3-6-95

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

#900AA

MAR - 6 1995

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: **THIODICARB**: Metabolism Study in the Rat

TO: Bonnie Adler
PM Team Reviewer (52)
Reregistration Branch, SRRD (7508W)

FROM: Linda L. Taylor, Ph.D. Linda Taylor Sup C 2/21/95
Toxicology Branch II, Section II,
Health Effects Division (7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 3/1/95
Section II Head, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D. Marcia Gemert 3/3/95
Chief, Toxicology Branch II/HFAS/HED (7509C)

Registrant: Rhône-Poulenc Secteur Agro
Chemical: Thiodicarb
Synonym: Larvin
Submission No.: S461924
DP Barcode: D201184
Caswell No.: 900AA
Case: 816454
Identifying No.: 114501-000264
Shaughnessey No.: 114501
MRID No.: 412500-06 and 412500-07
Action Requested: Please review the following metabolism data for the chemical thiodicarb.

Comment: The metabolism study on Thiodicarb submitted by the Registrant has been reviewed, and the DER is attached.

The absorption, distribution, elimination, and biotransformation of radiolabeled Thiodicarb were determined in rats following the administration of single oral doses of 2 or 16 mg [Acetyl-1-$^{14}$C] Thiodicarb/kg. The $C_{max}$ [0.82 µg equivalents/g (low)/4.7 µg equivalents/g (high)] and $t_{max}$ [1 hour (low)/4 hours (high)] in plasma were dose-dependent but not sex dependent. Since the elimination rates at the two dose levels were nearly equal, the difference in $t_{max}$ indicates a dose-dependence on absorption. The concentration of $^{14}$C in RBCs reached a $C_{max}$ considerably later [24

hours post dose] than in plasma, with the a $C_{max}$ range at the low dose of 4.1-6.4 μg equivalents/g and at the high dose of 28-41 μg equivalents/g, and the $^{14}C$ was eliminated at a much slower rate than from plasma. There was extensive absorption at both dose levels, as evidenced by the low [4-9%] fecal content of radiolabel. The major routes of elimination were expired $CO_2$ [13-24%], expired acetonitrile [13-38%], and urine [21-34%]. Tissue residues were 7-9% of the dose at 7 days post dose and may reflect the metabolism of $^{14}C$-Acetonitrile into the body's C-2 and C-1 pools and subsequent interaction with, or incorporation into natural products. Material balance was ≈88% at 7 days. The major metabolites of Thiodicarb in the rat are $CO_2$ and Acetonitrile. The major urinary metabolite is a labile unknown that represents ≈50% of the urinary radiolabel. It decomposed to ≈50% materials that were not condensed by dry-ice acetone, 25% volatiles that were condensed by dry-ice acteone, and 25% nonvolatile residues. The tissue residues consisted mainly of water-soluble materials and some insoluble residue. Hydrolysis of the residue resulted in solubilization of the residue in water but not in the formation of discrete metabolites. No acetamide was detected in any of the tissues. The RBCs contained only residue that cannot be extracted by organic solvents or water, indicating the presence of radiolabel incorporated into natural products or of material tightly bound to hemoglobin.

Classification: Acceptable. This study provides data on the metabolism of Thiodicarb following single low- and single high-dose exposures. Alone it does not satisfy the guideline requirement (85-1) for a metabolism study. Due to the recent finding of increased incidences of tumors [liver tumors in both sexes of mice and testicular tumors in male rats], a repeated dose metabolism study is required, with particular attention to the assessment of acetamide levels in the urine and tissues.

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Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 2/21/95
Tox. Branch II, Section II, HED (7509C)
Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 3/1/95
Head Section II, Tox. Branch II, HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rats TOX. CHEM. NO. 900AA

MRID No.: 412500-06 and 412500-07

TEST MATERIAL: Thiodicarb

TESTING FACILITY: Hazleton Laboratories America, Inc.

SPONSOR: Rhone-Poulenc Ag Company

STUDY NO.: HLA 6224-100

AUTHOR: RA Hiles

TITLE OF REPORT: The Metabolism of Thiodicarb (Acetyl-1-$^{14}$C) in Albino Rats; Phase I - $t_{max}$ of Plasma, Whole Blood, and Red Blood Cell Levels of $^{14}$C and Urine Collections; Phase II - Material Balance; Phase III - Urine, Plasma, Red Blood Cell, and Tissue Levels of Thiodicarb and Selected Metabolites; and Amendment No. 1

REPORT ISSUED: March 2, 1988; In-life study completed 11/2/87

QUALITY ASSURANCE: A quality assurance statement and a Good Laboratory Practice compliance statement were provided.

EXECUTIVE SUMMARY: The absorption, distribution, elimination, and biotransformation of radiolabeled Thiodicarb were determined in rats following the administration of single oral doses of 2 or 16 mg [Acetyl-1-$^{14}$C] Thiodicarb/kg. The $C_{max}$ [0.82 µg equivalents/g (low)/4.7 µg equivalents/g (high)] and $t_{max}$ [1 hour (low)/4 hours (high)] in plasma were dose-dependent but not sex dependent. Since the elimination rates at the two dose levels were nearly equal, the difference in $t_{max}$ indicates a dose-dependence on absorption. The concentration of $^{14}$C in RBCs reached a $C_{max}$ considerably later [24 hours post dose] than in plasma, with the a $C_{max}$ range at the low dose of 4.1-6.4 µg equivalents/g and at the high dose of 28-41 µg equivalents/g, and the $^{14}$C was eliminated at a much slower rate than from plasma. There was extensive absorption at both dose levels, as evidenced by the low [4-9%] fecal content of radiolabel. The major routes of elimination were expired $CO_2$ [13-24%], expired acetonitrile [13-38%], and urine [21-34%]. Tissue residues were 7-9% of the dose at 7 days post dose and may reflect the metabolism of $^{14}$C-Acetonitrile into the body's C-2 and C-1 pools and subsequent interaction with, or incorporation into natural products. Material balance was ≈88% at 7 days. The major metabolites of Thiodicarb in the rat are $CO_2$ and Acetonitrile. The major urinary metabolite is a labile unknown that represents ≈50% of the urinary radiolabel. It decomposed to ≈50% materials that were not condensed by dry-ice acetone, 25% volatiles that were condensed by dry-ice acteone, and 25% nonvolatile residues. The tissue residues consisted mainly of water-soluble materials and some insoluble residue. Hydrolysis of the residue

resulted in solubilization of the residue in water but not in the formation of discrete metabolites. No acetamide was detected in any of the tissues. The RBCs contained only residue that cannot be extracted by organic solvents or water, indicating the presence of radiolabel incorporated into natural products or of material tightly bound to hemoglobin.

Classification: Acceptable. This study provides data on the metabolism of Thiodicarb following single low- and single high-dose exposures to rats. Alone it does not satisfy the guideline requirement (85-1) for a metabolism study. Due to the recent finding of increased incidence of tumors following Thiodicarb exposure [liver tumors in both sexes of mice and testicular tumors in male rats], a repeated dose metabolism study is required, with particular attention to the assessment of acetamide levels in the urine and tissues.

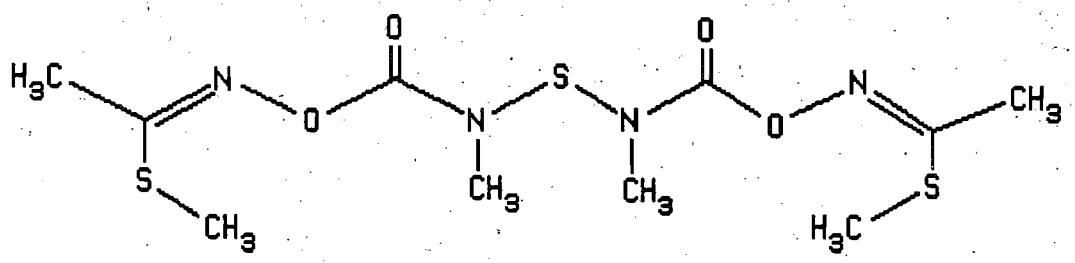
A. MATERIALS

1. Test Compound:

a. Labelled compound - [Acetyl-1-$^{14}$C] Thiodicarb; Purity: 87.5% Thiodicarb [redacted] [HPLC]-93% Thiodicarb [redacted] [TLC]; Batch #: none provided; Specific Activity: 23 mCi/mmol. Source: not provided.

