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

10/17/1994

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

SUBJECT: EPTC---Tox Data Submitted Under MRID 43230901
ID No. 041401

Chemical: 041401 (435)
RD Record: S468267
HED Project: D204573

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I
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10-07-94

TO: Walter Waldrop/Margery Exton, PM #71
Registration Branch
Special Review and Reregistration Division (7508W)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I
Health Effects Division (7509C)

10/17/94

Registrant: Zeneca, Wilmington, DE

Request: Review and evaluate the following subchronic (feeding) neurotoxicity study, submitted under cover of May 11, 1994, to satisfy data requirements under FIFRA TESTING GUIDELINE 82-7:

> Subchronic Neurotoxicity Study in Rats, performed at Zeneca's Central Toxicology Laboratory (CTL), Macclesfield (UK), Laboratory Project ID: [Final] Report No. CTL/P/3930 (Study No. PR0929) issued April 29, 1994. [MRID 43230901].

TB CONCLUSION: The May 11, 1994 cover to this submission states that this subchronic study is a follow-up to a prior submission (MRID 43039701) of acute oral neurotoxicity data (Zeneca CTL Report No. 4092; Study No. AR 5527) which has been reviewed by TB-1 (HED DOC. No. 010837), but considered only CORE-SUPPLEMENTARY since a NOEL was not established for males at the LDT, 200 mg/kg, at which dose brain neuronal cell necrosis was evident in two of five animals, as well as all other dose levels (1000, 2000 mg/kg).



10 of 10

The current study (MRID 43230901) is judged ACCEPTABLE, providing the following toxicological parameters (a detailed review is attached to this memo):

NOEL = 100 ppm (7.9/8.8 mg/kg/day for males/females).

LOEL = 500 ppm (39.4/43.8 mg/kg/day for males/females) resulting in decreased BW gain and reduced brain weight and size.

In addition, at the HDT, 2500 ppm: Urinary incontinence in females; increased incidence of neuronal cell necrosis in forebrain of both sexes.

ATTACHMENT: DER

Disk 3.5:Disk 2:041401:MAUER:MB

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Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I, HED (7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I, HED (7509C)

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DATA EVALUATION RECORD

MRID No.: 43230901
PC No.: 041401
RD Record No.: S468267
EPA ID No.: 041401
Tox Chem. No.: 435
Project No.: 204573

I. SUMMARY

STUDY TYPE: (82-7) Subchronic (90-day) oral (feeding) neurotoxicity-rat.

CHEMICAL: EPTC (s-ethyl dipropylthiocarbamate)

SPONSOR: Zeneca Agricultural Products, Wilmington, DE

TESTING FACILITY: [Zeneca] Central Toxicology Laboratory (CTL), Macclesfield (UK)

TITLE OF REPORT: EPTC; Subchronic Neurotoxicity Study in Rats

AUTHOR(S): D. J. Tinston

STUDY NUMBER: PRO 929; Report No. CTL/P/3930

DATE ISSUED: April 29, 1994

EXECUTIVE SUMMARY: Rats were fed diets containing 100, 500 and 2500 ppm EPTC for 13 weeks, and monitored monthly for signs of functional neurotoxic signs. Dose-related decreases in body weight gain, food consumption and brain weight and size were found in mid-dose and high-dose groups, as well as increased urinary incontinence in high-dose females, and increased incidence of neuronal cell necrosis in high-dose male and female brains.

TB-I EVALUATION: ACCEPTABLE

LOEL = 100 ppm (7.9 and 8.8 mg/kg/day in males and females)

LOEL = 500 ppm (39.4 and 43.5 mg/kg/day in males/females), resulting in decreased BW gain; and reduced brain weight and size

II. DETAILED REVIEW

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A. TEST MATERIAL: EPTC (Zeneca, Bracknell, UK)

| | |
|---|---|
| Description: | Yellow liquid |
| Batches (Lots): | P4/D7534/10 |
| Purity (%): | 98.4 |
| Solvent/carrier/diluent: | Rodent Lab Chow |

