataxia in all cases, and, for one dog, seizures). Symptoms were observed for at least 30 minutes (in one case, 3 hours) following the last injection.

The following analytical results from blood are reported for Deet (100 mg/kg):

Time after injection	range of values (ppm)	average of four replicates (ppm)
1 min after 1st injection	12.78-26.60	20.90
1 min after 5th "	40.54-69.57	55.20
5 min after 5th "	40.85-62.70	50.07
10 min after 5th "	39.00-63.12	48.27
15 min after 5th "	35.39-56.47	43.77
30 min after 5th "	28.76-48.63	35.45
45 min after 5th "	24.97-43.53	31.26
1 hr after 5th "	20.78-41.42	27.01
2 hr after 5th "	6.59-26.93	14.60
3 hr after 5th "	1.09-18.63	8.36
4 hr after 5th "	0.31-10.90	4.46
5 hr after 5th "	N.D.-5.40	2.18
6 hr after 5th "	N.D.-1.75	0.81
12 hr after 5th "	N.D.	N.D.

The dog (no. 855) which showed the greatest persistence of Deet was also the one in which unequivocal symptoms (ataxia) were still observed at 3 hrs after the last injection.

Dogs which recived injections with cumulative dosages of 30 mg/kg Deet and 3 mg/kg fenvalerate all showed symptoms (which included fasciculations and/or tremors in all four animals).

The following analytical results from blood are reported for Deet (cumulative dose of 30 mg/kg):

Time after injection	range of values (ppm)	average of four replicates (ppm)
1 min after 1st injection	3.06-10.32	6.54
1 min after 5th "	12.94-15.27	14.30
5 min after 5th "	9.79-12.21	10.33
10 min after 5th "	7.32-9.72	8.14
15 min after 5th "	5.47-8.51	6.63
30 min after 5th "	3.37-5.91	4.29
45 min after 5th "	1.99-4.13	2.80
1 hr after 5th "	1.07-3.05	1.97
2 hr after 5th "	N.D.-1.04	0.61
3 hr after 5th "	N.D.-0.46	N.D.
4 hr after 5th "	N.D.-0.24	N.D.
5 hr after 5th "	N.D.	N.D.

The following analytical results from the blood are reported for fenvalerate (cumulative dose of 3 mg/kg):

Time after injection	range of values (ppm)	average of four replicates (ppm)
1 min after 1st injection	2.07-4.13	2.98
1 min after 5th "	2.40-5.06	3.51
5 min after 5th "	1.20-1.70	1.46
10 min after 5th "	0.61-1.07	0.83
15 min after 5th "	0.52-0.85	0.68
30 min after 5th "	0.32-0.76	0.50
45 min after 5th "	0.30-0.65	0.41
1 hr after 5th "	0.22-0.60	0.32
2 hr after 5th "	0.11-0.27	0.16
3 hr after 5th "	0.05-0.18	0.10
4 hr after 5th "	0.05-0.08	0.07
5 hr after 5th "	N.D.-0.05	0.04
6 hr after 5th "	N.D.-0.04	0.03
12 hr after 5th "	N.D.-0.39	N.D.
24 hr after 5th "	N.D.-0.02	N.D.
48 hr after 5th "	N.D.	N.D.

Spraying with Blockade formulation:

No unequivocal symptoms were observed in any of the dogs which were sprayed with the Blockade formulation at cumulative dose levels of 1.5, 6, 4.5 and 18 g/kg. Although Deet was observed in some of the blood samples from these animals (detection limit of 0.2 ppm), no Fenvalerate was found (detection limit: 0.01 ppm).

For those dogs which were sprayed with the Blockade formulation at 1X (equivalent to 1.5 g/kg) no Deet was detected in the blood of any animal in the period from 1 to 30 minutes after spraying. One of the four dogs yielded low (but measurable) Deet blood levels in the period from 45 minutes to 3 hours after the spraying, as follows:

Time after spraying	Deet blood levels for one dog (#991) ppm
45 min	0.202
1 hr	0.378
2 hr	0.264
3 hr	0.308

For those dogs which were sprayed with four 1X applications of the Blockade formulation (cumulative dosage of 6 g/kg) the maximum Deet level (1.149 ppm) that was observed occurred in one dog at 45 minutes following the fourth spray. This same dog showed 0.274 ppm 3 hrs after the fourth treatment. This animal is reported as showing anxiety at about 30 minutes after the fourth application.