Unlabelled compound - Thiodicarb; Batch #: Lot 20-ARD-98; Purity: 97.5% Thiodicarb [redacted]; Description: not provided; Source: not stated.

b. Structure:



2. Test Animals:

Species: Rat
Strain: Charles River Crl:CD®(SD)BR
Age: not provided
Weight: ♀ 203-220 grams/♂ 218-248 grams
Source: Charles River Laboratories, Wilmington Massachusetts

B. STUDY DESIGN

1. Dose Administration: There are three phases to this study (described below), which involved 88 rats.

**PHASE I - $t_{max}$ of plasma, whole blood, and red blood cell levels of $^{14}$C and urine collections** - Five rats/sex/group were dosed via gavage with 2 or 16 mg [Acetyl-1-$^{14}$C] Thiodicarb/kg body weight to determine the time after dosing when the plasma and blood $^{14}$C levels reach a maximum and to obtain urine samples for metabolite identification work. These rats were sacrificed at 96-hours post dose.

**PHASE II - Material balance** - Five rats/sex/group were dosed as above with 2 or 16 mg [Acetyl-1-$^{14}$C] Thiodicarb/kg body weight to determine the absorption, distribution, elimination, and biotransformation of Thiodicarb in male and female rats. These rats were sacrificed 168-hours post dose.

**PHASE III - Urine, plasma, red blood cell, and tissue levels of Thiodicarb and selected metabolites** - Three rats/sex/group were dosed via gavage with (a) either 2 [Group 1] or 16 [Group 5] mg [Acetyl-1-$^{14}$C]

Thiodicarb/kg body weight and sacrificed pre-$t_{max}$ (0.5 hours post dose); (b) either 2 [Group 2] or 16 [Group 6] mg [Acetyl-1-$^{14}$C] Thiodicarb/kg body weight and sacrificed at $t_{max}$ (1 hour); (c) either 2 [Group 3] or 16 [Group 7] mg [Acetyl-1-$^{14}$C] Thiodicarb/kg body weight and sacrificed at 24 hours; or (d) either 2 [Group 4] or 16 [Group 8] mg [Acetyl-1-$^{14}$C] Thiodicarb/kg body weight and sacrificed at 48 hours. NOTE: $t_{max}$ refers to the time of maximum $^{14}$C concentration in plasma; pre-$t_{max}$ is one-half $t_{max}$, which are the values obtained in Phase I of the study.

For Phases I and III, test animals/phase arrived as one shipment and the rats were randomized via computer-generated random numbers for assignment to groups. For Phase II, two separate shipments of rats were used, with the rats used in the 2 mg/kg group arriving in May, 1987 and those for the 16 mg/kg group arriving in June, 1987. It is not clear how these latter animals were randomized to their group since there were only one low-dose group/sex and one high-dose group/sex. For all experimental groups, it is stated that the body-weight variation of each sex did not exceed ± 2 standard deviations of the mean weight and the mean weights of each/sex were not statistically different. The rats were acclimated for 8-10 days following arrival at the testing facility, and Certified Rodent Chow® #5002 [Ralston Purina Company, St. Louis, MO, Lot # 0205872A] and water were available ad libitum; exception: rats fasted overnight prior to dosing and for ≈4 hours post dose. Each rat was housed individually in a glass metabolism cage, to which they were acclimated overnight prior to dosing. In Phase II, the metabolism cages were equipped with a system to separate and collect urine, feces, carbon dioxide, and acetonitrile.

**Amendment I** - A further characterization of the $^{14}$C metabolites of Thiodicarb in the red blood cells, urine, and tissues from the rats in PHASE III was performed.

2. Dose Preparation and Analysis: The radiolabeled test material [known amount] was dissolved in acetone and pipetted into a glass tube from which the acetone was removed with a gentle stream of nitrogen gas. The unlabeled test material was weighed into the glass tube and both test materials were dissolved in an aqueous preparation of Emulphor® EL620 [GAF Corporation] by stirring and sonication [Phase I]. A similar procedure was described in Phases II and III, but after the unlabeled test material was placed into the glass tube [vial], an amount of water equal to one-half the total volume of dosing solution desired was added, the suspension was stirred rapidly, and one-half the desired total volume of Emulphor EL620 was added dropwise with continued stirring until homogeneous. The dosing solutions in all cases were stored overnight at room temperature to allow the air bubbles to dissipate. Each rat received a single dose via gavage at 8 mL/kg body weight [≈ 10 μCi $^{14}$C radioactivity]. A portion of each dose solution was analyzed by liquid scintillation counting [LSC] before use to verify that the concentration of $^{14}$C was within the desired range, and the actual amounts of $^{14}$C administered to each rat were determined by radioanalysis of duplicate weighed pre- and post-dose samples taken with the syringe used for dosing.

3. Observations: Following dosing, the rats were observed at least once an hour post dose until all clinical signs had ceased. Mortality and moribundity checks were made twice a day, with cageside observations for obvious signs of a toxic effect being made each morning. Body weights of each rat were obtained on arrival, at randomization, initiation of dosing, and at termination.

RESULTS

**PHASE I** - All rats survived to study termination. No overt signs of toxicity were displayed by any low-dose [Group 1] rat following dosing. On the day of dosing, all rats appeared lethargic and exhibited tremors during the first hour post dose. Some rats exhibited these signs for up to 5 hours post dose, and some exhibited excess salivation and urine stains during the first 12 hours post dose. The was an overall gain in body weight during the study at both dose levels [both sexes]. **PHASE II** - One high-dose rat was found dead due to suffocation on day 4 because of a frozen water vapor trap. Group 1 rats showed no clinical signs indicative of toxicity following dosing. Group 2 rats were lethargic and exhibited tremors during the first 2 hours post dose. Some also displayed excess salivation. All other rats were reported to be clinically healthy throughout the study. All rats gained weight during the study. **PHASE III** - There were no deaths during the study. Two low-dose rats [Group 3] displayed salivation following dosing, but all other low-dose rats [Groups 1-4] appeared normal on the day of dosing. At the high dose [Groups 5-8], tremors were exhibited during the first 2 hours post dose, and salivation, soft feces, rhinorrhea, and wheezing were also observed in females during the first 4 hours. Two rats displayed urine stains on day 2. All other rats were reported clinically healthy throughout the study. Rats of both sexes in Groups 1, 2, 5, and 6 displayed a negative body-weight gain following dosing. These latter rats were sacrificed at 0.5 to 4 hours post dose. Rats in Groups 3, 4, 7, and 8 [both sexes] gained weight during the study.

4. Sample Collection: **PHASE I** - Following dosing, urine and feces were collected [containers surrounded by dry ice] at 0-6, 6-12, 12-24, and 24-48 hours, and each sample was weighed. No attempt was made to collect any urine voided during the bleeding procedure. Two blood samples [≈0.50 µL each] were collected [exact time noted] from the tail vein of each rat at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, and 96 hours after dosing. One sample from each collection was centrifuged to separate red blood cells from plasma. At termination after the 96-hour bleeding, each rat was anesthetized and exsanguinated via cardiac puncture. The thyroid, parathyroids, heart, lungs, kidneys, liver, spleen, brain, adrenals, testes or ovaries, carcass, gastrointestinal tract with contents, bone (femur), muscle (thigh), fat (perirenal) were excised, weighed, and stored [<0°C] along with feces for possible metabolite identification. **PHASE II** - Following dosing, expired carbon dioxide [$CO_2$] and acetonitrile were collected at 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours. Carbon dioxide was collected in a mixture of 2 parts methyl cellosolve and 1 part ethanolamine, and acetonitrile was collected using butyl cellosolve at <-10°C. A cold trap [<-1°C] was placed between the animal cage and the acetonitrile traps.

