BB-1169
TXR-2369

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

JUL 27 1989

37369

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Tolerances and revised tolerances for Metribuzin on food use crops.
EPA Identifying Nos.: 8F3683, 8H5563, 3125-314, 3125-325, 2F2677, EPA MRID No's: 262892, 40255503, 40347701, 263761, 265366,40838401, 40838402, 258756, 258757, EPA Record Nos.: 230777 230778, 232598, 232599, 232593, 232600, 232601, HED Project Nos. 9-0271A and 9-0272, Caswell No. 33D.

TO: Robert Taylor/Vickie Walters (PM 25)
Herbicide-Fungicide Branch
Registration Division (H/505C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/13/89
Pharmacologist, Review Section I
Toxicology Branch-Herbicide, Fungicide, Antimicrobial Support/HED (H7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y.M. Ioannou 7/13/89
Section Head, Review Section I
and
Marcia van Gemert, Ph.D.
Chief, Toxicology Branch-Herbicide, Fungicide, Antimicrobial Support
Health Effects Division (H7509C)

Registrant: Mobay Corporation
Agricultural Chemcials division
P.O. Box 4913
Hawthorn Road
Kansas City, MO 64120-0013

Action Requested: Review tolerances and revised tolerance petition request and toxicology data submitted in response to the registration standard.

Recommendations: The Toxicology Branch-Herbicide, Fungicide, Antimicrobial Support does not recommend the establishment of the tolerances and revised tolerances requested until the deficiencies in the developmental toxicity (teratology) study in the rat with metribuzin are resolved.

I. Background

A. General Information

SENCOR (metribuzin) is a herbicide of the triazine class that is applied both pre- and post-emergence on a variety of sites including terestrial crops, ornamentals and noncrops. SENCOR is chemically 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one.

The proposed tolerances are as follows:

Raw Agricultural Commodities - 40 CFR 180.332

Commodity	Proposed Tolerance (PPM)
Alfalfa, seed	0.1
Alfalfa, chaff	1.0
Asparagus	0.1 (increased from 0.05)
Barley, forage	2.0
Barley, hay	7.0
Corn, silage	0.1
Corn, fresh, cannery waste	0.1
Pea, straw	4.0 (increased from 0.05)
Wheat, hay	7.0
Soybeans	0.25 (increased from 0.1)

Food Additive - 21 CFR 193.25

Tomato, processed products	0.2

Feed Additive - 21 CFR 561.41

Sugarcane, molasses	2.0 (increase from 0.3)

B. Regulatory Considerations

None at this time.

C. Previously Submitted Data on Metribuzin

This compound is a registered active ingredient. The following data were submitted prior to this application.

Guideline #	Study Type	Tox.Cat.	Core Classification
Acute Toxicity			
81-1	Acute oral toxicity in rats with technical material	III	
81-2	Acute dermal toxicity in rats with technical material		
81-2	Acute dermal toxicity in rabbits with technical material		
81-3	Acute inhalation toxicity in rats with technical material	II	Minimum
81-4	Primary eye irritation in rabbits with technical material		
81-5	Primary dermal irritation in rabbits with technical material		
81-6	Dermal sensitization in guinea pigs with end use product		Guideline
Subchronic Testing			
82-1	90 day feeding study - rodent (2 studies)		Supplementary
82-1	9 week feeding study - rodent		Supplementary
82-1	90 day feeding study - nonrodent		(IBT)

Chronic Testing	
83-1 2-year feeding - rodent	Minimum
83-1 2 year feeding - nonrodent	Minimum
83-2 Oncogenicity - rat	Minimum
83-2 Oncogenicity - mouse (2 studies)	Guideline
83-3 Teratology in rats	Supplementary
83-3 Teratology in rabbits (4 studies)	Guideline
83-4 Three generatior reproduction-rat	Supplementary
Mutagenicity Testing	
84-2 Dominant Lethal Test in mice (5 studies)	Acceptable
84-2 In vitro cytogenetic chinese hamster ovary	Acceptable
84-2 Gene Mutation (S.typh, E.col.,B.sub. assays)	Acceptable
84-2 Gene mutation (S.typh, and B.subtilis assays)	Acceptable
84-2 Micronuleus Assay - mice	Unacceptable
84-2 Gene Conversion/Mitotic recombination (Sacc. cerevisiae)	Unacceptable
84-2 DNA damage/repair - rat hepatocytes (Sacc. cerevisiae)	Acceptable
84-2 HGPRT-CHO	Acceptable
Special Testing	
85-1 General metabolism - rat	Supplementary
85-1 General metabolism - dog	Supplementary

II. Discussion

This chemical was a registration standard in 1985.

III: Summary of Toxicity Data

A. New toxicology Data on Metribuzin

Data Evaluation Records for the studies described below are attached.

1. Acute Toxicity for Metribuzin

No new data were submitted.

2. Sub-chronic Toxicity Studies with Metribuzin

No new data were submitted.

3. Chronic and Oncogenicity Studies with Metribuzin

Additional data on the chronic/oncogenicity in the rat were submitted; however, these data were reviewed in MEMORANDUM, November 19, 1985 (from Stephen C. Dapson to Robert Taylor). The sponsor had satisfactorily responded to all requests made in the review conducted for the 1985 Registration Standard of the chronic/oncogenicity rat study (Bayer AG Report No. 4888 and Mobay AG Chem No. 41816, September 25, 1974) and the study was upgraded to Minimum. Metribuzin is not oncogenic to the rat in dietary levels to 300 ppm (MTD). The systemic No Observed Effect Level is 100 ppm with a systemic lowest Observed Effect Level of 300 ppm based on decreased body weight gain, along with histopathological changes observed in the liver, kidneys, uterus and mammary glands.

There was a comment in the letter from MOBAY to the Agency dated July 7, 1988, pertaining to a deficiency related to the "completion of the review of the mouse oncogenicity study, Mobay Report No. 80050, submitted on May 31, 1983, EPA Accession No. 250446", this study is classified by HED as Core-Guideline Data.

4. Developmental Toxicity with Metribuzin

A study was submitted whereby metribuzin (SENCOR technical) was administered by gavage to pregnant rats from gestation days 6 through 18 inclusive (Mobay AG Chem No. 91330, October 3, 1986). Maternal toxicity was evident at all dose levels in the form of reduced body weight gain and decreased food consumption. Further, there was an effect on the thryoid gland in the mid and high dose groups with reduced T4 levels and in the high dose group with an increase in thyroid weight. Additional data are necessary before an assessment of the developmental toxicity can be made; the study is considered as Supplementary Data (see attached DER).

A two-generation reproduction study was submitted (Report No.: MTD0080, Mobay AG Chem No. 98295, September 23, 1988) which used 0, 30, 150 and 750 ppm of metribuzin in the diet. The parental generations showed signs of systemic toxicity in the form of statistically significantly decreased body weight gains in the males for the study period and the females prior to gestation. The F_1 females showed statistically significantly decreased body weight gains during the gestation period in the mid and high dose groups, and both the F_0 and F_1 females had statistically significantly increased body weight gains during the lactation period. There was a statistically significant decrease in food consumption in high dose males during the study period and in high dose females prior to gestation; however, the mean differences were very slight. Further, slight, not statistically significant decreases in food consumption were noted in high dose females during the gestation period. Lactation period data were not available due to spillage and consumption by pups. No other systemic effects were noted.

No effect of the test compound was noted on the measured parameters of reproductive performance.

The only treatment related effect noted on neonatal growth and development was slight, statistically significantly lower pup body weights at day 21 and body weight gain from birth to day 21 for F_1 high dose pups. The F_2 pups showed a similar pattern with lower body weights at day 14 in both the mid and high dose groups, although these changes were very small and it is apparent that no changes in body weight gain were seen until day 14 when pups began to consume the diet.

No treatment related necropsy findings were noted in F_0 and F_1 animals or F_1 and F_2 stillborns or culled neonates.

No treatment related effects were noted in microscopic examination of the reproductive organs, pituitary and tissues with gross pathology. Since the investigators noted elevations of GGT activity in F_1 females, livers were examined microscopically and a dose related increase in hypertrophy of hepatocytes of the centrilobular and midzonal regions was noted with the most relevant increases in high dose males and mid and high dose females. No other biologically relevant observations were noted.

The NOEL for Reproductive Toxicity is 30 ppm; the LOEL for Reproductive Toxicity is 150 ppm; the NOEL for Systemic Effects is 30 ppm and the LOEL for Systemic Effects is 150 ppm. The study is considered as Core Guideline Data.

5. Mutagenicity of Metribuzin

The registrant submitted a Saccharomyces cerevisiae D7 test for determination of point mutations (Study No. T 7023525, Mobay AG Chem No. 94786, May 5, 1987). Under the test conditions reported, metribuzin did not induce reverse mutation in the D7 strain of S. cerevisiae either in the presence or absence of metabolic activation at the concentrations tested (625 through 10000 mcg/ml). By contrast, the positive control compounds (MMS and CP) induced expected frequencies of reverse mutations in S. cerevisiae D7 that were greatly in excess of the vehicle control values under the nonactivation and activation systems. These positive responses indicated that the assay systems were functioning properly. However, the test material was dissolved in DMSO and not analyzed to confirm the intended concentrations for this study. Therefore, the study is not fully acceptable in the present form and may be upgraded upon resolution of this deficiency.

6. Metabolism of Metribuzin

The registant submitted a general metabolism study in the rat (Lab. Proj. ID SE4R, Mobay AG Chem No. 94605, June 25, 1987) to fulfill a data gap from the 1985 Registration Standard. The absorption of metribuzin (SENCOR) could not be determined since the low water solubility of the compound precluded the use of an IV dosed group. Excretion data indicated that radiolabeled metribuzin is rapidly excreted in the urine and feces (observed at 8 hours) and reached a plateau at 48 hours for the single dosed groups (low and high), except the high dose female feces which reached a plateau at 72 hours. The investigators found 27.3 to 43.4% of the label in the urine and 55.8 to 71.5% of the label in the feces at 96 hours. Pilot studies determined that negligible amounts of label were found in the expired air, therefore, it was not collected in this primary study. The high dose group excreted the greatest amount of the label in the feces with the least amount of label, in all 3 test groups, in the urine.

Blood levels at various time points were not determined, however, very small amounts were found at 96 hours (sacrifice) in the low dose and slightly higher levels in the high dose at sacrifice. No specific differences in tissue levels were noted in the low dose groups. The high dose group had higher tissue levels, which would be expected, with the GI tract having considerably higher levels.

The investigators identified metabolites in both the urine and feces with the most prevalent metabolite being DA-N-Ac-Cys. This study is acceptable for regulatory purposes.

7. Dermal Absorption with Metribuzin

This study was reviewed by Robert P. Zendzian of SACB/HED (MEMORANDUM, February 17, 1989, Robert P. Zendzian to Steve Dapson, attached). He determined the study to be "unacceptable in and of itself and the report is also unreviewable", see the MEMORANDUM attached.

8. Additional Studies with Metribuzin

A metabolism study of metribuzin in the mouse liver was also submitted (Mobay AG Chem No. 88750, March 1984), however, this was a published literature document and does not follow EPA Pesticide Assessment Guidelines for a metabolism study. Pretreatment with PB to inhibit the cytochrome P450 apparently reduced the acute toxicity observed with metribuzin. This may indicate that reactive metabolites of metribuzin are responsible for the acute toxicity observed. The action of metribuzin on the liver was indicated by the increase in SGPT levels observed along with the liver histopathologic observations and the decrease in liver GSH content. Further testing found that metribuzin (radiolabelled) was bound to liver and blood proteins and to a lesser extent, brain proteins. Excretion levels of metribuzin were apparently determined at daily intervals; however, this information is not adequate to fulfill the Guideline recommendations for a metabolism study. Further, only one metabolite was identified in the urine with no attempt at identification of other metabolites.

An analytical methodology document was submitted (Tox Report No. 640, Mobay AG Chem No. 90273, July 2, 1985). The investigators found a linear response for Metribuzin from 0-15 mcg/ml and a linear response for Metolachlor from 0-150 mcg/ml. The Toxicology Branch - Herbicide, Fungicide, Antimicrobial Support defers this submission to the Dietary Exposure Branch for consideration. This submission does not fulfill a Toxicology Guideline recommendation.

007369

GUIDELINE: 83-3

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/11/89
Pharmacologist, Review Section I, Toxicology Branch/HED (H7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D. Y.M.I. 7/13/89
Section Head, Review Section I, Toxicology Branch/HED (H7509C)

DATA EVALUATION RECORD

Study Type: Developmental Toxicity
Teratology - Rat
Guideline: 83-3

EPA Identification No.s: EPA MRID (Accession) No. 265336
EPA ID No. 3125-270
EPA Pesticide Chemical Code 101101
Caswell No. 33D
HED Project No. 9-0271A

Test Material: Metribuzin

Synonyms: SENCOR, technical
4-amino-6-(1,1-dimethylethyl)-3-(methylthio)- 1,2,4-triazin-5(4H)-one

Study Number(s): Mobay Ag Chem #91330

Sponsor: Mobay Corporation
Agricultural Chemicals Division
Kansas City, Missouri 64120

Testing Facility: Toxicology Department
Central Research Services
Miles Laboratories, Inc.,
Elkhart, IN 46515

Title of Report: A Teratology Study with SENCOR Technical (Metribuzin) in the Rat

Author(s): R.L. Kowaski
G.R. Clemens
J.J. Bare
R.E. Hartnagel, Jr.

Report Issued: October 3, 1986

Conclusions: Additional data are required (see page 8).

Core Classification: Core-Supplementary Data

A. Materials

A copy of the "Materials and Methods" section from the investigators report is appended.

Test Compound: Purity: 92.6% a.i.
Description: not provided
Lot No.: (Batch No.) 77-297-50
Contaminant: not provided

Vehicle(s): Aqueous Emulphor
(0.5% v/v Emulphor EL-719 in distilled water)

Test Animal(s): Species: Rat, male and female
Strain: Charles River Crl:CD BR
Source: Charles River Breeding Laboratories, Portage, Michigan
Age: 12 weeks
Weight: 209-292 gm
Animals were acclimated for at least 7 days prior to study initiation.

B. Study Design

This study was designed to assess the developmental toxicity potential of Metribuzin, when administered orally by gavage from gestation days 6 through 15, inclusive.

Mating:

Natural: 2 females with 1 male overnight
Vaginal smears taken to determine successful copulation. Positive smears were designated as Day 0 gestation.

Group Arrangement:

Test Group	Dose Level (mg/kg/day)	Number Assigned Phase I	Number Assigned Phase II
Control (10 ml/kg aqueous emulphor)		5	28
Low Dose	25	5	28
Mid Dose	70	5	28
High Dose	200	5	28

Dosing:

All doses were in a volume of 10 ml/kg of body weight/day prepared from stock solutions during the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on gestational day 6 body weight.

Observations

The animals were checked daily for mortality or abnormal condition. Dams from Phase I were sacrificed on day 16 of gestation and those for Phase II were sacrificed on day 20 of gestation.

Examinations at sacrifice consisted of: For Phase I dams, blood was collected (by open chest cardiac puncture) for measurement of maternal serum T3 and T4 levels by solid phase radioimmunoassay with ^{125}I. Thyroids were removed, weighed and fixed in a "modified Millonig's phosphate buffered 10% neutral formalin". The investigators further examined abdominal and thoracic viscera, confirmed pregnancy and recorded any gross changes.

For the first 20 pregnant Phase II dams, blood was collected for maternal T3 and T4 measurements. The thyroids were again collected and treated as mentioned above. The abdomen was opened, ovaries removed and corpora lutea counted. The gravid uterus was removed and weighed. The fetuses were removed, and implantation and resorption sites determined. The abdominal and thoracic organs were examined grossly.

The fetuses were examined in the following manner: After removal from the uterus, the viability of the fetus was determined, then each fetus was sexed and weighed. Individual placenta were also weighed. The fetuses were examined for gross external anomalies. The palate was also examined. One half of the fetuses were examined internally for abdominal and thoracic organ anomalies. The fetuses were then placed in Bouin's solution for later examination of eyes and cranium by free-hand razor blade sectioning. The remaining fetuses were fixed in alcohol, eviscerated and processed for skeletal examination by a "refinement of the KOH Alizarin Red-S method (Staples and Schnell, 1965)".

Statistical Analysis

The following statistical analysis methods were used (extracted from the investigators report):

> "Statistical analysis of the data consisted of application of one or more of the follcwing tests:
> student's t, Dunnett's (1955, 1964), Fisher's exact (Pagano and Halvorsen, 1981), Kruskal-Wallis (1952), and Dunn's (1964)."

A summary of the statistical analysis conducted was provided as a appendix.

Compliance
<NOTE: Pages 1 through 29 of the report are stamped DRAFT.>

A signed Statement of Confidentiality Claim was not provided.

An unsigned Statement of compliance with EPA GLP's was provided (prior to GLP's).

An unsigned Quality Assurance Statement was provided.

C. Results

1. Maternal Toxicity

Mortality

No animals were reported to have died in either Phase.

Clinical Observations

For both phases, the investigators reported hypoactivity and ptosis in "many" treated animals and ataxia in all mid and high dose animals. There was a treatment related increase in hypoactivity and ptosis in the high dose with ataxia noted in mid and high dose. Individual animal data were provided.

