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

SEP 29 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carbaryl: Review of a Rat Metabolism Study Submitted by the Registrant.

P.C. Code: 056801
Submission: S472242
MRID No: 433321-01
DP Barcode: D206775

FROM: Timothy F. McMahon, Ph.D., Pharmacologist 9/27
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

TO: Linda Propst / PM 73
Special Review and Reregistration Division (7508W)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

and

Marcia Van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (7509C) 9/28/94

Registrant: Rhone Poulenc Ag Company

Action Requested: Review of a rat metabolism study submitted by the registrant for carbaryl. Address metabolism issues raised by the Health Effects Division Carcinogenicity Peer Review Committee.

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Summary:

A study entitled, "Metabolism of ^{14}C Carbaryl in Rats(Preliminary and Definitive Phases)" was submitted for review (MRID # 433321-01) in response to the carbaryl Data Call-in.

In this study, ^{14}C-Carbaryl was administered orally in carboxymethylcellulose or intravenously in sodium phosphate buffer (pH 6.8) to groups (5 sex/dose) of male and female Sprague-Dawley rats at nominal doses of 1 mg/kg (single and repeated low oral doses; intravenous dose) and 50 mg/kg (single high oral dose).
Absorption was essentially complete for all dose groups and for male and female rats. At 168 hours post-dose, there were negligible percentages of th dose found in any tissue examined. On a µg/g tissue basis, kidney and blood were found to contain the highest concentrations of residual radioactivity, with female rats showing slightly higher values than males. Excretion of carbaryl derived radioactivity was largely through urine, where 88-95% of th dose was recovered for all dose groups. There were no significant dose- or sex-related differences in excretion.

Conjugated metabolites of carbaryl identified in this study included the glucuronic acid conjugate of dihydro-dihydroxy carbaryl (2.2% of the dose), the S(N-acetylcysteine) conjugate of dihydro-hydroxy carbaryl (3.7% of the dose), naphthyl glucuronide (2.0% of the dose), and naphthyl sulfate (6.4% o the dose). Non-conjugated metabolites identified were 1-naphthol, 5-hydroxycarbaryl, 5,6-dihydro-5,6-dihydroxycarbaryl, 4-hydroxycarbaryl, and N (hydroxymethyl)-hydroxycarbaryl. These accounted for 14.5%, 12.8%, 8.2%, 6.3%, and 5.7% of the administered dose, respectively. Three new urinary metabolites were identified in this study which were the N-(hydroxymethyl)-hydroxycarbaryl metabolite, hydroxy-desmethylcarbaryl (0.5% of the dose), and the S-(N-acetylcysteinyl)-dihydro-dihydroxycarbaryl conjugate. Fecal metabolites identified included 5,6-dihydro-5,6-dihydroxycarbaryl, which accounted for approximately 0.82% of the dose, and parent carbaryl, which accounted for approximately 0.15% of the dose. Based on these data, a metabolic scheme for carbaryl was proposed.

Classification: minimum

This study satisfies the guideline requirement for a metabolism study in rat (§85-1).

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Peer Review of Carbaryl

In the May 12, 1994 Peer Review Document on Carbaryl, metabolism studies wer requested which would provide information on the dose-dependent metabolism o carbaryl. Specifically, studies demonstrating saturation of metabolic pathways and any subsequent alteration in metabolic profile were requested. This request was reiterated in the September 19, 1994 DCI Notice for Carbary (i.e. identification of doses of carbaryl at which metabolic saturation or pathway shift occurs and possible epoxide formation from carbaryl metabolism).

The present study, while fulfilling the §85-1 guideline requirement, provide no data to fulfill the request made by the Peer Review Committee and the subsequent DCI notice. There was no alteration in the metabolic profile observed at any dose level tested in the present metabolism study, suggestir that saturation of metabolism was not achieved. The registrant is required t submit the additional data addressing the issues raised by the Peer Review Committee and in the DCI notice.

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Reviewed by: Timothy F. McMahon, Ph.D. 9/27/94
Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 9/27/94
Section I, Toxicology Branch II (7509C)

Data Evaluation Record

Study type: Metabolism (85-1)

EPA identification numbers: EPA MRID numbers: 433321-01
Submission: S472242
DP Barcode: D206775
P.C. Code: 056801

Laboratory Project ID: RP Ag. Study No. EC-92-222

Test materials: 1-naphthyl [1- ^{14}C] N-methylcarbamate

Other names: ^{14}C-Carbaryl

Testing Facilities: Hazleton Wisconsin, Inc.

Sponsor: Rhone Poulenc Ag Company, Research Triangle Park, N.C.

Title of report: Metabolism of 14-C Carbaryl in Rats (Preliminary and Definitive Phases)

Author(s): Craig B. Struble, Ph.D.

Report issued: August 5, 1994

Executive Summary:

In a rat metabolism study (MRID # 433321-01), ^{14}C-Carbaryl was administered orally in carboxymethylcellulose or intravenously in sodium phosphate buffer (pH 6.8) to groups (5 sex/dose) of male and female Sprague-Dawley rats at nominal doses of 1 mg/kg (single and repeated low oral doses; intravenous dose) and 50 mg/kg (single high oral dose).
Absorption was essentially complete for all dose groups of male and female rats. At 168 hours post-dose, there were negligible percentages of the dose found in any tissue examined. On a µg/g tissue basis, kidney and blood were found to contain the highest concentrations of residual radioactivity, with female rats showing slightly higher values than males. Excretion of carbaryl-derived radioactivity was largely through urine, where 88-95% of the dose was recovered for all dose groups. There were no significant dose- or sex-related differences in excretion.