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Urine and feces were collected as above at 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours post dose, and the weight of the sample was recorded. At 168 hours post dose, the rats were anesthetized [Halothane] and exsanguinated via cardiac puncture, and blood was collected for the analysis of whole blood, plasma, and red blood cells. The thyroid, parathyroids, heart, lungs, kidneys, liver, spleen, brain, adrenals, testes or ovaries, carcass, gastrointestinal tract with contents, bone (femur), muscle (thigh), fat (perirenal) were excised, weighed, and assayed for total radioactivity. After sacrifice, the cages were washed [aqueous solution of trisodium phosphate, wiped with gauze, and the cagewashes were weighed and analyzed for radioactivity. **PHASE III** - Urine and feces were collected as above at sacrifice for the rats sacrificed at pre-$t_{max}$ and $t_{max}$ [Groups 1, 2, 5, and 6] and for the remaining rats [Groups 3, 4, 7, and 8] at 0-6, 6-12, and 12-24 hours post dose and for Groups 4 and 8 at 24-48 hours post dose also. At termination [not specified], the rats were anesthetized [Halothane] and exsanguinated via cardiac puncture, and blood was collected for the analysis of whole blood, plasma, and red blood cells. The thyroid, parathyroids, heart, lungs, kidneys, liver, spleen, brain, adrenals, testes or ovaries, carcass, gastrointestinal tract with contents, bone (femur), muscle (thigh), fat (perirenal) were excised, weighed, and assayed for total radioactivity.

5. Sample Preparation for Radioanalysis: Duplicate aliquots of all samples were analyzed. **Phase I** - Whole blood and RBCs were weighed for combustion in a sample oxidizer [Packard Model 306 automatic; Carbo-Sorb and Perma-Fluor V scintillation cocktail], and urine and plasma were weighed and analyzed by direct counting in Insta-Gel scintillation cocktail. Whole blood, plasma and RBCs were analyzed immediately, and urine samples were stored frozen [<0°C] until preparation for radioanalysis. **Phase II** - Whole blood was weighed for combustion in a sample oxidizer [as above], and RBCs were prepared for combustion by centrifugation of the remaining blood. Urine, cagewash, and plasma were weighed and analyzed by direct counting in Insta-Gel scintillation cocktail, and $CO_2$ and acetonitrile trapping solutions were weighed and analyzed by direct counting in Permafluor scintillation cocktail. Fecal samples were mixed with a known weight of deionized water to facilitate homogenization, and weighed aliquots were prepared for combustion. Aliquots of cagewash residue were weighed for combustion also. Small tissue samples were either combusted directly as a single sample or were split into two portions and combusted directly. All remaining tissues were homogenized and aliquots of the homogenates were weighed for combustion. Fat was homogenized in a 1:1 mixture of methanol:deionized water. Blood, RBCs, and plasma were refrigerated prior to analysis, and all other samples were frozen until preparation for radioanalysis. All sample combustions and the scintillation cocktail were as described under PHASE I above. **PHASE III** - Whole blood, plasma, RBCs, urine, fecal samples, and tissue samples were prepared as described under PHASE II above. **Biotransformation Products in RBCs, Urine, and Tissues** - Analysis by Thin-Layer Chromatography [TLC] was performed using glass plates prelayered with silica, and chromatograms were developed using the following 2-dimensional systems:

SYSTEM 1 1st dimension - ethyl acetate:diisopropyl ether (3:2)
2nd dimension - ethyl acetate:acetone (4:1)
SYSTEM 2 1st dimension - butanol:acetic acid:water (11:4:5)
2nd dimension - ethyl acetate:isopropanol:water (11:6:3)
SYSTEM 3 1st dimension - propanol:water (85:15)
2nd dimension - ethyl acetate:isopropanol:water (55:30:15)

Migration of radioactive materials was determined using a linear TLC analyzer. Various RBC, urine, and tissue extracts were characterized using reversed-phase HPLC, using a Nucleosil®-C18 (Machery-Nagel) column and the following solvent systems:

SYSTEM 1 water:acetonitrile
SYSTEM 2 $KH_2PO_4$:acetonitrile
SYSTEM 2 $KH_2PO_4$:methanol

Aliquots of aqueous extracts were counted directly in an appropriate liquid scintillation cocktail, and aliquots of organic solvents were counted directly. Aliquots of chlorinated solvents were kept small or evaporated to dryness under nitrogen to minimize quenching. Aliquots of solids were combusted in a sample oxidizer before counting. Gas chromatography was performed using a SP-2100 column with helium as a carrier. The effluent from the column was split into 2 portions, with one portion introduced into the flame ionization detector and the other introduced into the gas-proportional counter for detection of radioactivity.

Characterization of $^{14}C$ Residues in Urine: Urine was diluted with water and extracted with dichloromethane [4X], and the combined organic layers were dried and then filtered. Duplicate aliquots of both the aqueous and organic layers were analyzed for radioactivity. The organic layer was evaporated to dryness, and the condensate was collected with a dry ice condenser. The residue was redissolved in dichloromethane, and duplicate aliquots of both the condensate and residue were analyzed. A portion of the concentrated organic extract was injected onto HPLC System 1, and a portion of the aqueous layer remaining after extraction was injected onto HPLC System 2. The aqueous layer remaining after extraction was treated with a B-glucuronidase-sulfatase mixture, and a portion was buffered and incubated. After hydrolysis, the incubation mixture was extracted with dichloromethane and analyzed using HPLC. Characterization of $^{14}C$ Residues in Liver, Kidney, and RBCs: Initially, liver homogenate was treated as above with dilution with water, extraction with dichloromethane, centrifugation, extraction of homogenate [3X] with dichloromethane, and the combined organic extracts dried and filtered. The aqueous layers were filtered also, and duplicate aliquots of the organic and aqueous layers were analyzed. Duplicate aliquots of the residue were combusted, and the resulting $CO_2$ was analyzed for radiolabel. The aqueous fraction of the liver was buffered and incubated with protease, extracted with dichloromethane, and duplicate aliquots of both the organic and aqueous layers were analyzed. All subsequent tissue samples [homogenates] were extracted with water:methanol:chloroform, and the extractions were shaken and centrifuged. The organic and aqueous layers from two extractions were pooled and filtered, and duplicate aliquots of each sample were analyzed by LSC. Duplicate aliquots of the residue for each tissue were combusted, and the resulting $CO_2$ was

analyzed. The organic layers were evaporated to just dryness, and duplicate samples of the condensate and residue [after reconstitution in chloroform] were analyzed, The aqueous layer was concentrated and filtered, and duplicate aliquots of the filtered concentrate were analyzed for radiolabel and injected onto HPLC solvent System 2. Some of the aqueous concentrates were also analyzed by TLC using Systems 2 and 3. The liver residue was subjected to acid hydrolysis and neutralized, and the hydrolyzate was extracted as above [water:methanol:chloroform], and duplicate aliqouts of the organic and aqueous layers were analyzed. Some of the aqueous layer remaining after extraction was concentrated and filtered, and duplicate aliquots of the filtered concentrate were analyzed [HPLC System 2]. Attempted Purification of Major Metabolite from Urine: Initial attempt involved multiple injections of the aqueous layer remaining after dichloromethane extraction onto HPLC System 2 and the collection of fractions, which were combined, and the mobile phase was removed by lyophilization. The residue was reconstituted in acetonitrile and portions were injected onto HPLC Systems 2 and 3. The remainder of the isolated metabolite was methylated with ethereal diazomethane, evaporated to just dryness and the residue reconstituted in acetonitrile. A portion of the solution was injected onto HPLC System 2. Another portion was reconstituted in methanol and a portion was injected onto a gas chromatograph equipped with flame ionization and radiochemical detectors. The solution was then subjected to gas chromatography-mass spectrometry analysis. To obtain a purer sample, the aqueous layer from the initial extraction was acidified and extracted with anhydrous diethyl ether, which was evaporated to dryness and reconstituted in ether. A portion of this was injected onto HPLC System 2, and the remainder was methylated with diazomethane. The ethereal solution of the methylated metabolite was concentrated and duplicate aliquots were analyzed. A portion of the methylated metabolite was injected onto HPLC System 2, and it was also analyzed by TLC using ethyl acetate:diisopropyl ether. To characterize the decomposition products of the major metabolite, the aqueous layer remaining after dichloromethane extraction was injected onto HPLC System 2 and fractions were collected. Fractions 18-22 were pooled and evaporated; the residue remaining was reconstituted in acetonitrile, and the solution was acidified and allowed to sit for 8 days. Additional acetonitrile was added and duplicate aliquots of the condensate and the residue were analyzed.

