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

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JAN 11 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: N-cyclopropyl-1,3,5,-triazine-2,4,6-triamine (Trigard): Review of a Rat Metabolism Study Submitted by the Registrant.

Caswell No: 1678
HED Project No: 0-1076
MRID No: 414421-01

FROM: Timothy F. McMahon, Ph.D., Toxicologist 1/7/91
Review Section I, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

TO: Phil Hutton/PM 17
Registration Division (H7505C)

THRU: Ylannakis M. Ioannou, Ph.D., Section Head J.M. Ioannou 1/9/91
Review Section I, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

and

Marcia Van Gemert, Ph.D., Branch Chief M. Van Gemert 1/9/91
Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

Registrant: Ciba-Geigy Corporation

Action Requested: Review of the following Toxicology study with N-cyclopropyl-1,3,5,-triazine-2,4,6-triamine (Trigard):

Rat Metabolism Study

1728

Conclusions:

In study # HLA 6117-160, the disposition and metabolism of ^{14}C-cyromazine was investigated in male and female rats at a low oral dose (3 mg/kg), a low intravenous dose (3 mg/kg), repeated low oral doses (3mg/kg) and a high dose (300 mg/kg). Comparison of oral and i.v. disposition data demonstrated that cyromazine was well absorbed after oral administration. Excretion was also rapid at the low dose, with the majority of radioactivity eliminated in the urine by 24 hours. No apparent differences were observed between dosing regimens in the total urinary radioactivity eliminated when cage washes and rinses were taken into consideration. At the high dose, however, there was an apparent delay in elimination of ^{14}C-cyromazine derived radioactivity, suggesting either some type of renal pathology or a shift in biotransformation kinetics of cyromazine.

Fecal elimination of ^{14}C-cyromazine derived radioactivity was equivalent between dose groups and sexes, with the exception that in the high dose males, a greater percentage of radioactivity was apparently eliminated by this route. The origin of fecal radioactivity from administration of ^{14}C-cyromazinewas apparently biliary in nature, as an equivalent percentage of ^{14}C-cyromazine derived radioactivity was eliminated by both orally dosed and intravenously dosed rats.

Residual ^{14}C-cyromazine derived radioactivity was minimal in all dose groups, with non-detectable levels in all tissues examined except liver, red blood cells, and carcass.

In study # ABR-89108, urinary and fecal metabolites of ^{14}C-cyromazine were isolated and identified by TLC, HPLC, and GC/MS. The N-dealkylated product melamine, hydroxycyromazine, and unmetabolized cyromazine were definitively identified by GC/MS, while tentative identification was given to methylcyromazine, based upon co-chromatography with TLC and HPLC. A proposed pathway for cyromazine biotransformation was given based upon these data.

Classification:

core minimum

This study satifies the guideline requirements (85-1) for a metabolism study in rats.

Reviewed by: Timothy F. McMahon, Ph.D. 1/9/91
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 1/9/91
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Data Evaluation Report

Study type: Metabolism (85-1) Tox. Chem. No.: 167B

EPA identification numbers:
EPA MRID number: 414421-01
EPA identifying number: 100-654
EPA record numbers: 262, 735
Caswell number: 167B
HED project number: 0-1076

Laboratory Project numbers: ABR-89108; HLA 6117-160

Test material: ^{14}C-Cyromazine

Synonyms: N-cyclopropyl-1,3,5-triazine-2,4,6-triamine; Trigard

Testing Facilities:
Ciba-Geigy Corporation
Metabolism Department
Greensboro, NC 27419

Hazelton Laboratories America, Inc.
Madison, WI 53707

Sponsor:
Ciba-Geigy Corporation
Agricultural Division
410 Swing Road
Greensboro, NC 27419

Title of reports: Laboratory Project no. ABR-89108, "Characerization and Identification of ^{14}C-Cyromazine and Metabolites in Rats;" Laboratory Project no.HLA 6117-160, "Metabolism of ^{14}C-Cyromazine in Rats."

Author(s): Thomas M. Capps

Report issued: ABR-89108: January 1990; HLA 6117-160, October 4, 1989

Conclusions:

In study # HLA 6117-160, the disposition and metabolism of ^{14}C-cyromazine was investigated in male and female rats at a low oral dose (3 mg/kg), a low intravenous dose (3 mg/kg), repeated low oral doses (3mg/kg) and a high dose (300 mg/kg). Comparison of oral and i.v. disposition data demonstrated that cyromazine was well absorbed after oral administration. Excretion was also rapid at the low dose, with the majority of radioactivity eliminated in the urine by 24 hours. No apparent differences were observed between dosing regimens in the total urinary radioactivity eliminated when cage washes and rinses were taken into consideration. At the high dose, however, there was an apparent delay in elimination of ^{14}C-cyromazine derived radioactivity, suggesting either some type of renal pathology or a shift in biotransformation kinetics of cyromazine.

