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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

~~October 27, 1992~~

MEMORANDUM

SUBJECT: DEET: Reviews of 2 Neurotoxicity Studies

TO: Robert S. Brennis
Insecticide/Rodenticide Branch/PM 10
Registration Division (H7505C)

FROM: William F. Sette, Ph.D.
Peer Review Section (H7509c)
Science Analysis Branch
Health Effects Division

THRU: Kerry Dearfield, Ph.D. Acting Section Chief
Peer Review Section
Science Analysis Branch
Health Effects Division

The purpose of this memorandum is to provide you with reviews of two neurotoxicity studies of DEET. The first was an acute oral exposure study (MRID # 413685-01) in which I conclude there is a LOAEL of 200 mg/kg based on decreased vertical motor activity and a NOAEL of 50 mg/kg. The second study was a multi-generation chronic dietary exposure study (MRID # 413684-01) in which neurotoxicity measures were made and in which there was a LOAEL of 5,000 ppm (roughly 225 mg/kg) based on increased motor activity and a NOAEL of 2,000 ppm (roughly 90 mg/kg). Both studies were considered of acceptable quality and are considered valid. If you wish to discuss these reviews or data further, my phone number is 305-6375.

Review by: William F.Sette, Ph.D.
Science Analysis Branch
Health Effects Division (H7509c)

HED PROJECT NO. 1-1514

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DATA EVALUATION REPORT

TEST MATERIAL: DEET CAS NO.: 134-62-3

TOX. CHEM NO: 346 SHG NO: 080301

SYNONYMS: N,N-Diethyl-meta-toulamide

STUDY TYPE: Acute Oral Neurotoxicity Study in Rats

TESTING FACILITY: International Research and Development Corporation, Mattawan MI 49071

TITLE OF REPORT: Neurotoxicity Evaluation in Rats Following Acute Oral Exposure to DEET.

AUTHOR(S): James L. Schardein

REPORT ISSUED: January 23, 1990

STUDY NUMBER: 555-017 MRID NO: 413685-01

SPONSOR: DEET Joint Venture/ Chemical Specialties Manufacturers Association, Washington, DC 20036

CONCLUSIONS:

This is a valid and well conducted study. At 500 mg/kg, rats showed piloerection, increased vocalizations, a significantly increased response time to heat, and significantly decreased horizontal and vertical activity. The piloerection and vocalization were seen 1 and 24 hours after dosing, while the effects on thermal response time and activity were seen one hour after dosing, with recovery 24 hours after the dose. However, at 14 days after dosing, suggestive, i.e., less consistent, increases in horizontal and vertical activity were noted for 500 mg/kg rats. At 200 mg/kg, a significant decrease in vertical motor activity was seen one hour after dosing.

LOAEL = 200 mg/kg, based on significantly decreased vertical motor activity one hour after dosing.

NOAEL = 50 mg/kg

A. MATERIALS:

1. Test compound: pale yellow liquid, Undiluted DEET: 98.3%; 98% meta isomer, 0.174% ortho, 0.127 % para isomers.

2. Test Subjects Charles River Crl:CD VAF/Plus rats, more than 70 days old, weighing 312-359g, males; and 231-254g, females; were matched for body weight variance homogeneity and randomly assigned to treated or control groups. Subjects were housed individually in wire mesh cages.

B. STUDY DESIGN: 10 rats/sex were given 0, 50, 200, or 500 mg/kg DEET by gavage. Controls received white mineral oil.

C. METHODS: Rats were observed twice daily for clinical signs of morbidity or mortality. They were weighed weekly and had their weekly food consumption measured. Detailed observations were performed once weekly following dosing. A functional observational battery and a motor activity test 1 hour, 24 hours, and 14 days after dosing.

