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

007157

MAY -3 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Azinphos-Methyl

Project No. 9-0147
TOX Chem No.: 374

FROM: Ray Landolt 4/28/89
Review Section I
Toxicology Branch II - Herbicide, Fungicide, and
Antimicrobial Support
Health Effects Division (H7509C)

TO: Dennis H. Edwards, Jr., PM 12
Insecticide-Rodenticide Branch
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THRU: Mike Ioannou, Acting Section Head J.M. Ioannou 5/1/89
Review Section I
Toxicology Branch II - Herbicide, Fungicide, and
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and

Marcia van Gemert, Acting Chief M van Gemert 5/1/89
Toxicology Branch II - Herbicide, Fungicide, and
Antimicrobial Support
Health Effects Division (H7509C)

Registrant: Mobay Corporation, letter of September 30, 1988

Action Requested: Review a General Metabolism Study (85-1) in rats submitted in response to the Registration Guidance Document dated September 11, 1986.

Conclusion: Classification of Data- Guideline

Data evaluation by Dynamic Corporation April 25,1989 (copy attached).

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EPA No.: 68D80056
DYNAMAC No.: 144-B
TASK No.: 1-44B
April 25, 1989

DATA EVALUATION RECORD

AZINPHOS-METHYL

Metabolism in Rats

STUDY IDENTIFICATION: Kao, L. R. M. Disposition and metabolism of azinphos-methyl in rats. (Unpublished study No. 98327 performed and submitted by Mobay Corporation, Stilwell, KS; dated September 30, 1988.) MRID No. 408365-01.

APPROVED BY:

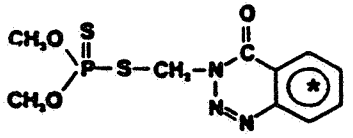
Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 4/25/89

1. CHEMICAL: Azinphos-methyl; 0,0-dimethyl-S-[(4-oxo-1,2,3-benzotriazin-3-(4H)-yl-methyl] phosphorodithioate; Guthion.

2. TEST MATERIAL: [^{14}C]Azinphos-methyl was uniformly labeled in the phenyl ring and had a specific activity of 22.32 mCi/mmol and a radiochemical purity of >99 percent. The chemical structure of the test material is as follows:



The asterisk (*) indicates the position of the ^{14}C label

3. STUDY/ACTION TYPE: Metabolism in rats.

4. STUDY IDENTIFICATION: Kao, L. R. M. Disposition and metabolism of azinphos-methyl in rats. (Unpublished study No. 98327 performed and submitted by Mobay Corporation, Stilwell, KS; dated September 30, 1988.) MRID No. 408365-01.

5. REVIEWED BY:

Nicolas P. Hajjar, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Nicolas P. Hajjar
Date: Apr. 26, 1989

William L. McLellan, Ph.D.
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Signature: William L. McLellan
Date: April 26, 1989

6. APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
Date: April 26, 1989

Ray Landolt
EPA Reviewer, Section I
Toxicology Branch II
(H7509C)

Signature: Ray Landolt
Date: 4/28/89

Mike Ioannou, Ph.D., D.A.B.T.
EPA Acting Section Head
Toxicology Branch (H7509C)

Signature: M. Ioannou
Date: 5/1/89

7. CONCLUSIONS: The metabolism of [^{14}C]azinphos-methyl was studied in groups of five male and five female Sprague-Dawley rats following oral administration. The test material was administered in a single dose at 0.125 and 2.5 mg/kg or following repeated dosing with unlabeled material at 0.125 mg/kg/day for 14 days. Approximately 92 to 109 percent of the dose was recovered within 72 hours postdosing. Little if any radioactivity was eliminated as [^{14}C]CO_2. Approximately 63 to 79 percent of the dose was eliminated in the urine and 20 to 27 percent in the feces. Residue levels in tissues ranged from 3.3 to 4.9 percent of the dose. There were no major differences between sexes or dosing regimens, although a dose-dependent increase was noted in tissue residues of rats dosed with 0.125 and 2.5 mg/kg. The highest residues were found in blood, kidney, liver, and lung ($\leq$0.32 ppm). Approximately 75 percent of the total dose was identified. The metabolites in urine were cysteinylmethylbenzazimide, cysteinylmethylbenzazimide sulfoxide, cysteinylmethylbenzazimide sulfone, methylsulfinylmethylbenzazimide, methylsulfonylmethylbenzazimide, benzazimide, glutathionyl methylbenzazimide, and desmethyl isoazinphos-methyl. The cysteinyl methylbenzazimide sulfone and the methyl-sulfonylmethylbenzazimide were the major metabolites and accounted for 13 to 30 and 14 to 20 percent of the dose, respectively. Some minor sex-related differences were noted. Fecal metabolites were identified as the azinphos-methyl oxygen analog, cysteinylmethylbenzazimide sulfoxide, methylthiomethylbenzazimide, methylsulfonylmethylbenzazimide and desmethyl isoazinphos-methyl. No azinphos-methyl or glucuronic or sulfate conjugates were found in urine or feces. In vitro metabolism of azinphos-methyl also supports the in vivo data, indicating that the metabolism of azinphos-methyl in rats is largely attributed to glutathione-transferase (GSH transferases) and mixed function oxidases (MFO).