For those dogs which received one "heavy" spray application of Blockade at 4.5 g/kg the maximum Deet blood level measured was 3.267 ppm in one dog at 2 hours after exposure; blood from this same animal showed 0.403 ppm Deet at 24 hours after treatment. One other dog had 0.817 ppm at 2 hours after exposure.

For those dogs which received four heavy applications of Blockade (total: 18 gm/kg) the maximum measured Deet blood level was 2.203 ppm in one dog 30 minutes after the final exposure. The concentration in another dog "peaked" (for that animal) at 1.119 ppm 6 hrs after the fourth exposure.

D. DISCUSSION:

It is emphasized that the Agency did not request this study, and had no opportunity to examine or comment on the protocol before its initiation.

It is not immediately evident why the intravenous administration of the combination of Deet (30 mg/kg) and Fenvalerate (3 mg/kg) involved a 10:1 ratio of these two actives, since the Blockade formulation is approximately 10% Deet and 0.1% Fenvalerate (a 100:1 ratio). Also, 3 mg/kg dosage of Fenvalerate had been demonstrated to cause symptoms, while findings with 30 mg/kg Deet alone had been extremely equivocal, so it could be assumed that the symptoms observed were primarily from the Fenvalerate. However, it is noteworthy that symptoms observed from this combination of Deet and Fenvalerate (all dogs showing tremors, with symptoms up to 4 hours after the last injection) were substantially greater than those observed from Fenvalerate (at 3 mg/kg) alone (ataxia in a single dog up to 1 hour after the last injection, emesis in another dog, and equivocal findings in the other two). This appears to be contradictory to the statement made in the report (p. 8) that: "IV injections of a combination of the two ingredients did not reveal any enhancement of reactions..." It is also noted that the conclusions embedded in the statement (p. 10) that "DEET does not enhance transdermal penetration of Fenvalerate. In fact, this study suggests an inhibition effect." do not appear to be supported by the data, particularly as the mixture tested by IV administration had a 10:1 ratio of these two materials.

Another area of concern is the amount of Deet measured in the blood relative to the total material injected. For example, dog 859 had 26.60 ppm of Deet in the blood 1 minute after the first injection of 20 mg of Deet/kg body weight. From p. 117 this dog weighed 41 lbs or 18.6 kg, which means it received 18.6 x 20 mg = 372 mg of Deet in that first injection. If it is assumed that this dog's blood volume was 2000 mls (in humans there is approximately 60 ml blood/kg body weight, so this value of 2000 mls is probably high) then the 26.60 ppm would represent 53.2 mg of Deet, or about one-seventh of the administered dose. It is not immediately apparent where the remainder (over 300 mg) of the Deet went. If the total blood volume was 1200 mls (using the 18.6 kg weight of the dog and the 60 ml blood/kg approximation for humans) then the total amount of Deet in the blood would have been 31.9 mg, and the amount not accounted for would be 340 mg, or over 90% of the administered dose.

If this reviewer has interpreted the toxicity graphs correctly, symptoms did occur in dogs receiving IV dosage of 100 mg/kg Deet before the full dosage had been administered. For one dog (#855, see p. 37) emesis appears to have been present immediately following administration of a single dose of 20 mg/kg, and ataxia was observed following the second IV injection (cumulative total of 40 mg/kg). In the case of dog #984 (refer to p. 36) ataxia was present following the first injection of 20 mg/kg. In this dog the measured blood level of Deet was 12.78 ppm at one minute after the first injection (although the other dogs in this group had levels greater than 20 ppm at this time). This dog is reported (p. 118) as weighing 50 lbs (=22.7 kg). Each of the five doses administered to this animal would then have been 20 mg/kg x 22.7 kg = 454 mg. Assuming 2000 mls of blood in this animal, 12.78 ppm would represent 26 mg of the test material, or less than 6% of the total dose received to that time. It is noteworthy that in those animals injected with both Deet and Fenvalerate (in a 10:1 ratio) at one minute following the first injection there were means of 6.54 ppm Deet and 2.98 ppm Fenvalerate (an approximately 2.2:1 ratio) in the blood. The methodology used to determine the Deet level was High Pressure Liquid Chromatography (HPLC), and for Fenvalerate was Gas Chromatography. It is not known (at least, not from the information provided in this report) whether one or both of these substances could be bound to plasma, and so might not be detectable.