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Conjugated metabolites of carbaryl identified in this study included the glucuronic acid conjugate of dihydro-dihydroxy carbaryl (2.2% of the dose), the S(N-acetylcysteine) conjugate of dihydro-hydroxy carbaryl (3.7% of the dose), naphthyl glucuronide (2.0% of the dose), and naphthyl sulfate (6.4% o the dose). Non-conjugated metabolites identified were 1-naphthol, 5-hydroxycarbaryl, 5,6-dihydro-5,6-dihydroxycarbaryl, 4-hydroxycarbaryl, and N (hydroxymethyl)-hydroxycarbaryl. These accounted for 14.5%, 12.8%, 8.2%, 6.3%, and 5.7% of the administered dose, respectively. Three new metabolites were identified in this study which were the N-(hydroxymethyl)-hydroxycarbaryl metabolite, hydroxy-desmethylcarbaryl (0.5% of the dose), and the S-(N-acetylcysteinyl)-dihydro-dihydroxycarbaryl conjugate. Based on these data, a metabolic scheme for carbaryl was proposed.

Core Classification: minimum

This study satisfies the data requirements for a metabolism study in rats under Subdivision F guideline §85-1.

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MATERIALS

A. Test Materials

[1]: 1-naphthyl [1-^{14}C] N-methylcarbamate
Lot no: CSL-92-360-5-31 Source: Chemsyn Science Laboratories
Batch No. IHR 1465
Radiochemical Purity: > 98.0%
Specific Activity: 22.04 mCi/mmol

[2]: Unlabelled carbaryl
Lot no: 85068 Source: Rhone-Poulenc Ag Company
Chemical purity: 99.8%

[3]: Reference standards:

1) 4-hydroxycarbaryl, purity 96.1%
2) 3-hydroxycarbaryl, purity 99.5%
3) 1-naphthol, purity 99.8%
4) N-(hydroxymethyl)carbaryl, purity 83.6%
5) 5-hydroxycarbaryl, purity 99.6%
6) ß-naphthyl alpha-D-glucopyranoside, purity >99.0%
7) alpha-naphthyl-ß-D-glucuronide, sodium salt, purity >99.0
8) 5,6-dihydro-5,6-dihydroxycarbaryl, purity ~ 95.0%
9) 5,6-dihydro-5,6-dihydroxy-1-naphthol, purity 98.5%
10) desmethylcarbaryl, purity ~95.0%
11) alpha-naphthyl sulfate, purity > 99.0%
12) ß-naphthyl sulfate, potassium salt, purity ~ 99.0%
13) 1,4-naphthoquinone, purity 94.7%
14) S-(5-hydroxynaphthyl)cysteine, purity 85%
15) S-(4-hydroxynaphthyl)cysteine, purity 95%
16) 5-methoxy-6-hydroxycarbaryl, purity 95%
17) 7-hydroxycarbaryl, purity 100%
18) 1,5-dihydroxynaphthalene, purity 89.8%

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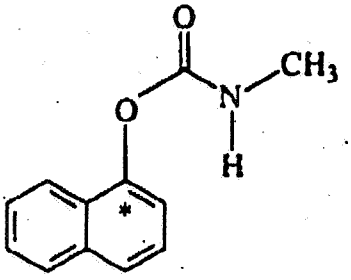
Structure of radiolabelled carbaryl:(* indicates position of label)



B. Vehicles: aqueous methylcellulose (1.0% w/v); sodium phosphate buffer (pH 6.8) containing 5% ethanol.

C. Test Animals: Species: rat
Strain: HSD:SD
Source: Harlan Sprague-Dawley, Inc.
Age: approximately 4-8 weeks on arrival
Weights (mean and range):

Dose groups (Definitive Study)

	males	females
Low Intravenous Dose (Group A)	190.4g (184-197g)	197.8 (191-206g)
Low Oral Dose (Group B)	200.8 (195-208g)	182.9 (166-196g)
Mult. Low Dose (Group C)	197.8 (193-206g)	190.6 (177-201g)
High Dose (Group D)	209.8 (200-224g)	200.8 (176-226g)

II. METHODS

A. Study Design

A total of 44 male and 44 female rats were assigned to this study. Study design was as follows:

Group	Phase	Dose Level (mg/kg)	Route	No. Animals Males	Females
P	Preliminary	1	Oral[a]	2	2
A	Definitive	1	IV	5	5
B	Definitive	1	Oral	5	5
C	Definitive	1	Oral[b]	5	5
D	Definitive	50	Oral	5	5

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[a]single oral dose
[b]14-day repeated nonradiolabelled dose, followed by a single radiolabelled dose.

Results of the preliminary oral dose experiment indicated that collection of expired carbon dioxide and organic volatiles was not necessary for the definitive experiment. Originally, the high dose was selected as 100 mg/kg, but at this dose, severe toxicological effects were observed. Thus, the high dose was lowered to 50 mg/kg.

