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

JAN - 4 1996

MEMORANDUM

SUBJECT: RfD/Peer Review Report of DEET [N,N-diethyl-m-toluidine]

CASRN. 134-62-3
EPA Chem. Code: 080301
Caswell No. 346

FROM: George Z. Ghali, Ph.D.
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Health Effects Division (7509C)

G. Ghali 1-4-96

THRU: William Burnam
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W. Burnam

TO: Rick Keigwin, PM 10
Insecticide-Rodenticide Branch
Registration Division (7505C)

The Health Effects Division-RfD/Peer Review Committee met on September 7, 1995 to discuss and evaluate the existing and recently submitted toxicology data in support of DEET reregistration and to assess a Reference Dose (RfD) for this chemical.

Material available for review consisted of data evaluation records (DERs) for a chronic toxicity/carcinogenicity study in rats **(83-5 or 83-1a and -2a)**, a carcinogenicity study in mice **(83-2b)**, a chronic (one-year) toxicity study in dogs **(83-1b)**, developmental toxicity studies in rats and rabbits **(83-3a and -3b)**, a multi-generation reproductive toxicity study in rats **(83-4)**, subchronic toxicity studies (3-month) in rats, mice, hamsters **(82-1a)** and dogs **(82-2b)**, acute and subchronic/multi-generation exposure neurotoxicity studies in rats **(81-8 and 82-7)** and a mutagenicity battery **(84-2)** including gene mutation, structural chromosomal aberrations and other genotoxic effects, in addition to other special studies including renal toxicity study in rats, dermal absorption study in human volunteers, subchronic dermal toxicity study in micropigs and subchronic dermal toxicity study in castrated male rats.

A. Chronic and Subchronic Toxicity:

The Committee considered the chronic toxicity studies in rats (83-1a, MRID No. 43514203) and dogs (83-1b, MRID No. 43320101) to be acceptable as Core-minimum data and the data evaluation records for these studies (HED Doc. No. 011625; 011630) to be adequate.

In the rat study, the NOEL/LOEL were considered to be 100 and 400 mg/kg/day, respectively, in females based on decreased body weight and food consumption and increased cholesterol levels. In males, the NOEL was considered to be 100 mg/kg/day, the highest dose level tested in males.

In the dog study, the NOEL/LOEL were considered to be 100 and 400 mg/kg/day, respectively, based on decreased food intake and body weights in males and females, decreased cholesterol level in males, increased platelets and incidences of hyperplasia of uterine epithelium in females. Tremors were also observed in one dog of each sex at this dose level.

The Committee did not discuss the subchronic studies in rats, dogs, mice and hamsters in detail. Most of these studies were conducted for range-finding purposes. An issue has emerged concerning the observation of testis and epididymis effects seen in male hamsters at 10,000 and 15,000 ppm. A slight decrease in testis/epididymis weight was also seen in male dogs at 400 mg/kg/day.

B. Carcinogenicity:

The Committee questioned the adequacy of the dose levels used in the carcinogenicity phase of the combined chronic toxicity carcinogenicity study in rats (MRID No. 43514203, HED Doc. 011525) and the rationale used for the dose selection. Females in the high dose group (400 mg/kg/day) exhibited a statistically significant decrease in body weight which progressed from about 9% at week 26 to 18% by week 104 accompanied by about 8% decrease in food consumption. In males, the highest dose level tested was only 100 mg/kg/day and there were no treatment-related effects observed for the study duration at any dose level up to and including the highest dose level tested.

The highest dose level tested in males was selected based on the findings of renal lesions observed in male rats in the range finding study. The renal lesions (characterized by inflammation, regeneration and the presence of granular mass and hyaline droplets in the kidney tubules) were found in all treated males at doses as low as 500 ppm (35 mg/kg/day). These kidney lesions were considered to be related to α-2μ-globulin nephropathy. In the range-finding study, body weight gain reductions were observed in the males and females at 500 mg/kg/day.

Prior to the initiation of the study, the registrant had consulted with the Agency on this issue emphasizing the kidney nephropathy observed in males in the subchronic range-finding study in rats and determined that the high dose level for the combined chronic toxicity/carcinogenicity study would be limited accordingly to 100 mg/g/kg. The Agency determined that the highest dose of 100 mg/kg/day might not be high enough (memo, W. Phang to J. Mitchell, 7/11/1991).