6. Sample Analysis and Data Calculations: Samples were analyzed by liquid scintillation counting [LSC] for at least 5 minutes or 100,000 accumulated counts [Tri-Carb® liquid scintillation spectrometer, Models 460CD and 4640, Packard Instrument Co.]. Scintillation counting data [cpm] were converted automatically to dpm using the external standardization technique and an instrument-stored quench curve generated from a series of sealed quenched standards. Concentrations of radioactivity in samples were calculated at $^{14}C$ dpm/g using the following formula:

$$\text{dpm/g} = \frac{^{14}\text{C dpm in SAMPLE aliquot}}{\text{SAMPLE aliquot weight [g]}}$$

Concentrations of radioactivity expressed as µg-equivalents $^{14}C$-Thiodicarb/g sample were calculated as follows:

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$$\mu g \text{ Equivalents/g} = \frac{\text{Mean dpm/g}\blacktriangledown}{\text{Specific activity [dpm/}\mu g\text{]}\blacklozenge}$$

♦ PHASE I Group 1: 36,700 dpm/µg; Group 2: 5230 dpm/µg;
PHASE II Group 1: 44,900 dpm/µg; Group 2: 5590 dpm/µg
PHASE III Groups 1-4: 40,700 dpm/µg; Groups 5-8: 5320 dpm/µg

▼ PHASE III dpm/g represents tissue concentration of radioactivity prior to addition of $H_2O$ for homogenization

The total amounts of radioactivity in samples of urine [PHASE I], samples of tissues and excreta [PHASE II], and samples of urine and feces [PHASE III] were calculated as percent of total dose as follows:

$$\% \text{ of total dose} = \frac{\text{Total sample } ^{14}C \text{ [dpm]}}{\text{Total } ^{14}C \text{ dose [dpm]}} \times 100$$

or

$$\frac{\text{SAMPLE } ^{14}C \text{ [dpm/g]} \times \text{SAMPLE weight}\blacklozenge \text{ [g]}}{\text{Total } ^{14}C \text{ dose [dpm]}} \times 100$$

♦ for tissues and organs, the entire weight was used except for those samples in which the aliquot analyzed represented an unknown fraction of the total body content [bone, fat, and muscle]; for these, the sample weight used was that obtained at necropsy

Validation of Radioanalyses: Triplicate aliquots each of control urine and whole blood [PHASE I]/urine, feces, and representative tissues [PHASES II and III] to which $^{14}C$-Thiodicarb at levels representative of those in samples from dose rats had been added were analyzed. Recoveries were calculated by comparing the mean radioactivity [dpm] recovered in these samples with the amount determined by direct counting of the same volume of the sample. Since overall recoveries at each added level were greater than 95%, the measured dpm values were not corrected for recovery.

7. Statistics: Statistical comparisons were limited to simple expressions of variation [mean ± standard deviation].

C. RESULTS

1. Dose Suspensions: The concentrations of radioactivity in the dosing solutions [dpm/µg Thiodicarb] are listed above in the calculations section [#6].

2. Administered Dose: The mean dose [mg/kg] for each of the phases of the study are presented in Table 1.

Table 1. Administered Dose

| DOSE LEVEL Study Group | PHASE I | | PHASE II | | PHASE III | |
|---|---|---|---|---|---|---|
| | males | females | males | females | males | females |
| LOW dose(mg/kg) | 2.5 | 2.5 | 1.9 | 1.9 | 2.4-2.5 | 2.4-2.5 |
| HIGH dose(mg/kg) | 18.5 | 18.6 | 15.9 | 15.9 | 18.8-19.0 | 18.7-19.1 |

3. **PHASE I Sample Analysis** - Radiolabel in Urine: The cumulative percent of the dose excreted in the urine following dosing at 2 and 16 mg/kg is shown in Table 2. NOTE: Rats were sacrificed at 96 hours post dose, but urine data were presented for the first 48 hours only. **LOW DOSE:** The majority of the radiolabel excreted via the urine within 48 hours post dose by both sexes was eliminated within the first 12 hours post dose. At the low dose level, 49% [♂♂]/15% [♀♀] of the amount of $^{14}C$ eliminated via the urine by 48 hours was excreted by 6 hours post dose. **HIGH DOSE:** At the high dose level in males, 45% of the dose eliminated by 48 hours post dose via the urine had been excreted by 6 hours post dose and 34% had been excreted by the females. In males, 73% of the amount excreted in the urine by 48 hours post dose was eliminated by 12 hours post dose, while for females only 50% had been eliminated by this time point. By 24 hours post dose, 90% [♂♂]/85% [♀♀] of the radiolabel eliminated via the urine by 48 hours had been excreted. Males at the high dose excreted a slightly greater amount [≈28%] of radiolabel via the urine than the females [≈22%]. Males at both dose levels displayed a more rapid elimination via the urine than did the females initially, but by 24 hours post dose, comparable amounts had been excreted by this route for both sexes.

Table 2. Cumulative Radiolabel in Urine [% of total dose]

| Sex/Dose/Interval (hours) | MALES | FEMALES |
|---|---|---|
| **Low dose** | | |
| 0-6 | 4.96 [49%]♦ | 1.58 [15%] |
| 6-12 | 7.21 [71%] | 6.79 [65%] |
| 12-24 | 8.81 [87%] | 8.91 [86%] |
| 24-48 | 10.13 | 10.41 |
| **High dose** | | |
| 0-6 | 12.7 [45%] | 7.42 [34%] |
| 6-12 | 20.63 [73%] | 10.85 [50%] |
| 12-24 | 25.33 [90%] | 18.54 [85%] |
| 24-48 | 28.19 | 21.89 |

♦ [% of total dose excreted in urine]

Radiolabel in Whole Blood, RBCs, and Plasma: Whole blood Concentrations of radiolabel in whole blood increased rapidly during the first hour post dose and then more slowly thereafter [Figures 3-6, appended]. The maximum concentration [$C_{max}$] was reached at 8 [♂♂]/12 [♀♀] hours post dose at the low dose and at 24 hours post dose at the high-dose. Both the time to maximum concentration [$t_{max}$] and the $C_{max}$ were dose-dependent. The rate of elimination of $^{14}C$ from whole blood was slow but not sex-dependent. The high-dose $C_{max}$:low-dose $C_{max}$ ratio was 6.4 ♂♂/6.1 ♀♀ vs 8 for the dose levels [Table 3].

TABLE 3. Radiolabel in Whole blood [µg Equivalents/g]

| Sex/Dose/Time (hours) | MALES | | FEMALES | |
|---|---|---|---|---|
| | Low Dose | High Dose | Low Dose | High Dose |
| 0.25 | 0.417 | 1.54 | 0.513 | 2.17 |
| 0.5 | 0.987 | 3.30 | 1.20 | 3.44 |
| 1 | 1.58 | 4.58 | 1.94 | 5.88 |
| 2 | 1.92 | 6.77 | 2.26 | 8.19 |
| 4 | 2.13 | 9.88 | 2.76 | 12.1 |
| 8 | 2.51 | 10.5 | 3.24 | 15.3 |
| 12 | 2.13 | 13.7 | 3.37 | 14.9 |
| 24 | 2.28 | 16.1 | 3.21 | 20.4 |
| 48 | 2.23 | 15.2 | 2.93 | 19.9 |
| 72 | 2.05 | 14.6 | 2.92 | 19.3 |
| 96 | 1.77 | 11.1 | 2.53 | 16.0 |

**RBCs** At both dose levels, radiolabel concentrations in RBCs were greater than those observed in whole blood [Figures 7-10, appended]. The maximum concentrations were reached over a prolonged time period, with the $t_{max}$ of 24 hours post dose for all groups [Table 4].

TABLE 4. Radiolabel in RBCs [µg Equivalents/g]

| Sex/Dose/Time (hours) | MALES | | FEMALES | |
|---|---|---|---|---|
| | Low Dose | High Dose | Low Dose | High Dose |
| 0.25 | 0.437 | 1.90 | 0.782 | 2.15 |
| 0.5 | 1.21 | 3.96 | 1.65 | 3.71 |
| 1 | 1.97 | 5.18 | 2.41 | 5.94 |
| 2 | 3.37 | 9.22 | 3.21 | 12.0 |
| 4 | 3.95 | 15.1 | 4.92 | 21.9 |
| 8 | 3.83 | 17.8 | 5.72 | 25.0 |
| 12 | 3.88 | 23.3 | 6.03 | 30.6 |
| 24 | 4.07 | 28.5 | 6.39 | 40.6 |
| 48 | 3.88 | 26.7 | 5.65 | 40.4 |
| 72 | 3.68 | 23.0 | 4.53 | 29.0 |
| 96 | 3.39 | 23.9 | 5.28 | 35.6 |

**Plasma** The maximum concentration of radiolabel occurred at 1 hour post dose at the low dose [both sexes] and at 4 hours post dose at the high dose [both sexes]. The high-dose $C_{max}$:low-dose $C_{max}$ ratio was 5.5 ♂♂/5.9 ♀♀ vs 8 for the dose levels. There was a rapid elimination of radioactivity after peak plasma concentration had been attained, followed by a slower phase of elimination. No differences were noted between the sexes [Table 5. data from text table 5, page 83].