Body Weight

The investigators supplied the following data: Mean and individual animal body weight. Gravid uterine weights were not provided.

Table I: Body Weight Gains (grams)[a]

Group:	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gains Entire Gest. Per.[1]
Control	28.1	48.4	69.0	145.5	68.4
LDT	29.4	30.6	70.9	130.9**	56.5**
MDT	26.0	29.7	69.3	125.0**	53.7
HDT	28.6	21.2	63.3	113.0**	42.6**

** P<0.01

[1] = corrected body weight gain for entire gestation period = body wgt gain for entire gestation period - gravid uterus wgt.
[a] = Data extracted from Mobay Ag Chem No. 91330, Table I.

From the data presented, it is apparent that all treated groups gained less weight during the dosing period when compared to control (see section on food consumption, following). The low and mid dose group gained weight similar to control after the dosing period, while the high dose showed continued reduced weight gain.

Food Consumption

The investigators supplied the following data: group mean and individual animal data.

Table II: Food Consumption Data (mg/kg/day)[a]

	Days	1	6	8	12	15	20
Group:	Control	21.4	24.2	24.2	27.5	25.5	27.7
	LDT	21.1	24.3	20.2*	23.2*	23.0*	28.1
	MDT	21.3	22.9	15.4*	23.1*	22.5*	27.8
	HDT	21.6	23.4	14.3*	21.4*	21.1*	26.2

* = $P < 0.05$

[a] = Data extracted from Mobay Ag Chem No. 91330 Table II.

All treated animals consumed less food during the dosing period, when compared to control. This observation coupled with the reduced body weight gain during the dosing period in all dosed animals may indicate a problem with palatability of the dosing solutions.

Clinical Pathology

The investigators supplied the following data: individual animal group mean values for maternal total serum thyroxine (T4) and triiodothyronine (T3) levels.

Table III: Thyroid Hormone Levels and Organ Weights for Metribuzin-Treated Pregnant Rats Mean ± S.E.M. (n)

Gestation Day	Control	25 mg/kg	70 mg/kg	200 mg/kg
		Triiodothyronine (T3) - nanograms/dl		
16	78.3 ± 4.8 (5)	80.5 ± 5.2 (5)	80.4 ± 7.7 (4)	60.6 ± 12.8 (5)
20	90.1 ± 4.8 (20)	81.3 ± 3.7 (20)	85.1 ± 4.5 (20)	82.5 ± 3.8 (20)
		Thyroxine (T4) - micrograms/dl		
16	2.24 ± 0.05 (5)	2.20 ± 0.16 (5)	1.08 ± 0.18* (4)	0.34 ± 0.08* (5)
20	1.33 ± 0.07 (20)	1.61 ± 0.10 (20)	1.67 ± 0.10* (20)	1.97 ± 0.10* (20)
		Thyroid Weight - grams		
16	0.021 ± 0.004 (5)	0.022 ± 0.002 (5)	0.024 ± 0.003 (4)	0.033 ± 0.003*(5)
20	0.018 ± 0.001 (20)	0.020 ± 0.001 (20)	0.020 ± 0.001 (20)	0.024 ±0.001*(20)

* Significantly different from control at the 0.05 level (Dunnett's test)

The levels of T3 were reduced slightly at both gestation days 16 and 20 but did not achieve statistical significance (wide variability was apparent). T4 levels were statistically significantly reduced in the mid and high dose groups at gestation day 16 but were not reduced at gestation day 20 (this was after the dosing period, maybe the reason for rebound increase). The thyroid weights were statistically significantly increased at both gestation days 16 and 20 in the high dose groups.

Gross Pathological Observations

The investigators supplied the following data: Thyroid weight data was discussed in the previous section, no other organs were weighed. They also provided individual gross observation data, no specific treatment related findings were noted.

Cesarean Section Observations

Table III: Cesarean Section observations[a]

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	33	33	33	33
#Animals Mated/Inseminated	32	31	31	31
Pregnancy Rate (%)	97.0	93.9	93.9	93.9
Maternal Wastage				
#Non pregnant	1	2	2	2
Number of litters	27	26	27	26
Corpora Lutea/dam	15.5	15.7	15.1	15.5
Total Implantation	394	385	381	373
Implantations/Dam	14.6	14.8	14.1	14.3
Total Live Fetuses	367	353	351	341
Live Fetuses/Dam	13.6	13.6	13.0	13.1
Total Resorptions	27	32	30	32
Resorptions/Dam	1.0	1.2	1.1	1.2
Total Dead Fetuses	0	1	0	0
Dead Fetuses/Dam	0	0.03	0	0
Median Fetal Weight (gm)[h]	3.8	3.6**	3.6**	3.1**
Preimplantation Loss(%)	6.5	6.0	7.5	7.6
Postimplantation Loss(%)	6.7	8.1	8.2	8.0
Sex Ratio (% Male)	47.1	50.0	46.7	50.0

** = $P < 0.01$

[h] = historical control range = 3.4 to 4.0

[a] = Data extracted from Mobay AG Chem. No. 91330, Table IV.

No specific treatment related effects were noted from the above measured parameters, except for the statistically significant reduction of median fetal weight in the high dose group. Although the low and mid dose groups were statistically significantly different, the biological relevance of the 5% decrement is unclear; further these weights were within the historical control range for this laboratory (data provided from 15 studies).

2. Developmental Toxicity

Table IV: External and Visceral Findings on Fetuses at Termination (Day 20)+

Dose (mg/kg/day)	#litters/ #fetuses	Observation
Control	1/1	Kidney, pelvis, bilateral, dilation; ureter, bilateral, dilation
	1/1	Brain, rt, cerebrum brownish discoloration, decreased tissue mass, possible MIL from injection
25	1/1	Runt (less than 2.0 g)
	1/1	Kidney, pelvis, bilateral, dilation
	1/1	Brain, diminished in size
	1/1	Non-viable
70	1/1	Artery, shortened innominate
	1/1	Kidney, pelvis, bilateral, dilation; ureter bilateral, dilation
	1/1	Brain, diminished in size
	1/2	Brain, diminished in size, rt hemisphere
200	1/1	Tail, curved
	1/1	Kidney, pelvis, bilateral, dilation; ureter bilateral, dilation
	3/3	Runt (less than 2.0 g)

+ = Data extracted from Mobay AG Chem No. 91330, Table VI.

No treatment related observations were noted in the above presented data.

Skeletal Examinations

Data could not be adequately evaluated since litter incidence was not provided on summary tables. The litter is the statistical unit as it is the mother that is treated directly with the test compound. The Agency directs the registrant to provide summary tables of both the litter and fetal incidence for all skeletal observations.

D. Discussion/Conclusions

a. Maternal Toxicity:

Maternal toxicity was evident at all dose levels in the form of reduced body weight gain and decreased food consumption (possible palatability problem). Further, there was an effect on the thyroid gland in the mid and high dose with reduced T4 levels and in the high dose with an increase in thyroid weight.

b. Developmental Toxicity:

i. Deaths/Resorptions:

No treatment related effects were noted.

ii. Altered Growth:

There was a statistically significant reduction of median fetal weight in the high dose group. Although the low and mid dose groups were statistically significantly different, the biological relevance of the 5% decrement is unclear; further these weights were within the historical control range for this laboratory (data provided from 15 studies).

iii. Developmental Anomalies:

Additional data are required for evaluation.

iv. Malformations:

Additional data are required for evaluation.

E. Study Deficiencies:

The Agency requests that the registrant provide the following information:

1. Gravid uterine weights (individual animal and group means)
2. Group mean fetal weights (not median)
3. Numbers of fetuses and litters examined for external, visceral and skeletal anomalies
4. Litter (and fetal) incidence of skeletal observation

E. Core Classification: Core-Supplementary Data.

Maternal and Developmental Toxicity NOEL's and LOEL's could not be determined with the available data.

METRIBUZIN

RIN: 3187-91

Page____ is not included in this copy.

Pages 18 through 27 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

GUIDELINE: 83-4

Primary Review by: Stephen C. Dawson, Ph.D. Stephen C. Dawson 7/11/89
Pharmacologist, Review Section I, Tox Branch-HFAS/HED (H7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D. Y.M.I. 7/13/89
Section Head, Review Section I, Tox Branch-HFAS/HED (H7509C)

DATA EVALUATION RECORD

I. Study Type: Two - Generation Reproduction - Rat
Guideline: 83-4

Study Title: A Two-Generation Reproduction Study in Rats with SENCOR Technical (Metribuzin)

EPA ID Number(s): EPA MRID No. 408384-01
EPA Pesticide Chemical Code 101101
Caswell No. 33D
HED Project No. 9-0271A
Document No.

Sponsor: Mobay Chemical Company
Agricultural Chemicals Division
P.O. Box 4913
Hawthorn Road
Kansas City, MO 64120-0013

Testing Laboratory: Toxicology Department
Miles Inc.
P.O. Box 40
Elkhart, IN 46515

Study Number(s): Laboratory Project ID Report No. 98295
Report No: MTD0080
Mobay AG Chem No. 98295

Study Date: September 23, 1988

Study Author(s): M.C. Porter, V. Jasty, R.E. Hartnagel, Jr.

Test Material: SENCOR Technical (also known as Metribuzin)
4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one
Purity = 92.6% a.i.
BATCH No. 77-297-50
Description: white crystalline solid

Test Animal: Male and Female Charles River Crl:CD BR rats
Age: 56 days old
Body weight 186-239 gm for males
150-205 gm for females
Supplier: Charles River Breeding Laboratories
Portage, Michigan

II. Materials and Methods: A copy of the "Materials and Methods" section from the investigators report is appended. Comments and highlights on these "materials and methods" are as follows.

The test chemical was mixed with pulverized commercial dry basal laboratory diet (Purina Certified Rodent Chow #5002) on a weight/weight basis at concentrations of 0, 30, 150 and 750 ppm (doses based on subchronic studies). These doses were discussed between the registrant and the Agency and found to be acceptable on the basis of preliminary data presented. Diets were prepared at 1 to 4 week intervals and stored frozen. Diet mixtures and water were provided to animals ad libitum. Diet mixtures were analyzed for stability, homogeneity and concentration.

The basic study schematic is as follows:

The investigators randomly assigned (by body weight) the F_0 animals into 4 study groups consisting of 30 animals/sex (after a 1 week quarantine period). This procedure was also used for the F_1 growth and development segment of the study.

The F_0 generation animals were given the test diets for 10 weeks prior to mating and continued throughout lactation until lactation day 21, when they were sacrificed. Any female that did not become pregnant (F_0 or F_1) was sacrificed 21 days after last mating. Dams who lost all pups, all culled F_1 pups (at 4 days of age) and nonselected for mating F_1 pups (at day 21) were sacrificed.

The selected F_1 animals were given the test diets for 10 weeks prior to mating to obtain the F_2 pups. The F_1 males were sacrificed when F_2 litters were born. The F_1 females were sacrificed at day 21 of lactation. F_2 culled pups were sacrificed at day 4 and the remaining pups at day 21.

All animals were kept under standard animal care conditions. The adult animals were individually caged and all animals were observed daily for "overt changes in appearance and behavior." Food consumption was recorded twice weekly. Body weights were obtained twice weekly for F_0 and F_1 adults and for females on Days 0, 7, 14 and 20 of gestation and Days 0, 7, 14 and 21 of lactation.

The F_0 and F_1 selected animals were paired randomly overnight for mating (data provided). The breeding took place over a 28 day period. No sibling mating was allowed. If insemination did not take place by 21 days another male was used for an additional 7 days. A sperm positive vaginal smear was designated at Day 0 of gestation.

Successfully mated females were placed in "littering boxes". They were observed for "evidence of premature or prolonged labor, dystocia, or atypical nesting habits...". The day of delivery was considered Day 0 of lactation. Pups were checked for viability,and gross malformations, and were sexed and weighed. All stillborn or early deaths were necropsied to determine cause of death, if possible. Viability and weaning indices were determined.

On lactation day 4 all litters were culled to 4 animals per sex, if possible. All culled pups were sacrificed and necropsied. All pups were weighed on lactation days 0, 4, 7, 14 and 21 and observed daily for general appearance and behavior.

The investigators randomly selected 10 animals per sex per dose group from the F_0 and F_1 generations to obtain open-chest cardiac puncture blood samples for an analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) activities. According to the investigators "All adults and pups were necropsied at death or sacrifice". They further removed and saved the vagina, uterus, cervix, ovaries, testes, epididymides, seminal vesicles, prostate, pituitary, liver and any tissue with significant pathology in the F_0 and F_1 parental animals. The tissues from control and high dose animals along with any low and mid dose tissues with gross lesions were examined histologically. Further, all livers from low and mid dose animals and prostrate glands from low and mid dose males of the F_0 generation were examined.

Statistical Analysis

The investigators used the following statistical methodology:

Dunnett's (1955; 1964), Fisher's exact (Pagano and Halvorsen, 1988), Kruskal-Wallis (1952), and Dunn (1964).

"All statistical comparisons of reproductive efficiency were done on median values using the dam as the unit of comparison."

Compliance:

A signed "Statement of No Data Confidentiality Claims" was included.

A signed "Statement of Compliance" with 40 CFR Part 160 (GLP's) was included.

A signed Quality Assurance Unit audit statement was included.

III. Results:

A. Parental Generations

1. Clinical Observations

The investigators supplied individual animal data. They stated that "No behavioral effects or signs of toxicosis were attributed to daily consumption of the test article at any of the 3 dietary concentrations". No treatment related effects were noted in the individual animal data provided.

2. Mortality

No animals were reported to have died in those "F_0 animals F_1 rats selected for the growth and development phase.

3. Body Weight

The investigators provided group mean and individual animal data. The following table provides mean body weight gain of F_0 and F_1 adults.

Table I: Body Weight Gains (gm)+

Dose(ppm)	F_0 Male[a]	F_0 Female[b]	F_1 Male[c]	F_1 Female[d]
Control	339.6 ±10.2	93.8 ±4.3	345.8 ±8.1	127 ±5.5
30	319.7 ±9.4	91.3 ±4.8	345.1 ±8.7	118.6 ±3.9
150	344.0 ±7.8	86.9 ±3.5	353.9 ±11.0	117.1 ±4.7
750	301.5* ±9.0	61.1** ±3.6	313.8* ±9.7	98.7** ±5.0

*=P<0.05 ** P<0.01

[a] = 142 days; [b] = 69 days; [c] = 105 days; [d] = 70 days

+ = Data extracted from Report MTD0080, Tables II, III, VI and VII.

As can be seen from the above data the high dose adult males and females of both the F_0 and F_1 parental generations gained statistically significantly less body weight than that of the control.

The following table presents female gestation and lactation body weight gains for F_0 and F_1 parental generations.

Table II: Body Weight Gains for Females during Gestation and Lactation+

Dose (ppm)	F_0 Gest.	F_0 Lact.	F_1 Gest.	F_1 Lact.
Control	106.8	3.4	110.8	9.8
	±3.3	±3.2	±3.5	±4.2
30	96.8*	8.6	101.7	13.7
	±2.7	±3.1	±2.4	±2.4
150	99.0	20.7**	95.7**	20.5*
	±3.0	±3.6	±3.1	±1.8
750	105.2	33.7**	98.9*	31.7***
	±2.4	2.5	±2.9	±3.1

* = P<0.05, ** = P<0.01

+ = Data extracted from Report No. MTD0080, Tables IV, VI, VIII and IX.

The above data indicate that for the F_0 females during gestation, little effect was noted on the body weight gain, for the F_1 females during gestation. There was a slight, but statistically significant decrease in body weight gain in the mid and high dose animals. During the lactation period, there was a dose related increase in body weight gain of all 3 dose groups, statistically significant in the mid and high dose groups for both the F_0 and F_1 females.

4. Food Consumption

The investigators provided group mean and individual animal data. Food efficiency was not calculated.

The data on food consumption during the lactation period was unavailable "due to spillage and consumption of test diet by pups".

Table IV: Food Consumption (gm)+

Dose(ppm)	F_0 Male[a]	F_0 Female[b]	F_1 Male[b]	F_1 Female[b]
Control	23.7	17.2	27.6	19.7
	±0.3	±0.3	±0.4	±0.3
30	23.2	16.7	26.8	19.3
	±0.2	±0.4	±0.5	±0.4
150	23.4	16.5	27.2	19.3
	±0.3	±0.3	±0.5	±0.4
750	21.6*	14.6*	25.3*	17.7*
	±0.5	±0.7	±0.4	±0.3

*=P<0.05

[a] = 143 days; [b] = 66 days

+ = Data extracted from Report MTD0080, Tables X, XI, XIII and XIV.

As can be seen in the provided data, the high dose animals (F_0 and F_1) consumed statistically significantly less food than that of the control.

Table V: Food Consumption in Females during Gestation+

Dose(ppm)	F_0	F_1
Control	20.7	22.6
	±1.0	±1.1
30	19.8	21.7
	±1.1	±1.1
150	19.9	21.3
	±0.8	±1.4
750	17.8	19.6
	±1.5	±1.1

+ = Data extracted from Report No. MTD 0080, Tables XII and XV.

