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

JUN 30 1988

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

TO: Portia Jenkins, PM Team # 12
Insecticide/Rodenticide Branch
Registration Division TS-767C

THRU: R. Bruce Jaeger, Section Head
Rev. Sec. # 1/Toxicology Branch
Hazard Evaluation Division TS-769C 6/28/88

THRU: Dr. T. M. Farber, Chief
Toxicology Branch
Hazard Evaluation Division TS-769C

FROM: D. Ritter, Adjuvants Toxicologist DLR 6-24-88
Rev. Sec. # 1/Toxicology Branch
Hazard Evaluation Division TS-769C 6/30/88

Subject: 3125-108 - Azinphos-Methyl Registration Standard: Submission of 28 Day Range-Finding Study in Rats.

Caswell #: 374.

TOX Project #: 8-0596

Sponsor: Mobay Corporation, Kansas City, MO.

The Mobay Corporation is submitting a range-finding study in order to determine the doses required for a chronic feeding study in rats. The DER of this study is appended.

1. "Toxicity Study on Rats With Particular Attention to Cholinesterase Activity". Study # 95608. MRID 40543201. CORE Supplementary.

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DDR 6-24-88

Reviewer: D. Ritter, Toxicologist Caswell #: 374.
Rev. Sec. # I/Toxicology Branch
Secondary Reviewer: R. Bruce Jaeger, Section Head
Rev. Sec. # I/Toxicology Branch

DATA EVALUATION RECORD

Study: 28 Day Toxicity/Cholinesterase Study in Rats.

MRID: 40534201

Performing Laboratory: BAYER AG Inst. Fuer Tox. Wuppertal-Elberfeld Fed. Rep. Germany.

Author(s): R. Eiben, W. Schmidt, E. Loeser.

Study ID Number: 95608.

Date of Study: 5/18/93.

Title: "Toxicity Study on Rats With Particular Attention to Cholinesterase Activity".

Test Material: Azinphos-Methyl (Guthion; R 1582) O,O-dimethyl S-[4-oxo-1,2,3-benzotriazin-3(4H)-yl methyl] phosphorodithioate

CORE Rating: Supplementary - No requirement for this type of study.

QA Statement: Present.

CONCLUSIONS: The overall NOEL is 5 ppm based on ChE inhibition at the higher levels.

METHODS:

Animals - SPF BOR:WISW male & female rats, approximately 13 weeks old, weighing 233 gm (males) or 158 gm (females).

Husbandry - Standard GLP.

Feed and Water - Available ad libitum.

Test Material -

R-1582 was mixed into standard laboratory rat chow at levels of 0, 5, 20 or 50 ppm in 1 % peanut oil.

Administration -

The mixed diets were offered ad libitum to groups of 5 males and 5 females each.

Cageside Observations -

Mortality and Signs of Toxicity - Twice daily; once daily on weekends and holidays.

Body Weights and Feed Consumption - Once weekly.

Clinical Observations -

Blood -

Red cell and plasma ChE activity was determined using Ellman's Method on samples obtained by orbital sinus puncture on days 1, 4, 14 and 28. A brief description of this procedure is appended.

Brain -

ChE activity was determined on brains obtained at termination. Whether these were whole brains or portions was not stated.

Post-Mortem Observations and Pathology -

All surviving animals were were killed and the following organs and tissues were reserved in Bouin's Solution:

Heart*	Liver*	Lungs*
Spleen*	Kidneys*	Adrenals*
Gonads*	Pituitary	Stomach

Gross necropsy findings were recorded.

RESULTS:

Animals -

Behavior and appearance of treated rats was comparable to that of the control rats.

Feed and Test Material Consumption -

The 5 ppm females consumed more feed than the other groups; however, this is not considered to be related to compound ingestion.

Ingestion of test material was at the expected levels.

* = Organs Weighed

Cageside Observations -

Mortality -

One 5 ppm female died following blood collection at day 14.

Signs of Toxicity -

None reported.

Body Weights -

There was no discernable effect on body weights at any level tested.

Clinical Observations -

Cholinesterase -

5 ppm groups showed no effect on ChE activity at any time.

20 ppm males showed significantly reduced plasma ChE levels ($P < 0.01$; 26%) at day 28.
20 ppm females showed significantly reduced red cell activity ($P < 0.05$; 27%) at 14 and 28 days. There was no reduction of brain ChE activity in this group.

statistically significant

50 ppm males showed significantly reduced plasma (21%) and red cell activity (32%);($P < 0.01$) at 14 and 28 weeks.
50 ppm females showed significantly reduced plasma activity ($P < 0.05$; 60%) at days 4, 14 and 28; significantly reduced red cell activity ($P < 0.01$; 35%) at days 14 and 28, and significantly reduced brain ChE activity at termination ($P < 0.01$; 53%).