TABLE 5. Radiolabel in Plasma [µg Equivalents/g]

| Sex/Dose/Time (hours) | MALES | | FEMALES | |
|---|---|---|---|---|
| | Low Dose | High Dose | Low Dose | High Dose |
| 0.25 | 0.103 | 1.38 | 0.246 | 1.37 |
| 0.5 | 0.478 | 2.95 | 0.630 | 2.24 |
| 1 | 0.819 | 3.27 | 0.826 | 3.07 |
| 2 | 0.706 | 3.42 | 0.714 | 4.28 |
| 4 | 0.526 | 4.49 | 0.567 | 4.87 |
| 8 | 0.493 | 2.37 | 0.577 | 4.15 |
| 12 | 0.459 | 3.64 | 0.506 | 3.34 |
| 24 | 0.268 | 4.10 | 0.340 | 3.67 |
| 48 | 0.197 | 2.83 | 0.238 | 2.68 |
| 72 | 0.132 | 2.18 | 0.178 | 1.98 |
| 96 | 0.0908 | 1.37 | 0.112 | 1.35 |

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The calculated values for $C_{max}$ and $t_{max}$ are listed below.

Table 6. Calculated Values for $C_{max}$ and $t_{max}$

| Parameter/ Dose/ Sex | MALES | | FEMALES | |
|---|---|---|---|---|
| | 2 mg/kg | 16 mg/kg | 2 mg/kg | 16 mg/kg |
| $C_{max}$ [µg equiv./g] | | | | |
| whole blood | 2.51 | 16.1 | 3.37 | 20.4 |
| RBCs | 4.07 | 28.5 | 6.39 | 40.6 |
| plasma | 0.819 | 4.49 | 0.826 | 4.87 |
| $t_{max}$ [hours] | | | | |
| whole blood | 8 | 12 | 24 | 24 |
| RBCs | 24 | 24 | 24 | 24 |
| plasma | 1 | 1 | 4 | 4 |
| Ratio $C_{max}^{\text{high dose}}/C_{max}^{\text{low dose}}$ | | | | |
| whole blood | 6.41 | | 6.05 | |
| RBCs | 7.00 | | 6.35 | |
| plasma | 5.48 | | 5.90 | |

4. **PHASE II Sample Analysis** - Material Balance: Overall recovery of radiolabel was ≥ 85.8%, [one exception: low-dose male 80.8%], with an average of 88% for the low dose and 89% for the high dose. The highest levels of $^{14}C$ were observed in the urine, followed by those in $CO_2$ Trap 1 and Acetonitrile Trap 1. At the high-dose level in both sexes, in all but one low-dose male, and in one low-dose female, the next highest levels were observed in the tissues, followed by the levels in the feces. In one low-dose male and 4 low-dose females, higher values were observed in the feces than were found in the tissues.

TABLE 7. Summary of Elimination and Distribution of Radiolabel

| Parameter/Dose/ Sex/Study | % AD Urine | % AD Feces | % AD $^{14}CO_2$ | % AD Tissues | % AD $^{14}C$-Acetonitrile | % AD Cage Wash | % AD Cold line Trap | % AD Total Excreted |
|---|---|---|---|---|---|---|---|---|
| PHASE II LOW | | | | | | | | |
| ♂♂ | 34.4 | 5.29 | 24.02 | 8.72 | 13.48 | 0.352 | 0.062 | 86.33 |
| ♀♀ | 20.9 | 9.23 | 12.76 | 7.53 | 37.59 | 0.876 | 0.11 | 89.04 |
| PHASE II HIGH | | | | | | | | |
| ♂♂ | 34.5 | 4.37 | 15.60 | 7.96 | 26.74 | 0.464 | 0.108 | 89.78 |
| ♀♀ | 30.8 | 4.25 | 22.8 | 6.87 | 20.16 | 3.575 | 0.105 | 88.60 |

Carbon Dioxide: The major route of elimination [range: 12% to 29% of the dose] was the conversion of acetyl-1-$^{14}C$-Thiodicarb to radiolabeled carbon dioxide [$^{14}CO_2$]. At the low dose, males eliminated ≈ twice as much of the dosed radiolabel as $^{14}CO_2$ as did females at this dose level, while high-dose females eliminated the greater amount of radiolabel via this route [≈1.5 times compared to the high-dose males. The conversion of $^{14}C$-Thiodicarb to $CO_2$ was rapid with 58-74% of the total $^{14}CO_2$ being eliminated during the first 6 hours post dose and 89-95% being eliminated by 24 hours.

| Table 8. Mean $^{14}C$ in Carbon Dioxide Trap 1♦ | | | | |
|---|---|---|---|---|
| Time interval (hours)/ Sex/Dose | MALES | | FEMALES | |
| | 2 mg/kg | 16 mg/kg | 2 mg/kg | 16 mg/kg |
| 0-6 | 17.8 | 9.69 | 7.96 | 13.2 |
| 6-12 | 3.94 | 3.65 | 2.31 | 4.83 |
| 12-24 | 0.920 | 1.29 | 1.08 | 3.30 |
| 24-48 | 0.434 | 0.320 | 0.474 | 0.560 |
| 48-72 | 0.246 | 0.170 | 0.200 | 0.305 |
| 72-96 | 0.176 | 0.172 | 0.142 | 0.265 |
| 96-120 | 0.168 | 0.112 | 0.108 | 0.158 |
| 120-144 | 0.144 | 0.088 | 0.114 | 0.130 |
| 144-168 | 0.112 | 0.084 | 0.170 | 0.130 |
| TOTAL | 23.89 | 15.57 | 12.56 | 22.82 |

♦ % of total dose

Acetonitrile: From 10 to 42% [range] of the administered radiolabel was found in acetonitrile traps, with low-dose females eliminating nearly three times the amount eliminated by the low-dose males via this route. Males excreted more via this route than females at the high dose level. Greater than 90% of the acetonitrile was trapped within the first 48 hours post dose. Elimination of the radiolabel as acetonitrile was slower compared to that of $CO_2$, with 9-16% being eliminated within the first 6 hours [Table 9.]

| Table 9. Mean $^{14}C$ in Acetonitrile Traps 1 and 2♦ | | | | |
|---|---|---|---|---|
| Time interval (hours)/ Sex/Dose | MALES | | FEMALES | |
| | 2 mg/kg | 16 mg/kg | 2 mg/kg | 16 mg/kg |
| TRAP 1 | | | | |
| 0-6 | 1.99 | 2.38 | 4.31 | 2.22 |
| 6-12 | 3.36 | 5.80 | 9.23 | 3.88 |
| 12-24 | 3.81 | 8.62 | 12.7 | 6.51 |
| 24-48 | 1.85 | 5.73 | 5.58 | 4.33 |
| 48-72 | 0.452 | 1.45 | 1.06 | 0.980 |
| 72-96 | 0.176 | 0.398 | 0.234 | 0.323 |
| 96-120 | 0.0900 | 0.134 | 0.066 | 0.168 |
| 120-144 | 0.062 | 0.0740 | 0.028 | 0.135 |
| 144-168 | 0.052 | 0.036 | 0.012 | 0.160 |
| TOTAL | 11.84 | 24.63 | 33.25 | 18.69 |
| TRAP 2 | | | | |
| 0-6 | 0.116 | 0.0700 | 0.208 | 0.0975 |
| 6-12 | 0.200 | 0.204 | 0.366 | 0.188 |
| 12-24 | 0.610 | 1.07 | 1.50 | 0.590 |
| 24-48 | 0.588 | 0.606 | 1.86 | 0.395 |
| 48-72 | 0.132 | 0.158 | 0.370 | 0.153 |
| 72-96 | 0.0300 | 0.0360 | 0.088 | 0.0300 |
| 96-120 | 0.0040 | b | b | 0.005 |
| 120-144 | b | b | b | b |
| 144-168 | b | b | b | b |
| TOTAL | 1.68 | 2.14 | 4.39 | 1.46 |

♦ % of total dose; b=below level of detection

Urine: From 18 to 41% [range] of the administered $^{14}C$ was excreted in urine, with the low-dose females displaying the lowest mean value. Of the amount of $^{14}C$ excreted in the urine, ≈49% was excreted within the first 6 hours post dose by males at both dose levels. The low-dose

females excreted ≈20% and the high-dose females excreted ≈39% within the first 6 hours. With the exception of the low-dose females, greater than 64% of the $^{14}C$ excreted via the urine had been excreted by 12 hours post dose. Low-dose females had excreted ≈ 37% by this time point. Excretion via the urine was rapid initially, but low amounts of $^{14}C$ were detectable at the 144-168 hours time interval.

