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

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MAY 10 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Dennis Edwards,
Product Manager (12)
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FROM: Roger Gardner, Toxicologist
Section 1 *Roger Gardner 5-22-89*
Toxicology Branch 1, Insecticides/Rodenticides
Health Effects Division (H7509C)

THRU: Robert P. Zendzian, Ph. D., Acting Section Head
Review Section 1
Section 1
Toxicology Branch 1, Insecticides/Rodenticides
Health Effects Division (H7509C)

*Byrd
7/7/89*

SUBJECT: Review of a Metabolism Study with Clofentezine in Rats (MRID
No. 409420-01) (EPA Reg. No. 45639-RGL; Caswell No. 593A; HED
Project No. 9-0754).

Actions Requested

Review of the metabolism study cited in the Appendix below.

Recommendations and Conclusions

1. Treated rats partially absorb single oral doses of clofentezine with maximum accumulation of residues in the liver and kidney. Those residues were subsequently cleared within 24 hours after treatment. Almost all of a single oral dose (10 to 1000 mg/kg) is excreted within 24 to 48 hours after administration regardless of its amount, and the major route of excretion is the feces.
2. Metabolites identified in the urine and feces of treated rats were free and conjugated hydroxylated derivatives of clofentezine, and approximately half of an administered dose (10 mg/kg) was excreted as unchanged clofentezine.
3. Repeated dosing of rats demonstrated that tissue levels of clofentezine residues reach a plateau after 5 to 15 daily doses. Residue concentrations in the liver and kidneys were 2 to 4 times those reported after a single dose. One study also indicated that the chemical does not readily pass across the placenta in pregnant rats.
4. No additional metabolism data are required at this time.

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I. General Information

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Clofentezine is proposed for use as a miticide on almonds, nectarines, peaches, pears and other food crops. The compound is approved for experimental use on those crops. Its chemical name is 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine.

In a letter dated December 21, 1988, the Registrant (Nor-AM Chemical Co.) acknowledged that the Agency required additional information on identification of fecal metabolites in clofentezine treated rats to support registration of the pesticide. The Registrant further stated that the required information had previously been submitted to the Agency on March 27, 1986 (MRID number 00159109). Another copy of the report accompanied the letter (MRID number 409420-01). The resubmitted report is reviewed in a Data Evaluation Record which is included in the Appendix below.

II. Summary of Previously Submitted Data

The Appendix includes the Toxicology Branch "One-Liners" for the studies described in this Section.

Studies with rats, mice, dogs, rabbits, and baboons demonstrated that the feces was the major route of excretion (70-80% of the administered dose in all species).

Almost all of the administered dose was recovered within 24 to 48 hours after treatment, and in rats the proportion of fecal excretion at high doses (1000 mg/kg) increased to 95-98% of the dose. The plasma half life of clofentezine in rats after administration of 1000 mg/kg was 3.6 hours.

The metabolites identified in the urine of treated rats and baboons were free and conjugated hydroxylated derivatives which accounted for approximately 70-80% of the radiolabel extracted.

In the rat, intravenous administration resulted in approximately the same proportions of excreted residues in the feces and urine as were noted after oral dosing suggesting excretion in the bile as the major route. Whole body radiography of treated rats also demonstrated the accumulation of residues in the liver and kidney which were subsequently cleared within 24 hours after treatment. Repeated dosing of rats at 20 mg/kg/day demonstrated that tissue levels of clofentezine residues reached a plateau after 5 to 15 daily doses. Residue concentrations in the kidneys and liver were 2 to 4 times those reported after a single dose.

The fetuses of pregnant rats treated with 3200 mg/kg/day doses of clofentezine during gestation had residue concentrations which were only one-fifth that of maternal blood or tissues which indicates that the chemical does not readily pass across the placenta.

III. Discussion

A. New Data

A Data Evaluation Record for the rat metabolism study discussed in this section is included in Appendix II below.