1. Functional Observational Battery and Motor Activity.

Rats were evaluated in platoons, such that an equal number of rats of each sex from each group were evaluated each day and at the same time each day. Evaluators were unaware of the treatment group of the rats. They were evaluated for a variety of signs in an open field, including: tremors (resting or during movement/ coarse/fine; convulsions (tonic/clonic); lacrimation (increased/colored); respiration (type/intensity); urination/defecation; vocalizations; piloerection; pupil size; other signs, i.e., diarrhea, salivation, abnormal body position, gait and coordination, stereotyped or abnormal behaviors.

More specific functional and sensory indices were: time to a licking response when standing on a heated platform; 3 determinations forelimb and hindlimb grip strength on a grip bar (average taken as datum); and motor activity in 40 minute sessions after a 10 minute dark adaptation period in a Digiscan (Columbus Instruments) activity meter. The motor activity session length was selected to yield asymptotic performance in the last 20% of the session. Both vertical and horizontal activity were measured.

2. Gross necropsy

After sacrifice by CO2 asphyxiation, all animals were examined grossly both externally and internally (abdominal and thoracic cavities), and abnormal tissues saved in 10% buffered formalin.

3. Statistical Analyses

A number of different statistical tests, involving tests of homogeneity of variance, some transformations, analyses of variance, and post-hoc comparisons were made.

D. RESULTS

In a previous study (IRDC 555-016), briefly noted in this study report, rats given 1,000 mg/kg showed mortality, and rats given either 500 or 1,000 mg/kg showed ataxia, abnormal carriage, and excessive grooming in the first hour after dosing.

Mortality, body weight, and food consumption

In this study, there were no deaths. High dose females ate significantly less in week 2 than control females on a g/day basis,(17.3 vs 19.5 g/day), but not on a g/kg basis. It was concluded that body weight gain and food consumption were not affected by exposure.

Necropsy

No treatment related lesions were noted in any animals on terminal necropsy.

Clinical signs

Mild increased salivation occurred in 50-80% of the animals in all dose groups, but not in control animals, immediately after dosing. This was interpreted as possibly related to the taste of the material.

No treatment related clinical signs were noted from daily observations or weekly examinations.

FOB results (Table 4)

Increased piloerection in both sexes 1 and 24 hours after the dose at 500 mg/kg was seen with a greater effect at 24 hours.

The mean number of vocalizations in males at the 2 higher doses was somewhat increased at one and 24 hours, with the largest increase at the highest dose at 24 hours (4.5 vs 1.0).

The increase in incidence of these effects is clear, if not, apparently, statistically significant. I conclude that piloerection in both sexes and number of vocalizations in males, both at 24 hours after the dose at 500 mg/kg was an effect of treatment.

Functional measurements

Thermal response time

After 500 mg/kg, subjects showed an increased response time to heat in the thermal response test.

The analyses were complex; because of lack of homogeneity of variances, a logarithmic transformation of the data was performed and separate analyses of variance conducted.

A dose x sex x time ANOVA with time as a repeated measures factor showed significant main effects of dose.

A dose x sex ANOVA at each time showed effects at one hour and 14 days. Group comparisons showed the 1 hour effect was attributable to 500 mg/kg group longer response times; the 14 day effect was attributable to the 200 mg/kg group's longer times.

The dose ANOVAs for time for each sex separately revealed no dose group effects, although females at one hour were close ($p=0.06$).

The study authors concluded that since only the one hour high dose effect was found in both analyses, (the $p=0.06$ in the females), that only this effect was considered a reliable treatment related effect.

Grip strength

No statistically significant effects on grip strength were found.

Activity measures

Horizontal activity

At one hour after treatment, a significant decrease in motor activity in interval 3 of the session was found and attributed to rats given 500 mg/kg. At 14 days after treatment, a significant effect was found for time interval 4 of the session and attributed to an increase in rats given 500 mg/kg. Given the large number of comparisons made, i.e., 8 intervals/session, and 3 sessions, these effects were not considered significant. However, these effects at 500 mg/kg at both times is consistent with effects noted below on vertical activity.

