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WASHINGTON, D.C. 20460

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APR 16 1993

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Picloram, Triisopropanolamine Salt Metabolism Study in Male F344 Rats (A Special Study)

Tox Chem No.: 039
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PC code: 005102

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Brian Dementi 2/1/93

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THRU: Karen Hamernik, Ph.D.
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K. Hamernik 4/13/93 HB 4/14/93

The Data Evaluation Review for the Picloram, triisopropanolamine salt metabolism study, MRID # 42343101, submitted by DowElanco toward satisfying reregistration GL85-1 data requirements, is herewith submitted to SRRD.

The essential finding in this study was that whether Picloram is administered as the free acid or as the Picloram triisopropanolamine (TIPA) salt, the metabolism of the Picloram moiety is the same in either case. In other words, the presence of TIPA in salt linkage with Picloram does not alter the metabolism of the latter. The reason for this lack of effect of TIPA on metabolism is that TIPA quickly dissociates from Picloram in vivo and under such circumstances exercises little or no influence upon its metabolism.

The study is rated core supplementary and in and of itself does not satisfy Guideline 85-1.

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FINAL

DATA EVALUATION REPORT

Picloram, Triisopropanolamine Salt

Study Type: Metabolism

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

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Contract Number: 68D10075
Work Assignment Number: 2-13
Clement Number: 44
Project Officer: James Scott

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GUIDELINE SERIES 85-1: Metabolism

EPA Reviewer: Paul Chin, Ph.D.
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Date: 1/25/93

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DATA EVALUATION REPORT

STUDY TYPE: Metabolism in rats

EPA IDENTIFICATION NUMBERS:

Tox. Chem. Number: 39

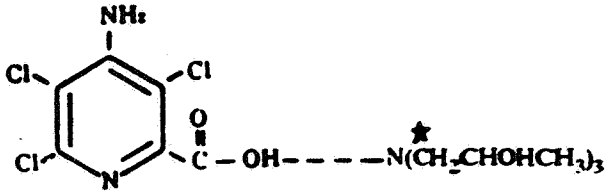
P.C. Code: 005101

MRID Number: 423431-01

TEST MATERIAL: Picloram, triisopropanolamine (TIPA) salt (AGR# 0276453)

SYNONYM: Picloram-TIPA; 4-amino-3,5,6-trichloropicolinic acid-TIPA

STRUCTURE:



picloram TIPA * denotes the position of the ^{14}C label

COLOR: Not reported

SPONSOR: DowElanco, 9002 Purdue Road, Indianapolis, IN 46268-1189

TESTING FACILITY: The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI 48674

AUTHORS: J.Y. Domoradzki, M.F. Hiser, G.A. Bormett, and R.J. Nolan

REPORT: Picloram, Triisopropanolamine Salt: Dissociation and metabolism in male Fischer-344 rats. Laboratory Study No. K-049877-013.

STUDY COMPLETION DATE: May 26, 1992

CONCLUSIONS: The objective of this study was to determine whether the fate of picloram is influenced by the co-administration of picloram with TIPA. The absorption, metabolism, and excretion of triisopropanolamine-1-^{14}C (^{14}C-TIPA)

were studied in male rats after administration of single oral doses of 9.5 mg ^{14}C-TIPA/kg and 9.8 mg picloram/kg. ^{14}C-TIPA was readily absorbed, with peak plasma radioactivity at 0.25 hours postdosing. The administered dose of radioactivity was recovered primarily in urine and feces (76% and 9%, respectively). The mean total radioactivity recovered in urine, feces, expired $^{14}CO_2$, tissues/carcass, and final cage rinse was 94%.

The 0-12-hour pooled urine was analyzed for metabolites; unchanged TIPA accounted for 80% of the radioactivity excreted in the urine. No other metabolites were identified in the 0-6-hour pooled urine samples. No treatment-related clinical signs of toxicity were observed in male rats. The data suggest that the conversion of picloram-TIPA salt to picloram was not affected by the presence of TIPA.

STUDY CLASSIFICATION: Supplementary. The objective of this metabolism study of picloram-^{14}C-TIPA salt was limited to examining whether the conversion of picloram-salt to picloram in rats was affected by the presence of TIPA. This study adequately demonstrated that the co-administration of TIPA and picloram did not affect the fate of picloram. Study deficiencies included lack of protocol, insufficient numbers of animals, use of a single sex (male) and dose.