Table 10. $^{14}C$ in Urine PHASE II (% of total dose)

| Time interval (hours)/ Sex/Dose | MALES | | FEMALES | |
|---|---|---|---|---|
| | 2 mg/kg | 16 mg/kg | 2 mg/kg | 16 mg/kg |
| 0-6 | 16.7[48.6]♦ | 17.1[49.5] | 4.28[20] | 12.1[39] |
| 6-12 | 10.3[78.5] | 6.53[68.5] | 3.43[36.8] | 7.68[64] |
| 12-24 | 3.73[89] | 4.75[82] | 4.71[59] | 5.43[81.8] |
| 24-48 | 1.76 | 2.69 | 4.05[78.7] | 3.01 |
| 48-72 | 0.686 | 1.23 | 1.72 | 1.36 |
| 72-96 | 0.434 | 0.866 | 1.04 | 0.488 |
| 96-120 | 0.280 | 0.580 | 0.708 | 0.380 |
| 120-144 | 0.226 | 0.456 | 0.566 | 0.223 |
| 144-168 | 0.212 | 0.362 | 0.414 | 0.160 |
| TOTAL | 34.38 | 34.52 | 20.93 | 30.82 |

♦ [% of total excreted via the urine]

Feces: Low amounts of radiolabel were detected in feces, and there was wide variability in the values among the groups. No samples were obtained from any of the low-dose females or from 3 of the 5 low-dose males within the first 12 hours post dose. Additionally, for the other rats, samples were not always obtained during this time period. Of the amount of $^{14}C$ eliminated via the feces, a higher percent had been eliminated by 96 hours post dose by males at both levels [≈70% at low dose and ≈51% at high dose] compared to females [≈39% at low dose and ≈37% at high dose]. For most of the rats, the greatest amount of $^{14}C$ was eliminated during the 144-168 hours interval.

TABLE 11. Elimination of Radiolabel in Feces PHASE II (range % AD(mean % AD))

| Group time interval (hours) | MALES [range(mean)] | | FEMALES range(mean) | |
|---|---|---|---|---|
| | Low Dose | High Dose | Low Dose | High Dose |
| 0-6 | <0.01 | 0.03-0.05(0.04) | NS | 0.1-0.23(0.095) |
| 6-12 | 0.01-0.06(0.035) | 0.01-0.09(0.05) | NS | NS |
| 12-24 | 0.48-0.90(0.728) | 0.27-1.55(0.75) | 0.02-1.14(0.518) | 0.18-0.67(0.425) |
| 24-48 | 0.17-2.12(0.666) | 0.35-2.54(0.898) | 0.70-2.51(1.39) | 0.17-0.71(0.475) |
| 48-72 | 0.06-0.29(0.160) | 0.07-0.41(0.216) | 0.06-4.85(1.23) | 0.46-0.63(0.548) |
| 72-96 | 0.05-10.05(2.12)[70%]♦ | 0.02-0.98(0/262)[51%]♦ | 0.02-1.14(0.458)[39%]♦ | 0.05-0.24(0.158)[37%] |
| 96-120 | 0.04-0.75(0.280) | 0.03-1.08(0.402) | 0.15-2.20(1.16)[52%]♦ | 0.5-1.08(0.423)[46%] |
| 120-144 | 0.01-0.19(0.066) | 0.05-0.88(0.446) | 0.06-0.53(0.258) | 0.21-1.61(0.595) |
| 144-168 | 0.53-2.15(1.26) | 0.55-3.24(1.36) | 0.75-6.19(4.21) | 1.20-2.67(1.85) |

♦ cumulative % of total eliminated via feces; NS=no sample

Blood, Red Blood Cells, and Plasma: At 168 hours post dose, the highest levels of $^{14}C$ were found in the RBCs, with whole blood displaying the next highest values. The ratio of RBCs:whole blood was constant among the groups [≈2], which would be expected, and the RBCs:plasma ratios ranged from 10 to 50. The ratios of the high dose:low dose average concentrations for RBCs, plasma, and whole blood for males are 4.2,

10.5, and 4.3, respectively and for females, 15.99, 5.1, 15.7, respectively [Table 12].

Radiolabel in Tissues: All tissues monitored displayed measurable amounts of $^{14}C$, but the values in the adrenals, thyroid/parathyroid, gonads [♀♀ only] and fat [on a % of total dose basis] were <0.01% for all rats. The carcass, GIT + wash, kidneys, and liver accounted for ≥95% of the $^{14}C$ found in the tissues. Approximately 8% of the administered dose was found in the tissues at 168 hours post dose. The amount of $^{14}C$ found in the tissues on a µg equivalents/ gram basis is shown in Table 12. The ratios [high dose:low dose] of the amount of $^{14}C$ in the various tissues in males ranged from 4 to 11 and in females from 4 to 13.

TABLE 12. Distribution of Radiolabel in Tissues and Blood (µg equivalents/g) PHASE II

| DOSE/ TISSUE | MALES | | FEMALES | |
|---|---|---|---|---|
| | LOW | HIGH | LOW | HIGH |
| Whole Blood | 1.68 | 7.20 | 0.826 | 13.0 |
| Plasma | 0.0608 | 0.639 | 0.153 | 0.777 |
| RBC | 3.02 | 12.7 | 1.47 | 23.5 |
| Adrenals | 0.206 | 0.851 | 0.164 | 1.23 |
| Bone | 0.0906 | 0.491 | 0.0732 | 0.517 |
| Brain | 0.135 | 0.555 | 0.0536 | 0.643 |
| Carcass | 0.143 | 1.06 | 0.120 | 0.993 |
| Fat | 0.0492 | 0.386 | 0.0484 | 0.484 |
| GIT | 0.0858 | 0.604 | 0.109 | 0.866 |
| GIT wash | 0.0198 | 0.215 | 0.0536 | 0.237 |
| Gonads | 0.105 | 0.568 | 0.123 | 0.877 |
| Heart | 0.235 | 0.982 | 0.128 | 1.64 |
| Kidney | 0.361 | 1.35 | 0.259 | 1.84 |
| Liver | 0.281 | 1.40 | 0.204 | 1.81 |
| Lung | 0.305 | 1.22 | 0.206 | 2.16 |
| Muscle | 0.0482 | 0.266 | 0.0318 | 0.329 |
| Spleen | 0.376 | 1.39 | 0.230 | 2.59 |
| Thyroid/parathyroid | 0.166 | 0.982 | 0.113 | 1.36 |

4. **PHASE III Sample Analysis** - Whole blood, RBCs, and plasma: The RBCs displayed higher levels of radiolabel than either whole blood or plasma in both sexes at both dose levels [Table 13]. At both dose levels, females displayed higher amounts of radiolabel in whole blood and plasma at all time points and in plasma at all time points except at 24 hours [high-dose] and at 48 hours [low dose] than males. The ratios for RBCs:whole blood and RBCs:plasma are listed in Table 14.

Radiolabel in Tissues: All tissues monitored displayed measurable amounts of $^{14}C$, as was observed in PHASE II. In general, the amount of $^{14}C$ observed at 1 hour following low-dose exposure was greater than that observed at 0.5 hours for each tissue, but with a few exceptions, the levels at 24 and 48 hours were below those at the 1-hour and in several cases, below those at the 0.5-hour time points [Table 13]. At 1 hour post dose, the highest levels of $^{14}C$ [$\mu$g equivalents/g] at the low dose were observed in the GIT, GIT wash, kidney, liver, and adrenals of both sexes and also in the lung and gonads of the females. At 48 hours post dose, the adrenals of low-dose females and the kidney of the low-dose males displayed the highest values. In general, the highest amount of $^{14}C$ in most of the tissues in males at the high-dose level was observed at 24 hour post dose, but in the high-dose females the time at which the highest levels were observed varied considerably. At 48 hours, the liver of both sexes at the high dose displayed the highest value.