Oral administration of a single 10 mg [¹⁴C]-clofentezine per kg body weight dose to male and female rats indicated that there are two metabolic pathways for the test substance. One results in the formation of 3-(2'-methylthio-3'-hydroxyphenyl)-6-(2'-chlorophenyl)-1,2,4,5-tetrazine in free and conjugated forms (35% of the urinary radioactivity), and the other pathway results in the formation of 3-, 4-, and 5-hydroxyclofentezine (34% of the urinary radioactivity and 1-2% of the fecal radioactivity). Approximately half of the administered clofentezine was recovered in the feces unchanged.

The authors noted that each of the two major pathways could lead to a variety of minor metabolites. They indicated that the hydroxylation of phenyl rings in clofentezine could result in free and conjugated di-hydroxy clofentezine. In addition, the replacement of chlorine with a methylthio group could result in the formation of glutathione, mercapturic acid, and cysteine conjugates of clofentezine or 3-hydroxyclofentezine.

B. Data Gaps

The information submitted on the identification of fecal metabolites in clofentezine treated rats is acceptable, and no additional data are required at this time.

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APPENDIX I

Toxicology "One-Liners" on Previously
Submitted Metabolism Studies with Clofentezine
(Caswell No. 593A)

CITATION ACCESSION/ MRID NO. MATERIAL RESULTS TOX CAT COREGRADE/ DOCUMENT#

Metabolism Species: baboon FBC Limited METAB/83/24; 6/10/83	071714	NC 21314 47.7 uci/g spec. activ purity unspecified	After a single oral dose of 10 mg/kg urinary metabolites were identified as free and glucuronide conjugated 4-OH NC 21314 (75%). Urinary residues accounted for 5% of the administered dose. No fecal residues were identified.	003879
Metabolism Species: rat FBC Limited METAB/81/26; 7/81	070965	NC 21314 > 98% (sp. act. 86.4 uci/mg)	50-60% of an oral dose (0.1 mg/kg) was recovered in the feces of rats during the first 17 hours after dosing. Approximately 20% is recovered during that time in the urine.	003879
Metabolism Species: rat FBC Limited METAB/83/14; 5/3/82	070965	NC 21314 > 98% (sp. act. 86.4 uci/mg)	60-70% of an intravenous dose (0.1 mg/kg) is excreted in the feces during the first 24 hours after dosing. Approximately 20-25% is recovered in the urine during that time.	003879
Metabolism Species: rat FBC Limited METAB/81/58; 12/81	070965	NC 21313 > 98% (sp. act. 86.5 uci/mg)	The rats were fed a diet containing 27000 ppm for 10 weeks prior to the administration of 0.1 mg/kg i. v. The excretion profile is similar to that reported in study no. METAB/83 /14 above.	003879
Metabolism Species: rat FBC Limited METAB/82/1; 1/82	070965	NC 21314 > 98% (sp. act. 86.4 uci/mg)	60-70% of an oral dose (10 mg/kg) was excreted in the feces during the first 24 hours after treatment. Approximately 20% was excreted in the urine during that time. The liver and the kidney were found to have the highest tissue concentrations 72 hours after dosing.	003879
Metabolism Species: rat FBC Limited METAB/83/27; 6/16/83	071714	NC 21314 > 98% (sp. act. 86.4 mci)	The rats were given a 10 mg/kg dose of radiolabelled test substance. 35% of radioactivity recovered in urine was described as a monochloro-hydroxylated derivative of the parent found free or conjugated. Approximately 34% of the recovered activity was identified as free or conjugated 3- or 4-hydroxy NC 21314. 9% of activity associated with unidentified metabolites and 3% was associated with unmetabolized parent	003879
Metabolism Species: rat FBC Limited METAB/82/22; 3/82	070965	NC 21314 > 98% (sp. act. 86.4 mci/g)	1000 mg/kg dose. Majority excreted in the feces (98% of administered dose; approximately 1 to 2% of dose excreted in urine. Highest tissue concentrations in the liver and plasma. Moderate levels were found in adrenals and fat. These levels were slightly above those found in all other tissues.	003879
Metabolism Species: rat FBC Limited METAB/81/52; 11/81	070965	NC 21314 > 98% (sp. act. 86.4 mci/g)	1-25 consecutive daily doses of 20 mg/kg administered by gavage to rats. Tissue levels rose to a plateau in kidney, liver, heart (females only), skin, and ovaries after 5 to 15 days. These levels are 2 to 4 times those found after a single dose.	003879