A. MATERIALS

1. Test Substance

The unlabeled liquid test material (Picloram-TIPA salt, DowElanco, AGR #0276453, specific activity 31.9 mCi/mmol) was assayed by nuclear magnetic resonance (NMR) and found to contain 31.6% H_2O, 37.7% picloram, and 33.5% TIPA (Redwine, 1991). The molar ratio of amine to acid was 1.12±0.05. ^{14}C-TIPA was diluted with equal parts of hexane/benzene solvents before purity analysis was performed. Radiochemical purity of ^{14}C-TIPA was 97.5% as determined by gas chromatography (GC)/radiogas.

2. Test Animals

Male Fischer-344 rats (10 weeks old) were obtained from Charles River Laboratories, Kingston, NY. Animal weights ranged from 171 g. to 175 g. at the first dosing. According to the study authors, only male rats were tested because no major sex-related differences in the pharmacokinetics or metabolism of picloram or in the toxicity of TIPA were known.

B. METHODS

1. Acclimation

Animals were acclimatized for at least 7 days prior to administration of the test material. An indwelling jugular vein cannula was implanted under methoxyflurane anesthesia in each rat 1 day prior to dosing. All animals were acclimated for 1 day prior to surgery in glass Roth-type metabolism cages, and recuperated in the cages for 24 hours before

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dosing. Animal rooms were kept on a 12-hour photocycle. Temperature and humidity data were not provided. Animals were provided a diet of Rodent Chow® 5002 (Purina Mills, Inc., St. Louis, MO) and tap water ad libitum throughout the study, except for a period of fasting (17 hours prior to dosing until approximately 4 hours postdosing). Feed and water were reportedly analyzed for contaminants, however the results were not provided.

2. Dosing Solutions

A measured volume of ^{14}C-TIPA which had previously been diluted with hexane/benzene solvent was evaporated to remove the solvent. A measured volume of non-labeled picloram-TIPA was added and diluted to final volume with distilled water. The dose solution contained 4.75 mg TIPA/mL, 5 mg picloram/mL, and 75 uCi ^{14}C/mL. Animals were to receive 2 ml/kg of the dose solution, resulting in a target dose of 9.5 mg TIPA/kg, 10 mg picloram/kg, and 20-30 µCi per animal. The dose solution was quantified using a liquid scintillation counter (LSC), and found to contain 0.431 mg ^{14}C-TIPA/mL. Using high-performance liquid chromatography (HPLC) with ultraviolet detection, the dose solution was also found to contain 5.01 mg picloram/mL. The total concentration of TIPA (labeled and unlabeled) in the dose solution was calculated to be 4.88 mg/mL. The specific activity of TIPA in the dose solution was 32,702 dpm/µg TIPA. The actual mean dose administered to rats was 9.8 mg picloram/kg and 9.5 mg TIPA/kg. The targeted dose of 10 mg picloram/kg was chosen because it is equal to the dose used in previous metabolism studies, and 9.5 mg TIPA/kg was chosen because it is well below the LD_{50} of ≈6000 mg/kg for TIPA.

A group of 4 male rats was given a single oral dose (gavage) using a glass syringe and stainless steel feeding needle. The syringe was weighed before and after dosing to quantify the dose administered. All animals were euthanitized with CO_2 and exsanguinated at 72 hours post-dosing.

3. Sample Collection

Blood samples (0.2 ml) were drawn from each jugular cannula at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 18, 24, 48 and 72 hours postdosing. Radioactivity in expired $^{14}CO_2$ was measured using traps of mono-ethanolamine:1-methoxy-2-propanol (3:7) while expired organic ^{14}C was measured using charcoal traps. The $^{14}CO_2$ traps were analyzed at 12, 24, 36, 48 and 72 hours postdosing. Organic ^{14}C traps showed insufficient radioactivity to quantify after 12 hours, so charcoal-trap collection was terminated. Urine was collected over dry ice at 6, 12, 24, 48 and 72 hours postdosing. Metabolism cages were rinsed with distilled water following collection. Cage washings and urine were combined for each interval to give the amount of radioactivity excreted in the urine. Feces were collected over dry ice at 24-hour intervals, homogenized in water, and prepared for scintillation. Glacial acetic acid was added to minimize chemical luminescence. Liver, kidneys, perirenal fat, skin and carcass were analyzed for radioactivity.

Radioactivity in blood, urine, feces, expired CO_2, and tissues was analyzed using a Beckman LS liquid scintillation counter (Beckman Instruments, Fullerton, CA). Counts/minute were corrected for background and quench (H# technique) and converted to disintegrations/minute (dpm). Methods for statistical analysis were limited to means and standard deviations. Aliquots of the 0-6- and 6-12-hour pooled urine samples were analyzed for picloram and ^{14}C-TIPA using a Hewlett Packard Model 5890 GC/5970 MSD system (Hewlett Packard, Palo Alto, CA).