Post-Mortem Observations and Pathology -

Organ Weights -

No effects at any level tested.

Gross Necropsy Findings -

None reported for any level tested.

DISCUSSION:

The only parameter affected in this range-finding study was ChE activity in the 20 and 50 ppm groups, with the 50 ppm females showing reduced brain ChE activity.

The study was well performed and generally supports proposed dose levels of 5, 15 and 45 ppm for a chronic feeding study. A better description of the method used to assay the brain ChE activity would have been useful.

The study is rated CORE Supplementary since there is no requirement for a 28 day study, AND NO description of BRAIN ChE ASSAY method.

flat layer at the interface between the two phases. When more than 10 mg of brain homogenate are used, it is advisable to extract with 3 ml of ketone. The ketone layer, containing acetylcholine, is transferred by a Pasteur pipette to another tube and is washed once with 4 ml of 10 mM sodium phosphate buffer, pH 7.4, containing 2 mg of sodium tetraphenylboron. After recentrifugation, a sample of the ketone layer (usually 0.5 ml) is transferred to a scintillation vial containing 2 ml of acetonitrile and 10 ml of toluene scintillation mixture. The radioactivity is determined in a liquid scintillation spectrometer.

2 *Micro procedure based on (1-^{14}C) acetate.*

This procedure is used for reaction volumes less than 50 μl. The incubation mixture contains 2 mM sodium (1-^{14}C) acetate, extract from 2.5 μg of acetone-dried pigeon liver/μl of incubation mixture, and other components as described above for the "macro procedure." The incubation mixture (100 μl) is preincubated for 10 min, and 1 μl is transferred via a pipette to a small conical tube (2 mm diameter) containing the enzyme preparation. After incubation, the tube is transferred to a 10-ml centrifuge tube containing 7 ml of 10 mM sodium phosphate buffer (pH 7.5) and 0.25 mg of ACh chloride. The phosphate buffer solution is flushed into the conical micro tube three times using a Pasteur pipette. This washes the contents of the micro tube into the large tube. The ACh is extracted with 1 ml of butyl ethyl ketone containing 15 mg of tetraphenylboron. The ketone layer is isolated by centrifugation, transferred to a new tube, and washed with 4 ml of 10 mM sodium phosphate buffer containing 2 mg of sodium tetraphenylboron. After recentrifugation, the radioactivity of the ketone layer is determined as described above.

Acetylcholinesterase (AChE)

A wide variety of methods are available for the determination of AChE. The hydrolysis of the ester is accompanied by the release of one equivalent of acid. This can be measured by Warburg manometry as CO_2 liberated from a bicarbonate buffer by the acid (119) or determined titrimetrically with an automated pH-stat (61). Radiochemical assays, in which the ^{14}C-acetic acid produced by hydrolysis of acetyl-1-^{14}C-ACh is measured, also have been used (33,90). The most commonly used method is that of Ellman et al. (26). The method involves the formation of the colored 5-thio-2-nitrobenzoate anion by the reaction of thiocholine with 5,5'-dithiobis-2-nitrobenzoic acid. This method is described below.

Solutions. Phosphate buffer, 0.1 M, pH 8.0; acetylthiocholine iodide, 0.075 M (21.67 mg/ml, this substrate solution is used successfully for 10–15 days if kept refrigerated); 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), 0.01 M, 39.6 mg are dissolved in 10 ml pH 7.0 phosphate buffer (0.1 M) and 15 mg of sodium bicarbonate are added (this reagent is made up in buffer of pH 7, in which it is more stable than in that of pH 8).

Tissue preparations. The tissue is homogenized (approximately 20 mg of tissue per ml of phosphate buffer) (pH 8.0, 0.1 M) in a Potter-Elvehjem homogenizer.

Assay procedure. An aliquot of the homogenate (0.4 ml) is added to a cuvette containing 2.6 ml of phosphate buffer (pH 8.0, 0.1 M). DTNB reagent (100 μl) is added to the photocell. The absorbance is measured at 412 mμ: when this has stopped increasing, the photometer slit is opened so that the absorbance is set to zero. Substrate (20 μl) is added and changes in absorbance are recorded, from which the change in absorbance per minute is calculated. The rates are calculated as follows:

$$R = \frac{\Delta A}{1.36 \times 10^4} \times \frac{1}{(400/3120)C_0}$$
$$= 5.74 \times 10^{-4} \frac{\Delta A}{C_0}$$

where R is the rate, in moles substrate hydrolyzed per minute per gram of tissue, ΔA is the change in absorbance per minute, and C_0 is the original concentration of tissue (mg/ml).

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