B. Experimental

a. Animal Husbandry

Rats were acclimated for 7 days prior to use, during which health status was monitored. Rats were also observed at least twice daily for moribundity, mortality, or toxicity. During acclimation and conditioning with the non-radiolabelled dose (Group C), rats were housed individually in stainless steel, wire mesh, screen bottom cages suspended on racks with absorbent paper liners. During the preliminary test, rats used were housed in all glass metabolism cages for collection of carbon dioxide and volatiles. During the definitive phase of the study, rats were housed individually in Nalgene metabolism cages designed for separation and collection of urine and feces for all groups. Animal room temperature was maintained at 19-25 ^{o}C with a relative humidity of 50±20% and a 12 hour light/dark cycle. Food (Certified Rodent Chow # 5002) and tap water were provided *ad libitum*, except for an overnight fast prior to oral dosing through approximately 4 hours post-dose.

b. Dosing

Doses received in this study are as follows:

Group	Labelled Material (mg)	Nonradiolabelled Material (mg)	Volume Vehicle (ml)	Dose Conc. (mg/ml)
P	1.80	0	9	0.20
A	3.48	0	14	0.25
B/C	7.50	0	37.5	0.20
C (cold)	0	4.03	20	0.20
D	5.14	182.5	18.75	10.0

Actual doses received by the various groups were stated on page 14 of the report. These are: Single intravenous dose (**1.02 mg/kg**); Single oral low dose (**1.21 mg/kg**); Repeated low oral dose (**1.21 mg/kg**); Single high oral dose (**48.0 mg/kg**).

Radiolabelled dose solutions were prepared and analyzed for concentration an homogeneity before dosing. All dose solutions were prepared 24 hours before dosing and were refrigerated until use. Concentration of radiolabelled dosin solutions were determined by radioanalysis of predose and postdose aliquots. Concentration of non-radiolabelled dose solutions was determined by HPLC jus prior to and following the treatment period.

Each intravenous dose was administered into the tail vein with a disposable syringe and a stainless steel needle. Prior to removing the needle from the tail vein, a gauze pad was placed over the dose site, slight pressure applied, and the needle removed. Each oral dose was administered via gavage with a disposable syringe and a stainless steel ball-tipped needle. Dose amount was based on individual body weight. Actual dose administered was determined by weighing the syringe before and after dosing.

c. Sample Collection and Analysis

Rats in groups A to C were placed in individual all-glass metabolism cages for collection of urine and feces following dosing. Containers for urine and feces collection were surrounded by ice. Collection times for urine and feces were stated as: 0-6, 6-12, 12-24 hours post-dose, and then daily thereafter until 7 days post-dose. Cages were rinsed with water after the 24 hour collection time point, and were rinsed with a 1% trisodium phosphate solution after the last collection time point.

Urine

A group composite urine sample was prepared by combining proportional aliquots of urine from collection intervals that contained > 95% of the urinary radioactivity for each sex/group. Samples were mixed and duplicate aliquots were analyzed directly by liquid scintillation counting. An aqueous extract of urine was treated with a combination of ß-glucuronidase/aryl sulfatase solution (*Helix pomatia* juice; 338,000 units ß-glucuronidase/g; 16,600 units aryl sulfatase/g). Approximately 46 ml 0.1 M sodium acetate buffer (pH 4.6) was added to approximately 45 ml aqueous urine extract. Enzyme activity was demonstrated by the use of two positive control samples (0.1ml substrate solution in 1 ml water or 1ml control urine) and one negative control (0.1ml substrate solution in 1ml of water) followed by HPLC analysis. Samples were incubated at 37 ^{o}C for 18 hours. Metabolites in urine were characterized and identified by the use of either 2D-TLC, HPLC, or mass spectrometry. A scheme for the isolation of

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urinary metabolites was presented in the report (Figures 9, 10, 11, 20, 24, and 25). These figures are attached to this review for clarification of the procedures used in urinary metabolite identification.

Feces

Fecal samples were homogenized with a measured amount of deionized water (approx. 2-3x the weight of the feces). Duplicate aliquots (approx. 0.2g) were combusted and analyzed by LSC. For metabolite analysis, composite samples were prepared for males and females for the 0-48 hour collection period. Approximately half of each composite sample was extracted 3 times with methanol (150ml). Duplicate aliquots were analyzed by LSC, while duplicate aliquots of the residue were also analyzed by LSC. A combination c TLC, HPLC, and mass spectrometry was used in the identification of fecal metabolites. Figure 66 of the report is attached to this review for clarification of the procedures used in fecal metabolite identification.

Tissues

At sacrifice, the following tissues were collected, rinsed with water, blotted dry, weighed, and processed for radioanalysis:

bone (femur)	
brain	fat (reproductive area)
testes	heart
kidney	liver
muscle	pancreas
spleen	lung
uterus	residual carcass

Tissues were analyzed for total radioactivity only.

D. Compliance

A signed statement of No Data Confidentiality claims was provided.

A signed statement of GLP compliance was provided.This study was conducted compliance with 40 CFR 160.35, U.S. Environmental Protection Agency Pesticic Programs Good Laboratory Practice.

A signed statement of quality assurance was provided.

III. RESULTS

1. Dose Solution Stability and Doses Received

Appendix D of the report, pages 217-219, demonstrated the stability of 14-C carbaryl in a 1% aqueous methylcellulose solution for at least one week. Appendix E, pages 220-221 of the report, presented verification of the radiolabelled doses received by each dose group. Appendix F, pages 222-223, presented results of non-radiolabelled test material analysis as formulated for the repeat dose group. These data showed that the non-radiolabelled dose corresponded to the nominal dose for the duration of the repeat dose study.