Vertical activity

Subjects in the two higher dose groups showed decreased rearing activity during the middle of the session one hour after the dose. A significant dose effect was noted for intervals 1-6, and this effect at intervals 2-5 attributed to the 500 mg/kg dose group. Comparisons revealed a significant decrease in interval 3 for the 200 mg/kg group as well. By visual inspection of the data tables, there were marked decreases in vertical activity for both sexes in intervals 2-7 in comparison to controls at both 200 and 500 mg/kg.

At 14 days after dosing, a significant increase in high dose animals was noted for interval 8.

Total distance decreases were noted from analyses for 500 and 200 mg/kg animals one hour after treatment for interval 3.

On movement time, again, significant decreases were seen for interval 3 attributable to 500 and 200 mg/kg groups. Significant

increases after 14 days were attributed to the 500 mg/kg group in interval 1, and the 50 mg/kg group interval 2, and no group in interval 4.

On rest time, the same pattern was seen, i.e., for mid and high dose animals, more rest time in interval 3; and at 14 days after dosing.

On vertical time, the effect was attributed only to the high dose. By visual inspection, the decrease, however, is of similar magnitude for both mid and high dose males, but not females.

Last, for the 200 mg/kg group, at one hour after the dose, a significant increase in number of clockwise turns was seen in interval 3.

In summary, there was a decrease in vertical activity for rats given 200 and 500 mg/kg one hour after dosing, a decrease in horizontal activity for 500 mg/kg rats one hour after dosing, and suggestive increases in horizontal and vertical activity for 500 mg/kg rats 14 days after dosing. These changes were seen in both the raw data and the pattern of statistically significant findings among the related measures of this behavior.

E. DISCUSSION

This study was conducted prior to the publication of the EPA Subdivision F Neurotoxicity Test Guidelines in 1991 on a voluntary basis on behalf of the registrant and is a well conducted study that would exceed the guidelines in terms of the behavioral measures and the sophistication of the statistical analyses. It does not however, contain histopathology of the nervous system, which our guidelines would require. Nonetheless, it is otherwise a valid and well conducted acute behavioral study.

On methods, I have a minor question with respect to what the diluent was. I assume, since vehicle controls received mineral oil, that this was the diluent, but it is not explicitly stated.

With respect to the results, I take issues with the conclusions of the study authors in that I find that it seems appropriate to consider 200 mg/kg as an effect of treatment, specifically with respect to decreases in vertical activity one hour after dosing. This effect is apparent in the data tables and is noted in the statistical analyses consistently among several of the related measures of vertical activity and I see no good reason to discount it.

With respect to the duration of effects, there are suggestive effects 14 days after dosing in animals given 500 mg/kg: a horizontal activity increase in interval 4; an increase in vertical activity in interval 8; and increased movement time in interval 1; and related measures. Since the pattern of these findings is inconsistent across intervals, they can only be regarded as suggestive.

Signs were seen 1 and 24 hours after dosing, while the quantitative measures on thermal response time and activity were seen one hour after dosing, with apparent recovery 24 hours after the dose.

I conclude that the LOAEL for this study was 200 mg/kg and the NOAEL 50 mg/kg.

Review by: William F.Sette, Ph.D.
Science Analysis Branch
Health Effects Division (H7509c)

HED PROJECT NO. 1-1514

DATA EVALUATION REPORT

TEST MATERIAL: DEET CAS NO.: 134-62-3

TOX. CHEM NO: 346 SHG NO: 080301

SYNONYMS: N,N-Diethyl-meta-toulamide

STUDY TYPE: Multi-generation Exposure Neurotoxicity Study in Rats

TESTING FACILITY: International Research and Development Corporation, Mattawan MI 49071

TITLE OF REPORT: Neurotoxicity Evaluation in Rats Following Multi-generation Exposure to DEET.

AUTHOR(S): James L. Schardein

REPORT ISSUED: January 23, 1990

STUDY NUMBER: 555-015 MRID NO: 413684-01

SPONSOR: DEET Joint Venture/ Chemical Specialties Manufacturers Association, Washington, DC 20036

CONCLUSIONS:

This is a valid and well conducted study. The sole neurotoxicologically significant effect was seen in high dose rats as an increase in motor activity at the beginning of the session. Body weight decreases of 10% and 15%, respectively, were seen in rats during all periods measured at the two highest doses.