Fecal elimination of ^{14}C-cyromazine derived radioactivity was equivalent between dose groups and sexes, with the exception that in the high dose males, a greater percentage of radioactivity was apparently eliminated by this route. The origin of fecal radioactivity from administration of ^{14}C-cyromazine was apparently biliary in nature, as an equivalent percentage of ^{14}C-cyromazine derived radioactivity was eliminated by both orally dosed and intravenously dosed rats.

Residual ^{14}C-cyromazine derived radioactivity was minimal in all dose groups, with non-detectable levels in all tissues examined except liver, red blood cells, and carcass.

In study # ABR-89108, urinary and fecal metabolites of ^{14}C-cyromazine were isolated and identified by TLC, HPLC, and GC/MS. The N-dealkylated product melamine, hydroxycyromazine, and unmetabolized cyromazine were definitively identified by GC/MS, while tentative identification was given to methylcyromazine, based upon co-chromatography with TLC and HPLC. A proposed pathway for cyromazine biotransformation was given based upon these data.

Core Classification: minimum

This study satisfies the guideline requirements (85-1) for a metabolism study in rats.

I. MATERIALS

A. Test Material:

Metabolism Studies (# HLA-6117-160):

^{14}C-Cyromazine, CGA-72662
Lot no: CL-XVIII-78 (3 mg/kg dose)
CL-XVIII-77 (300 mg/kg dose)
Radiochemical Purity: 97.2% (3.0 mg/kg dose)
97.7% (300 mg/kg dose)
Specific Activity: 9.8 µCi/mg (3.0 mg/kg dose)
0.8 µCi/mg (300 mg/kg dose)

Unlabelled Cyromazine, S83-0379-1
Chemical Purity: 96.3% (cyromazine standard)
98.8% (3.0 mg/kg dose)
99.0% (300 mg/kg dose)

Metabolite Characterization and Identification (# ABR-89108):

^{14}C-Cyromazine, CGA-72662
Radiochemical Purity: 97.2% (3.0 mg/kg dose)
97.7% (300 mg/kg dose)

B. Vehicle: 1% carboxymethylcellulose plus 4mg/ml Hi Sil 233

Test Animals: Species: rat
Strain: Crl:CD(SD)BR
Source: Charles River Laboratories (Portage, MI facility)
Weight: males, 191-276g; females, 164-216g.

II. METHODS

A. Study Design

1) Metabolism Study

The bioavailability and disposition of ^{14}C-Cyromazine was assessed in male and female rats following both oral and intravenous administration of the test compound. Rats received either a single oral dose of 3 or 300 mg/kg, a single intravenous dose of 3 mg/kg, or 14 repeated daily doses of unlabelled test material at

3 mg/kg followed by a single radiolabelled dose. Dose groups were as follows:

Group	Dose (mg/kg)	Dose Route	Number of Animals Male	Female
1	0 (vehicle control)	IV[a]	1	1
2	3	IV[a]	5	5
3	3	Oral[a]	5	5
4	3	Oral[b]	5	5
5	300	Oral[a]	5	5
6	0 (vehicle control)	Oral[a]	1	1
7	0 (vehicle control)	Oral[c]	1	1
8	0 (vehicle control)	Oral[a]	1	1

[a]single dose

[b]14 daily unlabelled doses followed by one radiolabelled dose.

[c]group 7 was dosed with the vehicle each time group 4 received a dose.

2) Metabolite Characterization and Identification Study

Major urinary metabolites of cyromazine were characterized and identified in this study. In addition, urinary metabolites were compared with those found in fecal extracts, and from these data a major metabolic pathway for biotransformation of cyromazine in rats was proposed.

Metabolites in rat urine were resolved using either normal phase or reversed phase TLC. Cyromazine and metabolites were isolated from rat urine and characterized by co-chromatography on TLC and HPLC. GC/MS or LC/MS of isolated metabolites was employed to confirm structural identity.

C. Experimental

1) Metabolism Study

a. Animal Husbandry

Animals were acclimated to the laboratory environment for 7 days (groups 4 and 7) or 8 days (all other groups) before study initiation. Animals were examined for health abnormalities during acclimation and at least twice daily for moribundity and mortality. Animals were given food (Purina Certified Rodent Chow #5002) and water *ad libitum*, except for an overnight fast prior to dosing and approximately 4 hours post dosing. Animals were housed in a temperature (72 ± 3 °F), humidity (50 ± 20%) and light (12 hour light/dark)

controlled room during the entire course of the study.

b. Dosing

Groups 1 and 2 were administered cyromazine in deionized water *via* intravenous injection into the tail vein at a dose volume of 2 ml/kg, while groups 3 through 8 received cyromazine in 1% carboxymethylcellulose *via* gavage with a disposable syringe and a steel ball tipped needle at a dose volume of 5 ml/kg. Radiolabeled dose was determined by weighing the dosing syringe before and after dosing.