2. Validation of Radioassay Procedures

Appendix F, pages 224-225 of the report, showed that the mean recovery of radioactivity from fortified samples of blood, fat, feces, kidney, muscle, and urine was 98.1% (95.2-100% range of recovery). Therefore, the values obtained from analysis of samples were not corrected for recovery, as the recovery was acceptable for all matrices.

3. Absorption

Summary results of intravenous data were presented in Tables 9, 13, and 1 of the report, pages 72, 76, and 77. When compared to the data for the sing low oral dose group (Table 10, page 73 of the report), it is observed that there was no significant difference in the percentage of the dose excreted either urine or feces, or that remaining in tissues. Thus, it can be concluded that at this dose, carbaryl was completely absorbed when given as an oral bolus.

	% Dose Excreted	
Group	Males	Females
A (IV dose)		
urine	90.0	88.6
feces	10.2	8.71
B (Oral dose)		
urine	92.1	91.5
feces	9.06	8.40

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In addition, the time course of excretion for both the low oral dose group (Tables 15 and 16, pages 78-79) and the intravenous dose group showed that the majority of radioactivity was eliminated in urine within the first 24 hours post-dose for both groups. Examination of the repeated low oral dose data for the time course of excretion (Tables 17 and 18, pages 80-81 of the report) showed no significant effect on the time course of excretion as compared to the single low dose. Examination of the single high dose data (Tables 19-20, pages 82-83 of the report) indicated that excretion might be saturated at this dose, as the percentages found in urine during the first 2 hours were more equivalent to each other. In addition, a more significant component of urinary radioactivity was observed between 24-48 hours post-dos vs the single or repeated low dose.

2. Distribution

Distribution data were found in Tables 21-28, pages 84-91 of the report. These data presented the amount of residual radioactivity in the tissues examined from all dose groups at 168 hours post-dose (Tables 21-24), as wel as the concentration of residual radioactivity (µg equivalents/g tissue; Tables 25-28). As a percentage of the dose administered, residual carcass wa found to be the only tissue examined which contained any significant amount of residual radioactivity, but this was less than 1% of the administered dos in all dose groups.

While all tissues examined showed negligible percentages of residual radioactivity, examination of the data showing residual radioactivity on a µg/g tissue basis showed that in all dose groups, the kidney and blood show the highest concentration of radioactivity in relation to the other tissues examined within any given dose group. These data are shown below:

Table 1

Concentration of ^{14}C-Labeled Carbaryl Derived Radioactivity in Male and Female Rats (µg equivalents/g tissue)

	IVM	IVF	LDM	LDF	RDM	RDF	HDM	HDF
kidney	0.003± 0.0004	0.006± 0.0005	0.002± 0.0005	0.004± 0.0005	0.004± 0.0013	0.005± 0.0022	0.188± 0.035	0.333 0.040
blood	0.002± 0.0	0.003± 0.0	0.002± 0.0	0.003± 0.0005	0.002± 0.0	0.003± 0.0007	0.103± 0.0065	0.170± 0.025

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3. Excretion

The excretion of ^{14}C-labeled Carbaryl in urine and feces at the low and high dose levels in this study (1 mg/kg and 50 mg/kg) is summarized below for male and female rats. Data were obtained from Tables 9-12, pages 72-75 of the report.

Table 2

Excretion of ^{14}C-Labeled Carbaryl Derived Radioactivity in Male and Female Rats[a]

	LDM	LDF	RDM	RDF	HDM	HDF	IVM	IVF
urine[b]	92.1± 1.35	91.5± 2.01	95.0± 2.39	95.0± 1.10	84.5± 2.69	88.2± 2.75	90.0± 2.9	88.6 2.0
feces	9.06± 1.15	8.40± 1.56	8.57± 0.99	7.68± 0.78	12.5± 2.05	6.98± 1.22	10.2± 2.65	8.71 3.43
tissues[c]	0.1± 0.045	0.24± 0.106	0.15± 0.075	0.22± 0.115	0.61± 0.36	0.91± 0.68	0.14± 0.04	0.36 0.2
Total	101± 0.9	100± 0.9	104± 1.9	103± 0.8	97.6± 2.50	96.1± 1.40	100± 1.4	97.7 1.56

[a]data represent the mean percent dose excreted at 168 hours post-dose for all dose groups. Abbreviations used are : LD, 1 mg/kg single oral low dose; RD, repeated low oral dose of 1 mg/kg; HD, single high oral dose of 50 mg/kg IV, single intravenous dose of 1 mg/kg.

[b]includes cage rinse, cage wash, and cage wipe.

[c]includes blood and carcass

Urine represented the major route of excretion for carbaryl derived radioactivity in all dose groups, with between 88-95% of the administered radioactivity excreted by this route. There were no significant differences between males and females in the percentage of radioactivity excreted by urine or feces, and no significant effects of repeated oral dosing or a single high dose on the total percentage excreted by urine or feces. Examination of the time course of excretion between the single low dose (Tables 15 and 16, pages 78-79 of the report) and the time course for the single high oral dose (Tables 19-20, pages 82-83 of the report), shows that at the high dose, a larger percentage was excreted in urine between 12-24 hours post-dose than at the low dose, indicating a possible saturation of excretion at the high dose.