LOAEL: Increased motor activity at 5,000 ppm (roughly 225 mg/kg)

NOAEL: 2,000 ppm (roughly 90 mg/kg).

A. MATERIALS:

1. Test compound: pale yellow liquid, Undiluted DEET: 98.3%; 98% meta isomer, 0.174% ortho, 0.127 % para isomers.
Homogeneity and stability of DEET in the diet was determined in the reproduction study and not repeated here. Fresh diet was prepared weekly.

2. Test Subjects Rats, weighing 466-884g, males; and 242-513g, females. Subjects were housed individually in wire mesh cages and given diet and water ad libitum.

B. STUDY DESIGN:

Subjects in this study were the second generation (F2) offspring from a rat multi-generation study (IRDC 555-004), in which rats received DEET in their diets over two generations at concentrations of 0, 500, 2000, or 5,000 ppm (roughly 22.5, 90, and 225 mg/kg for 670g males). The high dose level was established to cause decreased body weight gain. In the original study there were 28 males and females in each group. Selected animals included all remaining control offspring and, wherever possible, 2 males and 2 females from all F2 litters from each dietary group. F2 animals were selected after weaning and maintained for 9 months on the diets. Exposure then was in utero, lactation, and 9 months.

Following the 9 month exposure period, rats were selected for this study. One male and one female from each litter still containing one male and one female (from the 2/sex selected from the original 28 and all controls) were selected. Then 20 "litters"/group for each dose and control group were selected. An additional ten males and females were selected subsequently as a "sham" control group for the passive avoidance task.

At this point, subjects were roughly 40 weeks of age.

C. METHODS: Rats were observed twice daily for clinical signs of morbidity or mortality. They were weighed weekly and had their weekly food consumption measured. Detailed observations were performed once weekly.

Statistical Analyses

A number of different statistical tests, involving tests of homogeneity of variance, some transformations, analyses of variance, and post-hoc comparisons were made.

D. RESULTS

1. Clinical observations, mortality, and body weight

One female in the 2,000 ppm group died at week 48 in emaciated condition.

No observations were found that were considered to be treatment related.

Mean body weights were significantly lower for both male and female rats receiving 2,000 or 5,000 ppm (approx.10, 15%, respectively) at all time points assessed. Food consumption was not reduced, for the most part, among these groups. The report authors

observe that this was not seen in the 5000 ppm group in the reproduction study and suggest that this difference may have been a result of the process of selection of the animals.

2. Neurotoxicity Evaluations

All rats from each group were evaluated on the tests described below. Rats were evaluated in platoons, such that an equal number of rats of each sex from each group were evaluated each day and at the same time each day. Evaluators were unaware of the treatment group of the rats.

Evaluations on the tests listed below took place over an 8 week period of time in the following order: functional observational battery, grip strength, thermal response, week 1; motor activity, weeks 1-2; M maze, weeks 3-5; Startle reflex, weeks 6-7; Passive avoidance, week 8.

A. Functional Observational Battery

Subjects were evaluated for a variety of signs in an open field, including: tremors (resting or during movement/ coarse/fine; convulsions (tonic/clonic); lacrimation (increased/colored); respiration (type/intensity); urination/defecation; vocalizations; piloerection; pupil size; other signs, i.e., diarrhea, salivation, abnormal body position, gait and coordination, stereotyped or abnormal behaviors.

Results

There were no effects whose incidence appeared related to treatment.

B. Licking latency to heat stimulus, grip strength, motor activity

More specific functional and sensory indices were: time to a licking response when standing on a heated platform; 3 determinations of forelimb and hindlimb grip strength on a grip bar (average taken as datum); and motor activity in 40 minute sessions after a 10 minute dark adaptation period in a Digiscan (Columbus Instruments) activity meter. The motor activity session length was selected to yield asymptotic performance in the last 20% of the session. Both vertical and horizontal activity were measured. Some minor deviations resulting in failure to measure the first ten minutes of sessions for some animals in each dose group were noted; data were adjusted from group means.