Data indicate that the high dose females in both the F_0 and F_1 groups consumed slightly less food during the gestation period, when compared to control (however, the values did not achieve statistical significance).

B. Matings of the Parental Generations

1. Reproductive Performance

Males

According to the investigators, "the time required for males to inseminate females during a 28-day breeding period was similar for test article and control groups in the F_0 and F_1 phases of the study". This is supported by the data provided. Mating occurred in nearly all pairings except for one mid dose pair who did not mate.

Females

No differences were noted in data pertaining to estrous cycles in F0 and F_1 generation females. The attached Tables XX and XXI from the investigators report show that no treatment related effects were noted on copulation index, fertility index, gestation index or gestation length in either the F_0 or F_1 females. Further, no specific treatment related observations were noted for total number of pups born, litter size, birth index, or implantations (although F_1 animals in the mid and high dose groups had slightly lower numbers of implantations and subsequent smaller litter sizes, this is not believed to be treatment related (historical data were provided).

2. Neonatal Growth and Development

The attached Tables XX and XXI from the investigators report show no specific treatment related effects in terms of total numbers of dead pups, pup deaths after livebirth, viability index, weaning index and sex ratio for the F_1 or F_2 neonates. However, the high dose F_1 neonates had slightly (statistically significant) lower pup body weights at day 21 and body weight gain from birth to day 21. F_2 neonates also showed a similar pattern, with lower body weights at day 14 also, however, the low and mid dose pups had statistically significantly lower body weights, but these changes were small and were not apparent across generations. No changes in body weight gain were seen until pups began consuming test diets (around day 14).

No treatment related clinical observations were seen in the data provided.

Necropsy Findings

The investigators provided individual animal data for necropsy observations of the F_0 and F_1 parental generations as well as F_1 and F_2 stillborns and neonates. No treatment related effects were noted in data provided.

3. Clinical Pathology Findings

The investigators provided group mean and individual animal data for clinical chemistry determinations. The following Table VI presents the data from 9 to 10 selected animals. Although some statistically significant differences were noted, the biological relevance of the differences is unclear.

Table VI: Mean Clinical Chemistry Results+

DOSE		AST	ALT	GGT
		F_0 Generation		
		Males		
O PPM	MEAN	70.9	49.4	9.13
	SE	4.0	1.2	0.25
30 PPM	MEAN	99.6*	71.2*	8.75
	SE	8.8	8.2	0.32
150 PPM	MEAN	76.6	65.8	8.62
	SE	4.9	8.1	1.02
750 PPM	MEAN	81.0	54.5	8.77
	SE	6.2	4.1	0.74
		Females		
O PPM	MEAN	109.0	88.9	4.81
	SE	5.1	4.9	1.10
30 PPM	MEAN	120.3	82.1	5.60
	SE	11.8	4.3	1.18
150 PPM	MEAN	108.9	92.1	4.63
	SE	6.4	7.3	1.04
750 PPM	MEAN	123.7	103.9	7.01
	SE	9.4	8.4	1.33
		F_1 Generation		
		Males		
O PPM	MEAN	86.7	63.47	9.71
	SE	5.6	3.66	0.55
30 PPM	MEAN	82.0	62.71	11.22
	SE	4.1	2.28	1.16
150 PPM	MEAN	80.1	58.42	7.39
	SE	4.9	1.66	1.11
750 PPM	MEAN	86.3	61.53	4.79*
	SE	6.9	4.23	0.93
		Females		
O PPM	MEAN	108.7	90.8	5.96
	SE	6.7	8.5	1.18
30 PPM	MEAN[a]	149.4*	115.1	8.56
	SE	11.8	4.3	0.81
150 PPM	MEAN	141.7*	102.4	10.15*
	SE	10.7	8.0	0.64
750 PPM	MEAN[a]	109.9	93.1	11.81*
	SE	7.4	6.2	1.24

[a] = 9 animals

*Significant at the 0.05 Level (TWO TAILED DUNNETT T)

+ = Data extracted from Report No. MTD 0080, Tables XXII and XXIII.

4. Microscopic Pathology Findings

The investigators supplied a written summary and individual animal data. No treatment related effects were noted in the reproductive organs, pituitary and tissues with gross pathology. Elevations of GGT activity were noted in F_1 females, and upon examination the livers showed a dose related increase in hypertrophy of hepatocytes of the centrilobular and midzonal regions as noted on the following table.

Table VII: Liver: Hypertrophy[a]

Dose (ppm)	Males	Females
Control	0/60	3/60
30	0/60	6/60
150	1/60	31/60
750	8/60	44/60

[a] = Data extracted from Report No. MTD0080, Text.

Other findings of note are presented on the following Table (VIII).

Table VIII: Microscopic Findings[a]

Dose(ppm)		Control	30	150	750
Observation:					
Mammary adenocarcinoma[+]	F	0	1	0	0
Embryonal nephroma[+]	F	0	1	0	0
Hyperplasia of the prostatic epithelium	M	4/60[++]	0/29	3/30	7/59
Hepatitis - subacute/chronic	M	39/60	39/60	36/60	50/60
	F	29/60	34/60	25/60	28/60

[+] = single incidence observations
[++] = number of tissues examined
[a] = Data extracted from Report No. MTD0080, Tables 6 and 6a of Appendix K.

None of the above findings were statistically significantly different. The biological relevance of the slight increase in hyperplasia of the prostatic epithelium or the subacute/chronic incidence of hepatitis is unknown.

IV. Discussion and Conclusions

The parental generations showed signs of systemic toxicity in the form of statistically significantly decreased body weight gains in the males for the study period and the females prior to gestation. The F_1 females showed statistically significantly decreased body weight gains during the gestation period in the mid and high dose groups, and both the F_0 and F_1 females had statistically significantly increased body weight gains during the lactation period. There was a statistically significant decrease in food consumption in high dose males during the study period and in high dose females prior to gestation, however, the mean differences were very slight. Further, slight, not statistically significant decreases in food consumption were noted in high dose females during the gestation period. Lactation period data were not available due to spillage and consumption by pups. No other systemic effects were noted.

No effect of the test compound was noted on the measured parameters of reproductive performance.

The only treatment related effect noted on neonatal growth and development was slight, statistically significantly lower pup body weights at day 21 and body weight gain from birth to day 21 for F_1 high dose pups. The F_2 pups showed a similar pattern with lower body weights at day 14 in both the mid and high dose groups, although these changes were very small and it is apparent that no changes in body weight gain were seen until day 14 when pups began to consume the diet.

No treatment related necropsy findings were noted in F_0 and F_1 animals or F_1 and F_2 stillborns or culled neonates.

No treatment related effects were noted in microscopic examination of the reproductive organs, pituitary and tissues with gross pathology. Since the investigators noted elevations of GGT activity in F_1 females, livers were examined microscopically and a dose related increase in hypertrophy of hepatocytes of the centrilobular and midzonal regions were noted with the most relevant increases in high doses males and mid and high dose females. No other biologically relevant observations were noted.

NOEL for Reproductive Toxicity = 30 ppm
LOEL for Reproductive Toxicity = 150 ppm
NOEL for Systemic Effects = 30 ppm
LOEL for Systemic Effects = 150 ppm

V. Core Classification: Core Guideline Data

METRIBUZIN

RIN: 3187-91

Page____ is not included in this copy.

Pages 38 through 51 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

37569

Guideline Series 84: MUTAGENICITY

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/12/89
Pharmacologist, Review Section I, TB-HFAS/HED (H7509C)

Secondary Review by: John Chen, D.V.M. John H. Chen 7/12/89
Microbiologist, Review Section I, TB-HFAS/HED (H7509C)

DATA EVALUATION RECORD

Chemical: Metribuzin

Tox. Chem. No. 33D
HED Project No.: 9-0271A

Study Type: Saccharomyces Cerevisiae D7 Test for Determination of Point Mutations

MRID Number(s): 40347701

Synonyms/CAS NO.: SENCOR, DIC 1468
4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5-(4H)-one

Sponsor: Mobay Corporation, Agricultural Chemicals Division, P.O. Box 4913, Kansas City, MO. 64120

Testing Facility: Bayer AG, Fachbereich Toxikologie, Wuppertal-Elberfeld, Federal Republic of Germany

Title of Report: DIC 1468
Common Name: Metribuzin
S. CEREVISIAE D7 Test for Determination of Point Mutations

Author(s): Dr. B. Herbold

Study Number(s): Bayer Report No: 15760, Study No: T 7023525, Mobay Report No: 94786

Report Issued: May 5, 1987

Conclusion(s) - Executive Summary: Under the test conditions reported, Metribuzin did not induce reverse mutation in the D7 strain of Saccharomyces cerevisiae either in the presence or absence of metabolic activation at the concentrations tested (625 through 10000 mcg/ml). By contrast, the positive control compounds (MMS and CP) induced expected frequencies of reverse mutations in S. cerevisiae D7 that were greatly in excess of the vehicle control values under the nonactivation and activation systems. These positive responses indicated that the assay systems were functioning properly. However, the test material was dissolved in DMSO and not analyzed to confirm the intended concentrations for this study. Therefore, the study is not fully acceptable in the present form and may be upgraded on the resolution of this deficiency.

A. Materials: A copy of the "materials and methods" section from the investigators report is appended.

1. Test Material: Name: DIC 1468
Description: White Powder
Batch #: 238 603 171 Purity: 94.7%(7/10/86)
94.8%(1/19/87)
Contaminants: None provided
Solvents used: DMSO
Other comments: None

2. Control Materials:
Negative: DMSO
Solvent/final concentration: DMSO
Positive: Non-activation: Methylmethane sulfonate (MMS) obtained from E. Merck batch 4209279
solvent: DMSO
Activation: Cyclophosphamide in the form of Endoxan 100 mg injection vials (ASTA) batch 044438
solvent: Soerensen buffer (pH 7.4)

3. Activation: S-9 was derived from Arochlor 1254 enzyme induced livers of male Sprague-Dawley rats weighing 200-300 gm (process of Ames et al, 1975).

The metabolic activation mixture consisted of, for 100 ml:

30% S-9 fraction	
$MgCl_2 \times 6\ H_2O$	162.6 mg
KCl	246.0 mg
Glucose-6-phosphate, disodium salt	179.1 mg
NADP, disodium salt	315.0 mg
phosphate buffer	100.0 mg

4. Test organism: Saccharomyces cerevisiae D7
Properly maintained? Yes

According to the investigator, this tester strain "was developed by Zimmermann et al., (1975), and possesses the following genotype:

ade 2-40,	trp 5-12,	ilvI-92
ade 2-119,	trp 5-27,	ilvI-92

Via alleles ade 2-40 and ade 2-119 it permits the detection of induced mitotic crossing-over, and via alleles trp 5-12 and trp 5-27, the detection of induced mitotic gene conversion. The marker ilvI-92 permits detection of induced point mutation".

5. Test compound concentrations used:
Non-activated conditions: 625, 1250, 2500, 5000 and 10000 mcg/ml
Activated conditions: 625, 1250, 2500, 5000 and 10000 mcg/ml

B. TEST PERFORMANCE

1. Type of assay: Test for Point Mutagenic Effect

a. Protocol:

The test procedure followed the procedure of Zimmerman (1975). The test strain was incubated for 5 hours at 28°C and 150 rpm in YEP medium. The culture has then adjusted to a cell density of approximately 100 million cells/ml and used immediately for the test. The yeast suspension, YEP media, test compound solution, buffer and S-9 mix (as necessary) were then incubated for 16 hours at 37°C and 150 rpm. Finally, the suspensions were centrifuged and washed.

The suspensions of tester strain and test compound were streaked onto isoleucine-free nutrient plates and incubated for 6-8 days at 28°C. Ten (10) replicate plates per dose have used.

2. Test for Cytotoxicity:

Dilutions from each suspension were streaked onto complete medium plates at approximtely 200 colonies per plate (if no toxicity) and incubated for 3 to 6 days at 28°C. Ten (10) replicate plates per test dose were used. The first test used the following concentrations:

1.	Negative Control	9 mcg/ml
2.	DIC 1468	10000 mcg/ml
3.	DIC 1468	5000 mcg/ml
4.	DIC 1468	2500 mcg/ml
5.	DIC 1468	1250 mcg/ml
6.	DIC 1468	625 mcg/ml
7.	Positive Control Cyclophosphamide	60 mcg/ml
8.	Positive Control MMS	30 mcg/ml

The results were evaluated in the following manner: "A reproducible, dose-related increase in the revertant counts over the negative control is regarded as a positive result".

3. Mutagenicity assay:

Attached Table 1-4 presents the results of one assay (Table 1, 2) and a repeat (Table 3, 4) assay conducted 5 weeks later. Dose related cytotoxicity (statistically significant), both with and without metabolic activation was noted at 625 mcg/ml and above. There was no increase in isoleucine revertants in any of the treated groups under either metabolic activation condition, whereas positive controls did produce a statistically significant increase in mitotic recombinants.

4. Reviewer's discussion/conclusions:

Although the study was conducted in accordance with the acceptable procedures for performing the S. cerevisiae (D7) reverse mutation assay recommended by Zimmermann et. al. (Mutation Research 28, 381-388, 1975), the test material was dissolved in DMSO and not analyzed to confirm the intended concentrations. Therefore, the study is not fully acceptable in the present form and may be upgraded on resolution of this deficiency.

5. Was test performed under GLPs (is a quality assurance statement present)? - Yes, for OECD and CFR 40.160.

6. CBI appendix attached? No.

METRIBUZIN

RIN : 3187-91

Page_____ is not included in this copy.

Pages 56 through 67 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

37369

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/11/89
Pharmacologist, Review Section I, TB-HFAS/HED (H7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D. 7/13/89
Section Head, Review Section I, TB-HFAS/HED (H7509C)

DATA EVALUATION RECORD

I. Study Type: General Metabolism - Rat
Guideline: 85-1

Study Title: The Excretion and Metabolism of SENCOR by Rats

EPA Identification Numbers: EPA MRID No. 40255503
EPA Pesticde Chemical Code 101101
Caswell No. 33D
HED Project No. 9-027 A
Document No.

Sponsor: Mobay Corporation
Agricultural Chemicals Division
P.O. Box 4913
Hawthorn Road
Kansas City, MO 64120-0013

Testing Laboratory: Mobay Corporation
Agricultural Chemicals Division
Research and Development Department
Box 4913, Hawthorn Road
Kansas City, MO 64120

Study Number(s): Laboratory Project ID: SE4R
Mobay AG Chem No. 94005

Study Date(s): June 25, 1987

Study Author(s): K.S. Cain, C.M. Hanlon, J.B. Lane

Test Material: ^{14}C - SENCOR
Vial No. C-380B
Specific Activity = 20.8 mCi/mmol
Purity = 98.4% to 99.4%
Unlabeled SENCOR
Lot # 51025
Purity = 99.0%

Test Animals: Young adult male and female Wistar rats
(Rattus norvegicus)
Supplier: Charles River Breeding Laboratories,
Inc., Boston, MA

II. Materials and Methods: A copy of the "Materials and Methods" section from the investigators report is appended. The comments and highlights on these "materials and methods" are as follows.

Animals were fasted 24 hours prior to radiolabelled compound administration, water was available. The animals received food and water, ad libitum, after administration of the test compound.

Animals weighed 146 to 263 gms at time of dosing and were approximately 9 weeks of age. They were acclimated 7 days prior to dosing. Separate plastic metabolism cages were used after dosing (except for preliminary study where glass cages were used).

Dosing solutions were freshly prepared for each test group. The investigators followed the recommendation in the Pesticide Assessment Guidelines for choice of test groups: a low dose group treated with a single dose corresponding to the NOEL; a high dose group receiving a single administration; a repeated dose group receiving unlabelled compound daily for 14 days (low dose level) followed by a single labelled dose. Oral dosing was the chosen route, they did not employ intravenous dosing since SENCOR (Metribuzin) does not have adequate water solubility.

Preliminary studies were conducted on the volatility of SENCOR and its metabolites. Two female rats were used. They were treated with a 5.0 mg/kg single dose and placed in all glass metabolism cages. Urine, feces and expired air were collected. Urine and feces collection times were 8, 12, 24, 48, 72, and 96 hours. Urine was collected under various conditions, see attached "materials and methods" for additional information.

For the primary study, urine and feces were collected at 8, 12, 24, 48, 72 and 96 hours. Urine was collected in the presence of 5 ml isopropanol (based on results of preliminary study). Urine volumes were measured. Samples of urine and feces were handled for analysis as described in the attached "materials and methods."

Rats were anesthetized 96 hours post treatment. Blood was collected from the descending aorta. The liver, heart, gonads, gastrointestinal tract, spleen and kidneys were removed. Further, the brain was removed and samples of fat, bone and muscle were taken. Weights of all samples were recorded and treated as described in the attached "materials and methods."

Cages were rinsed with 150 ml of methanol followed by 150 ml of 2N HCl. The samples were then analyzed.

Samples of urine were used for metabolite isolation and identification. The investigators prepared separate composite samples of each sex for 0 through 48 hours for the low dose groups and 0 through 72 hours for the high dose group. Aqueous and organic extractions including enzymatic and acid hydrolysis treated samples were analyzed by thin layer chromatography (TLC), high pressure liquid chromatography (HPLC) or gas chromatography (GC). GC-Mass Spectromety (GC-MS) was also carried out on some samples. See attached "materials and methods" for more detail.