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d. Plasma Levels of 14-C Fipronil Derived Radioactivity

Pharmacokinetic parameters were not examined in this study, but were not required for fulfillment of the guideline requirement.

2) Metabolite Characterization and Identification

a) Urinary Metabolites

Composite urine samples were subjected to analysis by 2D-TLC using 2 different solvent systems. Analysis in solvent system 1 (ether:hexane [3:1 methanol:ethyl acetate:water [60:50:5]) indicated that most of the metabolites were polar in nature, as little movement from the solvent fron was observed in the first development, but good mobility was observed in t second development using this system. Using solvent system 2 (ether:acetonitrile [1:1]; methanol:ethyl acetate:water [60:50:5]), eight radioactive areas were observed. Examination of the data from this analysi (Table 31, page 94 of the report; below) showed little difference in the percentage of these eight areas among the various dose groups. Therefore, was decided that further identification would be performed using Group D (high dose) urine from 0-48 hours, as this would provide for a larger quantity (mass) of metabolites for identification. Note: Differences can observed in regions 3 and 4 from solvent system 2 between male and female rats.

Radioactive Area	Percent of Administered Dose							
	Group A		Group B		Group C		Group D	
	Male	Female	Male	Female	Male	Female	Male	Female
Urine Solvent System 1								
1	1.4	1.8	0.6	1.2	2.0	1.3	1.1	1.6
2	15.0	5.9	22.2	9.8	13.9	6.7	15.7	15.0
3	10.6	NF	10.6	NF	12.2	6.1	7.0	17.4
4	9.9	28.5	14.2	28.9	18.2	27.0	13.4	11.1
5	46.8	46.1	40.0	41.3	45.2	43.5	37.1	32.1
Total	83.7	82.3	87.6	81.2	91.5	84.5	74.3	77.2
Urine Solvent System 2								
1	6.7	6.1	4.7	4.1	5.0	6.1	4.0	3.1
2	2.8	2.8	3.0	2.3	3.8	3.1	4.1	NF
3	4.2	5.6	4.1	5.3	5.0	5.4	21.5	6.3
4	29.6	22.4	35.0	27.8	37.7	27.7	14.3	29.6
5	14.4	13.9	17.6	19.3	21.4	18.5	14.5	14.6
6	6.0	7.8	5.3	7.6	3.8	7.2	4.7	10.2
7	9.6	8.6	4.9	11.3	13.0	6.9	9.2	12.1
8	11.8	16.0	10.3	3.4	2.1	9.5	1.9	2.7
Total	86.1	82.2	87.8	81.8	91.8	84.4	74.2	78.8

NF Not found.

In this study, two isolation procedures were employed for urinary identification of carbaryl metabolites. Scheme "A", (figure 9, page 105 o the report) was used to isolate intact conjugated metabolites of carbaryl From group D urine, 37 peaks were detected. Individually, these peaks accounted for between <0.1% to 6.4% of the dose. Four conjugated metaboli were identified by LC/MS in this procedure. These are as follows:

1) glucuronic acid conjugate of dihydro-dihydroxy carbaryl (2.2% of dos
2) S(N-acetylcysteine) conjugate of dihydro-hydroxy carbaryl (3.7% of d
3) naphthyl glucuronide (2.0% of dose)
4) naphthyl sulfate (6.4% of dose)

The percentages for these metabolites were obtained from pages 107 and 11 the report. However, they may not be accurate. For example, the percentag stated for naphthyl sulfate on page 95 (0.9%) does not correspond to that stated on page 116 (6.4%).

The second isolation scheme (Scheme "B") had the objective of isolation a identification of extractable and hydrolyzed urinary metabolites. Identification in this scheme was by mass spectral analysis or TLC and HP co-chromatography with authentic reference standards. Using scheme "B", t initial organic extraction showed few, if any, metabolites in the "free" (unconjugated) state. It is noted in the report that the initial extracti was done within 2 months of the sample collection. The urine was apparent stored frozen for 16 months before further analysis was done. After stora the "free" metabolites accounted for 14.1% of the dose, indicating hydrol during storage.

Using the scheme B isolation procedure, a total of 10 labelled metabolite well as labelled parent chemical were identified. These results were presented in Table 32 of the report, page 95, which is reproduced below:

		Percent of Administered Dose			
			Conjugated Metabolites		
Metabolite Number	Metabolite	Free Metabolites[a]	Enzyme-Hydrolyzed Metabolites[b,d]	Acid-Hydrolyzed Metabolites[c,e]	Total
-	carbaryl	0.2	NF	2.7	2.9
M1	5,6-dihydro-5,6-dihydroxycarbaryl	1.7	6.5	NF	8.2
M2	3,4-dihydro-3,4-dihydroxycarbaryl	0.9	NF	NF	0.9
M3	α-naphthyl sulfate	0.9	NF	NF	0.9
M4	N-(hydroxymethyl)hydroxycarbaryl	1.3	4.4	NF	5.7
M5	1,5-dihydroxynaphthalene	0.3	NF	2.7	3.0
M6	hydroxy-desmethylcarbaryl	NF	NF	0.5	0.5
M7	5-hydroxycarbaryl	4.3	2.1	6.4	12.8
M8	4-hydroxycarbaryl	0.9	3.1	2.3	6.3
M9	1-naphthol	0.3	12.8	1.6	14.5
M10	dimer of 1,4-naphthoquinone[f]	NF	NF	1.5	1.5
Total		10.8	28.7	17.7	57.2

NF - Not found

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The predominant metabolites observed from the analysis of urine for "free" metabolites were 1-naphthol, 5-hydroxycarbaryl, 5,6-dihydro-5,6-dihydroxycarbaryl, 4-hydroxycarbaryl, and N-(hydroxymethyl)-hydroxycarbaryl, accounting for 14.5%, 12.8%, 8.2%, 6.3%, and 5.7% of the administered dose, respectively. Novel metabolites identified in this study included N-(hydroxymethyl)-hydroxycarbaryl and hydroxy-desmethylcarbaryl (0.5% of the dose).

