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

007037

FEB 17 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Azodrin (Monocrotophos) - Submission of a Metabolism Study With a Request for a Data Waiver of the Low Dose Requirements of the Study (EPA Registration No. 352-249)

TOX Chem No.: 377
Project No.: 8-0437
Record No.: 212636

FROM: William B. Greear, M.P.H. William B. Greear 2/13/89
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THRU: Marion P. Copley, D.V.M., Section Head Marion P. Copley 2/15/89
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Toxicology Branch I - Insecticide, Rodenticide Support
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Conclusions

The metabolism study is acceptable and fulfills the requirements for a general metabolism study. The waiver requests for the low-dose metabolism studies are acceptable.

1 of 30

Background

Under a cover letter dated January 7, 1988, D.M. Stanley of E.I. du Pont de Nemours & Company, Inc. submitted a metabolism study in compliance with the Registration Standard on Azodrin.

This single high-dose metabolism study is acceptable (the Data Evaluation Report is attached). The sponsor has requested a waiver of the low-dose studies. These studies are: 1) a single low-dose study; 2) an intravenous dose study; and 3) multiple low-dose study. The sponsor states that technical difficulties arose when trying to qualify and quantitate the small amount of residues in the excreta and tissues. If the low-dose was selected at 10 percent of the high-dose, the amount administered would be less than 40 ug per animal. In addition, the primary reviewer stated that "If the dose level is too low it may pose difficulties in monitoring radioactivity and analyzing metabolites." The Dynamac reviewer concluded that the use "of lower doses would probably provide similar results since neither dose is high enough to cause a saturation effect."

Based on the rationale provided by the sponsor and the primary reviewer, it is the opinion of TB-I that the data be waived.

Attachment

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EPA: 68D80056
DYNAMAC No. 135-A
December 16, 1988

# DATA EVALUATION RECORD

## AZODRIN

## Metabolism Study in Rats

STUDY IDENTIFICATION: Lee, P. W. Rat metabolism study of $^{14}$C-DPX-Y2034. (Unpublished study Nos. AMR-653-87 and RTI-3852, prepared by E. I. du Pont de Nemours and Company, Wilmington, DE, and Research Triangle Institute, Research Triangle Park, NC; dated December 22, 1987.) MRID No. 404712-09.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 12-16-88



3

1. CHEMICAL: Azodrin; DPX-Y2034; monocrotophos; SD 9129; crotonamide, 3-hydroxy-N-methyldimethylphosphate.

2. TEST MATERIAL: [$^{14}$C]DPX-Y2034 was from batch No. E-48043-32 and had a specific activity of 23.7 µCi/mg. It was purified by high-pressure liquid chromatography (HPLC) to a chemical purity of 98.4 percent. Unlabeled DPX-Y2034 was from batch No. 14-1-0-0 and was > 97 percent pure.

3. STUDY/ACTION TYPE: Metabolism in rats.

4. STUDY IDENTIFICATION: Lee, P. W. Rat metabolism study of $^{14}$C-DPX-Y2034. (Unpublished study Nos. AMR-653-87 and RII-3852, prepared by E. I. du Pont de Nemours and Company, Wilmington, DE, and Research Triangle Institute, Research Triangle Park, NC; dated December 22, 1987.) MRID No. 404712-09.

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7. CONCLUSIONS: The metabolism of [$^{14}$C]DPX-Y2034 was studied in male and female Wistar rats following oral administration at 2 mg/kg (approximately 1/10 the $LD_{50}$). The rats showed signs of organophosphate poisoning, but none died. No apparent sex-related differences were noted in the elimination and metabolism of [$^{14}$C]DPX-Y2034. Approximately 82 percent of the dose was eliminated in the urine, 6 percent as radiolabeled carbon dioxide ([$^{14}$C]$CO_2$), and 3.5 percent in the feces within 4 days postdosing. Most of the radioactivity was eliminated within 12 hours postdosing, indicating rapid absorption and elimination. Residues in tissues accounted for less than 1 percent of the dose 96 hours postdosing. The highest residues were found in fat ($\leq$0.09 µg/g) and liver ($\leq$ 0.052 µg/g). Approximately 34 and 41 percent of the dose in males and females, respectively, were detected in urine as the unchanged parent compound; 3-hydroxy-N-methylbutyramide was also detected in urine. N-Methyl-acetoacetamide accounted for 16 to 18 percent of the dose and was present only as a conjugate, as indicated by enzyme and acid hydrolysis. None of the fecal metabolites was identified.