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TOX CAT COREGRADE/ DOCUMENT#

RESULTS

ACCESSION/ NRID NO.

CITATION

MATERIAL

003879

Metabolism
Species: rat
FBC Limited
METAB/82/23; 4/82

NC 21314 > 98% (sp. act.
86.4 uCi/g)

070965

Whole-body autoradiography of rats given a single 10 mg/kg dose of radiolabelled test substance indicated that there is poor gastrointestinal absorption and organs and tissues are cleared of residues within 48 hours after dosing.

Metabolism
Species: rat
FBC Limited
METAB/81/19; 4/81

NC 21314 > 98% (sp. act.
10 uCi/g)

070965

Residues in fetuses of rats given a single oral dose of 20 mg/kg were one-fifth the level found in the maternal blood 6 hours after dosing. 24 hours after treatment, the liver, kidneys, and fat had 1 ppm concentrations of radioactive residues. No other tissues contained significant residue concentrations. The test substance did not readily cross the placenta, and is rapidly cleared from pregnant rats.

Metabolism
Species: mice
FBC Limited
METAB/82/11; 1/82

NC 21314 > 98% (sp. act.
86.4 uCi/g)

070965

Approx. 65% of a single 10-mg/kg oral dose is excreted in the feces of treated mice. Almost all of the administered dose is excreted within 48 hours after treatment.

Metabolism
Species: dog
FBC Limited
METAB/82/6; 1/82

NC 21314 > 98% (sp. act.
86.4 uCi/g)

070965

94-97% of the 10mg/kg oral dose was excreted by treated dogs within 48 hours after treatment.

Metabolism
Species: dog
FBC Limited
METAB/81/37; 12/81

NC 21314 > 98% (sp. act.
86.4 uCi/g)

070965

After an i. v. injection of a single dose of 0.1 mg/kg approximately 70% of the administered radioactivity was recovered in the feces and 20% was found in urine. Most of the excretion was noted during the first 48 hours after dosing. Tissue residues were at or below the level of detection (0.01 ppm).

Metabolism
Species: rabbit
FBC Limited
METAB/82/21; 4/82

NC 21314 > 98% (sp. act.
86.4 uCi/g)

070965

Approximately 60% of a single oral dose of 10 mg/kg to rabbits was recovered in the feces, and 40% was found in the urine. Almost all of the recovered residues were found during the first 48 hours after dosing. At 96 hours after treatment from 0.5 to 1.43 ppm was found in the stomach, bile, and large intestine of treated rabbits.

Dermal absorption
Species: rat
Huntingdon Res. Centre, Eng.
METAB/86/1; 2/3/86

Apollo radiolabelled
> 90%, sp. act. 47.4 mCi
(g) in a simulated 50 SC
formulation

262268

Doses tested: 4.8, 44, or 180 mg/kg applied to skin of male rats. Less than 1% was absorbed through the skin during the 10 hr. exposure pd.

Metabolism
Species: rat
FBC Limited
METAB/85/2; 3/15/85

Apollo radiolabelled
(> 99%, sp. act. 47.7)

262268

Doses tested: 1000 mg/kg. Peak levels in plasma were 15.6 ppm for males & 14.1 ppm for females. These peak levels were observed 6 to 8 hours after dosing and declined to approx. 3 ppm, 24 hrs after dosing. Plasma half life = 3.6 hrs. approx. 40% of radioactivity is associated with metabolites of test substance.