4. Metabolite Analysis

The 0-6- and 6-12-hour urine samples were pooled by mixing 0.5 ml urine samples from each animal, and stored frozen (-80°C) until analysis. Analyses for metabolites of ^{14}C-TIPA in urine were performed by GC and mass spectroscopy (MS) using a Finnigan® Model 9611 GC/4615 MS system (Finnigan MAT, San Jose, CA) equipped with a Radiomatic FLO-ONE/BETA® Model GCR radioactivity-GC-detector (Radiomatic Instruments and Chemical Company, Inc., Meriden, CT). Fecal and blood samples were not analyzed for metabolites.

5. Protocol

The study protocol was not provided.

C. REPORTED RESULTS

1. Elimination and Recovery

A summary of the distribution of recovered radioactivity is presented in Table 1. The mean total recovery of radioactivity was 93.7% of the administered dose. After 72 hours postdosing, the mean radioactivity recovered in the urine was 77.7% of the administered dose, of which 73.6% was recovered during the first 12 hours postdosing. There were considerable variations among animals particularly during the 6-12-hour interval postdosing. Mean fecal recovery was 9.1%, but there was a large standard deviation (9.23) among animals in all collection intervals. Recovery of expired $^{14}CO_2$ occurred primarily during the first 12 hours postdosing, and the 3-day recovery was 4.09% of the administered dose. Mean final cage wash accounted for 2.06% of the administered dose, while recovery in the tissue and carcass was 0.73% of the administered dose.

2. Tissue Distribution

After 72 hours postdosing, the tissues with the highest percentage of radioactivity were the carcass (0.36%), skin (0.21%), and liver (0.15%). Kidney and perirenal fat combined accounted for only 0.011% of the administered dose. Mean concentration of radioactivity (% dose/g tissue) was almost seven times greater in liver (0.0200) and 2.6 times greater in kidney (0.0075) than in carcass (0.0029).

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3. Pharmacokinetics

The radioactivity in the blood peaked at 0.25 hour postdosing in all animals, with a mean concentration of 4.72 µg eq ^{14}C-TIPA/g plasma. The concentration of radioactivity in the plasma decreased triexponentially, according to the authors. Half-lives were estimated to be 0.96, 4.3, and 28.9 hours for the initial, middle and terminal phases of the study, respectively; area under the curve (AUC), volume of distribution (V_d), and whole body and renal clearance were calculated as 18.33 hr.• µg equiv ^{14}C-TIPA/g plasma, 2.1 L/kg body weight, and 8.64 and 6.71 mL/min./kg body weight, respectively (see Appendix).

4. Metabolism

MS and GC analyses of pooled urine samples were compared to TIPA standards, with characteristic peaks and retention times found to be almost identical. No metabolites appear to have been excreted in the 0-6-hour urine. Unchanged TIPA accounted for ≈80% of the radioactivity in the 0-12-hour pooled urine. Metabolite analysis was not performed in the feces. The presence of expired ^{14}C-CO_2 indicates that some metabolism of ^{14}C-TIPA occurred. However, the $^{14}CO_2$ could have arisen from impurities in the ^{14}C-TIPA.

D. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES

^{14}C-TIPA was rapidly absorbed following the administration of single oral doses of 10 mg picloram/kg and 9.5 mg ^{14}C-TIPA/kg as demonstrated by peak blood plasma concentration occurring at 0.25 hour postdosing. The majority of the radioactivity was excreted in the urine within the first 24 hours after dosing, with lesser amounts being eliminated in feces and expired $^{14}CO_2$. This rapid elimination suggests that ^{14}C-TIPA does not bioaccumulate in the tissues of the rat. The authors suggest that the fate of picloram was unaffected by TIPA administration, since picloram was excreted in a similar pattern to those found in a previous study (Nolan et al; 1980). They further asserted that the metabolic fate of TIPA appeared to be unaffected by co-administration with picloram since the metabolism of ^{14}C-TIPA in this study was similar to that of a structural analog ^{14}C-diisopropanolamine (Frantz et al; 1986). However, there were no data provided to support this suggestion or the suggestion that TIPA and its analog would necessarily follow similar metabolic pathways.

Quality assurance statements and statements of compliance with Good Laboratory Practices for the study were signed on May 20, 1992, and on May 13, 25, 26, 1992, respectively. A statement of no confidentiality claims was signed May 13, 1992.