Body weights were recorded for all animals at randomization and study initiation. Body weights in groups 4 and 7 were recorded daily. During the study period, animals were observed twice daily for mortality and moribundity.

Comment: The type of cage used for collection of urine and feces was not mentioned in this study. In addition, no preliminary study was mentioned which examined possible excretion of test compound as volatiles.

c. Sample Collection and Analysis

Urine was collected on ice at 4, 8, 12, 24, 36, and 48 hours following oral or intravenous administration of test material. Feces were collected at the same times as urine. Cages of treated animals were rinsed with tap water at 24 hours following dosing. Following the last collection time point, cages were rinsed with 1% trisodium phosphate solution and the wash was collected. Urine and feces samples were stored in a freezer before and after analysis.

At sacrifice, rats were anesthetized with halothane and approximately 2 to 5 ml of blood was collected *via* cardiac puncture. After sacrifice, the following tissues were collected, weighed, and saved for analysis of radioactivity: bone (femur), brain, fat (urogenital), ovaries, testes, heart, liver, kidneys, lungs, muscle (thigh), spleen, uterus, and residual carcass.

Small tissue samples (bone, heart, spleen, ovaries, and uterus) were split into two portions and analyzed for radioactivity by direct combustion. Remaining tissues were homogenized and aliquots of approximately 2g were analyzed in duplicate by combustion. Carcasses were ground using a Hobart meat grinder and Cuisinart food processor, and duplicate aliquots of approximately 0.5g were analyzed by combustion. Plasma was analyzed for radioactivity by liquid scintillation counting, while aliquots of blood cells (approximately 0.5g) were analyzed by combustion. Fecal samples were homogenized in 2 volumes of water and duplicate 0.5g samples analyzed by combustion. Duplicate aliquots of urine, cage rinse, and cage wash samples were analyzed by liquid scintillation counting.

d. Statistics

According to the registrant, statistical procedures were limited to simple expressions of variation such as mean and standard deviation.

2) Metabolite Characterization and Identification Study

According to the registrant (page 14), high dose urine gave essentially the same metabolic profile as low dose urine. Thus, because of the greater mass per volume of high dose urine, this dose was chosen for isolation and identification of metabolites.

Whole urine, urine components, and fecal extracts were analyzed by both normal and reversed phase TLC. Normal phase TLC consisted of 3 solvent systems, identified as follows:

SS1: chloroform: methanol: formic acid: water (75:20:4:2)
SS2: ethyl acetate: ethanol: ammonium hydroxide (80: 15: 5)
SS3: toluene: p-dioxane: methanol: ammonium hydroxide (40: 40: 30: 10)

Reversed phase TLC solvent system consisted of 100% water.

Urine and feces were prepared for TLC through application of urine or feces to a Sephadex A-25 anion exchange column followed by a methanol rinse. Rinse from the urine wash was concentrated to a suitable volume for TLC, while the feces rinse was concentrated to approximately 2ml and then applied to Bio-Rad AG50W-8X cation exchange column. The extract was eluted with 1% NH_4OH in 3:1 water:methanol. This eluate was then concentrated to a suitable volume for TLC application.

Following TLC, plates were scraped at the major areas of radioactivity, then sonicated three times with approximately 10ml methanol. The supernatants from each extraction were pooled and concentrated to dryness.

Whole urine or fecal extract was also subjected to HPLC with both UV and radiochemical detection. Five different procedures were employed for complete metabolite separation and identification, as listed on pages 17-18 of the registrant report. Electron impact mass spectrometry and Thermospray LC/MS were employed for comfirmation of metabolite idenitity and structure.

Standards of cyromazine (96.3%), methylcyromazine (97.8%), hydroxcyromazine (93 and 99%), and melamine (99%) were employed for comparison to unknown radioactive zones on TLC and for mass spectral analysis.

D. Compliance

A signed statement of no data confidentiality claims was provided with both studies.

A signed statement of GLP compliance (40 CFR 160.35) was provided with both studies.

A signed statement of quality assurance was provided with both studies.

III. RESULTS

1) Metabolism Study

The stability of the dose solution for the repeated low-dose study was confirmed by comparative analysis of the dose solution and freshly prepared cyromazine standard using HPLC. It was stated in the report (page 75) that stability of the low dose solution was verified by the consistent retention times observed between the low dose solution and the cyromazine standard. While consistent retention times are desirable, information regarding the concentration of test article in the dose solution over time would be more appropriate in judging stability of the dose solution.