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CITATION	MATERIAL	ACCESSION/ NRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Metabolism Species: rat FBC Limited METAB/85/36; 12/20/85	Apollo radiolabelled (>99%, sp. act. 47.7 mCi/g)	262268	No accumulation of radioactivity in the thyroid after 1 or 10 daily oral doses of 20 mg Apollo/kg body wt. in rats. Before dietary dosing, thyroxine half-lives for control and treated groups of male rats were 16.7 and 17.05 hrs, respectively. Those values after a 4 week feeding period (0 or 30,000 ppm) were 17.61 and 16.42 hrs. After 4 weeks on diets containing 0 or 30,000 ppm, iodine uptake by thyroid in male and female rats was increased and blood levels were decreased.		Acceptable 006232
Metabolism Species: mice FBC Limited METAB/85/36; 12/20/85	Apollo radiolabelled 99% sp. act. 47.7 mCi/g)	262268	In mice, blood iodine levels were comparable & thyroid iodine levels were higher in treated mice when compared with untreated mice.		Acceptable 006232
Photosensitization Species: ? FBC Limited TOX/81/167-17; 5/81	MC 21314 Tech.	070961	No data were presented. A theoretical discussion of the potential of the test substance to be phototoxic was presented.		003879
Mutagenic-Ames Species: salmonella FBC Limited TOX/80/167-3; 7/80	MC 21314 Tech 98.5%	070961	No mutagenic activity was found in bacteria with or without metabolic activation at doses ranging from 3.3 ug/plate to 33 ug/plate		003879
Mutagenic-gene conv/mit recomb Species: Yeast FBC Limited TOX/83/167-36; 6/6/83	MC 21314 Tech	071714	The vehicle (dimethylformamide/ ethanol) was found to be cytotoxic and a reduced concentration had to be used. Concentrations >125 ug per ml culture medium were insoluble. No increases in gene conversions or the frequency of mitotic recombinations were observed at 12.5, 25, 100, or 200 ug/ml above that in vehicle controls was found.		003879
Mutagenic-micronucleus assay Species: mice FBC Limited TOX/82/167-32; 4/82	MC 21314 tech	071085	There was no compound-related increase in the number of micronuclei in mice given 800, 1600, or 3200 mg/kg doses above that observed in untreated control mice.		003879
Mutagenic-dominant lethal test Species: rat FBC Limited TOX/83/167-45; 3/29/83	MC 21314 Tech	071714	No dominant lethal mutations were induced in rats after 10 weeks of feeding diets containing 0, 4, 40, or 400 ppm. Slightly elevated cholesterol levels, absolute and relative liver weights were observed in rats given the 400 ppm diet.		003879
Mutagenic- Lymphoma mutation Species: mice Life Science Research TOX/82/167-38; 10/14/82	Apollo Tech. (98.8% +- 1.4%)	262268	Doses tested: 15, 30, 70, 100 & 128 ug/ml without microsomal activ.; 2, 10, 30, 80, 100, & 128 ug/ml with activation. No increase in frequency of mutation at the TK locus in L5178Y cells in vitro. Highest dose tested doubled the mutation frequency above current control cultures, but report stated that frequency was within historical range. No data on historical controls were included.		Provis. Accep- 006232

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APPENDIX II

Data Evaluation Record for

Challis, I. R., and Needham, D. November 4, 1985. The Metabolism of Clofentezine in the Rat. Unpublished report no. METAB/85/5 prepared by FBC Limited, Chesterford Park Research Station, Saffron, Walden, Essex CB10 1XL, England. Submitted by Nor-Am Chemical Co., Wilmington, DE.
MRID No. 409420-01

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Reviewed by: Roger Cardner *Roger Jordan* 5-22-89
Section 1, Toxicology Branch 1
Insecticides/Rodenticides (H7509C)
Secondary Reviewer:
Section 1, Toxicology Branch 1
Insecticides/Rodenticides (H7509C)

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Byrd
5/27/89

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rat (Guideline §85-1)

MRID NUMBER: 409420-01

TEST MATERIAL: Technical grade Clofentezine (Batch no. CR 20099/5, purity >98%), radiolabeled clofentezine (C¹⁴) (batch no. CFQ 2533, specific activity of 86.6 mCi/g; and batch no. CFQ 2874, specific activity of 47.7 mCi/g).