Subsequently, in a meeting between the Agency and registrant representatives, Dr. K. Baetcke presented the Agency's position and concern that male rats might be able to tolerate dose levels higher than the 100 mg/kg/day proposed by the registrant because α-2μ-globulin nephropathy does not, in itself, result in life threatening kidney effects as demonstrated by the d-limonene bioassay where survival of the high dose (150 mg/kg/day) was higher than the control (80 and 60%, respectively for the high dose and controls). The registrant representatives presented their rational for selecting the 100 mg/kg/day as the highest dose level to be used in males testing. After a lengthy discussion the Agency reluctantly conceded that the registrant may employ 100 mg/kg/day as the highest dose level to be tested in male rats. However the results of combined toxicity/carcinogenicity study in rats clearly indicated that male rats in this study could have tolerated doses far higher than the highest dose level tested.

Members of the Committee tried to find some support for the dose selection for male rats based on increased motor activity observed in the acute and/or subchronic neurotoxicity studies in rats (MRID No. 41368501, 41368401), but again these effects are considered transient and not life threatening effects.

In conclusion, the Committee considered the highest dose level tested in female rats (500 mg/kg/day) to be adequate or, at least, approaching an adequate dose level for carcinogenicity testing. On the other hand, the highest dose level tested in males (100 mg/kg/day) was considered to be inadequate. The Committee indicated that males could have tolerated higher dose levels. However, based on the kidney lesions observed in the subchronic study, the Committee believed that the type of tumors that might be induced in males, if higher dose levels were used, would be kidney tumors related to the accumulation of α-2μ-globulin which is species and sex specific and also irrelevant to human risk assessment. On this basis the Committee determined that a new carcinogenicity study in rats would not be required at this time.

The Committee considered the mouse carcinogenicity study (83-2b, MRID No. 43320101) to be acceptable and the data evaluation record (HED Doc. No. 011630) to be adequate. The high dose levels tested in males and females in the mouse carcinogenicity study were considered to be adequate.

Overall, the Committee concluded that the treatment did not alter the spontaneous tumor profile in both sexes of rats and mice. However, because the Committee believed that male rats could have tolerated higher dose levels, the Committee recommended that the chemical be classified as a "Group D", not classifiable as to human carcinogenicity. It should be noted that this group is generally used for agents with inadequate human and animal evidence of carcinogenicity or for which no data are available.

C. Developmental and Reproductive Toxicity:

The Committee considered the developmental toxicity study in rats (83-3a, MRID No. 41351401) to be acceptable as Core-minimum data. The developmental toxicity study in rabbits (83-3b, MRID No. 42141101) was considered to be Core-supplementary data. The data evaluation records of the two developmental toxicity studies (HED Doc. No. 008117; 009470) were considered to be generally adequate.

In the rat study, the maternal toxicity NOEL/LOEL were considered to be 250 and 750 mg/kg/day based on clinical signs (including possible neurotoxic effects), mortality, reduced maternal body weight gain and food consumption, and increased mean liver weight. The reproductive toxicity NOEL/LOEL were considered to be 250 and 750 mg/kg/day based on a statistically significant decrease in the mean fetal body weight. However, litter size data for the rat study should be compared to historical control data before a final conclusion could be made regarding the developmental toxicity NOEL/LOEL.

In the rabbit study, the NOEL for maternal and developmental toxicity was considered to be 350 mg/kg/day, the highest dose tested. The results indicate that animals could have tolerated higher dose levels. The report fails to provide a rationale for dose selection.

The Committee considered the reproductive toxicity study in rats (83-4, MRID No. 40979001) to be acceptable and the data evaluation record (HED Doc. 007645) to be adequate. The reproductive toxicity NOEL was considered to be 250 mg/kg/day, the highest dose level tested. The parental toxicity LOEL, according to the data evaluation report prepared by the scientific reviewer, was considered by 25 mg/kg/day based upon signs of kidney effects which included mottling, presence of hyaline droplets, granular cast formation, and tubular regeneration observed in males.

D. Acute and Subchronic Neurotoxicity:

The Committee considered the acute (81-8, MRID No. 41368501) and subchronic/multigeneration exposure (82-7, MRID No. 41368401) neurotoxicity studies to be acceptable and the data evaluation records (HED Doc. No. 009829) to be adequate.