Verification of dose for rats in the 3 and 300 mg/kg dose groups was presented by comparison of predose and postdose aliquots. Recovery of radiolabel from urine and tissues ranged from 95-103%.

a. Absorption

In those rats dosed orally with 3 mg/kg ^{14}C-cyromazine, 82-86% of the dose was excreted in urine, while approximately 86% of a dose of ^{14}C-cyromazine was excreted in urine in those rats dosed i.v. at this same dose level. Thus, the absorption of ^{14}C-cyromazine appeared complete.

b. Distribution

Analysis of tissue and blood levels of ^{14}C-cyromazine derived radioactivity 7 days following a 3 mg/kg intravenous dose showed undetectable levels of radioactivity in all tissues examined except liver and residual carcass for both male and female rats. Liver levels of ^{14}C-cyromazine derived radioactivity in males ranged from < 0.01 % of the dose to 0.03% (mean value < 0.01%), while levels in residual carcass ranged from 0.002-0.24% of the dose (mean value 0.15%). Liver and carcass values for female rats in this dose group were similar.

The distribution of ^{14}C-cyromazine derived radioactivity in rats given an oral dose of 3 mg/kg ^{14}C-cyromazine was similar to that observed in intravenously dosed rats. Mean liver levels of ^{14}C-cyromazine derived radioactivity were 0.03%

of the dose in males, and 0.02% in females. Mean residual carcass levels were 0.16 and 0.13% of the dose in males and females, respectively. In both male and female rats in this dose group, some residual ^{14}C-cyromazine derived radioactivity was observed in red blood cells in a few rats, but amounted to < 0.01% of the dose of ^{14}C-cyromazine.

In rats given 14 repeated oral doses of cyromazine followed by a single dose of ^{14}C-cyromazine, distribution of ^{14}C-cyromazine derived radioactivity showed a similar pattern as that observed in the single dose oral and i.v. studies. The only exception to this was found in female rats in this dose group, where the mean level of ^{14}C-cyromazine derived radioactivity in the carcass was 0.58% of the dose.

Rats in the high dose oral group (300 mg/kg ^{14}C-cyromazine) showed similar residual levels of ^{14}C-cyromazine derived radioactivity in blood and liver, but slightly higher levels of radioactivity in the carcass (mean values of 0.3 and 0.2% of the dose in males and females, respectively) when compared to the single low dose oral groups.

c. Excretion

The excretion of ^{14}C-cyromazine in urine and feces at both 3 and 300 mg/kg is summarized in the following Table:

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

	LDM	LDF	IVM	IVF	PCM	PCF	HDM	HDF
urine (+cage wash, rinse, wipe)	82.4± 18.6	86.4± 4.9	86.5± 5.0	86.5± 7.5	91.9± 7.0	90.1± 3.0	83.5± 6.1	86.4± 2.2
feces	4.07± 3.5	3.77± 1.9	5.16± 3.7	6.43± 5.2	3.31± 1.5	2.69± 1.0	7.52± 3.3	6.36± 2.7
carcass (mean)	0.19	0.15	0.16	0.13	0.18	0.6	0.3	0.2
Total	86.5	90.2	91.7	93.0	95.3	93.3	91.3	92.9

Abbreviations are: LD, low dose (3 mg/kg); IV, intravenous dose (3mg/kg); PC, pre-conditioned dose (3mg/kg x 14days); HD, high dose (300 mg/kg).

[a]data represent the mean percent dose excreted at 168 hours post-dosing

With the exception of the males in the low dose oral group, >90% of a given dose of ^{14}C-cyromazine was excreted within 5 days. Examination of the time course of urinary excretion in all dose groups (Figure 1) shows that urinary excretion in males and females was essentially complete in all dose groups by 24 hours, with the exception of the high dose groups, where urinary excretion was not complete until 48 hours.

Fecal elimination of ^{14}C-cyromazine derived radioactivity was a minor route of excretion, representing between 3 and 8% of a given dose in all dose groups. Excretion in the single low dose groups and repeated low dose groups appeared similar (between 3-4% of a given dose), while fecal excretion in the intravenous and high dose groups was somewhat higher (between 5-7% of a given dose). The similarity between the intravenous and oral dose groups in fecal elimination of ^{14}C-cyromazine derived radioactivity supports the conclusion that the fecal radioactivity arises from biliary excretion of the test material. Although recovery of test material was >90% in most cases, the possibility of excretion as volatiles or $^{14}CO_2$ in minor amounts was apparently not explored in this study.

2) Metabolite Characterization and Identification Study

a. Preparative TLC of urine

Rat urine from the high dose groups was separated into 5 zones by preparative TLC. Radioactivity from zone 1 was further resolved by reversed phase TLC into at least 3 components. These components accounted for no more than 0.4% of total urinary radioactivity.