According to the data presented above, "free" metabolites of carbaryl accounted for 14.1% of the administered dose (which may be an exaggeration, due to decomposition in storage), conjugated metabolites accounted for 37.7% of the dose, and acid hydrolyzed metabolites (i.e. those representing a conjugate, but not glucuronic acid or sulfate) accounted for 28.2% of the dose. The totals presented at the bottom of Table 32 from the report indicat some differences in recovery of radioactivity. For the free, enzyme-hydrolyzed, and acid-hydrolyzed metabolites, the differences are approximately 4%, 9%, and 10%, respectively.

b) **Fecal Metabolites**

Extraction of feces yielded a methanol extract, a hexane extract, and a residue for each dose group and sex. The recovery and distribution of the radioactivity were presented in Appendix P, page 446 of the report. These data show that recovery accounted for all of the radioactivity originally excreted by this route for each dose group and sex. The majority of radioactivity in feces was extractable into methanol. HPLC analysis of the methanol extract indicated that for dose groups A-C, there was one major radioactive peak observed at approximately 16 minutes. For group D males and females, radioactive peaks were also observed at 14.7 minutes and 27.9 minutes. Because Group D feces contained all the peaks of interest, it was used for metabolite isolation.

Isolation of fecal metabolites was attempted by TLC, but inconsistent resul were obtained. Because of depletion of the methanol extract from group D males, the metabolite identification in feces was completed using group D female rat feces. The isolation scheme was presented in Figure 66, page 162 of the report. HPLC analysis of the methanol extract resulted in five groups comprising between 0.16-1.19% of the dose. Further HPLC analysis of Group 3 yielded 2 peaks, one of which comprised 0.82% of the dose. This peak was identified by positive ion electrospray MS and co-chromatography as 5,6-dihydro-5,6-dihydroxycarbaryl. The glucuronic acid conjugate of carbaryl ha been previously identified as a major radioactive metabolite in the bile of rats administered 14-C carbaryl. The results of this study are consistent with previous results, in that the glucuronic acid conjugate would be hydrolyzed in the g.i. tract through bacterial action, and would thus appea as the free metabolite in feces.
Group 5 analysis showed the presence of a single peak, accounting for 0.15% of the administered dose. This peak was identified through co-chromatograph as the parent chemical, carbaryl.

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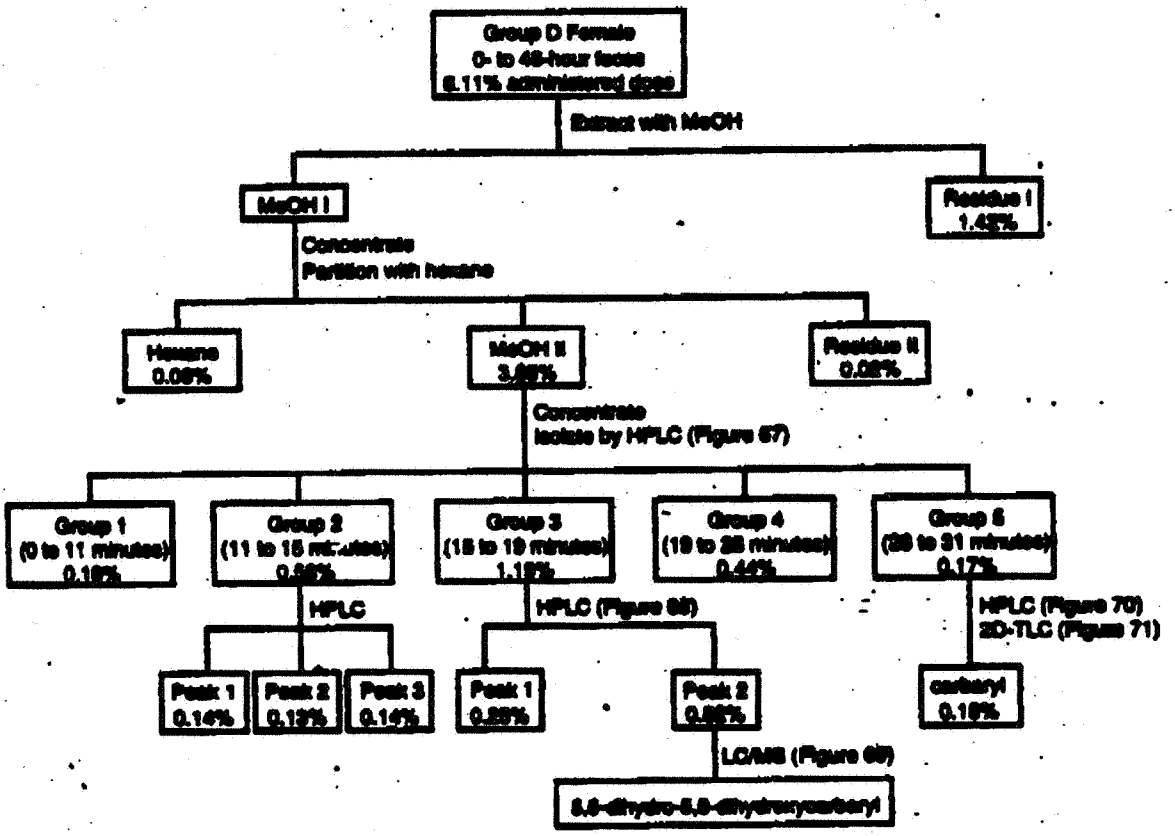
Of interest is the observation that other peaks were observed in the isolation scheme but were not identified. Although they comprised a low percentage of the dose, these peaks were similar in percentage to the parent carbaryl.