Sample of feces were also used for metabolite isolation and identification. The investigators used separate composite samples for each sex for the single dose low and high dose groups at 0 to 48 or 72 hours, respectively. For the mulitple dose animals, composite samples of females of 0 to 72 hours and for males of 0 to 48 hours. The samples were treated in a similar manner as with the urine samples.

A signed statement of "no claim of confidentiality" was included.

A signed "Good Laboratory Practice Certification" was included.

A signed "Certificate of Authenticity" was included.

III. Results

A. Preliminary Study

The investigators found "insignificant amounts of radioactivity (<0.1% of administered dose)" in expired air, therefore, expired air was not collected during the primary study (as allowed by the EPA Guidelines).

The investigators found that approximately 60.4% of the label was excreted in the feces and 38.4% in the urine. Further, they found that greater than 95% of the administered dose was excreted by 96 hours, therefore, they limited the primary study to this time frame.

Urine was collected under 2 conditions, either at $0^{o}C$ or in the presence of isopropyl alcohol. Both were intended to inhibit microbial activity. No major differences were noted, so the investigators used isopropyl alcohol based on "ease of handling".

B. Primary Study

1. Clinical Observations

The high dose group (500 mg/kg) presented with labored breathing and either rapid blinking or closed eyes. Some animals were reported to "tremble and then become very still". Two female rats died, one on day 2 and the other on day 3. The investigators used two replacement females. No Male rats were reported to have died. In the low dose group (5 mg/kg), one female was eliminated due to gavage error and one male from diarrhea (before and after treatment). No animals were replaced, however, as there were still 5 animals per sex left, which meets the EPA Guidelines recommendation.

No clinical observations were reported for the repeated dose group.

2. Radiolabel Recovery

The following table extracted from the investigators report presents the label recovered.

Percent of Administered Radioactivity

	Group B		Group C		Group D	
	Male	Female	Male	Female	Male	Female
Urine	37.6	37.7	40.0	43.4	27.3	32.2
Feces	60.5	61.0	59.1	55.8	71.5	66.3
Blood	0.1	0.2	0.1	0.1	0.2	0.1
Tissues	0.2	0.2	0.2	0.1	0.2	0.3
Carcass	0.6	0.4	0.2	0.2	0.3	0.4
Cage Rinse	1.0	0.5	0.4	0.4	0.5	0.7
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0
Actual % of Radiolabel Recovered	106.1	106.1	95.2	95.8	104.7	102.5

Mean Recovery = 101.7
Standard Deviation = 5.0
Data extracted from Table IV, Mobay Ag Chem# 94605.

The investigators "normalized" the above data for ease of reporting. The major route of excretion was the feces, ranging from 55.8 to 71.5% of administered label. The urine excretion was slightly less varying from 27.3 to 43.4%.

3. Radiolabel Excretion

The investigators stated that "no significant differences were detected in the rates and routes of ^{14}C-elimination between male and female rats", this pertains to the total excretion and the previous table supports this statement. Little residual radiolabel was found in the blood, tissues, carcass and cage rinse. The above results are similar to an earlier study. Mobay AG Chem # 33366 conducted in 1972 (DER attached).

The investigators further stated that there were "no significant differences in the rates and routes of radiocarbon elimination between male and female rats" in either the low or high dose single administration groups. This can be seen, on attached Figures 4 and 5 from the investigators report. The excretion levels appear to plateau at approximately 48 hours for the low dose and at approximately 72 hours for the high dose.

The investigators stated that the repeated dose group showed a difference in the rate of elimination between males and females. They stated that: "The percent of radiocarbon eliminated via the urine reached a plateau by 48 hours in the male and female rats". And further, "However, in the feces, the male rats' elimination of radiocarbon reached a plateau by 48 hours and the female rats' feces elimination of radioactivity did not reach a plateau until 72 hours post-administration of ^{14}C-SENCOR". Figure 6 from the investigators report, however, shows that elimination in both urine and feces reached a plateau at slightly greater than 70 hours. The grcup mean data also seem to support this 70 plus hours plateau, although one can stretch the point and say that the urine levels began the peak at 48 hours.

4. Tissue Distribution

Attached combined Tables V and VI presents the percent of and concentration of dose remaining in various tissues. As can be seen on the Tables, very little of the administered dose (in%) remained in the tissues and blood with the residual carcass containing the highest levels. Low dose group levels in ppm were found in very low amounts, with slightly greater amounts in ppm found in the high dose group.

5. Metabolite Identification

The investigators used pooled samples of urine and feces for metabolite isolation and identification by TLC, HPLC and GC.

a. Urine Metabolite Analysis

The investigators subjected the urine samples to organic extraction (after acidifying the samples to pH 2) with methanol extraction of the aqueous phase. They found 16 metabolites, 12 of which could be identified, see attached Table IX. No apparent differences were noted in percent distribution between sexes or among treatment groups. Very small amounts of the parent compound were recovered. According to the investigators, the most prevalent metabolites were the DA and DA-N-Ac-Cys followed by DADK, DK, t-BuOH-SENCOR, t-BuOH-DADK, t-BuOH-DA, t-BuOH-DK, Butylthione, SENCOR-N-Ac-Cys and 3-amino-DA.

Further analysis of Unknown 1 indicated that it may be comprised of more than one conjugated metabolite of SENCOR (metribuzin).

Treatment with beta-glucuronidase did not release any conjugated material in the urine sample. The investigators, therefore, deduced that the 4 unknowns were not beta-glucuronide conjugates.

The investigators included an open literature paper (as an appendix) that supports their findings (attached).

b. Fecal Metabolite Analysis

The pooled fecal material samples were also treated by organic solvent extraction with an extraction of the aqueous phase. The majority of the metabolites were found in the aqueous phase. After the solvent extraction, the fecal solids were treated by acid hydrolysis and then extraction of the filtrate. Attached Table XI presents the distribution of metabolites in percent. No specific differences were noted between sexes or among dose groups except for higher levels of unknown 1 in Groups C and D. The most prevalent metabolites were DA-N-Ac-Cys (this was also the most prevalent in the urine samples) and DADK followed by parent SENCOR, DA, DK, t-BuOH-SENCOR, t-BuOH-DADK, t-BuOH-DA, t-BuOH-DK, SENCOR-N-Ac-Cy and 3-amino-DA.

The unknowns were further treated with beta-glucuronidase, however, no conjugated materials were released. Therefore, the investigators concluded that the 4 unknowns were not beta-glucuronide conjugates of SENCOR (metribuzin) metabolites.

IV. Discussion and Conclusions

The absorption of metribuzin (SENCOR) could not be determined since the low water solubility of the compound precluded the use of an IV dosed group.

Excretion data indicated that radiolabeled metribuzin is rapidly excreted in the urine and feces (observed at 8 hours) and reached a plateau at 48 hours for the single dosed groups (low and high), except the high dose female feces which reached a plateau at 72 hours. The investigators found 27.3 to 43.4% of the label in the urine and 55.8 to 71.5% of the label in the feces at 96 hours. Pilot studies determined that negligible amounts of label were found in the expired air, therefore, it was not collected in this primary study. The high dose group excreted the greatest amount of the label in the feces with the least amount of label, of all 3 test groups, in the urine.

Blood levels at various time points were not determined, however, very small amounts were found at 96 hours (sacrifice) in the low dose and slightly higher levels in the high dose at sacrifice.

No specific differences in tissue levels were noted in the low dose groups. The high dose group had higher tissue levels, which would be expected, with the GI tract having considerably higher levels.

The investigators identified metabolites in both the urine and feces with the most prevalent metabolite being DA-N-Ac-Cys.

The investigators proposed a metabolic pathway, for Metribuzin in rats, see attached Figure 12. They further stated that:

> "The metabolism of SENCOR in rats appears to involve deamination, dethioalkylation, hydroxylation of the t-butyl side chain and conjugation.
>
> The appearances of SENCOR-N-Ac-Cys and DA-N-Ac-Cys suggest SENCOR is undergoing conjugation with glutathione. These glutathione-conjugates are subsequently processed through the mercapturic acid pathway. The n-acetyl cysteine conjugates can be excreted either in the urine or the bile, explaining their presence in both the urine and feces samples of the rats. The detection of Butylthione in the urine could be a result of cleavage of the acetyl cysteine groups in the kidneys".

V. Core Classification: Acceptable

METRIBUZIN

RIN: 3187-91

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Pages 75 through 88 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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unknown. The fact that 1 and the 6-chloro and 6-bromo analogues 19 and 21 were either inactive or had only slight activity in the wheat cylinder, pea segment, and pea curvature tests (James and Wain, 1968) suggests that these compounds do not possess auxin activity. Additional studies will be required to elucidate the mode of action of 6-methylanthranilic acid and analogues.

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Registry No. 1, 4389-50-8; 2, 2941-78-8; 3, 2305-36-4; 4, 4389-45-1; 5, 2840-04-2; 6, 2486-75-1; 7, 52130-17-3; 8, 2486-70-6; 9, 2458-12-0; 10, 118-92-3; 11, 66232-56-2; 12, 66232-47-1; 13, 66232-53-9; 14, 66232-54-0; 15, 53600-33-2; 16, 90321-28-1; 17, 90321-29-2; 18, 90321-30-5; 19, 2148-56-3; 20, 19407-42-2; 21, 20776-48-1; 22, 50573-74-5; 23, 6946-22-1; 24, 1885-31-0; 25, 56043-01-7; 26, 18595-13-6; 27, 65658-16-4; 28, 39967-87-8; 29, 90321-31-6; 30, 37777-66-5; 31, 66232-39-1; 32, 66232-55-1; 33, 90321-32-7; 34, 66232-45-9; 35, 66232-41-5; 36, 66232-48-2; 37, 66232-49-3; 38, 66232-50-6; 39, 66232-44-8; 40, 66232-60-8; 41, 567-61-3; 42, 17839-53-1; 43, 18239-19-5; 44, 21327-86-6; 45, 90259-31-7; 46, 54811-50-6; 47, 13506-76-8; 48, 15540-91-7; 49, 90321-33-8; 50, 5628-48-8; 51, 90321-34-9; 52, 90321-35-0; 53, 89977-14-0; 54, 89977-13-9; 55, 90321-36-1; 56, 90321-37-2.

LITERATURE CITED

Audus, L. J. "Plant Growth Substances"; Leonard Hill: London, 1972; Vol. 1, p 87.
Baker, B. R.; Schaub, R. E.; Joseph, J. P.; McEvoy, F. J.; Williams, J. H. *J. Org. Chem.* **1952a**, *17*, 164.
Baker, B. R.; Schaub, R. E.; Joseph, J. P.; McEvoy, F. J.; Williams, J. H. *J. Org. Chem.* **1952b**, *17*, 149.
Bullock, E.; Johnson, A. W. *J. Chem. Soc.* **1957**, 1602.
Courtin, A. *Chimia* **1975**, *29*, 260.
de Silva, W. H.; Bocion, P. F.; Eggenberg, P.; de Mur, A. *J. Plant Dis. Prot.* **1979**, *86*, 546.
Farkas, L.; Soti, F.; Incze, M.; Nogradi, M. *Chem. Ber.* **1974**, *107*, 3874.
Gabriel, S.; Thieme, A. *Chem. Ber.* **1919**, *52*, 1079.
James, C. S.; Wain, R. L. *Ann. Appl. Biol.* **1968**, *61*, 295.
Kovacic, P.; Levisky, J. A.; Goralski, C. T. *J. Am. Chem. Soc.* **1966**, *88*, 100.
Moll, H.; Musso, H.; Schroder, H. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 212.
Piper, J. R.; Stevens, F. J. *J. Org. Chem.* **1962**, *27*, 3134.
Stom, D. I.; Khutoryanskii, V. A. *Fiziol. Biokhim. Kul't. Rast.* **1972**, *4*, 183; *Chem. Abstr.* **1972**, *77*, 97678c.
Theilacker, W.; Hopp, R. *Justus Liebigs Ann. Chem.* **1963**, *669*, 85.

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Appendix H

Metribuzin Metabolites in Mammals and Liver Microsomal Oxidase Systems: Identification, Synthesis, and Reactions

Marian Saeman Bleeke and John E. Casida*

Mercapturic acid derivatives are the major urinary metabolites of metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] in intraperitoneally treated mice and orally treated rats, accounting for ~20% of the dose. These mercapturates of metribuzin and deaminometribuzin, in which the methylthio substituent is replaced by an *N*-acetylcysteinyl moiety, are also the major products on incubation of mouse and rat liver microsomes with metribuzin in the presence of *N*-acetylcysteine and NADPH. Other NADPH-dependent metabolites are deaminometribuzin and protein-bound material, the latter formed in large amounts only when *N*-acetylcysteine is not added. Deamination appears to be more important in rat than in mouse metabolism, both in vivo and in vitro. These findings suggest the formation of metribuzin sulfoxide and deaminometribuzin sulfoxide as activated intermediates. Oxidation of metribuzin and deaminometribuzin with *m*-chloroperbenzoic acid yields the corresponding sulfoxides, which react readily with *N*-acetylcysteine or protein in neutral aqueous solutions. The *N*-amino group is also cleaved on peracid oxidation, but *S*-methyl sulfoxidation occurs more rapidly.

Metribuzin or Sencor [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] is a potent photosystem II inhibitor used as a pre- and postemergence herbicide against a variety of broadleaf and grass weeds in potatoes, soybeans, and other tolerant crops (Draber et al., 1968; Eue, 1972). Acid hydrolyzes the SMe substituent from the metribuzin ring to give a diketo derivative (Frear et al., 1983a), and metal-catalyzed oxidation with *tert*-butyl hydroperoxide cleaves either the C–SMe or N–NH_2 group depending on the catalyst to form the diketo or deamino derivative, respectively (Nakayama et al., 1982). The metabolic fate of metribuzin is reported in several plant systems but not in animals. Soybean, sugarcane, and/or tomato form metribuzin *N*-glucoside and malonyl *N*-glucoside, the homoglutathione conjugate, and the deamino-, diketo-, and deaminodiketo derivatives (Hilton et al., 1974; Mangeot et al., 1979; Frear et al., 1983a,b). Metribuzin sulfoxide is a possible intermediate in metabolic formation of the homoglutathione conjugate (Frear et al., 1983b).

This investigation considers the metabolism of metribuzin in rats and mice and their liver microsomal oxidase systems. It also develops a chemical model for the observed reactions with emphasis on the importance of metribuzin sulfoxide as an activated intermediate.

MATERIALS AND METHODS

Chromatography and Analysis. Thin-layer chromatography (TLC) utilized precoated silica gel 60 F-254 20

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Table I. Thin-Layer Chromatography R_f Values for Metribuzin and Related Compounds

unconjugated triazinones[a]	TLC R_f in indicated solvent system[b] CE	DEH
I-SMe	0.54	0.46
I-S(O)Me	0.09	
II-SMe	0.42	0.29
II-S(O)Me	0.04	
III-NH_2	0.23	0.10
III-H	0.32	0.20
IV-NH_2	0.60	0.59

conjugated triazinones[a,c]	TLC R_f in indicated solvent system[b] BAW	MBBW
I-SMA	0.50	0.69
I-SG	0.26	
II-SMA	0.50	0.69
II-SG	0.26	

[a] For compound designations, see Figure 1. [b] Silica gel chromatoplates developed with the following solvent systems: CE, chloroform–ethyl acetate, 1:1; DEH, dichloromethane–ether–hexane, 3:2:2; BAW, 1-butanol–acetic acid–water, 4:1:1; MBBW, methanol–benzene–1-butanol–water, 2:1:1:1. [c] R_f = 0.00 in CE and DEH.

× 20 cm chromatoplates (EM Laboratories, Inc., Elmsford, NY) with 0.25 and a 0.5 mm layer thickness for analysis and product isolation, respectively. Solvent systems and R_f values are given in Table I. Nonradioactive products were detected by ultraviolet (UV) quench and ^{14}C-labeled compounds by autoradiography. High-performance liquid chromatography (HPLC) was carried out on a μBondapak CN column (Waters Associates, Milford, MA). Radiocarbon content was quantitated by liquid scintillation counting (LSC) of liquid samples, radioactive ge' regions scraped from TLC plates, and combusted solid samples. Melting points (mp) are uncorrected.

Proton nuclear magnetic resonance (^1H NMR) spectra were obtained with either a Perkin-Elmer R32B 90-MHz or a Bruker WM 300-MHz spectrometer. ^{13}C NMR spectra were recorded with the Bruker WM 300 instrument (75.4 MHz for ^{13}C). Samples were dissolved in deuterated solvents containing 1% tetramethylsilane as the internal standard. IR spectra were taken with a Perkin-Elmer 457 grating spectrophotometer and UV spectra with a Perkin-Elmer 576 spectrophotometer. Chemical ionization mass spectra (CI-MS) were recorded with a Finnigan 3200 instrument with methane as the reagent gas.

Chemicals. Figure 1 gives the structures and designations for metribuzin (I-SMe) and its metabolites and reaction products and related compounds. The chemicals are *N*-aminotriazinones (I), triazinones (II), triazinediones (III), thioxotriazinones, (IV), or various N-2-Me (V), N-4-Me (VI), or O-Me (VII) derivatives. Abbreviations for the ring substituents are given in Figure 1.