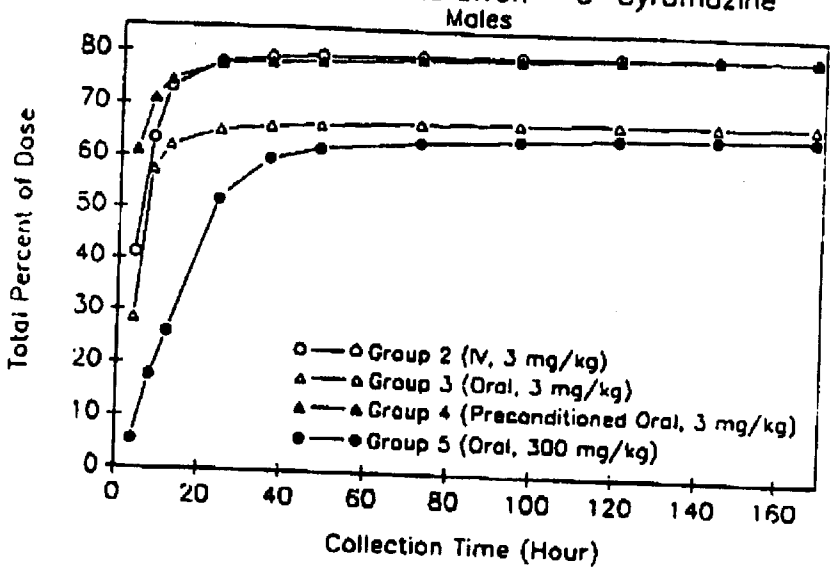
Radioactivity in zone 2 corresponded to the melamine standard. This was confirmed by isolation of zone 2 radioactivity and analysis by GC/MS (Figure 14, page 50 of report, and Figure 16, page 52 of report).

TLC zone 3 was separated into at least 4 components by TLC SS2. The major component of this separation was tentatively identified as hydroxycyromazine, based upon comparison to the percentage isolated from HPLC separation (Table VI, page 30 of report). A minor component of prep TLC zone 3 accounted for 1.1% of total urinary radioactivity, and was tentatively identified as methylcyromazine, based also on comparison to percentages isolated by HPLC (Table VI, page 30 of report). The major component of zone 3 was confirmed as hydroxycyromazine by GC/MS (Figure 14, page 50 of report, and Figure 17, page 53 of report).

Urinary radioactivity in zone 4 was separated into at least 4 additional components using TLC SS2. No component in this zone accounted for more than 1.6% of total urinary radioactivity, and no component of this zone was identified.

Zone 5 of prep TLC represented the largest percentage of total urinary radioactivity (Table III, page 25-26 of report). Isolation of this zone and analysis by GC/MS confirmed the identity of this radioactivity as cyromazine (Figure 14, page 50 of report, and Figure 15, page 51 of report).

Figure 1
Cumulative Percent Recovery of ^{14}C Residues
in Urine From Rats Given ^{14}C–Cyromazine