Figure 68. Isolation of Metabolites from Female Rat Feces



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IV. DISCUSSION

In this study, the disposition of radiolabelled carbaryl was examined in male and female Sprague-Dawley rats as part of the carbaryl Data Call-In. Groups of 5 rats/sex were administered a single intravenous or oral dose of mg/kg 14-C carbaryl, a single high oral dose of 50 mg/kg 14-C carbaryl, or a repeated low oral dose of 1 mg/kg x 14 days unlabelled carbaryl followed by single radiolabelled dose.

The absorption of carbaryl in this study was essentially complete. When the total recovery of radioactivity in urine, tissues, and residual carcass for the different oral dose groups was divided by the corresponding value fc the intravenous dose group, absorption values of 100% or slightly greater were obtained. This result is consistent with the data on excretion, which did not show any significant differences with dosing regimen.

Distribution data presented in this study (168 hours post-dose) showed that the residual carcass was the only tissue containing any significant amount of radioactivity, but this was less than 1% of the administered dose for all dose groups. The kidney and blood were found to contain the highest concentrations of residual radioactivity when measured on a µg/g tissue basis. Values ranged from 0.002-0.333 µg/g tissue for kidney, and from 0.002 0.170 µg/g tissue for blood. The largest values are from the high dose group In many cases, females showed higher residual levels than males for these 2 organs.

Urine was the major route for excretion of carbaryl-derived radioactivity, accounting for between 84-95% of the administered dose across all dose groups, with feces accounting for approximately 7-12% of the administered dose. Repeated low oral dosing or a single high dose did not result in any significant differences in the excretion pattern for carbaryl in male or female rats. The high dose used, 50 mg/kg, appears to be saturating, insofar as the examination of the time course of urinary elimination of radioactivit at this dose (Tables 18 and 19, pages 81-82 of the report) indicate a saturation of renal excretion during the first 24 hours post-dose. In addition, it was reported (page 26) that administration of 100 mg/kg resulte in significant and severe toxicity, making this dose unacceptable for use ir the main study.

Based on TLC, HPLC, and mass spectral analysis, four conjugated and ten non-conjugated metabolites of carbaryl were identified. The identification was made using high dose urine, as TLC analysis indicated no major differences in the radioactive bands observed from the various dose groups, with the possible exception of bands 3 and 4 from the high dose male and female rats, which appeared to show differences.

The major conjugated metabolites identified in this study were the glucuronic acid conjugate of dihydro-dihydroxy carbaryl (2.2% of the dose), the S(N-acetylcysteine) conjugate of dihydro-hydroxy carbaryl (3.7% of the dose), naphthyl glucuronide (2.0% of the dose), and naphthyl sulfate (6.4% c the dose).