A metabolic pathway for the in vitro and in vivo metabolism of azinphos-methyl was proposed.

These studies are acceptable.

Items 8-10--see footnote 1.

[1]Only items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. [^{14}C]Azinphos-methyl was dissolved in 0.25 mL of Cremophor EL, and the solution was diluted with deionized water to 5 mL. About 0.5 mL of the dosing solution was orally administered to each rat.

2. Male and female Sprague-Dawley rats weighing 200 g each (age not specified) were obtained from SASCO, Inc., Omaha, NE. The animals were acclimated for 6 days prior to dosing.

3. The following experiments were conducted:

a. Detection of Expired [^{14}C]CO_2: Three rats/sex were orally dosed with 0.125 mg/kg of [^{14}C]azinphos-methyl and then placed individually in glass metabolism cages. Expired [^{14}C]CO_2 was trapped in 400 mL of 1 N NaOH for a period of 24 hours. Aliquots of the solution were then radioassayed by liquid scintillation counting (LSC).

b. Elimination and Metabolism of [^{14}C]Azinphos-methyl: Three experiments were conducted to determine the elimination and metabolism of [^{14}C]azinphos-methyl following oral administration of a single low dose (0.125 mg/kg), a single high dose (2.5 mg/kg), or a single dose (0.125 mg/kg) after the animals had received single daily doses of unlabeled test material at 0.125 mg/kg/day for 14 days (repeated dosing). Groups of five rats/sex were dosed with each of the three dosing regimens and the animals were then placed in plastic metabolism cages for 72 hours.

Urine and feces were collected separately at 8, 24, 48, and 72 hours postdosing. At the end of each experiment, the cages were rinsed and the animals were sacrificed following the collection of a blood sample. The following tissues were trimmed and weighed: bone, brain, fat, gonad, heart, kidney, liver, lung, muscle, spleen, and gastrointestinal tract. All samples were analyzed in triplicate. Urine and cage rinse were analyzed directly, whereas blood, feces, and tissues were combusted prior to radioassay by LSC.

Urine samples collected at 0 to 48 hours postdosing were combined and analyzed for metabolites directly by high-performance liquid chromatography (HPLC). Similarly, feces were combined and one third of the composite sample was sequentially sonicated for 30 minutes with 100 mL each of acetone:water (7:3), methanol:water (7:3) twice, and 0.6 N aqueous HCl. The extracts were combined and concentrated in vacuo. The concentrate was fractionated by column chromatography into organic and aqueous soluble fractions, which were then analyzed by HPLC and thin-layer chromatography (TLC). Aliquots of urine and fecal extracts were subjected to sulfatase and ß-glucuronidase hydrolysis. In addition, the aqueous fecal fraction was subjected to acid hydrolysis and the metabolites identified by HPLC and TLC.

c. In-vitro metabolism: [^{14}C]Azinphos-methyl was also incubated with various subcellular fractions of rat liver in the presence or absence of various cofactors, e.g., NADPH and glutathione (GSH), for 2 hours at 37°C. The incubations were then terminated and the supernatants were analyzed for metabolites by HPLC.

B. Protocol: A protocol was not included in this report.

12. REPORTED RESULTS:

A. Detection of Expired [^{14}C]CO_2: The results from this preliminary experiment indicated that expired [^{14}C]CO_2 accounted for less than 0.2 percent of the administered dose. Most of the radioactivity was eliminated in the urine and feces.

B. Elimination and Metabolism of [^{14}C]Azinphos-methyl:

1. Rats receiving the high dose (2.5 mg/kg) exhibited salivation, lacrimation, and minor tremors. The rats recovered, but excreted very little feces in the first 8 hours postdosing. No toxic signs were noted in rats receiving the low dose.

2. Following the administration of the test material in a single low or high dose or following repeated dosing, approximately 92 to 109 percent of the

dose was recovered within 72 hours postdosing (Table 1). Approximately 20, 70, and 90 percent of the dose was eliminated 8, 24, and 48 hours, respectively, after dosing. Of the radioactivity eliminated, 63 to 79 percent of the dose was in the ~~feces~~ urine and 20 to 27 percent in the ~~urine~~ feces. Residue levels in tissues ranged from 3.3 to 4.9 percent of the administered radioactive dose. There were no apparent differences between sexes. Total recovery was slightly lower in animals receiving the repeated dose when compared to the single low dose, whereas the percent radioactivity in tissues was slightly higher in animals receiving the high or repeated dose (Table 1). However, when residues in individual tissues were compared, a dose-dependent increase was noted (Table 2). The highest residues were found in blood, kidney, liver, and lung.