Results

No treatment related effects on the latency of the licking response to the heated platform were apparent. However, the variability noted in the control animals was quite large, i.e., 11 seconds, relative to that in most exposed groups, i.e., 2-5 seconds. Still, no differences in means appeared systematically

related to treatment.

No treatment related effects on grip strength were apparent by any statistical analysis or observation of the data.

Rats given 5,000 ppm showed increased horizontal activity in the first two 5 minute intervals in the 40 minute session.

These rats also covered more distance in interval 1, although this is not pronounced in males or females.

In addition, some statistically significant increases in the first 3 intervals in counterclockwise circling were noted. For intervals 2 and 3, 5,000 ppm rats made significantly more counterclockwise revolutions; for interval 3, a significant dose x sex interaction was seen and the data further analyzed. For females, but not males, significant effects were noted. Here both the 5,000 ppm and 500 ppm rats made significantly more counterclockwise revolutions, but this seems a minor change.

C. Passive Avoidance

Each rat was tested in a shuttle box modified to have an opaque chamber on one side separated by a barrier from a lighted side. The floor of the chamber could deliver a mild electrical stimulus (0.8 milliamps, 3 secs).

Males and females were tested in separate boxes to avoid odor distraction and all subjects were tested in a darkened room. There was one 3 minute trial/day over 3 days.

On each trial, the subject was placed in the lighted side, the barrier removed, and the time until the rat crossed into the dark side recorded. (Rats prefer a darkened chamber). On the first trial and day, crossing to the darkened side is followed by a brief, mildly aversive electrical stimulus. Thus, on subsequent trials 2 and 3, passivity, i.e., not crossing into the darkened chamber avoids the aversive stimulus. A "sham" control group received no electrical stimulus, and would so be expected to shuttle on all 3 days.

For all groups, the percentage of animals shuttling and the mean latency were recorded.

Results

No treatment related effects were observed on the percentage of animals shuttling on days 2 or 3 and on the mean latency of response.

D. M maze

Rats received 4 sessions of 20 trials each in which they were placed in the central alley of a water maze in a darkened room. The task was to escape via a ramp at one end of the maze. Three measures were made for each trial: latency to escape (maximum, 60 secs); number of incorrect alleys entered; and the "first response

direction"(correct/incorrect). For the first two sessions, the escape ramp was placed at the end of a lighted alley; for the second two sessions, the ramp was placed at the unlighted alley. Thus, there was a reversal of the correct response between sessions 2 and 3, and subjects had to "relearn" the correct response. The correct side was randomly varied on each trial. Subjects were towel dried between trials and the minimum inter-trial interval was one minute.

Results

No treatment related effects were seen on acquisition measures of response time, errors, or initial choices. No treatment related effects were noted on the number or errors or response times on the reversal task. However, a statistically significant effect in all dose groups was noted on the number of correct initial choices, with the treated groups making fewer correct initial choices.

E. Auditory Startle Habituation

Each rat was placed in a plexiglas platform box connected to a strain gauge which could measure the amplitude of the subject's startle reflex in response to a 110 dB tone. Each session consisted of 51 trials separated by intertrial intervals (ITIs) (range 7.1-12.9 seconds). Data consisted of the maximum startle amplitude, the latency to the maximum startle amplitude, and the average startle amplitude of each response, all for the 125 milliseconds after the sound began.

Results

The only significant effect of treatment reported here was on the latency to the maximum startle amplitude. Both a significant dose term and a dose x sex x block interaction were found in the ANOVA-R. Only the interaction term was analyzed further, and this showed that rats in the 5,000 ppm group were significantly slower in block 5 than the animals in the 2,000 ppm and 500 ppm groups, but not the control animals. While it might have been more thorough to pursue the dose effect as well, this seems like a minor finding of little significance, particularly without a significant comparison with controls.