This study is acceptable. Although EPA guidelines require metabolic studies following administration of a low dose (i.e., no effect) and repeated dosing (14 days), the use of lower doses will most probably provide similar results since neither dose is high enough to cause a saturation effect. If the dose is too low, it may pose difficulties in monitoring radioactivity and analyzing metabolites. However, a repeated dosing study may be more appropriately conducted at 2 mg/kg to determine whether the compound causes induction of hepatic microsomes or bioaccumulation, if any.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. [$^{14}$C]DPX-Y2034 was mixed with unlabeled material and dissolved in water. The test material was administered to individual animals in a single dose of 2 mg/kg.

2. Male and female Wistar rats each weighing 117 to 169 g were obtained from Charles River Laboratories, Kingston, New York. Animals were acclimated to laboratory conditions for 1 week prior to dosing. Diet and water were provided ad libitum.

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

3. [$^{14}$C]DPX-Y2034 was administered to seven animals/sex. All dosed animals were individually housed in glass metabolism cages, which allowed for separate collection of urine, feces, and expired $CO_2$. Urine, feces, and expired $CO_2$ were collected 12 hours after dosing and daily thereafter. Animals were sacrificed 4 days after dosing with $CO_2$. A 3-mL sample of whole blood and 13 tissues were collected from individual animals (five/sex) at sacrifice.

   Radioactivity in 0.2- to 0.5-g urine samples was assayed directly by liquid scintillation counting (LSC). Fecal samples were homogenized in water and combusted prior to radioassay. Triplicate samples of all tissues (about 100 mg) were weighed and combusted prior to radioassay. The normal background level and combustion efficiency was determined from tissues of one male and one female control rat. Radioassay procedures for the sodium hydroxide/$CO_2$ traps were not reported.

4. The 12-hour urine samples were used for the quantitative and qualitative analyses of metabolites. The pH of the urine samples was adjusted to pH 5 with 10 percent HCl and extracted 3 times with two volumes of chloroform. The organic extract was dried over anhydrous sodium sulfate, concentrated and analyzed by two-dimensional thin-layer chromatography (TLC). The aqueous phase was incubated with a glucuronidase/sulfatase enzyme preparation at 37°C for 25 hours. The incubation mixture was extracted with two volumes of chloroform and the extract was analyzed by two-dimensional TLC as described above. The aqueous phase was then subjected to acid hydrolysis and extracted with chloroform, and the extract was analyzed by two-dimensional TLC. The final aqueous phase was adjusted to pH 11 with 50 percent NaOH and hydrolyzed. This sample was extracted with chloroform.

   Feces collected 12 to 48 hours after dosing were combined for all animals and extracted two times with 0.1 M sodium acetate buffer (pH 5) at a ratio of 1:5(w/v). The solution was centrifuged and the precipitate further extracted with methanol. The extracts were extracted 3 times with two volumes of chloroform and analyzed by two-dimensional TLC. The solid fecal materials after the methanol extractions were further subjected to a continuous Soxhlet extraction with chloroform for about 16 hours. The extracts were radioassayed by LSC.

5. Urinary and fecal metabolites were analyzed by TLC using the following solvent systems: dichloromethane-acetone-acetic acid (60:40:5, v/v/v) and acetonitrile-water-concentrated ammonium hydroxide (40:9:1, v/v/v). Samples were cochromatographed with available standards and radioactive spots were visualized by autoradiography on x-ray film. The standards were visualized with iodine vapor. The urinary extracts were also analyzed by HPLC. The major radiolabeled metabolites were subjected to further spectrometric analysis.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Toxicological signs of organophosphate poisoning were evident approximately 30 minutes after dosing. None of the animals died and the signs abated after about 3 hours.

B. Following oral administration of [$^{14}$C]DPX-Y2034, most of the radioactivity was eliminated during the first 12 hours. Approximately 77 percent of the dose was eliminated in the urine, 5 percent as [$^{14}$C]$CO_2$, and 1.5 percent in the feces. No apparent sex-related differences were noted. Greater than 90 percent of the radioactivity was eliminated from the animals 96 hours after dosing (Table 1).

C. The combined residue levels in 13 tissues from either male or female rats accounted for less than 1 percent of the dose. The highest residues were detected in adipose tissues and accounted for 0.08 and 0.06 ppm in females and males, respectively. There were no apparent sex differences (Table 2).