TABLE 13. Distribution of Radiolabel in Tissues ($\mu$g equivalents/g) PHASE III

| LOW DOSE | MALES | | | | FEMALES | | | |
|---|---|---|---|---|---|---|---|---|
| TISSUE/Sacrifice time [hours] | 0.5 | 1 | 24 | 48 | 0.5 | 1 | 24 | 48 |
| Whole Blood | 0.987 | 1.60 | 2.30 | 2.47 | 2.22 | 2.63 | 3.47 | 3.23 |
| Plasma | 0.838 | 1.11 | 0.381 | 0.392 | 1.67 | 1.75 | 0.576 | 0.336 |
| RBC | 2.01 | 2.36 | 4.50 | 4.86 | 2.68 | 3.57 | 6.54 | 6.27 |
| Adrenals | 0.791 | 1.13 | 0.730 | 0.762 | 1.16 | 1.34 | 1.03 | 1.05 |
| Bone | 0.260 | 0.436 | 0.300 | 0.280 | 0.352 | 0.444 | 0.269 | 0.193 |
| Brain | 0.249 | 0.302 | 0.249 | 0.223 | 0.385 | 0.397 | 0.218 | 0.182 |
| Carcass | 0.315 | 0.383 | 0.303 | 0.326 | 0.416 | 0.489 | 0.309 | 0.235 |
| Fat | 0.103 | 0.126 | 0.139 | 0.109 | 0.232 | 0.172 | 0.0683 | 0.0840 |
| GIT | 2.93 | 3.19 | 0.439 | 0.373 | 2.67 | 2.09 | 0.483 | 0.343 |
| GIT wash | 2.07 | 2.76 | 0.121 | 0.158 | 2.09 | 1.91 | 0.165 | 0.987 |
| Gonads | 0.236 | 0.309 | 0.276 | 0.261 | 1.13 | 1.08 | 0.582 | 0.496 |
| Heart | 0.391 | 0.620 | 0.356 | 0.375 | 0.715 | 0.867 | 0.520 | 0.488 |
| Kidney | 1.96 | 2.40 | 1.09 | 1.05 | 3.18 | 2.93 | 1.17 | 0.937 |
| Liver | 1.49 | 1.84 | 0.962 | 0.852 | 1.84 | 1.92 | 1.25 | 0.770 |
| Lung | 0.542 | 0.708 | 0.578 | 0.587 | 1.19 | 1.39 | 0.838 | 0.724 |
| Muscle | 0.207 | 0.268 | 0.130 | 0.149 | 0.338 | 0.391 | 0.133 | 0.103 |
| Spleen | 0.378 | 0.851 | 0.533 | 0.539 | 0.681 | 0.787 | 0.903 | 0.839 |
| Thyroid/parathyroid | 0.604 | 0.994 | 0.501 | 0.575 | 0.951 | 1.14 | 0.551 | 0.605 |
| **HIGH DOSE** | **MALES** | | | | **FEMALES** | | | |
| TISSUE/Sacrifice time [hours] | 2 | 4 | 24 | 48 | 2 | 4 | 24 | 48 |
| Whole Blood | 7.39 | 9.38 | 16.9 | 14.3 | 11.9 | 16.3 | 24.9 | 22.1 |
| Plasma | 6.09 | 5.27 | 7.20 | 3.09 | 8.86 | 7.25 | 6.38 | 3.73 |
| RBC | 9.83 | 15.3 | 27.3 | 27.5 | 14.9 | 24.2 | 45.6 | 42.1 |
| Adrenals | 3.97 | 3.77 | 5.48 | 3.55 | 5.52 | 5.82 | 5.00 | 5.70 |
| Bone | 1.94 | 2.30 | 2.98 | 1.77 | 2.30 | 3.26 | 2.60 | 2.02 |
| Brain | 1.39 | 1.79 | 1.83 | 1.14 | 2.35 | 1.80 | 1.72 | 1.38 |
| Carcass | 2.16 | 2.67 | 2.61 | 2.02 | 3.61 | 2.87 | 3.34 | 2.55 |
| Fat | 0.793 | 0.699 | 0.978 | 1.10 | 3.08 | 0.976 | 0.545 | 1.23 |
| GIT | 19.0 | 17.8 | 5.51 | 2.68 | 19.7 | 18.7 | 5.28 | 3.36 |
| GIT wash | 24.1 | 25.1 | 2.51 | 1.26 | 10.1 | 11.0 | 3.31 | 0.860 |
| Gonads | 1.60 | 1.67 | 3.07 | 1.70 | 3.92 | 3.15 | 3.07 | 3.65 |
| Heart | 2.22 | 2.45 | 3.24 | 2.96 | 3.40 | 2.81 | 3.45 | 3.44 |
| Kidney | 9.88 | 7.42 | 6.06 | 4.11 | 13.5 | 11.4 | 6.20 | 5.89 |
| Liver | 7.44 | 7.65 | 8.28 | 5.33 | 9.89 | 8.78 | 8.81 | 6.74 |
| Lung | 3.52 | 3.84 | 4.95 | 3.68 | 5.66 | 6.27 | 6.30 | 6.15 |
| Muscle | 1.21 | 1.53 | 1.51 | 0.892 | 1.78 | 1.31 | 1.05 | 0.768 |
| Spleen | 2.00 | 2.83 | 4.59 | 3.44 | 2.38 | 3.68 | 6.14 | 5.66 |
| Thyroid/parathyroid | 3.31 | 3.33 | 4.28 | 3.39 | 4.52 | 3.62 | 3.52 | 4.45 |

Table 14. Ratio of $^{14}C$ in RBCs:Whole Blood and RBCs:Plasma

| Time (hours)/ Sex/Dose | MALES | | FEMALES | |
|---|---|---|---|---|
| | 2 mg/kg | 16 mg/kg | 2 mg/kg | 16 mg/kg |
| RBCs:Whole Blood | | | | |
| 0.5/24 | 2.04 | 1.33 | 1.21 | 1.25 |
| 1/44 | 1.48 | 1.63 | 1.36 | 1.48 |
| 24 | 1.96 | 1.62 | 1.88 | 1.83 |
| 48 | 1.97 | 1.92 | 1.94 | 1.90 |
| RBCs:Plasma | | | | |
| 0.5/24 | 2.4 | 1.61 | 1.60 | 1.68 |
| 1/44 | 2.13 | 2.9 | 2.04 | 3.34 |
| 24 | 11.8 | 3.79 | 11.35 | 7.15 |
| 48 | 12.4 | 8.90 | 18.66 | 11.29 |

♦ low dose time/high dose time

5. Characterization of Biotransformation Products of Thiodicarb in RBCs, Urine, Liver, and Kidney: The report stated that the major metabolite in the organosoluble fraction of the urine, which constituted 1.31% to 6.56% of the total urinary radioactivity, was the volatile material [one-fourth to one-half of the organosoluble urinary $^{14}C$] condensed by the dry ice trap and identified previously by Andrawes and Bailey as acetonitrile. The major nonvolatile metabolites had the same retention times as methomyl, methomyl oxime, methomyl sulfoxide, and methomyl oxime sulfoxide and constituted ≤ 1% of the urinary radiolabel. The major nonvolatile metabolite was unidentified [$U_1$], which in absolute terms constituted only ≈1% of the urinary $^{14}C$, and no further efforts to identify $U_2$ were made. Hydrolysis of the aqueous layer that remained after extraction by B-glucuronidase-sulfatase resulted in only a small percentage of additional $^{14}C$ being extracted into organic solvents, and it was determined that any material formed was a result of the decomposition of a nonconjugated polar metabolite rather than hydrolysis of a small amount of conjugated metabolite. Water-soluble metabolites constituted 86.3% to 102% of the urinary $^{14}C$. Three peaks were resolved following injection of extracted urine onto HPLC System 2: $U_2$, $U_3$, and $U_4$, the latter being the major metabolite, which represented ≈half of the urinary radiolabel. It decomposed to ≈50% materials that were not condensed by dry-ice acetone, 25% volatiles condensed by dry-ice acetone [presumably acetonitrile], and 25% nonvolatile residues. No conjugated metabolites were found. Tissue data suggest that Thiodicarb in tissues is broken down to one or two carbon fragments, which are incorporated into natural products. The RBCs show a different picture. No radioactivity was removed from the RBCs by extraction with organic solvents, and less that 15% was found in the aqueous fraction. Since all of the radiolabel remained bound to the solid RBC residue, it was concluded that the radiolabeled carbon fragments were incorporated into natural products or tightly bound to the hemoglobin.