I-SMe (99% chemical purity) and [*carbonyl*-^{14}C]I-SMe (4.4 mCi/mmol; >99% radiochemical purity) were provided by Mobay Chemical Corp. (Kansas City, MO). Spectral data for I-SMe: ^1H NMR ($CDCl_3$) δ 4.94 (br s, 2 H, NH_2), 2.61 (s, 3 H, SMe), 1.43 (s, 9 H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 161.0, 160.7, 151.0, 37.6, 27.5, 14.2; UV (MeOH) λ_{max} 227 nm (log ε 3.9), 293 (3.9).

Metabolism of [^{14}C]Metribuzin in Rats and Mice. [^{14}C]Metribuzin was administered orally to male albino rats (180–200 g) at 200 mg/kg and intraperitoneally (ip) to male Swiss-Webster mice (20–25 g) at 75 mg/kg, in each case with methoxytriglycol (MTG) as the carrier vehicle. The animals were held for 5 days in all-glass metabolism cages with ground rat chow and water ad libitum. Expired

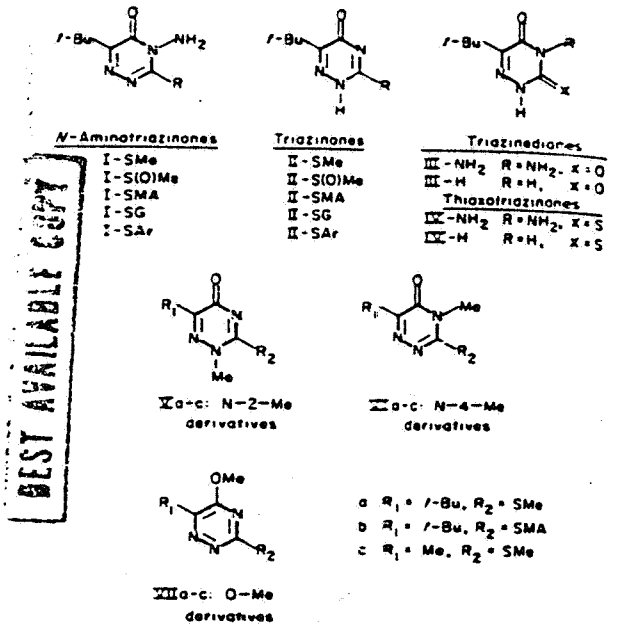


Figure 1. Structures and designations for metribuzin and related compounds. (Me = methyl; *t*-Bu = *tert*-butyl; Ar = 3,4-dichlorophenyl; SG = glutathionyl; SMA = *N*-acetylcysteinyl or mercapturic acid. The SMA derivatives Vb and VIIb are methylated).

$^{14}CO_2$ was monitored for the rats by collection in a monoethanolamine–methyl cellosolve, 2:1, mixture. The urine and a methanol extract of the feces were analyzed by LSC and TLC (CE and BAW). For isolation of the mercapturic acids, rat urine was diluted with an equal volume of saturated NaCl, acidified to pH 2 with 1 N HCl, and extracted with 3 volumes of ether–ethanol, 3:1, and the extract was subjected to preparative TLC (BAW) (mercapturic acids R_f = 0.50).

Metabolism of [^{14}C]Metribuzin in Rat and Mouse Liver Microsomal Enzyme Systems. Rats and mice as above were treated ip with phenobarbital at 80 mg/kg on each of 3 consecutive days with sacrifice on the fourth day. Livers from these phenobarbital-induced animals were homogenized at 20% (fresh weight/volume) in 0.1 M phosphate, pH 7.4, buffer. Centrifugal fractionation gave the soluble fraction (100000*g* supernatant) and the microsomal pellet (13000*g* supernatant and 100000*g* pellet washed once by resuspension and recentrifigation). These fractions were stored at −80 °C until used. Incubation mixtures in 0.1 M phosphate, pH 7.4, buffer (2.5 mL) contained the microsomal preparation (0.75 mL at 20% fresh liver weight equivalent) with one or more of the following additions: soluble fraction (0.50 mL), reduced nicotinamide adenine dinucleotide phosphate (NADPH) (2.2 mg), *N*-acetylcysteine (NAcCys) or glutathione (GSH) (5 mg), and [^{14}C]I-SMe (0.05 μCi, 20 nmol) added last in ethanol (20 μL). After incubation in a 25-mL Erlenmeyer flask with shaking for 2 h at 37 °C, the mixture was extracted with chloroform (3 × 2.5 mL). The aqueous phase was lyophilized to dryness and the residue extracted with methanol (3 × 2.5 mL). The dried ($MgSO_4$) chloroform fraction and the methanol fractions were concentrated under a stream of nitrogen and separated by TLC (CE, BAW). Methanol fractions from large-scale incubations (500 μg of I-SMe/flask) were subjected to TLC (BAW) for isolation of mercapturic acids. Solvent (Packard Instru-

ment Co., Downers Grove, IL) was used to solubilize protein fractions for LSC. A comparative study employed enzyme from rats and mice that received no phenobarbital pretreatment.

Hydrolysis and Methylation of Mercapturic Acids. The TLC-purified mercapturic acid metabolites were subjected to acid hydrolysis (2 N HCl, 100 °C, 3 h), neutralized with NaOH, and extracted into chloroform for TLC analysis (CE). They were also methylated with diazomethane (a hazardous chemical; handle with caution) followed by separation by TLC (chloroform–ethyl acetate, 13:10; Vb R_f = 0.09, VIIb R_f = 0.21) and HPLC (hexane–ethyl acetate, 2:1; Vb elutes completely before VIIb) and analysis by CI-MS and UV.

Syntheses and Spectral Features of Triazinones, Triazinediones, Thioxotriazinones, and Related Compounds (Figure 1). The syntheses are based on procedures referenced in Neunhoeffer and Wiley (1978).

II-SMe. IV-H (3.5 g, 19 mmol, described below) was added to a solution of sodium (1.38 g, 60 mmol) in methanol (30 ml). Methyl iodide (1.3 mL, 21 mmol) was added, and the solution was heated at reflux for 15 min. Most of the methanol was then removed under vacuum, water (50 mL) was added, and the solution was neutralized with 2 N HCl. A precipitate formed, and the mixture was extracted with ethyl acetate. After drying ($MgSO_4$), the ethyl acetate was removed under vacuum. Recrystallization from chloroform–hexane yielded II-SMe (3.3 g, 87%, mp 199–200 °C): ^{1}H NMR (CD_3COCD_3) δ 2.55 (s, 3 H, Me), 1.33 (s, 9 H, *t*-Bu); ^{13}C NMR (CD_3COCD_3) δ 164.2, 160.0, 157.8, 37.1, 27.4, 12.4; IR (2% MeOH–$CHCl_3$) 1650 cm^{-1} (C=O); UV (MeOH) λ_{max} 236 (log ϵ 3.1); CI-MS *m/e* 200 (M + 1).

III-NH$_2$. I-SMe (200 mg, 0.93 mmol) was refluxed in 2 N HCl (50 mL) for 3 h. The solution was cooled, neutralized with 10% NaOH, and extracted with ether. Washing the ether layer with saturated NaCl, drying ($MgSO_4$), and solvent evaporation yielded III-NH_2 (145 mg, 79%, mp 167–168 °C): ^{1}H NMR ($CDCl_3$) δ 5.20 (br s, 2 H, NH_2), 1.33 (s, 9 H, *t*-Bu); ^{13}C NMR (CH_3COCH_3 + CD_3COCD_3) δ 152.8, 149.7, 148.1, 37.4, 27.8; UV (MeOH) λ_{max} 212 nm (log ϵ 3.7), 262 (3.7); CI-MS *m/e* 185 (M + 1).

III-H. II-SMe (200 mg, 1.0 mmol) was treated as above to obtain III-H (120 mg, 70%, sublimed 260–265 °C): ^{1}H NMR (CD_3COCD_3) δ 1.35 (s, *t*-Bu); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 155.9, 150.7, 149.9, 36.0, 26.7; UV (MeOH) λ_{max} 208 nm (log ϵ 3.7), 261 (3.7); CI-MS *m/e* 170 (M + 1).

IV-NH$_2$. Addition of trimethylpyruvic acid (from oxidation of pinacolone with alkaline $KMnO_4$; Saeman, 1984) (1.6 g, 12.3 mmol) to thiocarbohydrazide (1.3 g, 12.3 mmol; Aldrich) dissolved in refluxing water (20 mL) gave a fine white precipitate. The suspension was stirred for 1 h and filtered to yield IV-NH_2 (2.1 g, 84%, mp 219–220 °C): ^{1}H NMR (CD_3COCD_3) δ 6.43 (br s, 2 H, NH_2), 1.37 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 204 nm (log ϵ 3.5), 270 (4.0); CI-MS *m/e* 201 (M + 1).

IV-H. Thiosemicarbazide (4.4 g, 48 mmol; Aldrich) in hot water (100 mL) was treated with trimethylpyruvic acid (6.3 g, 48 mmol) as above to obtain a white precipitate. NaOH pellets (3.8 g, 96 mmol) were added, the precipitate was dissolved, and the solution was heated for 30 min. After cooling and neutralization with 5 N HCl, the precipitate was filtered and recrystallized from ether to yield IV-H (3.9 g, 44%, sublimed 260–270 °C): CI-MS *m/e* 186 (M + 1).

Va, VIa, and VIIa. A solution of II-SMe (43 mg, 0.22 mmol) in methanol (5 mL) was treated dropwise with a diazomethane–ether solution until the bubbling stopped and the yellow color remained. The mixture was separated by preparative TLC (ether–hexane, 1:1) into three fractions with a combined yield of 32 mg (68%). Va: 8 mg (25% of products), R_f = 0.08; ^{1}H NMR ($CDCl_3$) δ 3.72 (s, 3 H, NMe), 2.55 (s, 3 H, SMe), 1.29 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 236 nm (log ϵ 4.1); CI-MS *m/e* 214 (M + 1). VIa: 14 mg (44% of products), R_f = 0.22; ^{1}H NMR ($CDCl_3$) δ 3.45 (s, 3 H, NMe), 2.67 (s, 3 H, SMe), 1.37 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 211 nm (log ϵ 4.0), 230 (3.8), 295 (3.2); CI-MS *m/e* 214 (M + 1). VIIa: 10 mg (31% of products), R_f = 0.47; ^{1}H NMR ($CDCl_3$) δ 4.03 (s, 3 H, OMe), 2.62 (s, 3 H, SMe), 1.37 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 208 nm (log ϵ 3.9), 248 (4.1), 298 (3.6); CI-MS *m/e* 214 (M + 1).

Syntheses, Spectral Features, and Reactions of Metribuzin and Deaminometribuzin Sulfoxides [I-S(O)Me and II-S(O)Me] (Figure 1). *Peracid Oxidation of I-SMe.* A solution of [^{14}C]I-SMe (6 mg) in chloroform (0.5 mL) was treated with appropriate amounts of *m*-chloroperbenzoic acid (MCPBA) with stirring at 0 °C. Analyses involved TLC (CE) and LSC.

I-S(O)Me. A solution of I-SMe (150 mg, 0.70 mmol) in chloroform (6 mL) at 0 °C was treated with MCPBA (65 mg, 0.35 mmol) dissolved in chloroform (6 mL) and stirred for 5 min at 0 °C. The chloroform was removed under vacuum and the residue taken up in ether. Solvent evaporation and cooling on partial removal of the ether under vacuum with no applied heat precipitated I-S(O)Me (30 mg, 37% based on MCPBA): ^{1}H NMR ($CDCl_3$) δ 5.92 (br s, 2 H, NH_2), 3.25 [s, 3 H, S(O)Me], 1.48 (s, 9 H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 166.9, 155.9, 149.8, 38.5, 37.2, 27.2. IR ($CHCl_3$) 1675 (C=O), 1050 cm^{-1} (S=O); CI-MS *m/e* 231 (M + 1).

II-S(O)Me. A solution of II-SMe (500 mg, 2.5 mmol) and MCPBA (700 mg, 3.8 mmol) in acetone (50 mL) at 0 °C was stirred for 2 h at 0 °C, the acetone was removed under vacuum, and the residue was taken up in ether. Partial removal of the ether gave II-S(O)Me as a precipitate (380 mg, 70%): ^{1}H NMR (CD_3COCD_3) δ 3.03 [s, 3 H, S(O)Me], 1.34 (s, 9 H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 164.6, 161.7, 159.3, 39.9, 37.7, 27.0; IR ($CHCl_3$) 1672 (C=O), 1050 cm^{-1} (S=O); CI-MS *m/e* 216 (M + 1).

I-SAr. 3,4-Dichlorobenzenethiol (6 μL, 0.05 mmol; Aldrich) was added to a solution of I-S(O)Me (10 mg, 0.043 mmol) in chloroform (0.5 mL). After 30 min at 25 °C, the reaction mixture was separated by TLC (0.5 mm silica gel, developed twice in ether–hexane, 1:1), and the band at R_f = 0.32 was scraped and extracted with ether to give I-SAr (9 mg, 60%): ^{1}H NMR ($CDCl_3$) δ 7.82 (br s, 1 H, aromatic), 7.60 (br s, 2 H, aromatic), 4.98 (br s, 2 H, NH_2), 1.43 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 213 nm (log ϵ 4.4), 294 (4.0); CI-MS *m/e* 345 (M + 1).

II-SAr. 3,4-Dichlorobenzenethiol (60 μL, 0.48 mmol) was added to an acetone solution (6 mL) of II-S(O)Me, prepared in situ from II-SMe (65 mg, 0.33 mmol) and MCPBA (80 mg, 0.43 mmol) as described above. The solution was stirred overnight. II-SAr (78 mg, 70%, mp 220–223 °C) was obtained both as a white precipitate on partial removal of the acetone under vacuum and by preparative TLC of the supernatant (0.5 mm silica gel, acetonitrile–chloroform, 1:7, R_f 0.67, recovered by chloroform extraction): ^{1}H NMR (CD_3COCD_3) δ 7.89 (br s, 1 H, aromatic), 7.65 (br, d, 2 H, aromatic), 1.30 (s, 9 H, *t*-Bu); UV (MeOH) λ_{max} 234 (log ϵ 4.2); CI-MS *m/e* 330 (M + 1).

I-SMA. MCPBA (150 mg, 0.90 mmol) in acetone (5 mL) at 0 °C was added to I-SMe (300 mg, 1.4 mmol) in acetone (10 mL) at 0 °C. After 10 min this cold solution was added dropwise to NAcCys (500 mg, 3 mmol) dissolved in 0.1 M

Table II. Metabolism of [^{14}C]Metribuzin by Phenobarbital-Induced Rat and Mouse Liver Microsomes in the Presence or Absence of *N*-Acetylcysteine and NADPH

^{14}C compd or ^{14}C fraction	radiocarbon recovery, %,[a] with indicated fortification[b]			
	none	NAcCys	NADPH	NAcCys + NADPH
Rat Liver Microsomes				
I-SMe	80	91	35	28
II-SMe	0.3	0.6	24	18
I-SMA + II-SMA[c]	0	3	0	45
unidentified[d]				
apolar	7	5	8	1
polar	1	0.2	19	7
bound	0.3	0.2	14	1
Mouse Liver Microsomes				
I-SMe	84	83	56	56
II-SMe	0.9	0.6	6	6
I-SMA + II-SMA[e]	0	2	0	28
unidentified[d]				
apolar	14	13	13	7
polar	0.6	0.8	8	3
bound	0.6	0.2	17	0.7

[a] Average of two independent studies with data for product yields varying by 0.8–1.2-fold between the experiments. [b] Mixtures of microsomes (150 mg fresh liver weight equivalent) with NAcCys (5 mg) and/or NADPH (2.2 mg) in 0.1 M phosphate, pH 7.4, buffer (2.5 mL) incubated for 2 h at 37 °C. [c] About equal amounts of I-SMA and II-SMA. [d] The unidentified metabolites are apolar (chloroform extractable), polar (methanol extractable), or protein bound. Apolar and polar unknowns include material at the origin or streaking on the plate. [e] >80% I-SMA with a small amount of II-SMA.

phosphate, pH 7.4, buffer (25 mL). After stirring 18 h at 25 °C, the solution was extracted with ether (2 × 20 mL), and the aqueous phase was then saturated with NaCl and extracted with ether–ethanol, 3:1 (3 × 40 mL), to recover crude I-SMA.

II-SMA. II-S(O)Me (100 mg, 0.47 mmol) was dissolved in acetone (5 mL) and treated as above to obtain crude II-SMA.

Derivative with BSA. A solution of [^{14}C]I-SMe (10 μg) and MCPBA (30–45 μg) was held in acetone (35 μL) for 30 min at 25 °C. After analysis for I-S(O)Me content by TLC (CE), the solution was diluted with acetone to 0.2 mL, added to BSA (10 mg) dissolved in 0.1 M phosphate, pH 7.4, buffer (2 mL), and stirred for several hours at 37 °C. An equal volume of 10% aqueous trichloroacetic acid was added. The precipitate recovered by centrifugation was redissolved in water (2 mL) and reprecipitated as above, and the procedure was repeated a third time to ensure that no soluble radioactivity remained. The precipitate was dissolved in Soluene for LSC.