D. Approximately 34 to 40 percent of the administered radioactivity was recovered in the initial chloroform extract of urine from dosed animals. An additional 5 to 7 percent was extractable after enzymatic hydrolysis, 13 to 15 percent after acid hydrolysis, and 1 to 2 percent after base hydrolysis. About 17 to 20 percent was unextractable (Table 3). There were no apparent sex-related differences in the distribution of urinary radioactivity or metabolites identified.

Table 1

Summary of Cumulative Elimination Rate of $^{14}$C-DPX-Y2034 Equivalent Radioactivity in the Excreta of Male and Female Test Animals

| Hour | Percent of Administered Radioactivity[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | | | Female | | | |
| | Urine | Feces | Breath | Total | Urine | Feces | Breath | Total |
| 12 | 76.7 ± 4.3[b] | 1.8 ± 0.9 | 5.2 ± 0.8 | 83.8 | 77.0 ± 2.8 | 1.4 ± 0.8 | 4.7 ± 0.6 | 83.0 |
| 24 | 80.2 ± 4.0 | 3.0 ± 1.2 | 5.7 ± 0.9 | 88.9 | 81.5 ± 1.8 | 2.4 ± 1.4 | 5.3 ± 0.6 | 89.2 |
| 48 | 81.1 ± 3.6 | 3.4 ± 1.4 | 6.1 ± 0.9 | 90.5 | 82.4 ± 1.9 | 2.7 ± 1.5 | 5.6 ± 0.6 | 90.7 |
| 72 | 81.6 ± 3.3 | 3.5 ± 1.4 | 6.2 ± 0.9 | 91.3 | 82.7 ± 2.0 | 2.9 ± 1.7 | 5.8 ± 0.6 | 91.4 |
| 96 | 81.7 ± 3.3 | 3.6 ± 1.5 | 6.3 ± 0.9 | 91.6 | 82.8 ± 2.0 | 3.1 ± 2.0 | 5.8 ± 0.6 | 91.7 |

[a] All values are calculated from DPM data, then rounded to least significant figure; columns may not add directly to yield values for totals.
[b] Mean ± standard deviation (number of animals = 5).

Source: CBI Table 3, CBI p. 59.

TABLE 2. Tissue [$^{14}$C] Residues (µg/kg tissue) Found in Male and Female Rats Following Oral Administration of [$^{14}$C]DPX-Y2034 at 2 mg/kg

| Tissue | [$^{14}$C] Residues[a] | |
|---|---|---|
| | Female | Male |
| Adipose, K[b] | 0.065 ± 0.033 | 0.089 ± 0.045 |
| Adipose, M[b] | 0.057 ± 0.023 | 0.077 ± 0.032 |
| Adipose, R[b] | 0.058 ± 0.032 | 0.072 ± 0.038 |
| Blood | 0.010 ± 0.001 | 0.011 ± 0.001 |
| Bone | 0.001 ± 0.001 | 0.001 ± 0.001 |
| Brain | 0.010 ± 0.001 | 0.011 ± 0.002 |
| Gonads | 0.045 ± 0.018 | 0.015 ± 0.005 |
| Heart | 0.014 ± 0.001 | 0.012 ± 0.003 |
| Kidney | 0.030 ± 0.003 | 0.026 ± 0.004 |
| Liver | 0.039 ± 0.004 | 0.052 ± 0.009 |
| Lung | 0.024 ± 0.002 | 0.020 ± 0.006 |
| Muscle | 0.011 ± 0.001 | 0.010 ± 0.002 |
| Skin | 0.046 ± 0.012 | 0.039 ± 0.010 |
| Spleen | 0.022 ± 0.003 | 0.019 ± 0.003 |

[a]Mean ± standard deviation.

[b]K = kidney; M = mesenteric tissues; R = reproductive tissues.

TABLE 3. Distribution of Radioactivity in Urine of Male and Female Rats Following Oral Administration of [$^{14}$C]-DPX-Y2034 at 2 mg/kg

| Fraction | Percent of [$^{14}$C] administered[a] | |
|---|---|---|
| | Female | Male |
| Total [$^{14}$C] in urine | 78.6 ± 8.9 | 76.7 ± 4.3 |
| Organic extractable | 40.6 ± 2.6 | 34.0 ± 5.4 |
| Water soluble | 38.0 ± 4.5 | 42.6 ± 5.8 |
| Unextractable | 17.1 ± 3.7 | 19.9 ± 4.4 |
| Enzyme hydrolysis | 5.2 ± 0.8 | 6.7 ± 2.1 |
| Acid hydrolysis | 13.3 ± 1.6 | 14.6 ± 3.5 |
| Base hydrolysis | 1.2 | 1.4 ± 0.5 |

[a] Mean ± standard deviation.