SYNONYMS: NC 21314; Apollo; 3,6-bis[2-chlorophenyl]-1,2,4,5-tetrazine; clofentezine

STUDY NUMBER(s): METAB/85/5

SPONSOR: Nor-Am Chemical Co., Wilmington, DE

TESTING FACILITY: FBC Limited, Chesterford Park Research Station, Saffron, Walden, Essex CB10 1XL, England

TITLE OF REPORT: The Metabolism of Clofentezine in the Rat

AUTHOR(S): Challis, I. R., and Needham, D.

REPORT ISSUED: November 4, 1985.

CONCLUSIONS: Oral dosing of male and female rats with 10 mg [¹⁴C]-clofentezine per kg body weight indicated that there are two metabolic pathways for the test substance. One resulted in the formation of 3-(2'-methylthio-3'-hydroxyphenyl)-6-(2'-chlorophenyl)-1,2,4,5-tetrazine in free and conjugated forms (35% of the urinary radioactivity), and the other pathway resulted in the formation of 3-, 4-, and 5-hydroxyclofentezine (34% of the urinary radioactivity and 1-2% of the fecal radioactivity).

Approximately half of the administered dose was excreted in the feces as unchanged clofentezine.

Core Classification: Acceptable

I. MATERIALS AND METHODS

A. MATERIALS

1. Test species: Male and female Charles River CD Sprague-Dawley rats were used. They were of various unspecified ages and weights according to the report.

2. Chemical standards: Potential metabolites were synthesized for use in the identification of metabolites in urine and feces of treated rats. The standards included the following:

3-hydroxyclofentezine	5-methoxyclofentezine
3-methoxyclofentezine	6-methoxyclofentezine
4-methoxyclofentezine	

3-(2'-methylthio-3'-hydroxyphenyl),6-(2'-chlorophenyl)-1,2,4,5-tetrazine
 3-(2'-methylthio-4'-hydroxyphenyl),6-(2'-chlorophenyl)-1,2,4,5-tetrazine
 3-(2'-methylthio-5'-hydroxyphenyl),6-(2'-chlorophenyl)-1,2,4,5-tetrazine
 3-(2'-methylthio-6'-hydroxyphenyl),6-(2'-chlorophenyl)-1,2,4,5-tetrazine

B. METHODS

1. Experimental design: The report described the experimental design as follows:

...male and female rats...were dosed orally with [¹⁴C]-clofentezine at 10 mg/kg body weight and placed in glass metabolism cages. Urine and feces for metabolite identification were collected at 24 hours after dosing.

In order to boost the amount of metabolites available for isolation, the urine was mixed with urine from rats fed diet containing clofentezine at 9000 or 27000 ppm in a 90-day toxicity study.

2. Chromatography: Thin layer chromatography (TLC) was carried out on silica with the following solvent systems:

1	Chloroform/methanol/acetic acid	10:2:0.1
2	Chloroform/methanol/amonia (0.88 S. G.)	10:2:0.1
3	Chloroform/methanol/amonia (0.88 S. G.)	10:4:0.1
4	Toluene/ethyl acetate	4:1

High performance liquid chromatography (HPLC) was performed on reverse phase or silica columns, and the metabolites to be identified determined which were used (see Section II. below)

3. Analytical procedures

- a. Urine: Urine samples from the 90-day study were collected (430 ml) and added to urine from rats in the metabolism study before extraction procedures were conducted.