B. TEST ORGANISM: Rodent

| | |
|---|---|
| Species: | Rat |
| Strain: | Alpk:APfSD (SPF) |
| Age: | 28 days |
| Weights - | males: 191-194 g |
| | females: 150-153 g |
| Source: | Zeneca, Macclesfield (UK) |

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the neurotoxic potential of the test article when administered for 13 weeks to male and female rats, according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: Based upon prior short-term feeding studies with EPTC stated to have been performed in this laboratory, groups of rats (12/sex/dose group) were fed diets containing 0, 100, 500 or 2500 ppm test article continuously for 13 weeks. Animals were observed daily, weighed weekly (and food consumption calculated), and subjected monthly (wks - 1, 5, 9, 14) to both a observational battery (FOB),[1] as well as a series of ten locomotor activity screens (five minutes each) on an automated activity recording apparatus.

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[1] FOB:

| | |
|---|---|
| Landing foot splay | Tail flick test |
| Limb grip strength | Autonomic functions |
| Convulsions/tremors | Arousal level |
| Sensorimotor responses | Reactivity to stimuli |
| Alteration in respiration | |

At scheduled termination, one-half of each group (6/sex/dose) were anesthetised with barbiturate ip and killed by perfusion fixation with modified Karnovsky's Fluid. The remaining animals were exsanguinated under terminal anesthesia with Halothane, and subjected to full necropsy[2], with selected tissues fixed in 10% neutral buffered formol-saline. Only tissues from the Karnowsky-perfused animals were processed for histological examination (including H and E), namely, brain and gastrocnemius muscle, vertebral column (spinal cord, dorsal root ganglia and spinal roots). In addition, where feasible, samples of some neural tissues (left Gasserian ganglion; left sciatic, sural and tibial nerves; cervical and spinal cord) were post-fixed in osmium tetroxide, embedded in resin and "semi-thin sections" stained in toluidine blue. All sections were examined by light microscopy.

Standard statistical analyses were applied to resulting data, as follows:

| | |
|---|---|
| Analysis of covariance: | for body weights, brain wts. and dimensions. |
| Analysis of variance: | for (1) food consumption and utilization (the latter, calculated as: $\frac{\text{BW (g) X 100}}{\text{Fd eaten (g)}}$ |
| | (2) Motor activity |
| | (3) FOB |

E. RESULTS: [Selective significant results are summarized on the following page].

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[2]Brain
Gasserian ganglia
Spinal roots
Sciatic nerve
Tibial nerve
Spinal Cord
Dorsal root ganglia
Gastrocnemius muscle
Sural nerve

Effects of Dietary EPTC Feed for 13 weeks in S-D Rats[3]

| Observation (12/sex/dose group) | Dietary Concentration (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 100 | | 500 | | 2500 | |
| | M | F | M | F | M | F | M | F |
| Mortality | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Urinary incontinence | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| Body weight gain: - Wk 1-14 (g) | 321 | 123 | 324 | 122 | 298 | 107[4] | 290[4] | 102[4] |
| F. O. B: | | | | | | | | |
| Reduced Splay Reflex[(a)] | 1 | 0 | 2 | 0 | 2 | 2 | 3 | 2 |
| Landing foot splaymm) | 81 | 72 | 84 | 74 | 83 | 72 | 80 | 63[4] |
| Time to tail flick (sec) | 4.7 | 4.6 | 5.4 | 4.6 | 6.3 | 3.5 | 6.9[4] | 4.3 |
| Pathology:: | | | | | | | | |
| Brain weight (g) | 2.15 | 1.99 | 2.15 | 1.98 | 2.09[4] | 1.89[5] | 2.06[5] | 1.78[5] |
| Brain length (mm) | 28.4 | 27.3 | 28.3 | 26.7[4] | 27.6[4] | 26.3[5] | 28.2 | 26.6[4] |
| Neuronal cell necrosis (n) | 1 | 0 | 1 | 0 | 2 | 1 | 4 | 5 |

Concentrations and homogenicity of test article at the various levels of the prepared dietary mixtures were considered satisfactory, never more than 9% of target levels, with deviations at mean concentrations of EPTC at different levels in the mixer at the LDT and HDT within 4% (Diet analysis: Report Table 2; APPENDICES I, J). Stability of the dietary mixtures for successive batches was achieved by freezing them immediately after preparation, then defrosting and feeding to animals for no more than three days per batch.