The initial chloroform extract of urine was found to contain the parent compound, which accounted for 26 percent of the dose in males and 33 percent in females. A minor metabolite (SD 11734) was identified by TLC and HPLC as 3-hydroxy-N-methylbutyramide (Table 4). Following glucuronide/sulfatase incubation of the aqueous phase, N-methylacetoacetamide (SD 9112) was identified and accounted for 3 to 4 percent of the dose. In addition, 3-hydroxy-N-methylbutyramide and other minor metabolites were detected (Table 4). Following acid hydrolysis, approximately 13 to 14 percent of the dose was identified as N-methylacetoacetamide.

Approximately 80 percent of the fecal radioactivity was extracted by the acetate buffer. Of that radioactivity, 15 percent was extracted by chloroform (Table 5), but further characterization by TLC was not possible. About 10 percent of the fecal radioactivity was extracted following acid hydrolysis. The remaining fractions contained very low amounts of radioactivity (Table 5).

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

A. The elimination of the administered radioactive dose was rapid via urinary (~77 percent), fecal (~2 percent), and respiratory (~5 percent as [$^{14}$C]$CO_2$) excretion during the initial 12-hour post dosing. Greater than 90 percent of the administered radioactivity was eliminated after 96 hours. A statistical difference in the amount of urinary and fecal elimination between the male and female animals was not observed.

Approximately 26 to 33 percent of the administered [$^{14}$C]DPX-Y2034 was recovered intact in the urinary samples from the first 12 hours postdosing, further supporting the conclusion that DPX-Y2034 is absorbed rapidly after dosing and immediately eliminated via renal clearance. In addition to DPX-Y2034, SD 9112 and SD 11734 were derived from the crotonamide moiety, which resulted from the ester cleavage of DPX-Y2034--a detoxification reaction. They were recovered in both the organic-extractable fraction and as conjugates recovered from the aqueous phase after enzyme, acid, and base treatments. The metabolic pathway of DPX-Y2034 in the rat is proposed in Figure 1 and it is consistent with its metabolic pathways in goat, plants, and soil.

TABLE 4. Distribution of Radioactivity in Chloroform Extracts of Urine from Male and Female Rats Administered [$^{14}$C]DPX-Y2034 at 2 mg/kg Prior To and Following Enzyme and Acid Hydrolysis

| Chloroform Fraction | Sex | [$^{14}$C] Compound, % of Administered Dose | | | | |
|---|---|---|---|---|---|---|
| | | DPX-Y2034 | SD 11734[a] | SD 9112[b] | Unidentified[c] | Total |
| Initial extraction | M | 26.0 ± 5.0 | 7.0 ± 2.0 | -- | 1.0 ± 0.7 | 34.0 |
| | F | 33.0 ± 2.0 | 7.0 ± 1.0 | -- | 1.0 ± 0.8 | 41.0 |
| After enzyme hydrolysis | M | -- | 0.7 ± 0.2 | 4.0 ± 1.0 | 2.0 ± 1.0 | 6.7 |
| | F | -- | 1.0 ± 0.9 | 3.0 ± 1.0 | 1.0 ± 0.9 | 5.0 |
| After acid hydrolysis | M | -- | -- | 14.0±4.0 | 1.0 ± 0 | 15.0 |
| | F | -- | -- | 13.0±2.0 | 0.8 ± 0.4 | 13.8 |

[a] 3-Hydroxy-N-methylbutyramide.

[b] N-Methylacetoacetamide.

[c] Includes all minor metabolites.