RESULTS

Metabolism of [^{14}C]Metribuzin in Rats and Mice. The major urinary metabolite in rats is II-SMA (identification discussed below) and in mice chromatographs (BAW) in the position of I-SMA + II-SMA, in each species accounting for ~20% of the administered dose within 5 days after treatment. Although other urinary products are not identified, they are polar compounds, such as conjugates, and no one of them accounts for more than 5% of the dose. Rat feces contain 0.1–1% of the administered radiocarbon as unmetabolized I-SMe. The other rat fecal products are not identified but do not include any of the nonconjugated compounds shown in Figure 1 based on TLC cochromatography (CE, BAW) (Table I). No radioactivity is expired by rats as [^{14}C]carbon dioxide.

Table III. Triazinediones from Acid Hydrolysis of [^{14}C]Metribuzin and the [^{14}C]Mercapturic Acids Formed on in Vivo and in Vitro Metabolism in Rats and Mice

material hydrolyzed[a]	triazinedione, %[b]	
	III-NH_2	III-H
I-SMe	89	11
II-SMe	0[c]	100[c]
I-SMA + II-SMA[d]		
rat urine	0.5	99
rat enzyme	51	49
mouse enzyme	84	16

[a] 2 N HCl, 3 h, 100 °C. [b] Yields of III-NH_2 and III-H normalized to 100%. Additional products were not recovered on chloroform extraction or not resolved by TLC, respectively, as follows: I-SMe, 3 and 2%; rat urine, 5 and 0.5%; rat enzyme, 11 and 11%; mouse enzyme, 5 and 5%. [c] Results with unlabeled II-SMe based on TLC and product visualization by UV quench. [d] The percent radiocarbon recovery as mercapturic acids (I-SMA plus II-SMA) was ~20% in rat urine (Bleeke et al., 1984) and 45 and 28% in rat and mouse enzyme systems, respectively (Table II).

Metabolism of [^{14}C]Metribuzin in Rat and Mouse Liver Microsomal Enzyme Systems. Comparative studies revealed that phenobarbital induction increases NADPH-dependent metabolism of I-SMe by 2–3-fold in rats and about 50% in mice. [^{14}C]I-SMe undergoes relatively little metabolism or decomposition on incubation with phenobarbital-induced rat or mouse liver microsomes alone or fortified with NAcCys (Table II). Metabolism is greatly facilitated and new metabolites are formed on fortification with NADPH or a combination of NADPH and NAcCys. Deamination to II-SMe is more prominent with rat than with mouse liver preparations. Fortification with both NADPH and NAcCys gives a mixture of I-SMA and II-SMA, as discussed later. Protein-bound ^{14}C derivatives are major NADPH-dependent products in the absence of NAcCys but not in its presence, suggesting that the activated intermediates forming the I-SMA plus II-SMA mixture with NAcCys are also trapped by reaction with protein. TLC analysis (CE and BAW, Table I) revealed that the apolar and polar unknowns do not include I-S(O)Me, I-SG, II-S(O)Me, II-SG, and IV-NH_2 and that little (<1%) III-H is present. III-NH_2 is occasionally detected as a minor (1–2%) NADPH-dependent metabolite formed in the absence of added thiols.

A GSH conjugate appears to form as a microsomal metabolite under suitable conditions. Thus, a product of appropriate TLC characteristics (BAW) is obtained with suitable mouse liver enzyme systems in yields as follows: microsome plus NADPH <1%; soluble fraction plus NADPH 2%; microsome plus soluble fraction plus NADPH 16%; microsome GSH plus NADPH 13%.

Identification of Mercapturic Acids in Urine and Enzyme Systems. I-SMA and II-SMA are not separated in the TLC systems examined (Table I). They were therefore identified as mercapturates by TLC cochromatography and as I-SMA or II-SMA by degradation and spectroscopic methods described below.

Acid hydrolysis of I-SMe and II-SMe gives primarily III-NH_2 and III-H, respectively (Table III). Analogous reactions of the mercapturic acids serve to distinguish I-SMA from II-SMA. The rat urinary mercapturic acid fraction yields almost only III-H, strongly indicating that it is almost entirely II-SMA (Table III). The mouse mercapturic acids were not isolated for identification, but direct acid hydrolysis of the urine yields 35% III-NH_2, 5% III-H, and 60% polar products. The isolated mercapturic acids from the mouse enzyme give III-NH_2 and III-H in a 5:1 ratio, approximating the 8:1 ratio for hydrolysis of [^{14}C]I-SMe, but in marked contrast to the 1:1 ratio for the

Table IV. Comparison of Dimethylated Mercapturic Acids (Vb and VIIb) with Related S-Methyl Derivatives

methylated deriv[a]			NMR, δ				UV, λ_{max}, nm
compd	R_1	R_2	S-Me	N-2-Me	N-4-Me	O-Me	
			N-2-Me Derivatives				
Va	*t*-Bu	SMe	2.55	3.72			236
Vb	*t*-Bu	SMA	*b*	*b*			239
Vc	Me	SMe	2.48	3.66			236
			N-4-Me Derivatives				
VIa	*t*-Bu	SMe	2.67		3.45		211, 230, 295
VIc	Me	SMe	2.60		3.35		231, 284, 295
			O-Me Derivatives				
VIIa	*t*-Bu	SMe	2.62			4.03	208, 248, 298
VIIb	*t*-Bu	SMA	*b*			*b*	211, 246, 294
VIIc	Me	SMe	2.58			4.02	247, 305

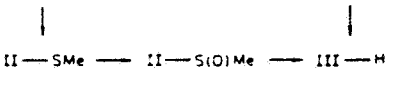
[a] Series a from synthesis, b from methylation of a rat urinary metabolite, and c from the literature (Daunis et al., 1971). [b] Not determined.

rat enzyme products (Table III). These findings suggest that the mercapturic acid fraction is >80% I-SMA with a small amount of II-SMA for the mouse but about equal amounts of I-SMA and II-SMA for the rat. Thus, the ratio of III-H to III-NH_2 both in vivo and in vitro suggests that deamination of I-SMe is more important in rats than in mice.

The UV spectra of triazinones give absorbances characteristic of the substitution pattern on the ring, allowing comparison of the mercapturic acid metabolites with I-SMe and II-SMe. The TLC-isolated mouse microsomal product is primarily I-SMA since the spectrum in methanol has two main absorbances [λ_{max} as nm (log ε)], i.e., 223 (4.0) and 296 (3.6), very similar to those of I-SMe with 227 (3.9) and 293 (3.9). The rat urinary mercapturic acid fraction is almost only II-SMA, giving 238 (4.2), much like that of II-SMe with 236 (3.1).

Methylation of the rat urinary mercapturic acid fraction gives two dimethyl derivatives with spectral features further supporting the identification of this metabolite as primarily II-SMA. Thus, treatment with diazomethane, followed by HPLC and TLC, results in the isolation of two products. The CI-MS for each compound gives a $(M + 1)^+$ signal of 343, the expected mass of II-SMA after methylation of both the ring and the carboxylic acid. Table IV compares the spectral data of the two methylated derivatives of the mercapturic acid (Vb and VIIb) with the three methylated products from II-SMe, V–VIIa, and the related compounds V–VIIc. The UV λ_{max} values of the methylated mercapturic acid derivatives correspond to those of the N-2-Me (239 nm) and the O-Me (246 nm) compounds. Clearly, the mercapturic acid metabolite is methylated at the N-2 and O positions as anticipated for II-SMA.

Peracid Oxidation of Metribuzin and Deaminometribuzin. The reaction of I-SMe with MCPBA in chloroform at 0 °C proceeds as follows (Figure 2):



I-S(O)Me is the principal product formed with 1 part of MCPBA to 2 parts of I-SMe (30–40% yield based on MCPBA). With increasing oxidant (1:1, 2:1, and 4:1

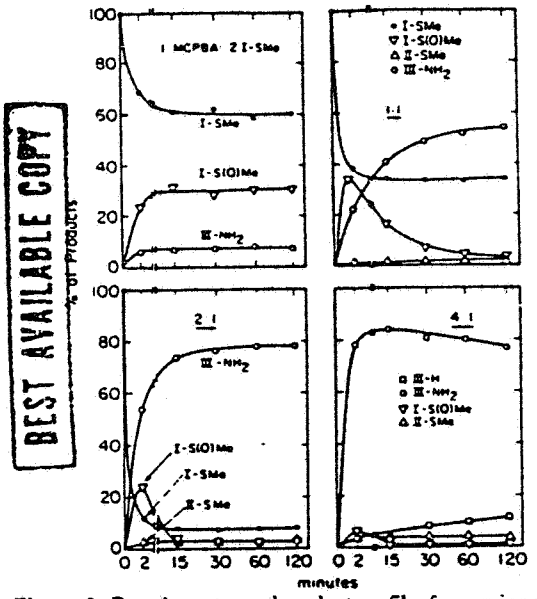


Figure 2. Reaction rates and product profiles for varying ratios of metribuzin to *m*-chloroperbenzoic acid in chloroform at 0 °C.

MCPBA–I-SMe), I-S(O)Me is rapidly converted to III-NH_2, which becomes the major final product. At the 4:1 ratio very little I-S(O)Me is detected, even at short reaction times. The product profile indicates that I-S(O)Me serves as the intermediate in forming III-NH_2, a proposal confirmed by finding that MCPBA directly converts I-S(O)Me to III-NH_2.

The deamino derivatives II-SMe and III-H are minor products. I-SMe is oxidatively deaminated to II-SMe but at a very slow rate compared with sulfoxidation. III-H is the final product resulting from oxidation at both the SCH_3 and NH_2 groups and becomes significant only with a large excess of MCPBA. Studies comparable to those shown in Figure 2 but with II-SMe establish a much slower conversion rate for II-SMe to II-S(O)Me and in turn to III-H than for the analogous reactions in the I-SMe series. II-S(O)Me is formed almost quantitatively on oxidation of II-SMe with a slight excess of MCPBA, showing that in this case sulfoxidation is much faster than subsequent cleavage to the diketo compound. II-S(O)Me is not detected as a product on treatment of I-SMe with MCPBA because little II-SMe is formed and its subsequent oxidation is slow.

Preparation, Properties, and Reactions of Metribuzin and Deaminometribuzin Sulfoxides. I-S(O)Me and II-S(O)Me are formed in 70–80% yields (NMR) based on MCPBA and can be isolated in 37 and 70% yields, respectively, on MCPBA oxidation of I-SMe in chloroform and of II-SMe in acetone at 0 °C. I-S(O)Me is rarely obtained in >80% purity due to decomposition to III-NH_2; partial decomposition occurs within 1 day either neat or in chloroform at 0 °C and complete breakdown is evident within 8 h at 25 °C. II-S(O)Me, obtained in >95% purity, is stable for weeks as a crystalline solid at 0 °C and for at least a few days at 25 °C.

Sulfoxides I-S(O)Me and II-S(O)Me are identified on the basis of their reactions, described below, and of their spectral features that are characteristic of sulfoxides (Silverstein et al., 1981). They each give CI-MS base peaks appropriate for monooxygenated derivatives, a strong IR band at 1050 cm^{-1} associated with the S—O absorption, a ^1H NMR spectrum with a signal for the S(O)Me protons

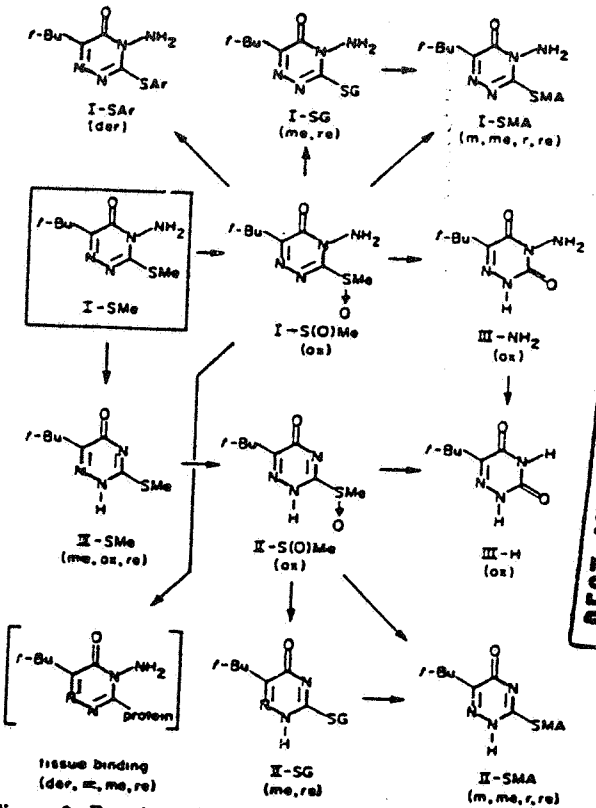


Figure 3. Reactions of metribuzin. Abbreviations: der, derivatization; m, mouse; me, mouse enzyme; ox, MCPBA; r, rat; re, rat enzyme.

shifted 0.5–0.6 ppm downfield from the SMe protons of the parent compound, and a ^{13}C signal for the S(O)Me substituent shifted 24–27 ppm downfield with respect to the parent SMe group.

The thiol 3,4-dichlorobenzenethiol reacts quickly with I-S(O)Me and II-S(O)Me, displacing the S(O)Me group to form thioethers I-SAr and II-SAr, respectively, which are crystalline or solid products; an analogous reaction was used to characterize cyanatryn sulfoxide (Bedford et al., 1975). I-S(O)Me and II-S(O)Me also react with NAcCys to give products with chromatographic properties identical with those of the mercapturate metabolites of I-SMe discussed above.

The protein BSA also reacts readily with I-S(O)Me at physiological pH. Thus, addition of the crude reaction mixture of [^{14}C]I-SMe and MCPBA [containing ~25% I-S(O)Me] to BSA leads to binding of about 5% of the total radioactivity compared to only 0.1% binding for an equivalent reaction of BSA and [^{14}C]I-SMe with no oxidant.

DISCUSSION

Several chemical and metabolic reactions of I-SMe are given in Figure 3. Sulfoxides I-S(O)Me and II-S(O)Me, from MCPBA oxidations of I-SMe and II-SMe, respectively, are reactive, electrophilic compounds. I-S(O)Me is harder to isolate and less stable than II-S(O)Me. Further oxidation of I-S(O)Me with MCPBA gives diketo derivative III-NH_2. The mechanism of this conversion (formally a replacement of S(O)Me with OH followed by tautomerization) is not known. Although a possible short-lived intermediate, no sulfone has been isolated or identified by TLC or NMR of the reaction mixture. A side reaction in MCPBA oxidation of I-SMe is the loss of NH_2, resulting in II-SMe. Reactions at both sites to give III-H is important only with a large excess of MCPBA. No intermediates are detected in the deamination reaction.

In vitro metabolism of I-SMe with rat or mouse liver microsomal enzymes occurs at both the N-NH_2 and SMe substituents. The enzymes involved require NADPH and are induced by phenobarbital, suggesting an oxidative mechanism for both processes. In contrast, deamination of I-SMe in plants appears to be a reductive cleavage (Fedtke and Schmidt, 1983). I-S(O)Me is the proposed intermediate in yielding thiol conjugates and protein derivatives. Analogous pathways are known in mammalian metabolism of other sulfur-containing pesticides (Casida et al., 1975; Bedford et al., 1975; Hubbell and Casida, 1977; Crawford et al., 1980; Hutson, 1981). Formation of I-SG requires oxidative activation by microsomes and is not dependent on the soluble fraction, indicating that conjugate formation is mediated by a microsomal GSH *S*-transferase or is due to direct chemical reaction between I-S(O)Me and GSH; the latter proposal is consistent with the demonstrated reactivity of chemically formed I-S(O)Me. Systems lacking thiols generally give more unidentified products and streaking on TLC, possibly due to decomposition of I-S(O)Me to III-NH_2 and other compounds. No IV-NH_2 is detected in the microsomal oxidations, suggesting that I-SMe does not undergo S-demethylation. No attempt was made to look for products resulting from oxidation of the *tert*-butyl group.

The findings on in vivo mammalian metabolism parallel the in vitro results. Deamination appears more important in rats than in mice, as is also the case for microsomal metabolism. The lack of [^{14}C]carbon dioxide formation by rats treated with [*carbonyl*-^{14}C]I-SMe suggests that ring opening followed by decarboxylation does not occur. Mercapturic acid formation is a major metabolic pathway, consistent with initial formation of a sulfoxide followed by conjugation with GSH. Sulfoxidation in mice appears to activate I-SMe for conjugation with GSH until the thiol is depleted and then for reaction with tissue proteins and associated hepatotoxicity (Bleeke et al., 1984).

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Luis Ruzo and Ian Holden of this laboratory assisted in the MS and NMR analyses, respectively.

Registry No. I-SMe, 21087-64-9; I-S(O)Me, 90269-30-0; I-SAr, 90269-27-5; I-SMA, 90269-25-3; II-SAr, 90269-28-6; II-SMe, 35045-02-4; II-S(O)Me, 90269-26-4; II-SMA, 90269-24-2; III-H, 52236-30-3; III-NH_2, 56507-37-0; IV-H, 66392-60-7; IV-NH_2, 33509-43-2; Va, 79988-50-4; VIa, 62036-60-6; VIIa, 90269-29-7; trimethylpyruvic acid, 815-17-8; thiocarbohydrazide, 2231-57-4; thiosemicarbazide, 79-19-6; 3,4-dichlorobenzenethiol, 5858-17-3.