In the acute exposure study, the NOEL/LOEL were considered to be 50 and 200 mg/kg/day, respectively, based on significantly decreased vertical motor activity.

In the subchronic/multigeneration exposure study, the NOEL/LOEL were considered to be 90 and 225 mg/kg/day, respectively, based on increased motor activity. Body weight decreases of 10 and 15%, respectively, were observed in rats of the two highest dose groups (90 and 225 mg/kg/day) during all periods. However, the significance of these changes in motor activity were discussed in the October 17, 1995 less than life time exposure meeting.

E. Mutagenicity:

The Committee considered the following mutagenicity studies to be acceptable:

1) Salmonella assay (MRID No. 41344801, HED Doc. No. 008111): the test is negative up to 8333 μg/plate, the highest concentration tested.

2) Chinese hamster ovary(CHO) cells/aberrations (MRID No. 41344401, HED Doc. No. 008111): the test is negative up to 1 μl/ml, the highest concentration tested without metabolic activation and up to 0.5 μl/ml, the highest concentration tested with metabolic activation.

3) Unscheduled DNA synthesis(UDS)/in primary rat hepatocytes (MRID No. 41344301, HED Doc. No. 008111): the test is negative up to 0.3 μl/ml, the highest concentration tested.

The Committee was also aware of additional mutagenicity information in the open literature, i. e. published studies, including the following:

1) Salmonella assay (Zeiger et al., Environ. Molec. Mutagen 19 (Suppl. 21): 2-141, 1992): the test is negative and the results are consistent with the submitted study described above.

2) Rat spermhead abnormality assay (MRID No.00149154, HED Doc. No. 000000): the test showed some positive response at dose levels $\geq$1500 mg/m^3. Administration of the test compound was done through inhalation exposure for 6 hours/day, 5 days/week over a period of 13 weeks.

3) Mouse dominant lethal study (MRID No. 00138397, HED Doc. No. 000000). The test results were reported as negative, but actual report was not located in Agency files (personal communication; K. Dearfield and C. Swentzel, author of the report).

Overall, the Committee concluded that based on submitted studies, the acceptable tests satisfy the minimal testing

requirements for mutagenicity in accordance with the pre-1991 guideline. There does not appear to be an overt mutagenicity concern. However, the rat spermhead abnormality finding may present some concern. The dominant lethal results, if substantiated by location of the actual study, would help lower any possible heritable genetic risk concern . It should be mentioned that reproductive performance in a multigeneration reproductive toxicity study in rats and fetal development as seen in developmental toxicity studies in two different species, rats and rabbits, partially, helped in alleviating some of this concern.

F. Reference Dose (RfD):

The Committee recommended that a Reference Dose (RfD) be established on the basis of a subchronic/multigeneration exposure neurotoxicity study with a NOEL of 90 mg/kg/day. Increased motor activity and decreased body weight were observed at the next higher dose level of 225 mg/kg/day. An Uncertainty Factor (UF) of 100 was applied to account for both the interspecies extrapolation and intraspecies variability. On this basis the RfD was calculated to be 0.9 mg/kg/day.

However, based on a subsequent less than life time (LTL) risk assessment meeting held on October 17, 1995, in which the critical subchronic/multigeneration study was reevaluated, the NOEL of the neurotoxicity study was revised to 225 mg/kg/day for the purposes of subchronic risk assessment. This indicated that the chronic RfD would be based on both the chronic toxicity studies in dogs and rats with NOEL/LOEL of 100 and 400 mg/kg/day, respectively, for both studies.

In the rat study, decreased body weight and food consumption and increased cholesterol levels were observed in females at 400 mg/kg/day. In the dog study, decreased food intake and body weights in both males and females, decreased cholesterol level in males, increased platelets and incidences of hyperplasia of uterine epithelium in females were observed at 400 mg/kg/day. Tremors were also observed in one dog of each sex at this level.

Using a UF of 100 to account for both the interspecies extrapolation and intraspecies variability, the RfD was calculated to be 1.0 mg/kg/day.

It should be noted that this chemical has not been reviewed by the FAO/WHO joint committee meeting on pesticide residue (JMPR) and that an acceptable daily intake (ADI) has not been established by that Committee.