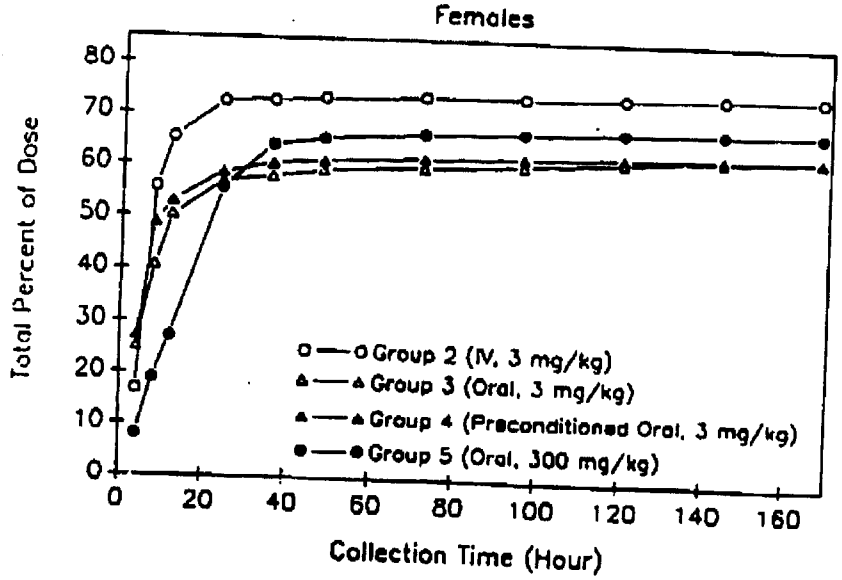
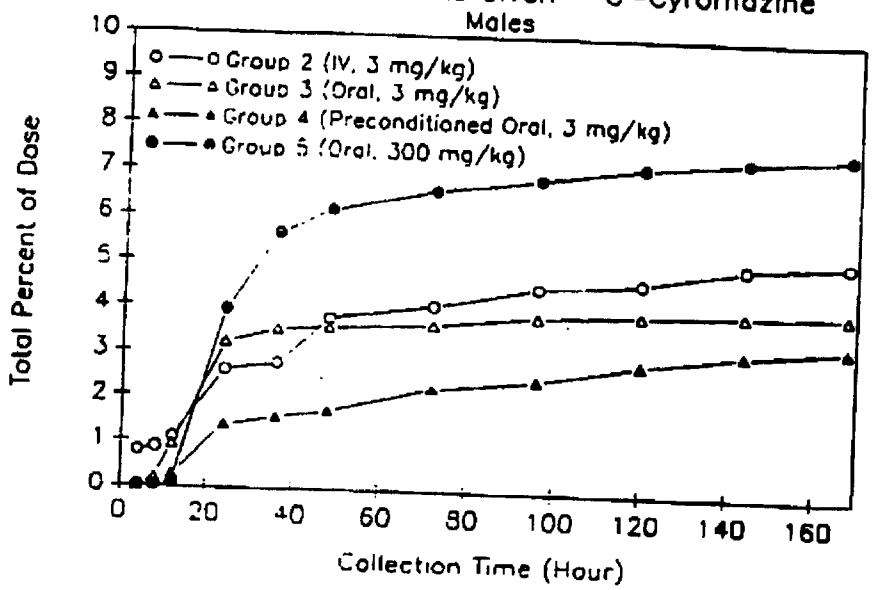
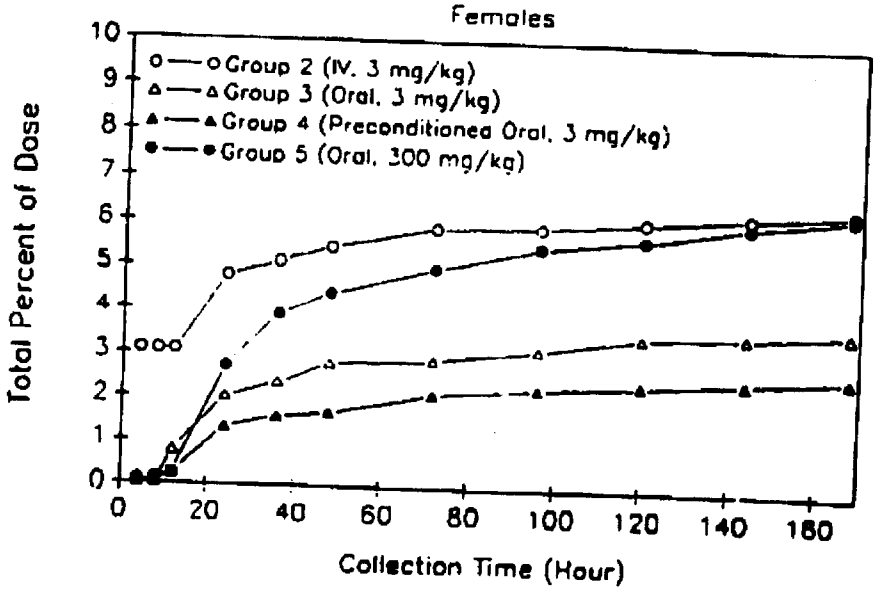


Figure 2
Cumulative Percent Recovery of ^{14}C Residues
in Feces From Rats Given ^{14}C-Cyromazine





b. HPLC of urine

Urinary metabolites of cyromazine were also separated and characterized by HPLC. In this system, urine could be separated into five clusters. Cluster 1 corresponded to the metabolite melamine, based on the retention time of the authentic standard and analysis by GC/MS (Figure 16, page 52 of report).

Cluster 2 retention time did not correspond to the retention times of any of the authentic metabolite standards of cyromazine, or to parent compound. This peak accounted for an average of 7% of total radioactivity in urine in all of the low dose groups, and approximately 3% of total radioactivity in the high dose groups.

Retention time of cluster 3 on HPLC corresponded to the authentic standard of melamine, Cluster 4 to that of hydroxycyromazine, and Cluster 5 to the parent compound, cyromazine.

Quantitation of urinary metabolites by TLC and HPLC is presented in the following Table:

TLC and HPLC Quantitation of Urinary Metabolites of Cyromazine
(values expressed as percent of urinary radioactivity)

	LDM	LDF	IVM	IVF	PCM	PCF	HDM	HDF
HPLC cluster 1 (methylcyromazine)	2.5	2.6	2.4	3.5	1.8	1.8	---	---
TLC zone 3 (methyl+hydroxy+ other)	19.3	15.7	17.6	16.0	19.6	17.0	8.8	8.9
HPLC cluster 2 (unidentified)	7.6	8.7	6.3	7.3	7.3	6.9	3.2	2.1
TLC zone 3 (methyl+hydroxy)								
HPLC cluster 3 (melamine)	7.6	9.2	6.4	8.6	7.8	12.1	4.3	2.8
TLC zone 2 (melamine)	8.5	12.3	6.3	7.5	5.5	7.3	5.3	5.0

(continued)