2. Neuropathology

One male and one female from each of ten randomly selected litters in each group were selected for histopathological examination. Following pentobarbital anesthesia, they were sacrificed and given gross examinations. They were then perfused in situ with paraformaldehyde /glutaraldehyde and sections from the following areas taken: forebrain; center of the cerebrum; midbrain; cerebellum; pons; medulla; proximal sciatic; sural n.; tibial n.; spinal cord at cervical and lumbar swellings (cross and longitudinal sections made); gasserian ganglia; dorsal root ganglia; dorsal and ventral root fibers. H&E stains, as well as

luxol fast blue, gallocyanin, and Bielchowsky's stain of sections of each nervous tissue were made. Teased fibers of sural nerve were also prepared.

Examination of all H&E sections were made of high dose and control animals by a Board certified pathologist without knowledge of the treatment group.

Results

No significant treatment related effects on the incidence or severity of macroscopic or histopathological changes were found.

E. DISCUSSION

It is important to bear in mind that rats eat all night, so that in feeding studies such as this, animals may be able to tolerate much higher doses than they can by doses where absorption is relatively rapid. Thus, the kinetics of a dietary feeding study may be quite different from that in a chronic daily dermal exposure situation. On the other hand, in a study such as this where the exposure duration is chronic, the differences in toxicity from cumulative exposure may be lessened by the long duration of exposure. However, this important difference needs to be borne in mind when considering the extrapolation of these data to dissimilar patterns of animal or human exposure.

This is a well conducted and thoroughly analyzed study, that, while it preceded the publication of our revised Neurotoxicity Test Guidelines, and was conducted voluntarily, would satisfy those guidelines, and exceed them in many respects. In general, I concur with the conclusions of the study authors in that the only toxicologically significant effects seen here was a minor effect of an increase early in the session on motor activity in rats given 5,000 ppm. I concur that the statistically significant effect on the initial choice measure in the water maze task was not a biologically significant effect. A number of general comments follow.

The results on circling are partially consistent with the high dose results seen on other measures, i.e., increased activity early in the session, and unique with respect to the low dose group. It is frankly difficult to see how one could consider those effects at other than the high dose to be treatment related. It is unfortunate that in a design such as this, in effect only one sample of behavior is taken, although the analysis may be extensive. We are left then with a difference whose reliability and duration are uncertain in a study where a long term exposure has been undergone.

The purpose and appropriateness of the "sham" control group in the passive avoidance task seems puzzling. The "sham" controls are from the control group and they are not given electrical shocks.

Authors suggest that "non-associative factors did not contaminate passive avoidance retention testing, since these non-reinforced controls failed to exhibit any evidence of dark-side avoidance (learning)." Thus, we would expect this group to shuttle quickly on all 3 days. This "sham" control group represents, at best, a limited control group, in that it seems only to provide control for the "non-associative" aspects of the task in untreated animals. It seems to me the appropriate non-associative control group should have been exposed. Were there an effect seen, we would be interested in whether it was related to treatment and unrelated to the associative aspects of the task. These "sham" controls didn't get exposed and didn't learn. Thus, their lack of retention, i.e., quick and consistent shuttling was not a function of forgetting what they never learned, and not a function of treatment which they never received. In other words, not a non-associative artifact of the non-shock portion of the procedure.

The report authors discounted the effect on initial choice on water maze reversal learning, in that it was not dose dependent and was not supported by other measures on the speed or accuracy of this task. Table 10 for males and females shows no consistent pattern in this endpoint, for the raw data. In addition, a complex transformation, 2 arcsin square root x was performed a priori, which may well have influenced this result. Last, it seems fair to ask what the significance of this measure might be anyway; the animal swims around, some first enter an incorrect alley, then quickly (since response time doesn't change) orient towards the correct alley and complete the task. In summary, I agree that this does not appear to be a biologically significant effect.