G. Individuals in Attendance

Peer Review Committee members and associates present were George Ghali (Manager, RfD/Peer Review Committee), Marion Copley ((Acting Chief, TB I), Karl Baetcke (Acting Chief, TB II), Stephen Dapson, Kerry Dearfield, Roger Gardner, Guruva Reddy, William Sette, and Rick Whiting. In attendance also were Kit Farwell and Paula Deschamp of HED as observers.

Scientific reviewer (Committee or non-committee member(s) responsible for data presentation; signature(s) indicate technical accuracy of panel report)

Whang Phang

James Rowe

Respective branch chief (Committee member; Signature indicates concurrence with the peer review unless otherwise stated)

Karl Baetcke

CC: Stephanie Irene (HED/OPP)
Debra Edwards (HED/OPP)
Karl Baetcke (HED/OPP)
Kerry Dearfield (HED/OPP)
James Rowe (HED/OPP)
Whang Phang (HED/OPP)
Karen Whitby (HED/OPP)
Albin Kocialski (HED/OPP)
Beth Doyle (HED/OPP)
Amal Mahfouz (OW)
RfD File (SAB/HED)
Caswell File (HED/OPP)

H. Material Reviewed:

1. Goldenthal, E. I. (1994). Evaluation of Deet in a two-year dietary and oncogenicity study in rats. MRID No. 43514203, HED Doc. No. 011625. Classification: Core-minimum for chronic toxicity, Core minimum for carcinogenicity testing in females and Core-supplementary for carcinogenicity testing in males. This study satisfies data requirement 83-1a of the Pesticide Assessment Guideline for chronic toxicity testing and **partially** satisfies data requirement 83-2a of Subpart F of the Pesticide Assessment Guideline for carcinogenicity testing in rats.

2. Goldenthal, E. I. (1990). Evaluation of Deet in an eighteen month dietary oncogenicity study in mice. MRID No. 41351501, HED Doc. No. 018117. Classification: Core-minimum data. This study satisfies data requirement 83-2b of Subpart F of the Pesticide Assessment Guideline for carcinogenicity testing in rats.

3. Goldenthal, E. I. (1994). Evaluation of Deet in a one year chronic oral toxicity in dogs. MRID No. 43320101, HED Doc. No. 011630. Classification: Core-minimum data. This study satisfies data requirement 83-1b of Subpart F of the Pesticide Assessment Guideline for chronic toxicity testing in dogs.

4. Schardein, J, L. (1989). Evaluation of Deet in a two generation reproduction/fertility study in rats. MRID No. 40979001, HED Doc. No. 007645. Classification: Core-minimum data. This study satisfies data requirement 83-4 of Subpart F of the Pesticide Assessment Guideline for reproductive toxicity testing in rats.

5. Neeper-Bradley, T. L. (1990). Developmental toxicity evaluation of Deet administered by gavage to CD (Sprague-Dawley) rats. MRID No. 41351401, HED Doc. no. 008117. Classification: Core-minimum data. This study satisfies data requirement 83-3a of Subpart F of the Pesticide Assessment Guideline for developmental toxicity testing in rats.

6. Chun, J. S. and Neeper-Bradely, T. U. (1991). Developmental toxicity evaluation of Deet administered by gavage New Zealand white rabbits. MRID No. 42141101, HED Doc. no. 009470. Classification: Core-supplementary data. This study does not satisfy data requirement 83-3b of Subpart F of the Pesticide Assessment Guideline for developmental toxicity testing in rats.

7. San, R. H. C. and Schadly, M. B. (1989). Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test) with a confirmatory assay. MRID No. 41344801, HED Doc. No. 008111. Classification:

Acceptable. This study satisfies data requirement 84-2 of Subpart F of the Pesticide Assessment Guideline for gene mutation testing.

8. Putman, D. L. and Morris, M. J. (1989). Chromosome aberrations in Chinese hamster ovary (CHO) cells. MRID No. 41344401, HED Doc. No. 008111. Classification: Acceptable. This study satisfies data requirement 84-2 of Subpart F of the Pesticide Assessment Guideline for chromosomal aberrations testing.

9. Curren, R. D. (1989). Unscheduled DNA synthesis assay in rat primary hepatocytes with a confirmatory assay. MRID No. 41344301, HED Doc. No. 008111. Classification: Acceptable. This study satisfies data requirement 84-2 of Subpart F of the Pesticide Assessment Guideline for other genotoxic effects.