Analysis of urine revealed the presence of 12 metabolites; 8 of these were identified by cochromatography with authentic standards. The amounts of each metabolite expressed as percent of dose are shown in Table 3. The major metabolites found were cysteinylmethylbenzazimide sulfone and methylsulfonylmethylbenzazimide and accounted for 13 to 30 and 14 to 20 percent of the dose, respectively. There were some differences between sexes in the amounts of individual metabolites, e.g., the concentration of the two major derivatives were slightly higher in males than females. Whereas, the concentrations of methylsulfinylmethylbenzazimide and cysteinylmethylbenzazimide sulfoxide were higher in females than males. In addition, higher amounts of the sulfone derivative and lesser amounts of the methylsulfonylmethylbenzazimide were detected in animals receiving the high or repeated doses than animals receiving the single low dose. Enzyme hydrolysis did not alter the chromatographic pattern of the radioactivity in the urine.

Approximately 65 to 82 percent of the fecal radioactivity was extracted and accounted for 16 to 25 percent of the dose in the six groups (Table 4). Of the extracted radioactivity, 1 to 2 percent was water soluble and 12 to 18 was soluble in organic solvents. The radioactivity in the water soluble fraction was not identified; however, it was not hydrolyzed by sulfatase or glucuronidase. Seven metabolites were determined in the organic fraction from feces of animals receiving the high dose, five of which were identified (Table 5).

Azinphos-methyl RIN: 7365-92

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_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

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_____ The product confidential statement of formula.

_____ Information about a pending registration action.

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3. In-vitro metabolism: Incubation of [^{14}C]azinphos-methyl with various subcellular preparations and cofactors resulted in rapid formation of several metabolites. The percentages of each of four metabolites formed and unchanged parent compound are listed in Table 6. A metabolic pathway for the in vitro metabolism of [^{14}C]azinphos-methyl was proposed.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Azinphos-methyl is rapidly absorbed and metabolized in Sprague-Dawley rats. More than 95 percent of the [^{14}C]azinphos-methyl-derived radioactivity was excreted in urine and feces 72 hours posttreatment. About 4 percent of the dose remained in the tissues and less than 1 percent of the dose was expired as [^{14}C]CO_2. The radioactive residue in highly perfused tissues was higher than other tissues. There was no difference in disposition and metabolism of azinphos-methyl between sexes of rats.

The in vivo and the in vitro metabolism studies revealed that azinphos-methyl is mainly metabolized by GSH-transferases and mixed function oxidases (MFO). Based on the results of this study, the metabolic pathway of azinphos-methyl in rats is proposed in Figure 1. Upon absorption, azinphos-methyl is rapidly metabolized by mixed function oxidases and GSH-transferases in the liver and other tissues, which results in the formation of azirphos-methyl oxygen analog, mercaptomethylbenzazimide, glutathionyl methylbenzazimide, and desmethyl isoazinphos-methyl. Further hydrolysis, methylation, and oxidation of mercaptomethylbenzazimide forms benzazimide, methylthiomethylbenzazimide, and its corresponding oxidized metabolites. Hydrolysis of glutathionyl methylbenzazimide may result in the formation of cysteinylmethylbenzazimide. Subsequent oxidation of cysteinylmethylbenzazimide forms its corresponding sulfoxide and sulfone. About 75 percent of the total dose was identified. The unidentified metabolites accounted for about 20 percent of the total dose; but none of the individual metabolites constituted more than 5 percent of the total dose.

B. A quality assurance statement was signed but not dated.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. The materials and methods used were adequate and conformed with EPA guidelines. The results suggest that the high dose selected was adequate since some toxic effects were

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noted. The reported LD_{50} for this chemical in rats is 10 mg/kg. At the doses tested (0.125 or 2.5 mg/kg), no apparent differences were noted in the metabolism or excretion of azinphos-methyl, although a dose-dependent increase in tissue residues was detected. The compound apparently does not bioaccumulate and does not induce hepatic enzymes following repeated dosing. A deficiency was noted in reporting the disposition data; all the results were normalized to 100 percent recovery. The data should have been reported as determined with no corrections (see Table 1). These studies are acceptable. The results from an intravenous study would have been helpful to determine biliary excretion. However, in view of the fact that 62.5 to 79 percent of the dose is absorbed and eliminated in the urine, the intravenous study is not necessary.

Items 15 and 16--see footnote 1.