HPLC cluster 4 (hydroxycyromazine) TLC zone 3 (methyl+ hydroxy)	16.3	10.9	8.2	6.8	9.2	4.6	6.1	5.9
HPLC cluster 5 (cyromazine)	63.2	65.4	71.5	70.8	70.6	69.8	83.6	83.3
TLC zone 5 (cyromazine)	55.8	59.5	67.0	68.1	66.8	70.6	80.7	81.6

Cluster 1

There was no apparent difference in the percentage of urinary radioactivity identified as methylcyromazine when comparing results from i.v. vs oral exposure to cyromazine. Repeated oral dosing resulted in an apparent decrease in the percentage of urina v radioactivity, as indicated by results of HPLC analysis. Because TLC zone 3 was a mixture of metabolites, the effects of different dosing regimens were not apparent.

Cluster 2

Radioactivity in this cluster was not identified by HPLC. This cluster of radioactivity was also a component of TLC zone 3. Because the radioactivity in TLC zone 3 could be further resolved into components identified as methylcyromazine and hydroxycyromazine, it is conceivable that some of the unidentified HPLC radioactivity could be stereoisomers of identified metabolites (e.g., methylcyromazine) or other polar metabolites of cyromazine. This becomes more apparent when the radioactivity in HPLC zones 1, 2, and 4 is added and compared to TLC radioactivity in zone 3 (see below).

	LDM	LDF	IVM	IVF	PCM	PCF	HDM	HDF
HPLC cluster 1+ 2+ 4	26.4	22.2	16.9	17.6	18.3	13.3	9.3	8.0
TLC zone 3	19.3	15.7	17.6	16.0	19.6	17.0	8.8	8.9

Cluster 3

Cluster 3 was identified as melamine. No apparent effects of i.v. or repeated dosing was

observed on formation of this metabolite. However, at the high dose, an apparent decrease in formation of this metaboltie was observed in both male and female rats relative to the low dose exposure. A decrease in the percentage of this metabolite could be observed in the repeated oral dose groups vs the low dose groups when evaluating TLC data; however, this effect was not observed from results of HPLC analysis.

Cluster 4

From HPLC analysis, there was an apparent decrease in formation of hydroxycyromazine in the repeated oral dose and high dose groups relative to the low dose groups.

Cluster 5

Increasing the oral dose of cyromazine resulted in an apparent increase in excretion of unchanged cyromazine, as seen both by HPLC and TLC analysis.

Overall, the most consistent effect observed in this study is a decrease in formation of the methylated and hydroxylated products of cyromazine as well as the N-dealkylated product melamine upon increasing the oral dose from 3 to 300 mg/kg. The apparent alteration in the pattern of cyromazine biotransformation may have some relationship to the altered pattern of urinary elimination observed at the high oral dose of cyromazine. However, the lack of statistical analysis and statement of recoveries of metabolites during sample preparation make a definitive analysis difficult.

c. TLC of fecal extracts

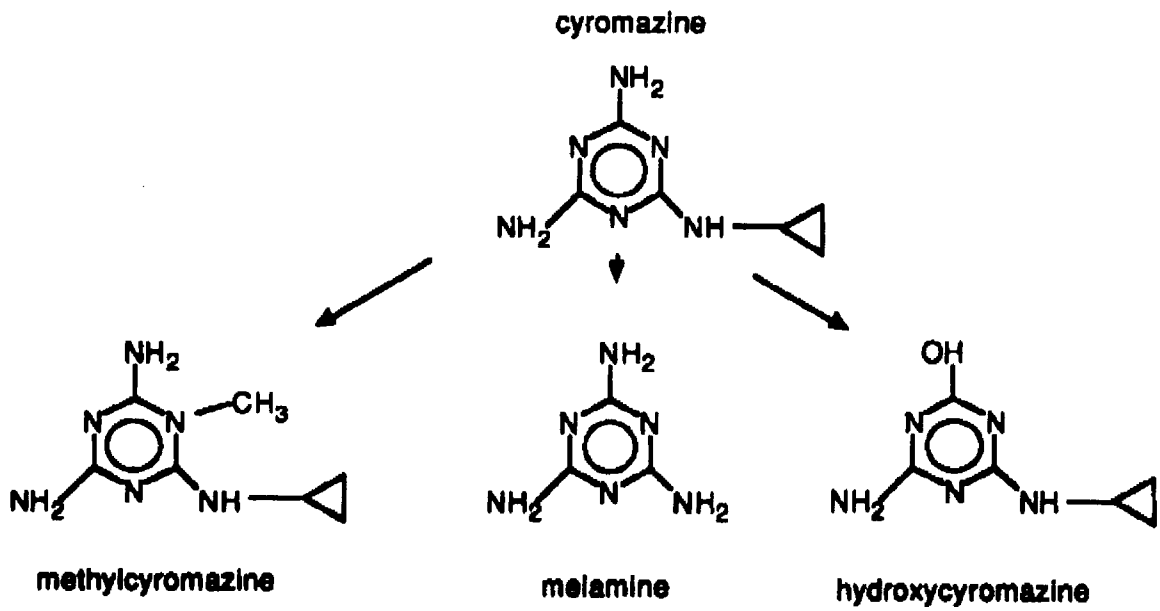
TLC quantitation of fecal extracts of cyromazine dosed rats is presented in Table VII, page 32 of the registrant report. Metabolites in feces were identified by co-chromatography with authentic standard metabolites of cyromazine. A summary of the findings of this aspect of the study is given here.