E. CONCLUSIONS BASED ON THE REVIEWER'S DISCUSSION AND INTERPRETATION OF DATA

Since only male rats and only one dose level were tested in this study, it was not possible to evaluate sex- or dose-related differences following oral administration of TIPA in rats. ^{14}C-TIPA was rapidly absorbed after oral administration, as evidenced by peak plasma

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concentration (4.72 µg eq TIPA/g plasma) at 0.25 hours postdosing. Extensive absorption of ^{14}C-TIPA was indicated by the high levels of radioactivity (83% of the administered dose) recovered in urine at 72 hours postdosing. The data appear to indicate that there is a biphasic elimination in which radioactivity is eliminated rapidly from the blood (0.25-8.0 hours postdosing) followed by a slower decline (8-72 hours), however, the authors felt that the elimination data best fit a tri-exponential equation. ^{14}C-TIPA was rapidly eliminated in urine, with 74% of the administered dose excreted within the first 12 hours postdosing. The majority of the radioactivity recovered in the feces and as expired $^{14}CO_2$ was also eliminated during the first 24 hours. Overall, the recovery of radioactivity in the feces was low, however, one of the four rats had ≈23% of the administered dose in the feces compared to ≈5% in the other 3 rats. A corresponding decrease in radioactivity in the urine was also observed in this rat.

The study indicates that TIPA and/or its metabolites do not bio-accumulate following oral exposure. Analysis of 0-12 hour pooled urine samples indicate that most of the TIPA dose was eliminated unchanged, with no apparent metabolism taking place. However, urine samples >12 hours postdosing and fecal samples were not analyzed for the presence of metabolites. Metabolite analysis revealed that ≈80% of the radio-activity in the 0-12-hour pooled urine samples was due to unmetabolized TIPA. However, since 20% of the radioactivity was not characterized in the urine, there is the possibility that other metabolites may have been formed.

The authors' suggestion that the co-administration of TIPA and picloram did not affect the fate (absorption and excretion) of picloram can be supported based on rapid absorption, and excretion of ^{14}C-TIPA primarily unchanged in the urine.

STUDY DESIGN/ REPORTING DEFICIENCIES, TAKING INTO CONSIDERATION THE NATURE OF THIS SPECIAL STUDY:

Minor deficiencies in study design or reporting occurred as follows:

(1) No female animals were used in the study, as per Guidelines.

(2) Insufficient numbers of animals were used. Guidelines recommend 5 animals/sex/dose, and only 4 animals were used in this study.

(3) Summary tables presented by the study authors contained discrepancies with individual animal data tables, thus the transcribing of the data appears to be less than reliable.

(4) It was not reported whether the analyses were performed in duplicate. This information would have been useful since there was considerable variations among animals (see Addendum pp. 28, 30, 31)

(5) Most data are reported as "Percentage Administered Dose"; raw data are not provided for most parameters.

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References:

1. Frantz, S.W., Spence, M.W., and Nolan, R.J. (1986). Diisopropanolamine: Pharmacokinetic Fate Following Dermal Application to Female Fischer 344 Rats. Report of the Dow Chemical Company, Midland, MI.

2. Nolan, R.J., Smith, F.A., Muller, C.J. and Curl, T.C. (1980). Kinetics of 14C-Labelled Picloram in Male Fischer 344 Rats. Report of the Dow Chemical Company, Midland, MI.

3. Redwine, O.D. (1991). NMR Assay of Picloram:Triisopropanol Amine (AGR 0276453) ML-A1-050169 Report of the Dow Chemical Company, Midland, MI.

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Table 1. Mean Percent Recovery of Radioactivity by 72 Hours after Single Oral Dose of 10 mg Picloram/kg and 9.5 mg ^{14}C-TIPA/kg to Male Rats[a,b]

	Percentage of Administered Dose					
	Urine[c]	Feces	Tissue & Carcass	$^{14}CO_2$	Final Cage Wash	Total Recovery
Animal Number						
90A-7478	83.87	5.20	0.75	4.21	1.53	95.56
90A-7479	83.78	3.97	0.67	4.00	1.36	93.78
90A-7480	58.86	22.92	0.79	4.23	4.00	90.80
90A-7481	84.31	4.30	0.69	3.91	1.34	94.55
Mean	77.71	9.10	0.73	4.09	2.06	93.69
S.D.	12.57	9.23	0.05	0.16	1.30	2.04

[a]Data extracted from Tables 2, 4, 5, and 6 (pp. 28, 30, 31, 32).

[b]These values were extracted from individual animal data by the reviewers; minor discrepancies exist between this table and the summary table from the Study Report (Table 2).

[c]Urine includes the radioactivity recovered from the cage rinses with distilled water.

Picloram

Tox Review 10165 RIN 1901-99

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