Pooled urine samples were freeze dried, and the solid residue was extracted with methanol which removed approximately 90% of the radioactivity according to the report. The methanol was evaporated, and the resulting red oil was dissolved in methanol/ethanol and cooled to produce a colorless solid residue. The report indicated that the extraction of the red oil was repeated 5 times. Following these procedures, the red oil was finally dissolved in methanol, and the solution was subjected to TLC in solvent system 1. Results of the

a. Urine (continued)

TLC autoradiography indicated 4 distinct bands which separated readily according to the report. Each of these bands was extracted from TLC plates with methanol and analyzed by further TLC as well as HPLC and mass spectroscopy for identification of metabolites.

b. Feces: The report described the extraction procedures as follows:

Feces collected 24 hours after dosing three rats with [¹⁴C]-clofentzine at 10 mg/kg were Soxhlet extracted for 22 hours with diethyl ether and the residual solid extracted for a further 5 hours with methanol.

The ether extract was shown to contain 49% of the total radioactivity present while a further 39% was in the methanol extract. The remaining 13% was retained in the fecal fibre.

The ether and methanol extracts were applied to...TLC plates and developed in solvent system 4. Autoradiography indicated the presence of 6 multicomponent bands from the ether extract and 4 from the methanol.

These bands were removed and the bands extracted from the silica with methanol. The isolated bands were investigated further by TLC/HPLC and mass spectral analysis.

c. Enzyme hydrolysis and methylation of metabolites: The report stated that purified metabolites were incubated for 15 to 17 hours at 35° C in acetate buffer (pH 5.0) and Helix pomatia digestive juice. After the incubation, the resulting solution was acidified with hydrochloric acid and extracted with ether. The extracts were purified with TLC.

Methylation of metabolites was described in the report as follows:

Samples of purified metabolites were dissolved in methyl iodide (ca. 1 ml) in a Reactivial. A small amount of silver oxide (approximately 10-20 mg) was added, the Reactivial was sealed and heated at 60° C in the dark for 4 hours with occasional shaking. The methyl iodide was evaporated off under a stream of nitrogen and the residue extracted with methanol. The extract was filtered and the methylated derivative was purified by TLC and/or examined further by TLC/HPLC.

II. REPORTED RESULTS

A. Isolation and Identification of Urinary Metabolites The report's description of the isolation and identification of metabolites is included in Addendum 1 below. The extractable metabolites identified are summarized as follows:

A. Isolation and Identification of Urinary Metabolites (continued)

<u>Metabolite</u>	<u>% total radio- activity in urine</u>
Unchanged clofentezine	
3-(2'-methylthio-3'-hydroxyphenyl)-6-(2'-chlorophenyl)-1,2,4,5-tetrazine	1
Conjugated 3-(2'-methylthio-3'-hydroxyphenyl)-6-(2'-chlorophenyl)-1,2,4,5-tetrazine	33
3-hydroxy-clofentezine	1
4-hydroxy-clofentezine	5
5-hydroxy-clofentezine	3
Conjugates of 3-, 4-, or 5-hydroxy-clofentezine	25
Unidentified metabolites	23
Total	94

The report noted that there were several other minor metabolites present, but no effort was made to identify them.

B. Isolation and Identification of Fecal Metabolites The results of fecal sample analysis were described in the report as follows:Ether Extract

The ether extract contained 49% of the total radioactivity in the feces. TLC examination of this extract showed that a major fraction (36% of the total radioactivity in the feces) consisted of unchanged clofentezine. Of the remaining radioactivity, 2% was identified as a mixture of 4 and 5-hydroxyclofentezine and the rest consisted of a multitude of minor compounds. Of the latter only 3 of the more polar components accounted for greater than 1% of the fecal radioactivity (1.3%, 1.6%, and 4.4%).

Methanol Extract

This extract contained 39% of the total radioactivity in the feces. TLC examination showed that once again a major part (26% of the total feces ¹⁴C) consisted of unchanged clofentezine. The remaining activity consisted of a number of materials ranging from 0.4-2.7% of the total radioactivity. None of these were identified.