D. DISCUSSION

PHASE I - The doses were selected to provide one dose at which no overt toxicity would be observed and one where overt toxicity but no deaths would be observed. Males at both dose levels displayed a more rapid elimination of radiolabel via the urine than did the females initially, but by 24 hours post dose, comparable amounts had been excreted by this route by both sexes. In whole blood, the $t_{max}$ and $C_{max}$ were dose-dependent, with $C_{max}$ being attained at 8 hours ♂♂/12 hours ♀♀ at the low dose and at 24 hours at the high dose [both sexes]. The rate of elimination from whole

blood was slow but not sex-dependent. In the RBCs, concentrations of radiolabel exceeded those in whole blood, and the $C_{max}$ was reached over a prolonged time period [$t_{max}$ of 24 hours] at both dose levels and in both sexes. The author stated that the relatively long time [compared to the plasma] required for the RBCs to reach $C_{max}$ implies that the radiolabel may represent a product of metabolism of Thiodicarb and not Thiodicarb or Methomyl. In plasma, the $C_{max}$ occurred at 1 hour post dose [low dose] and at 4 hours post dose [high dose]. There was a rapid elimination of $^{14}C$ after the peak plasma concentration, which was followed by a slower phase. There were no sex differences observed in plasma. The data indicate that most of the whole blood radiolabel is in the RBCs rather than in the plasma. The rate of elimination of radiolabel from the RBCs was very different from that of the plasma, and the plasma was used to judge the time of maximum tissue concentrations and the sacrifice times to be used in PHASE III of the study.

**PHASE II** - Overall average recovery of radiolabel was 88%. It is stated that the consistency of the data suggests a systematic loss of radioactivity, and the loss as acetonitrile was considered a reasonable explanation. Additionally, since only a sample of the blood was counted and the total weight of the blood was not determined, this may account for some of the apparent loss. The major elimination route was as gaseous products [$CO_2$ and acetonitrile], with the urine being the next important route, and ≤ 15% was found in the feces. The majority of the dose was eliminated within 24 hours, and the RBCs contained the majority of the dose. The author indicated that most of the radiolabel in the tissues was associated with organs of detoxification [liver], removal of damaged RBCs [spleen and liver], and elimination [kidneys and lungs]. After 7 days, ≈8% of the dose remained in the tissues.

**PHASE III** - It was concluded that Thiodicarb is extensively metabolized to $CO_2$ and acetonitrile, which can be further metabolized to intermediates in the body's C-2 and C-1 pool that interact with, or can be incorporated into natural products and ultimately be widely distributed in the body. It was noted also that the finding of the highest levels of radiolabel in the GIT, liver, and kidneys is not unexpected since exposure was <u>via</u> the oral route, there was extensive metabolism, and urine was a primary excretion route. It was stated that the high concentrations of radiolabel in the RBCs are not understood.

**AMENDMENT NO. 1** - The objective of this part of the study was to characterize further the $^{14}C$ metabolites of Thiodicarb found in the RBCs, urine, and tissues. The major metabolites of Thiodicarb in the rat following oral doses of either 2 or 16 mg/kg [single dose] are carbon dioxide and acetonitrile. The major urinary metabolite, which decomposed to ≈50% materials that were not condensed by dry ice/acetone, 25% volatiles that were condensed by dry ice/acetone, and 25% nonvolatile residues, is reported to be a labile unknown, representing about one-half of the urinary radiolabel. The tissue residues were reported to consist mostly of water-soluble materials and some insoluble residue. Hydrolysis of the residue resulted in solubilization of the residue in water but not in the formation of discrete metabolites. Since the RBCs contained only residue that could not be extracted by organic solvents or water, it was concluded that the radiolabel had been incorporated into natural products or was tightly bound to hemoglobin. No acetamide was detected in any of the tissues. NOTE: The author stated that there were no apparent differences between the sexes, but this reviewer noted large differences

in some of the values reported. For example, in Table 3 [Distribution of Nonvolatile Urinary Radioactivity; page 23 of the report], females show a ≈2-13 fold increase in Thiodicarb/metabolites compared to the males [see Table 15 below]. In Table 2, page 22 of the report, for collection times that are the same for each sex, the following differences are noted [see Table 16 below]:

Table 15. Distribution of Nonvolatile Urinary Radioactivity [% total urinary activity]

| Metabolite | MALES | FEMALES |
|---|---|---|
| Thiodicarb | 0.02 | 0.04 |
| Methomyl | 0.12 | 1.22 |
| Methomyloxime | 0.32 | 0.91 |
| $U_1$ | 0.16 | 2.15 |
| Methomyl sulfoxide and Methoxyl oxime sulfoxide | 0.29 | 0.34 |

Table 16. Distribution of Radioactivity in Dichloromethane Extracts of Urine

| Medium/collection time (hrs) | MALES | FEMALES |
|---|---|---|
| Volatiles | | |
| 0-6 | 27.8 | 54.8 |
| 6-12 | 44.0 | 26.2 |
| Residues | | |
| 0-6 | 82.4 | 38.6 |
| 6-12 | 28.2 | 97.6 |

E. CONCLUSION

The absorption, distribution, elimination, and biotransformation of radiolabeled Thiodicarb were determined in rats following the administration of single oral doses of 2 or 16 mg [Acetyl-1-$^{14}$C] Thiodicarb/kg. The $C_{max}$ and $t_{max}$ in whole blood were dose-dependent and $^{14}$C concentrations increased rapidly during the first hour and then more slowly thereafter. The $C_{max}$ at the low-dose was 8 hours [♂♂]/12 hours [♀♀] and 24 hours at the high dose [both sexes]. The rate of elimination from whole blood was slow in both sexes. In RBCs, $^{14}$C concentrations were greater than those in whole blood at both dose levels, and the $C_{max}$ was reached over a prolonged time period [$t_{max}$ = 24 hours]. In plasma, the $C_{max}$ occurred at 1 hour at the low dose and 4 hours at the high dose. There was a rapid elimination after peak plasma concentration was reached and then a slower phase. By 24 hours post dose, 85-90% of the $^{14}$C that would be eliminated via the urine in 48 hours had been eliminated. Most of the $^{14}$C in the whole blood was in the RBCs rather than in the plasma. The relatively prolonged time required for the RBCs to attain $C_{max}$ relative to the plasma indicates that the $^{14}$C may represent a product of metabolism of Thiodicarb and not Thiodicarb or Methomyl. Additionally, the differences in the terminal elimination phase rates for RBCs and plasma indicate that the $^{14}$C in the RBCs is tightly bound and not in rapid equilibrium with the plasma. The overall recovery of $^{14}$C was ≥ 85.8%. The highest levels of $^{14}$C were observed in the urine, followed by those in the $CO_2$ trap and Acetonitrile trap. The conversion of Thiodicarb to $CO_2$ was rapid, with 89-95% being eliminated by 24 hours. All tissues monitored had measurable amounts of $^{14}$C. The major metabolite in the organosoluble fraction of the urine was identified as acetonitrile, and the major nonvolatile metabolites had the same retention times as Methomyl, Methomyl oxime, Methomyl sulfoxide, and Methomyl oxime sulfoxide and constituted ≈1% of

the urinary $^{14}C$. The major nonvolatile metabolite was unidentified [$U_1$], which constituted ≈1% of the urinary $^{14}C$ and no further efforts were made to identify it. Water-soluble metabolites constituted 86-102% of the urinary $^{14}C$, and one of these [$U_4$] represented about half of the urinary label. $U_4$ decomposed to ≈50% materials not condensed by dry-ice acetone, 25% volatiles condensed by dry-ice acetone [presumable acetonitrile], and 25% nonvolatiles residues. No conjugated metabolites were found. Thiodicarb appears to be broken down in tissues to one or two carbon fragments, which are incorporated into natural products. No $^{14}C$ was removed from the RBCs by extraction with organic solvents, and less than 15% was found in the aqueous fraction. Since the radiolabel remained bound to the solid RBC residue, it appears that $^{14}C$ carbon fragments are incorporated into natural products or tightly bound to the hemoglobin. No acetamide was detected in any of the tissues. The proposed metabolic pathway for Thiodicarb is appended.

Thiodicarb

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Pages 23 through 47 are not included.

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