TABLE 5. Distribution of Radioactivity in Feces of Male and Female Rats Following Oral Administration of [$^{14}$C]DPX-Y2034 at 2 mg/kg

| Fraction | Percent of [$^{14}$C] Administered[a] | |
|---|---|---|
| | Female | Male |
| Total [$^{14}$C] in feces | 3.07 ± 1.96 | 3.57 ± 1.47 |
| Buffer extractable | 2.50 ± 1.40 | 2.78 ± 1.27 |
| Organic extraction | 0.68 ± 0.78 | 0.43 ± 0.31 |
| Enzyme | 0.09 ± 0.06 | 0.07 ± 0.04 |
| Acid | 0.26 ± 0.18 | 0.32 ± 0.12 |
| Base | 0.04 ± 0.02 | 0.04 ± 0.01 |
| Unextractable | 1.43 ± 0.41 | 1.93 ± 0.92 |
| Methanol extract of pellet | 1.09 ± 0.03 | 0.96 ± 0.02 |
| Solid pellet[b] | 0.48 ± 0.59 | 0.69 ± 0.24 |

[a]Mean ± standard deviation.
[b]Measured by difference.

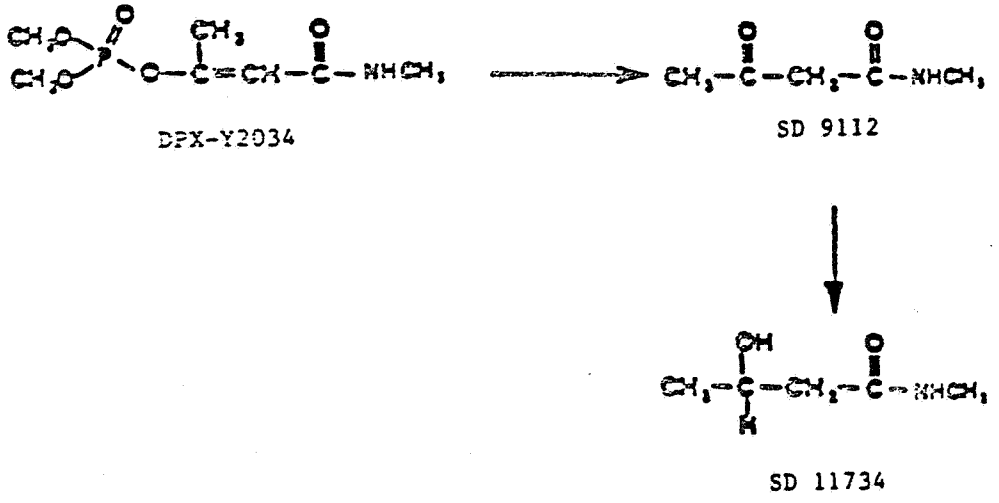


Figure 1. Proposed Metabolic Pathway of DPX-Y2034 in Rats Following Oral Administration

Source: CBI Figure 13, CBI p. 79

Tissue residue distribution data indicated the lack of bioconcentration of [$^{14}$C]DPX-Y2034 equivalent residues in the blood, lung, heart, gonad, kidney, muscle, brain, bone, and spleen tissues of the test animals. Significant level of [$^{14}$C]DPX-Y2034 equivalent residues (max., 0.08 ppm) was detected in the adipose and liver tissues.

B. A quality assurance statement was signed and dated December 3, 1987.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The results of this study indicate that [$^{14}$C]DPX-Y2034 is readily absorbed and that a large proportion of it is eliminated in the urine unchanged. The conclusions of the author are supported by the results presented, with one exception. It appears that the purity of one metabolite standard (SD 11734) was inadequate for proper identification by TLC, although identification was apparently accomplished by HPLC. Dose selection and the use of the extra animals were appropriate. Cholinesterase inhibition was observed at the dose selected (10 percent of the $LD_{50}$); a higher dose may have resulted in death or interfered with the metabolism of the test material. Elimination data were similar for all animals. The methods used were adequate. This study is acceptable.

Although the rationale and justifications submitted by the registrant in support of data waiver for the single oral low-dose, multiple oral low-dose, and the intravenous low-dose studies can be argued to various extents, from a metabolic point of view, the 2-mg/kg dose used in the "high-dose" study is not by any means approaching saturation kinetics, rather it is based on the demonstration of cholinesterase inhibition. Consequently, the use of 0.2 mg/kg will most likely lead to the same results and conclusions. However, studies conducted with lower doses (e.g., $\leq$ 0.02 mg/kg) will be quite difficult to monitor and obtain meaningful results. Similarly, a repeated design study (14 days) may not be feasible at the lower doses for the same reasons. However, a study could most likely be done adequately at 2 mg/kg to determine if bioaccumulation and/or microsomal induction, if any, may occur.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol, CBI pp. 67 to 81.

AZODRIN

Page_____ is not included in this copy.

Pages 16 through 30 are not included.

The material not included contains the following type of information:

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