In all cases of TLC with both ether and methanol extracts "streaking" of the parent compound on the plate was quite significant, and levels of unchanged clofentezine are almost certainly considerably higher than indicated by these results.

B. Isolation and Identification of Fecal Metabolites (continued)Residual Fibre

After extensive extraction with ether and methanol, approximately 13% of the total fecal radioactivity remained in the fibre.

Attempts to free the radiolabelled material were partially successful. Almost a quarter of the radioactivity (\approx 3% of the total fecal radioactivity) was released as o-chlorobenzoic acid (a hydrolysis product of clofentezine) after treatment with hydrobromic acid.

Helix pomatia digestive juice liberated approximately 2% of the total fecal radioactivity. This consisted of mainly polar materials, with small amounts of clofentezine and hydroxyclofentezine.

III. DISCUSSION

A. Investigators' Conclusions: The conclusions were presented in the report as follows:

Following oral dosing at 10 mg/kg with [14 C]-clofentezine, at least half of the dose was excreted unchanged in the feces. This may be unabsorbed material.

The remaining material appears to have been extensively metabolized with large numbers of (mainly minor) metabolites occurring in both urine and feces.

One of the major routes of metabolism involved hydroxylation and replacement of a chlorine atom with a methylthio group. Thus 3-(2'-methylthio-3'-hydroxyphenyl)-6-(2'-chlorophenyl)-1,2,4,5-tetrazine in free and conjugated forms accounted for 35% of the urinary radioactivity.

The other major pathway of metabolism involved hydroxylation of clofentezine at either the 3, 4, or 5 position. Thus free and conjugated 3-, 4-, and 5-hydroxyclofentezine isomers accounted for a further 34% of the urinary radioactivity and 1-2% of the fecal radioactivity.

The authors noted that each of the two major pathways could lead to a variety of minor metabolites. They indicated that the hydroxylation of phenyl rings in clofentezine could result in free and conjugated di-hydroxy clofentezine. The replacement of chlorine with a methylthio group could result in the formation of glutathione, mercapturic acid, and cysteine conjugates of clofentezine or 3-hydroxyclofentezine.

The proposed metabolic pathway is shown in Addendum 2 below.

- B. Reviewer's Discussion: The report did not include specific observations that were used in calculations of the proportions of recovered radioactivity associated with particular metabolites. However, the reported results supported the conclusions made by the investigators.

It should be noted that the investigators discussed the results of this study with those from other metabolism studies with clofentezine as follows:

Previous studies in rats have shown that following oral dosing with [¹⁴C]-clofentezine at 10 mg/kg, the bulk of radiolabelled material (80%) is excreted in the feces over 4 days (65% within the first 24 hours). Although extensive biliary excretion has been shown to occur from rats dosed intravenously with clofentezine at a lower level of 0.1 mg/kg, the aqueous solubility of the compound is very low (<1.0 mg/l), and the possibility of this resulting in poor absorption from the gut leading to a large portion of the dose being excreted in the feces cannot be discounted.

The findings of the present study tend to support the idea of low absorption with a large part (at least 62%) of the fecal radioactivity being present as unchanged parent compound....

The report did not include information on the proportion of administered radioactivity recovered in the urine and feces, and a determination of the extent of absorption could not be made from the results as reported. However, the previous rat metabolism studies indicated that 70-80% of an orally administered dose is excreted in the feces which suggests that approximately 46% ($0.62 \times 0.75 = 0.465 \times 100 = 46.5\%$) may not be absorbed. This result confirms the authors' statement that approximately 50% of the administered clofentezine was not changed and probably not absorbed.

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ADDENDUM 1

Description of Analytical Procedures for the
Identification of Metabolites in the Urine of
Clofentezine Treated Rats

CLOFENTEZINE TOXICOLOGY REVIEW

Page _____ is not included in this copy.

Pages 16 through 25 are not included in this copy.

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 - Identity of product impurities
 - Description of the product manufacturing process
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