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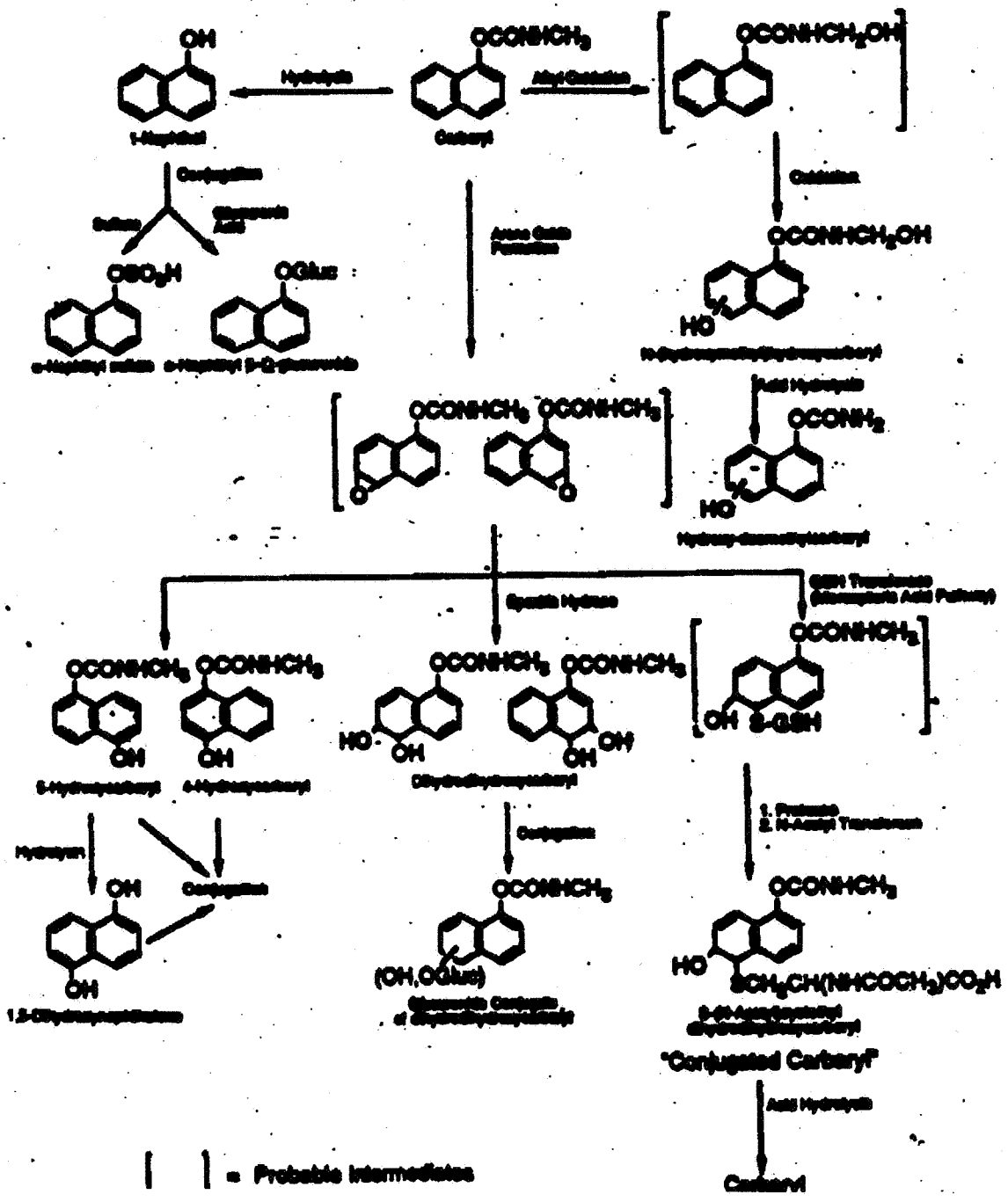
Of the non-conjugated metabolites identified, 1-naphthol, 5-hydroxycarbaryl, 5,6-dihydro-5,6-dihydroxycarbaryl, 4-hydroxycarbaryl, and N-(hydroxymethyl)-hydroxycarbaryl were identified as major metabolites. These accounted for 14.5%, 12.8%, 8.2%, 6.3%, and 5.7% of the administered dose, respectively.

In the analysis of carbaryl metabolites, 3 novel structures were identified. These were the N-(hydroxymethyl)-hydroxycarbaryl metabolite, hydroxy-desmethylcarbaryl (0.5% of the dose), and the S-(N-acetylcysteinyl)-dihydro-dihydroxycarbaryl conjugate. Based on these data, a metabolic scheme for carbaryl was proposed:



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In this scheme, three main metabolic pathways are observed: 1. Arene oxide formation with subsequent metabolism to dihydro-dihydroxycarbaryl and conjugation with glutathione via the mercapturic acid pathway; 2. Carbamate hydrolysis to form 1-naphthol; and 3. Oxidation of the N-methyl moiety (alky oxidation). The subsequent metabolites formed from these pathways can also undergo conjugation with glucuronic acid or sulfate.

CONCLUSIONS

In this study, ^{14}C-Carbaryl was given a a single low and high oral dose (1 and 50 mg/kg), a repeated oral dose (1 mg/kg x 14 days unlabelled carbaryl followed by a single radiolabelled dose), or a single low intravenous dose (mg/kg) to male and female Sprague-Dawley rats. Absorption was essentially complete for all dose groups of male and female rats. At 168 hours post-dose there were negligible percentages of the dose found in any tissue examined. On a µg/g tissue basis, kidney and blood were found to contain the highest concentrations of residual radioactivity, with female rats showing slightly higher values than males. Excretion of carbaryl derived radioactivity was largely through urine, where 88-95% of the dose was recovered for all dose groups. There were no significant dose- or sex-related differences in excretion. Conjugated metabolites of carbaryl identified in this study included the glucuronic acid conjugate of dihydro-dihydroxy carbaryl (2.2% o the dose), the S(N-acetylcysteine) conjugate of dihydro-hydroxy carbaryl (3.7% of the dose), naphthyl glucuronide (2.0% of the dose), and naphthyl sulfate (6.4% of the dose). Non-conjugated metabolites identified were 1-naphthol, 5-hydroxycarbaryl, 5,6-dihydro-5,6-dihydroxycarbaryl, 4-hydroxycarbaryl, and N-(hydroxymethyl)-hydroxycarbaryl. These accounted for 14.5%, 12.8%, 8.2%, 6.3%, and 5.7% of the administered dose, respectively. Three new urinary metabolites were identified in this study which were the N (hydroxymethyl)-hydroxycarbaryl metabolite, hydroxy-desmethylcarbaryl (0.5% of the dose), and the S-(N-acetylcysteinyl)-dihydro-dihydroxycarbaryl conjugate. Fecal metabolites identified included 5,6-dihydro-5,6-dihydroxycarbaryl, which accounted for approximately 0.82% of the dose, and parent carbaryl, which accounted for approximately 0.15% of the dose.

Core Classification: minimum

The data in this study satisfy the data requirements for a metabolism study in rats under Guideline §85-1.

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