Metabolites identified in feces were melamine, cyromazine, hydroxycyromazine, and methylcyromazine. Methyl- and hydroxycyromazine were not distinguishable by TLC. Unmetabolized parent compound accounted for between 68-76% of fecal radioactivity in all dose groups. Melamine accounted for an average of 7%, 7.6%, 6.1%, and 4.7% of fecal radioactivity in the low oral dose, intravenous dose, repeated oral dose, and high dose groups, respectively. Radioactivity comprising the mixture of methyl- and hydroxycyromazine accounted for an average of 12.5%, 7.5%, 5.5%, and 4.7% in the low oral dose, intravenous dose, repeated oral dose, and high dose groups, respectively.

It should be noted that male rats in the low oral dose group had what appeared to be greater percentages of melamine and the methyl- and hydroxylated metabolites of cyromazine in feces when compared to female rats. This was also apparent when

comparing metabolite percentages as a percentage of the total dose (Table VII, pages 32 and 33 of registrant report). However, urinary analysis did not show any sex difference in the percentage of these metabolites. While the sex difference in percentage of fecal metabolites found at the low oral dose may be real, it is not known whether some losses occurred for specific fecal metabolites during extraction and analysis.

GC/MS analysis confirmed the identity of parent compound (cyromazine) and the metabolites melamine and hydroxycyromazine in urine of male and female rats. TLC analysis supported the existence of the methyl- and hydroxy metabolites of cyromazine. Other minor metabolites are inferred based upon the small amounts (approximately 6%) of unidentified urinary radioactivity. Based on the results of this study, the proposed pathway of cyromazine biotransformation in rats is as follows:



IV. CONCLUSIONS

In this study, the disposition and metabolism of the insect growth regulator cyromazine was investigated in male and female rats. Data was presented in study # HLA 6117-160 demonstrating the absorption, distribution, and excretion of ^{14}C-cyromazine in male and female rats at a low oral dose (3 mg/kg), a low intravenous dose (3 mg/kg), repeated low oral doses (3mg/kg) and a high dose (300 mg/kg).

Comparison of oral and i.v. disposition data demonstrated that cyromazine was well absorbed after oral administration. Excretion was also rapid at the low dose, with the majority of radioactivity eliminated in the urine by 24 hours. No apparent differences

were observed between dosing regimens in the total urinary radioactivity eliminated when cage washes and rinses were taken into consideration. At the high dose, however, there was an apparent delay in elimination of ^{14}C-cyromazine derived radioactivity, suggesting either some type of renal pathology or a shift in biotransformation kinetics of cyromazine. The available evidence indicates that the latter possibility may be the case, as decreases in formation of melamine, methyl-cyromazine, and hydroxycyromazine were observed in high dose rats compared to low dose rats. This possibility deserves further investigation.

Fecal elimination of ^{14}C-cyromazine derived radioactivity was equivalent between dose groups and sexes, with the exception that in the high dose males, a greater percentage of radioactivity was apparently eliminated by this route. The origin of fecal radioactivity from administration of ^{14}C-cyromazine was apparently biliary in nature, as an equivalent percentage of ^{14}C-cyromazine derived radioactivity was eliminated by both orally dosed and intravenously dosed rats.

Residual ^{14}C-cyromazine derived radioactivity was minimal in all dose groups, with non-detectable levels in all tissues examined except liver, red blood cells, and carcass. However, only one time point was examined for tissue radioactivity in this study, and thus potential accumulation of ^{14}C-cyromazine derived radioactivity could not be demonstrated.

In study # ABR-89108, urinary and fecal metabolites of ^{14}C-cyromazine were isolated and identified by TLC, HPLC, and GC/MS. The N-dealkylated product melamine, hydroxycyromazine, and unmetabolized cyromazine were definitively identified by GC/MS, while tentative identification was given to methylcyromazine, based upon co-chromatography with TLC and HPLC. While some of the *in vivo* metabolites of cyromazine were identified in rats, the kinetics of these biotransformations was not studied. Given the altered urinary excretion seen at the 300 mg/kg dose and the apparent shift in percentage of cyromazine metabolites at this same dose, this avenue of cyromazine biotransformation should be explored.

Core Classification: minimum

This study satisfies the guideline requirements (85-1) for a metabolism study in rats.