LITERATURE CITED

Bedford, C. T.; Crawford, M. J.; Hutson, D. H. *Chemosphere* 1975, *4*, 311.

Bleeke, M. S.; Smith, M. T.; Casida, J. E. *Pestic. Biochem. Physiol.* 1984, in press.

Casida, J. E.; Kimmel, E. C.; Ohkawa, H.; Ohkawa, R. *Pestic. Biochem. Physiol.* 1975, *5*, 1.

Crawford, M. J.; Hutson, D. H.; Stoydin, G. *Xenobiotica* 1980, *10*, 169.

Daunis, J.; Jacquier, R.; Viallefont, P. *Bull. Soc. Chim. Fr.* 1971, 3658.

Draber, W.; Dickore, K.; Büchel, K. H.; Trebst, A.; Pistorius, E. *Naturwissenschaften* 1968, *55*, 446.

Eue, L. *Pflanzenschutz-Nachr.* 1972, *25*, 175.

Fedtke, C.; Schmidt, R. R. In "Pesticide Chemistry: Human Welfare and the Environment"; Miyamoto, J.; Kearney, P. C., Eds.; Pergamon Press: Oxford 1983; pp 177–181.

Frear, D. S.; Mansager, E. R.; Swanson, H. R.; Tanaka, F. S. *Pestic. Biochem. Physiol.* 1983a, *19*, 270.

J. Agric. Food Chem. 1984, 32, 755-759

94605

Frear, D. S.; Swanson, H. R.; Mansager, E. R. "Abstracts of Papers", 185th National Meeting of the American Chemical Society, Seattle, WA, March 1983; American Chemical Society: Washington, DC, 1983b; PEST 103.
Hilton, H. W.; Nomura, N. S.; Yauger, W. L., Jr.; Kameda, S. S. *J. Agric. Food Chem.* 1974, 22, 578.
Hubbell, J. P.; Casida, J. E. *J. Agric. Food Chem.* 1977, 25, 404.
Hutson, D. H. In "Sulfur in Pesticide Action and Metabolism"; Rosen, J. D.; Magee, P. S.; Casida, J. E.; American Chemical Society: Washington, DC, 1981; ACS Symp. Ser. No. 158, p 1.
Mangeot, B. L.; Slife, F. E.; Rieck, C. E. *Weed Sci.* 1979, 27, 267.
Nakayama, Y.; Sanemitsu, Y.; Yoshioka, H.; Nishinaga, A. *Tetrahedron Lett.* 1982, 23, 2499.
Neunhoeffer, H.; Wiley, P. F. *Heterocycl. Compd.* 1978, 33, 194-574.
Saeman, M. C. Ph.D. Dissertation, University of California, Berkeley, CA, 1984.
Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. "Spectrometric Identification of Organic Compounds", 4th ed.; Wiley: New York 1981.

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Interaction between γ-Hexachlorocyclohexane and the Gastrointestinal Microflora and Their Effect on the Absorption, Biotransformation, and Excretion of Parathion by the Rat

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Pretreatment of rats with the organochlorine insecticide lindane reduced the estimated absorption rate of parathion from the gastrointestinal tract. Lindane pretreatment also significantly reduced the metabolism of parathion to *p*-nitrophenol in vivo. Lindane pretreatment altered the gastrointestinal (GI) microflora by increasing the ratio of anaerobes to aerobes. Consiatent with this alteration was a significantly greater retention of unaltered parathion and the microbial metabolite aminoparathion in the GI tract of the lindane-pretreated rats 1 h after the administration of parathion. Enhanced conversion of parathion to aminoparathion together with a slower absorption rate may play a role in the antagonism parathion toxicity by lindane.

In the past it has been widely reported that pretreatment of animals with halogenated chemicals antagonizes the toxicity of organophosphate pesticides (Ball et al., 1954; Triolo and Coon, 1966; Townsend and Carlson, 1981; Iverson, 1976; Mendoza and Shields, 1976; Welch and Coon, 1964; Triolo et al., 1970). Moreover, this antagonism has generally been attributed to induction of either esterases or hepatic mixed function oxidases. While examining the dynamics of the absorption, biotransformation, and excretion of parathion [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphorothioate], we observed that pretreatment of rats with lindane (γ-hexachlorocyclohexane) impaired the estimated absorption rate of parathion. Corresponding to this impaired absorption, significantly lower excretion rates during the initial absorption were also observed in the lindane-pretreated rats. A series of experiments designed to determine the mechanism by which lindane elicited these effects produced data that indicate that altered gastrointestinal microflora may contribute to the protective effect of lindane and possibly other halogenated chemicals against the toxicity of ingested organophosphate pesticides.

MATERIALS AND METHODS

Apparatus. Gas–liquid chromatographic analysis was performed on a Tracor Model MT-220 gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) and a flame photometric detector (FPD). Urinary *p*-nitrophenol was determined by ECD on 1% SP1240-DA on 100–120-mesh Supelcoport at 170 °C with 60 cm^3/min of 95:5 methane–argon carrier gas. Derivatized diethyl phosphorothioic acid (DETP), diethylphosphoric acid (DEP), and paraoxon [*O,O*-diethyl *O*-(*p*-nitrophenyl) phosphate] were determined by FPD with 3% OV-1 on 80–100-mesh Chromosorb W at 210 °C. Aminoparathion and parathion were analyzed by FPD with 3% QF-1 on 80–100-mesh Gas-Chrom Q at 185 °C. Air, H_2, and N_2 carrier gas flows were regulated at 100, 50, and 60 cm^3/min, respectively.

Reagents. [*ring*-2,6-^{14}C]Parathion (specific activity 12.2 mCi/mmol and 98% purity) was obtained from Amersham Corp., Arlington Heights, IL. Parathion, aminoparathion, DETP, DEP, paraoxon, and lindane were obtained from the EPA, Health Effects Research Laboratory Analytical Reference Standards Repository, Research Triangle Park, NC. Pentafluorobenzyl bromide (PFB-Br) was obtained from Aldrich Chemical Co., Milwaukee, WI. Tetrahexylammonium hydrogen sulfate was obtained from Regis Chemical Co., Morton Grove, IL; 3% QF-1 on 80–100-mesh Gas-Chrom Q and 3% OV-1 on 80–100-mesh Chromosorb W were obtained from Applied Science Laboratories, Inc., State College, PA. 1% SP-1240 DA on 100–120-mesh Supelcoport was obtained from Supelco Inc., Bellefonte, PA. Thioglycollate medium was obtained from Becton, Dickinson and Co., Cockeysville, MD. Bacto nutrient broth was obtained from Difco Laboratories, Detroit, MI.

Procedures. Separate experiments were conducted to determine (1) the effects of pretreatment with lindane or

U.S. Environmental Protection Agency, Health Effects Research Laboratory, Developmental Biology Division (MD 67), Perinatal Toxicology Branch, Research Triangle Park, North Carolina 27711.

Data Review:

Study Identification:

Study Title: The Metabolism and Excretion of SENCOR in Rats.

EPA Identification Numbers:

Sponsor: Mobay Chemical Corporation
Chemagro Agricultural Division
Kansas City, Missouri 64120

Testing Laboratory: Chemagro Division of Baychem Corporation
Research and Development Department

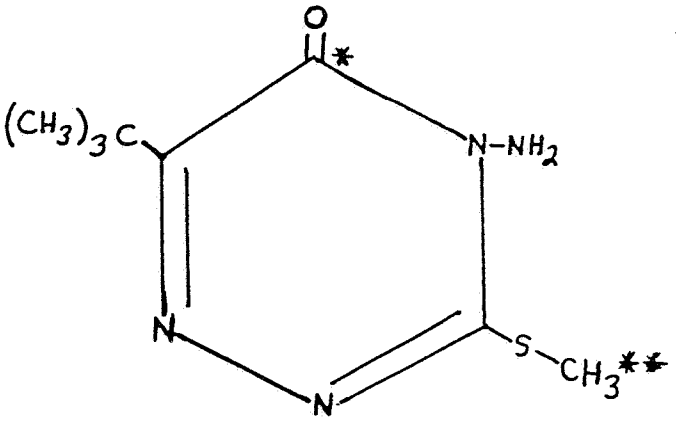
Report Number: 33366

Date of Study: May 1, 1972
Revised July 5, 1 73 (to add additional information)

Study Authors: D.R. Flint
R.R. Gronberg
F.E. Sandie

Study Director: T.B. Waggoner

Test Compound: SENCOR [4-Amino-6-t-butyl-3-(methylthio)-1-2,4-triazin-5(4H)-one] (below) initially labeled with carbon-14 in the carbonyl group* and with tritium in the s-methyl group**.



Radiolabelled SENCOR

07369

Dosages: First excretion study: 4 mg SENCOR - ^{14}C, ^{3}H in 0.8 ml 50% aqueous ethanol for a dose rate of 20 mg/kg in a 200 gm rat given orally by gavage (stomach tube).

Second excretion study and tissue residue studies: SENCOR (presumably ^{14}C labelled only , although not clearly stated) administered orally as a suspension in 0.5% aqueous gum tragacanth For animals weighing 150 to 165 gm, dosage volumes of 0.75 to 1.00 ml per animal were administered (presumably by gavage). The dose rates were calculated as 100 and 50 mg/kg for these studies.

Test Animals: Rats, Sprague-Dawley strain
Sprague-Dawley Company

Experimental: A copy of the experimental section from the investigators report is appended.

There was no clear indication as to how many animals were used at study initiation, however the results section states that one male and one female rat were used for the first excretion study, 2 males for the second excretion study and from the tables, 2 males and 2 females for the tissue residue studies. Also the age and sex of the animals was not given (although the results sections mentions male and female).

Apparently 2 excretion studies were conducted, one using glass metabolism cages with collection of respiratory gases and the other study using plastic with no collection of gases.

There was no mention of the purity of the test compound (a statement was made: "All equipment was standard except as listed and all chemicals were reagent grade or better.").

There was no indication of the time period for observation of animals (Guidelines state 7 days or until 90+% of the administered dose is excreted, with the animals in individual metabolism cages), although the individual tables in the final report state collection times.

The investigators examined expired air (only in the initial study for both ^{3}H and $^{14}CO_2$), urine, feces, blood, plasma, liver, kidney, heart, brain, muscle, testes, ovaries and fat. There was apparently no analysis of bone, lungs, spleen or residual carcass.

Results:

Excretion Studies:

The first study (using ^{14}C, ^{3}H labelled SENCOR) involved only 2 animals, one male and one female. The investigators reported sex related differences in excretion where in the male, 60.7% of the recovered radioactivity was found in the feces and in the female, 57.4% of the recovered radioactivity was found in the urine (over 90% of ^{14}C was recovered in urine and feces of both animals over a 16 day period). These values probably include measured ^{3}H levels as the total values on Table I do not totally agree. They further stated that no ^{14}C was recovered in the expired air. Sex related differences were also seen in the blood and tissue studies (to be discussed later).

The second study used 2 male rats (using only ^{14}C labelled SENCOR). The investigators found 45.89% of the radioactivity in the feces and 56.27% in the urine, from these finding they justified their reason for not collecting expired air, since the total was 102.16% of the administered radioactivity. See Table I. The excretion peak levels from this study were generally in agreement with the earlier study.

Table I: Excretion of Radioactivity (% of administered radioactivity)

Hours Post-Administration	Male		Female		2 Males	
	Urine	Feces	Urine	Feces	Urine	Feces
6.0	-	-	-	-	7.86	-
7.0	8.21	0.08	7.27	0.06	-	-
7.5	-	-	-	-	-	0.75
9.0	-	-	-	-	3.24	-
12.0	4.78	9.21	10.23	0.93	5.09	-
18.0	13.51	4.77	17.70	9.08	-	-
24.0	4.59	6.43	8.50	6.73	18.90	-
30.0	2.42	25.23	2.85	12.73	-	29.80
48.0	1.25	6.38	3.02	7.04	9.52	21.76
72.0	0.52	2.60	1.10	1.78	0.45	2.74
96.0	-	-	-	-	0.47	0.36
100.0	0.26	0.39	0.41	0.05	-	-
120.0	-	-	-	-	0.36	0.87
124.0	0.11	0.10	0.20	0.05	-	-
16 days	0.36	0.34	0.57	0.14	-	-
TOTAL	36.00	55.53	51.85	38.59	45.89	56.27

Data extracted from CHEMAGRO Report No. 33366 Tables I and II.

Tissue Residue Studies:

These determinations reportedly involved 2 male and 2 female rats. The investigators stated that the residue levels were "generally similar" between the male and female rats except at 28 hour (after administration) interval which showed the females retaining more of the radioactivity in all tissues examined. After this time point the decline is similar, however the females still show slightly higher levels. There were not enough animals for statistical evaluation. The investigators further state that this was due to "sex-related differences in rates of absorption, metabolism, distribution and/or excretion". The table which they present for "half-lives" compares different interval measurements. See Table II.

Table II: Radioactive Residues in Rat Tissues (estimated "half-lives" of total ^{14}C in hours)

Tissue	Male†	Female††
Brain	21.1	22.4
Heart	26.4	33.6
Liver	30.4	33.6
Kidney	26.9	31.2
Muscle	21.3	24.5
Testes or Ovaries	18.4	30.4
Fat	25.0†††	24.8
Blood Plasma	19.1	27.2

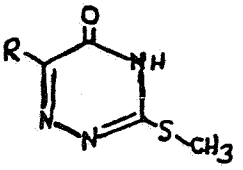
† - determined over a 24 to 96 hour interval.
†† - determined over a 48 to 96 hour interval.
††† - apparent biphasic decay curve after 24 hours.

Data extracted from CHEMAGRO Report No. 33366 Table III.

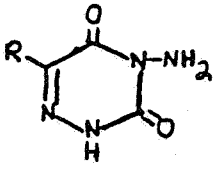
The investigators noted high tissue residue levels in liver and kidney (stated "presumably due to concentration in these organs for detoxification and elimination"). As can be seen in Table II the female rat presented with higher residue levels in heart, kidney, sex organs and blood plasma than the male rat.

Metabolite Identification:

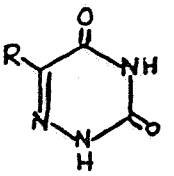
From earlier metabolism studies in the soybean plant, three metabolites have been identified.



A



B



C

R = tertiary butyl

A - DA, deaminated SENCOR, also called BAY Dic 2058
B - DK, diketo SENCOR
C - DADK, deaminated diketo SENCOR, also called BAY Dic 2164

These metabolites were also identified in the animal studies. However, not all the residues were accounted for in the present study and many of the methods employed by the investigators destroyed much of the primary metabolites; this was especially true for the conjugate hydrolysis methods. The investigators should have employed non-harsh methods which could have involved the pre-separation of the metabolites prior to analysis and then study each metabolite separately.

Urine:

The investigators employed thin-layer chromotography (TLC) methods for urine studies. They observed that very polar solvent systems were needed to separate the samples and stated that this indicated that there were "either highly polar metabolites or, more likely, conjugated metabolites".

Enzyme incubation did not substantially change the pattern urine metabolites. The investigators then employed acid hydrolysis and found that one third of the radioactivity in the urine was rendered organoextractable. The organoextractable fraction was submitted to gas chromotographic analysis and SENCOR, DA, DK and DADK metabolites were found.

From other experiments the investigators stated that they found that the conditions of hydrolysis (not given) can affect a near complete de-thiomethylation of SENCOR and the DA metabolite to produce the DK and DADK metabolites, therefore the procedure of using acid hydrolysis after enzyme treatment was not an accurate determination of urine metabolic distribution.

Studies with potatoes found that incubation in buffers of near neutral pH at 37°C could release significant amounts of SENCOR without other treatment.

A pooled 24 hour rat urine specimen was first deproteinized with perchloric acid and then extracted twice with isopropyl ether (IPE). The IPE extracts were analyzed by gas chromatography revealing small amounts of SENCOR and the 3 metabolites. The water soluble portion was analyzed by gel filtration. Two large fractions were found and were further treated by hydrolysis and ion-exchange chromatography. Although the investigators state that work in the area is not complete, they feel that significant amounts of the fractions they found from gel filtration are conjugates of SENCOR and its metabolites.

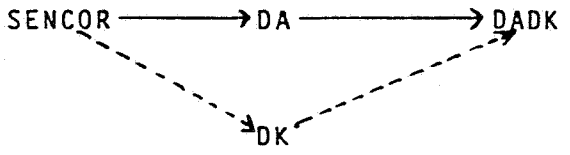
Tissues:

Liver and muscle tissues from male and female rats were homogenized in a two-phase water-chloroform system and each phase was assayed. They found slight differences in liver distribution of the compound between the male and female rats, whereas muscle distribution showed no sex related differences. The insoluble residue from the 28 hour female rat liver tissue extraction was also assayed using various enzymatic and hydrolytic methods (below).