Mean intake of test article over the 13-week feeding period was calculated in terms of mg EPTC/Kg B W/day as follows (extracted from Report APPENDIX K):

[3]Selected (statistically significant) effects extracted from Tables 3 through 13 and INDIVIDUAL ANIMAL DATA SUPPLEMENT of the Final Report.

[4]Significant at $p<0.05$

[5]Significant at $p<0.01$

| SEX | Nominal Dietary Level (ppm) | | |
|---|---|---|---|
| | 100 | 500 | 2500 |
| MALES | 7.9 | 39.4 | 193.1 |
| FEMALES | 8.8 | 43.5 | 205.1 |

One high-dose male was found dead and partially cannibalized on Study Day 3, but had manifested no previous adverse clinical signs; autopsy of the limited amount of relevant tissues available revealed (only) soft areas in the brain consistent with the general autolysis found. The author considered this single death to be unrelated to EPTC treatment. All other animals survived to study termination without apparent adverse clinical signs (Report Table 3).

Signs of urinary incontinence were evident in 5 of the 12 high-dose (2500 ppm) females, but not in males at that dietary level, nor at lower levels in either sex.

Results of the FOB and motor activity measurements indicated no consistent or dose-related changes in treated animals (Report Tables 3, 4, 5, 6 and 10). Isolated changes that were observed in single animals of different dose groups (including controls) were considered incidental to treatment.

Dose-related, statistically significant, decreases in body weight, however, persisted throughout the study among mid and high-dose male and female groups (Report Table 7; Figs. 1-4), correlated with comparably significant reductions in food consumption and utilization (Report Tables 8, 9; Figs. 5, 6). Minimal (insignificant) decrements were also found at the LDT (100 ppm) during the first few weeks of the study, but considered "...to be of no toxicological significance" [according to the author].

Gross pathological examination revealed statistically significant reductions in absolute brain weight for both mid and high-dose treatment in both sexes, but only for relative organ weight in high-dose males when adjusted for body weight (Report Table 11). In addition, for mid and high-dose females (but not males), brain width and length were reduced significantly. All other macroscopic changes treated groups were incidental and unrelated to EPTC treatment (Report Table 12).

Histopathological examination revealed dose-related incidence of neuronal cell necrosis in the brains of all treated groups, as well as controls, as follows:



46

4/6 males and 5/6 females at 2500 ppm; 2/6 ma[illegible]nd 1/6 females at 500 ppm; 1/6 low-dose and 1/6 [illegible]col males, but none so affected in th[illegible]emale 100 ppm or control groups (Report Table 13) Additionally, "mild" sciatic nerve and spinal cord degeneration were observed in high-dose groups, but discounted by the author, since these changes "are commonly seen in this strain of rat, and also in the controls, they were considered to be incidental to treatment with EPTC".

The investigator concluded that dietary EPTC at levels of 500 and 2500 ppm resulted in decreased body weight gain, food consumption, and in brain weight and size, but without any evidence of functional (neurotoxic) impairment, as determined by results from the FOB and motor actively measurements. High-dose females also manifested urinary incontinence, unassociated with any changes in kidneys or bladders. Both male and female high-dose animals also had increased incidence of neuronal cell necrosis in pyriform cortex and hippocampal areas of the brain.

Hence, the no-observed-effect level was considered to be 100 ppm EPTC.

F. TB EVALUATION: ACCEPTABLE. This study provides acceptable data to satisfy requirements for FIFRA TEST GUIDELINE 82-7 (subchronic neurotoxicity), and establishes the following toxicity parameters:

NOEL = 100 ppm (7.9 mg/kg/day for males; 8.8 mg/kg/day for females)
LOEL = 500 ppm (39.4 mg/kg/day for males: 43.5 mg/kg/day for females), producing decreased BW gain and food consumption in both sexes; reduced brain weight and size in females.

In addition, at the HDT, 2500 ppm (193.1 mg/kg/day in males; 205.1 mg/kg/day in females): urinary incontinence in females; and increased incidence of neuronal cell necrosis in parts of the forebrain of both sexes.

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10