All of the dogs which received a cumulative dosage of 30 mg/kg Deet and 3 mg/kg Fenvalerate were showing unequivocal symptoms (tremors or ataxia) at 1 hour after the last injection. Yet, at this time the mean blood concentration (see p. 39) of Deet was 1.97 ppm, and of Fenvalerate (see p. 40) was 0.32 ppm. One dog (965) was still showing symptoms at 3 and 4 hours after the last injection (p. 44). It is possibly significant that this dog was the only one in this group at this time with measurable levels of Deet in the blood, but the levels were low (0.46 ppm at 3 hours; 0.24 ppm at 4 hours).

The highest level of Deet in the blood observed following dermal exposure to Blockade was 3.267 ppm occurring in one dog (907, refer to p. 57) 2 hours after a single exposure to 4.5 g/kg treatment. Presumably, this correlates with the rate at which the Deet was absorbed through the skin. However, there were no evident symptoms of toxicity in this dog following this exposure to Blockade. None of the dogs injected with the combination of 30 mg/kg Deet and 3 mg/kg Fenvalerate had this high a level of Deet (maximum value observed was 1.04 ppm) two hours after the last injection.

It appears then that the appearance of symptoms following administration of Deet by the IV route correlates with several factors, including not only dose level, but the amount administered in a single injection (essentially no symptoms were observed following five injections over a period of 40 minutes with a cumulative dosage of 30 mg/kg, but a single injection containing 20 mg/kg Deet elicited symptoms). The fact that apparently most of the Deet was not (or was not detected) in the blood samples taken at one minute following injection suggests the possibility that this substance is taken up rather rapidly (stored?) by other tissues or other organs, which presumably includes the nervous system.

While the study findings are interesting, and the results (primarily the lack of symptoms in the dogs which were dermally exposed to Blockade) suggest that there is probably a safety factor associated with single dermal exposure of dogs to Blockade at 4.5 g/kg, and to four 4.5 g/kg dermal exposures (cumulative dosage of 18 g/kg), it is not readily apparent as to how (or even whether) a margin of safety could be calculated from comparison of the IV administration and dermal application findings. We would probably have recomended radioactive labelling of at least the Deet used in both the IV administration and dermal exposure, along with possibly collection and analyses of urine and feces, in order to get a better determination as to how much label was excreted (and the rate of excretion) and how much was retained, along with correlation of these values and the occurrence (or absence) of symptoms.

The study is classified as supplementary data.

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Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Blood levels of Deet & Fenvalerate in dogs & correlation with symptoms; laboratory claimed confidential; Hartz Test No. 1022; 11/17/88	Deet and Fenvalerate alone and in combination (by IV route); Blockade formulation by dermal route.	409279-01	Symptoms (including ataxia, tremors and emesis) after IV administration of cumulative amounts of 3 mg/kg Fenvalerate or 100 mg/kg Deet. However, these cumulative dosages were each divided into five equal doses and symptoms occurred in some dogs following a single dose of 0.6 mg/kg Fenvalerate or 20 mg/kg Deet. IV administration of Deet & Fenvalerate together (cumulative 30 mg/kg & 3 mg/kg respectively) resulted in more severe symptoms than 3 mg/kg Fenvalerate alone. Highest level of Deet observed in blood was 69 ppm at 1 minute after 5th injection in dog receiving total dosage of 100 mg/kg. Although Deet and Fenvalerate levels dropped rapidly, symptoms were still being observed in one dog which had received 30 mg/kg Deet & 3 mg/kg Fenvalerate at 3 and 4 hours after the 5th injection with blood Deet levels of 0.46 and 0.24 ppm at these respective times. Dermal exposure to single doses of 1.5 or 4.5 g/kg Blockade, or to 4 applications of these dose levels (cumulative 6 and 18 g/kg respectively) resulted in no symptoms; highest level of Deet in blood was 3.267 ppm 2 hrs after a single exposure to 4.5 g/kg.		Supplementary