The investigators evaluated several procedures and decided to employ a pepsin digestion followed by an acid hydrolysis of the aqueous phase. They were able to render 94-96% of the activity in the muscle, 55-78% of the activity in the liver and 43-58% of the activity in the kidney organosoluble. They could also render 63-96% of the activity of the brain and heart organosoluble without acid hydrolysis.

Tissues from male and female rats, collected at 4 and 28 hours after ^{14}C-SENCOR administration showed similar patterns of metabolites (no sex related differences). They determined that the DA metabolite appears early with the DK and DADK metabolite being produced at later intervals.

The scheme is as follows:



The investigators state that "the solid line indicates the more active pathway". However it is noted that on page 15 of the report both dotted lines point to "DK" and on page 16 one dotted line points to "DK" and the other to "DADK". The latter is consistent with the findings of the report.

Feces:

In a preliminary investigation the investigators tried organic extraction with acetone, methanol and then water. They were unable to extract the isotopes. TLC analysis yielded little information. No other procedures were tried.

Conclusions:

The excretion studies found sex related differences with the males excreting the radiolabel primarily in the feces and the females excreting the label primarily in the urine, however this reviewer feels that an inadequate number of animals was used in this study (one male and one female in one study and two males in another study). Tissue distribution studies also suggested slight sex related differences in distribution up to the 28 hour interval (after administration) with similar patterns of reduction in residue levels after that time point (however the females tended to present with higher overall levels at all time points measured). These studies also used an inadequate number of animals.

The investigators found a metabolic scheme for SENCOR in rats that was similar to what was found in an earlier study in soybeans. The metabolites that were identified are:

deaminated SENCOR (DA), also called BAY Dic 2058
diketo SENCOR (DK)
deaminated diketo SENCOR (DADK), also called BAY Dic 2164

Additional metabolites were not identified.

The following are the study deficiencies:

1. The numbers of animals used was inadequate.
2. The age of the animals was not provided.
3. The purity and clear isotope identification of the test compound was not given.
4. Rationale for time frame used for collection of urine, feces and expired air since there should have been some time points earlier than the 7.0 hour in one study and 6.5 in the other.
5. There was no tissue analysis of bone, lungs, spleen and residual carcass.

Core Classification: Core-Supplementary Data based on above deficiencies.

METRIBUZIN

RIN: 3187-91

Page____ is not included in this copy.

Pages 103 through 109 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

37369

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/11/89
Pharmacologist, Review Section I, TB-HFAS/HED (H7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. YMI 7/13/89
Acting Section Head, Review Section I, TB-HFAS/HED (H7509C)

DATA EVALUATION RECORD

I. Study Type: Analytical Methodology
No Guideline Requirement

Study Title: A Liquid Chromatographic Method for the Determination of Metribuzin and Metolachlor (SENCOR - Dual) in Inhalation Chamber Atmospheres

EPA ID Number(s): EPA ID No. 3125-270
EPA MRID No. 262892
EPA Pesticide Chemical Code 101101
Caswell No. 33D
HED Project No. 9-0271A
Document No.

Submitter: Mobay Corporation
Agricultural Chemicals Division
P.O. 4913
Hawthorn Road
Kansas City, Missouri 64120-0013

Testing Laboratory: Mobay Chemical Corporation
Environmental Health Research
Corporate Toxicology Department
17745 Metcalf
Stilwell, Kansas 66085

Study Number(s): Toxicology Report No. 640
Mobay AG Chem No. 90273

Study Date(s): July 2, 1985

Study Author(s): R.S. Schroeder
K.D. Moore
C.L. Rudy

Test Material: Metribuzin technical Standard
500 mcg/ml in acetonitrile
Metolachlor technical standard
500 mcg/ml in acetonitrile

II. Materials and Methods: A copy of the "materials and methods" section from the investigator's report is appended. The comments and highlights from these "materials and methods" are as follows.

> This submission is not a Guideline requirement, however, it affects Guideline 81-3.

The basic equipment and analytical methodology are described in detail on the attached "Methods and Materials." Acetonitrile was chosen as the solvent since it was compatible with the mobile phase of the HPLC work.

III. Results

The investigators found a linear response for Metribuzin from 0-15 mcg/ml and a linear response for Metolachlor from 0-150 mcg/ml.

IV. Discussion

The Toxicology Branch - Herbicide, Fungicide, Antimicrobial Support defers this submission to the Dietary Exposure Branch for consideration.

V. Core Classification: Not applicable, this is not a Guideline submission.

METRIBUZIN

RIN : 3187-91

Page_____ is not included in this copy.

Pages 112 through 115 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

37369

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/11/89
Pharmacologist, Review Section I, TB-HFAS/HED (H7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y.M.I. 7/13/89
Acting Section Head, Review Section I, TB-HFAS/HED (H7509C)

DATA EVALUATION RECORD

I. Study Type: Metabolism - Mouse Liver
Guideline: None

Study Title: Metabolism and Toxicity of Metribuzin in Mouse Liver

EPA Identification No(s).: EPA ID No. 3125-270
EPA MRID No. 262892
EPA Pesticide Chemical Code 101101
Caswell No. 33D
HED Project No. 9-0271A
Document No.

Submitter: Mobay Corporation
Agricultural Chemicals Division
Kansas City, Missouri 64120

Testing Facility: Pesticide Chemistry and Toxicology Laboratory
Department of Entomological Sciences
and
Department of Biomedical and Environmental Health Sciences
School of Public Health
University of California
Berkeley, CA 94720

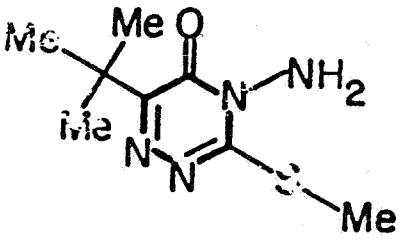
Study Number(s): Mobay AG Chem # 88750

Study Date(s): Approximately March 1984 (date paper submitted)

Study Citation: Pestic. Biochem. Physiol.
Vol 23, Iss. 1, 1985, Pgs. 123-30

Study Author(s): Marian Saeman Bleeke
Martyn T. Smith
John E. Casida

Test Material: Metribuzin
[^{14}C carbonyl]metribuzin (4.4 mCi/mmol)
obtained from Mobay Chemical Corp.,
Kansas City, MO.



Test Animals: Swiss Webster albino mice (male) obtained from
Charles River Breeding Laboratories.
Wilmington, MA
Albino rats
body weights: 20-25 gm
body weights: 100-200 gm

II. **Materials and Methods:** A copy of the "materials and methods" section from the investigators' report is appended.

This study was provided as a "galley proof" of a submitted journal article. The methods were described briefly and with references to published techniques. The comments and highlights on these "materials and methods" are as follows.

1. Acute toxicity testing was done to obtain a median lethal dose to male mice by IP injection. Methoxytriglycol (MTG) was used as a vehicle for metribuzin. Some of the mice were pretreated with either Piperonyl Butoxide i.p. (PB, at 150 mg/kg in MTG) 60 minutes prior to treatment with metribuzin or diethyl maleate i.p. (DEM, 250 mcl/kg in corn oil) 60 and 30 minutes prior to treatment with metribuzin. PB was used to inhibit cytochrome P450 and DEM was used to lower liver GSH levels.

2. Serum glutamic-pyruvic transaminase (GPT) activities were measured as an indicator of hepatotoxicity. A brief description of the procedure was included (see attached materials and methods for more detail).

3. The investigators conducted liver histopathology 24 hours after treatment. Liver slices were fixed and stained and examined by light microscopy.

4. Liver GSH content was measured 90 minutes post treatment by a technique described briefly in the attached materials and methods.

5. The investigators also measured the protein binding of radiolabeled metabolites of metribuzin in the liver, blood and brain. The technique is outlined in the attached materials and methods

6. The "metabolism" of metribuzin in male rats was determined as follows:
The rats were treated orally with labeled and unlabeled metribuzin at 1 and 200 mg/kg in MTG. They were kept for 5 days in glass metabolism cages. Feces and urine were collected and radiolabel levels were determined. Metabolites were identified by thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), ultraviolet (UV) spectroscopy, and gas chromatography-mass spectrometry (GC-MS). The "metabolism" of metribuzin in male mice was also determined, however, mice were treated with labeled and unlabeled metribuzin i.p. at 75 mg/kg in MTG. Urine and feces were collected analyzed as stated above. Further detail is available in the attached materials and methods.

III. Results

1. Acute Toxicity in Mice

Metribuzin administered i.p. to 10 male mice produced a Median Lethal Dose of 210 mg/kg with 90% confidence limits of 85 - 270 mg/kg. Sedation was observed, however, the time of this observation was not provided. Death occurred between 1 and 24 hours, according to the investigators.

Pretreatment with PB (60 minutes prior to the administration of metribuzin) to inhibit cytochrome P450 levels, reduced the toxicity of metribuzin to a median lethal dose of 620 mg/kg (490 - 770 mg/kg, confidence limits). No sedation was observed, however, convulsions were noted at dose levels greater than 400 mg/kg.

2. Serum GPT Activity

An increase in SGPT activity was noted at dose levels of metribuzin at 200 mg/kg or higher within 24 hours, according to the investigators. Pretreatment with PB reduced the effect by "2 fold".

3. Liver Histopathology

According to the investigators, "small changes" in the liver noted within 24 hours after treatment with metribuzin. At 200 mg/kg small white spots and a slightly pitted surface were noted on certain "portions", becoming more "uniformly distributed" over the liver at 250 mg/kg. Further, at 200 - 250 mg/kg, they stated that the intestines were "darker than normal" and there was apparent hemolysis of the blood.

Histopathologic slides photographs were provided, however, these were photocopies and not clear enough for an evaluation. According to the investigators, necrotic lesions were found after doses of 250 mg/kg "confined to the centrilobular region of the liver". Lower doses (100 - 200 mg/kg) apparently "caused progressively greater cloudy swelling and fatty change". No effect was noted at 50 mg/kg. Pretreatment with PB prevented the necrotic lesions at 400 mg/kg but not at 600 mg/kg. However, the investigators, noted a "fatty change" in the periportal region of the liver a 400 mg/kg rather than the centrilobular region. They stated that this reflects "a differential sensitivity of the two regions".

4. Liver GSH Content:

The investigators reported a dose related decrease in liver GSH content to less than 5% of control at dose levels above 200 mg/kg. PB protected against this effect, so that at 600 mg/kg, GSH levels were approximately 50% of control.

5. Protein Binding of ^{14}C Metribuzin Metabolites:

The investigators found increasing amounts of radiolabeled metribuzin bound to liver proteins at dose levels of 150 mg/kg and above. This appeared to correlate with decreased GSH levels in the liver. PB pretreatment blocked the protein binding effect. Data provided on tables was incomplete for evaluating equal doses in terms of liver fractions; however, it was apparent that less radiolabel was present after pretreatment with PB. The investigators stated that there was "less effect on the overall liver radiocarbon"; however, this is not apparent from Table 2 or Table 3 (attached). A similar pattern was observed in blood protein binding with large increases occurring at doses of 150 mg/kg and greater. Pretreatment with PB also reduced the radiolabel binding. Brain levels showed a slight, dose related

increase in binding, although less than that seen in the liver (Table 3). It was difficult to assess the effect of PB based on the dose levels provided; however, according to the investigators, the levels were "affected little, if at all, by PB" pretreatment.

6. "Metabolism" of ^{14}C-Metribuzin in Rats and Mice

The actual "metabolism" segment of this study was limited to excretion levels. No individual dose levels were provided; however, according to the investigators "about 90% of the"...label..."was excreted within the first five days after oral administration to rats at 1 or 200 mg/kg". They further stated that 80% was recovered in the first day and 95% by the second day finding approximately equal amounts in the urine and feces (Figure 3, attached). The major urinary metabolite identified was deaminometribuzin mercapturate, no attempt was made to identify other metabolites.

IV. Discussion

The submitted study is a published literature document and does not follow EPA Pesticide Assessment Guidelines for a metabolism study.

The acute toxicity testing in male mice yielded similar results to those submitted for regulatory purposes:

i.p. LD_{50} = 247 mg/kg in male mice.

Pretreatment with PB to inhibit the cytochrome P450 apparently reduced the acute toxicity observed with metribuzin. This may indicate that reactive metabolites of metribuzin are responsible for the acute toxicity observed.

The action of metribuzin on the liver was indicated by the increase in SGPT levels observed along with the liver histopathologic observations and the decrease in liver GSH content.

Further testing found that metribuzin (radiolabelled) was bound to liver and blood proteins and to a lesser extent, brain proteins.

Excretion levels of metribuzin were apparently determined at daily intervals; however, this information is not adequate to fulfill the Guideline recommendations for a metabolism study. Further, only one metabolite was identified in the urine with no attempt at identification of other metabolites.

V. Core Classification: Core-Supplementary Data, since does not fulfill a Guideline requirement. This study provides corroborative or supplementary information for the Metribuzin database.

Metabolism and Toxicity of Metribuzin in Mouse Liver[1]

MARIAN SAEMAN BLEEKE, MARTYN T. SMITH* AND JOHN E. CASIDA[2]

Pesticide Chemistry and Toxicology Laboratory,

Department of Entomological Sciences,

and

*Department of Biomedical and Environmental Health Sciences,

School of Public Health,

University of California, Berkeley, California 94720

Received March , 1984; accepted , 1984

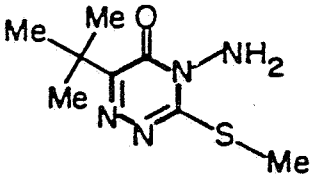
Metribuzin is hepatotoxic in mice when administered intraperitoneally (i.p.) at sublethal doses of 150 to 250 mg/kg. Four dose-dependent abnormalmalities are evident. Histopathological examination reveals a fulminant centrilobular hepatic necrosis. The serum glutamic-pyruvic transaminase (GPT) activity is elevated. The liver glutathione (GSH) content is almost completely depleted. There is extensive covalent binding of radiocarbon from [^{14}C-carbonyl]-metribuzin to liver proteins and also high blood levels of metribuzin fragments. Each of these four effects of metribuzin on the liver or blood is alleviated or blocked in mice pretreated with piperonyl butoxide (PB) which inhibits the cytochrome P-450-dependent monooxygenase. PB also reduces the lethality of metribuzin by 3-fold. In contrast, pretreatment with diethyl maleate to suppress the liver GSH content increases the lethality of metribuzin by 2-fold. The hepatotoxicity and acute lethality of metribuzin are probably due to reactive intermediates which are normally detoxified by GSH conjugation. The principal urinary metabolites of metribuzin in mice and rats are mercapturic acids which arise via metribuzin sulfoxide or deaminometribuzin sulfoxide reacting with GSH. Sulfoxidation therefore appears to activate metribuzin to an electrophilic metabolite which, in the absence of GSH, binds to tissue proteins producing hepatotoxicity.

/

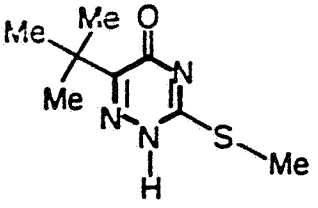
INTRODUCTION

Metabolism of the herbicide metribuzin by the liver microsomal mixed-function oxidase (MFO)[3] system yields a reactivate intermediate, probably metribuzin sulfoxide, that reacts quickly with available thiols or in their absence with proteins (1,2). Metribuzin also undergoes sequential enzymatic deamination to deaminometribuzin and sulfoxidation to deaminometribuzin sulfoxide, followed by nonenzymatic thiol or protein conjugation (2). The reactivity of metribuzin and deaminometribuzin sulfoxides is similar to that of cyanatryn sulfoxide (3-5) and thiocarbamate sulfoxides (6-8) which conjugate with glutathione (GSH), leading to mercapturic acid metabolites, or with protein, particularly with rat hemoglobin in the case of cyanatryn sulfoxide (4,5).

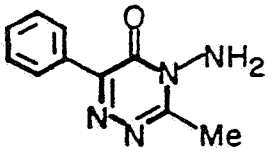
Metribuzin is of relatively low acute toxicity to mammals but gives dose-dependent liver enlargement on three-month feeding to rats at dietary levels of 50 to 1500 ppm (9). This study considers possible relationships between the hepatotoxicity and the metabolism of metribuzin. Deaminometribuzin and the herbicide metamitron are used as comparison compounds since they lack the N-amino and S-methyl substituents, respectively.



metribuzin



deaminometribuzin



metamitron

MATERIALS AND METHODS

Chemicals. Metribuzin, [^{14}C-carbonyl]metribuzin (4.4 mCi/mmol) and metamitron were provided by Mobay Chemical Corp. (Kansas City, MO). Deaminometribuzin was synthesized for this study (2). Piperonyl butoxide (PB) was provided by FMC Corp. (New York, NY).

Acute Toxicity in Mice. Median lethal dose (LD_{50}) values were determined for male Swiss Webster albino mice (20-25 g) from Charles River Breeding Laboratories (Wilmington, MA) 24 hr after intraperitoneal (i.p.) injection of the triazinones. The carrier vehicles (20-60 µl/mouse) were methoxytriglycol (MTG) for metribuzin and deaminometribuzin and dimethylsulfoxide (DMSO) for metamitron. Some of the mice were pretreated with PB (150 mg/kg in 20-30 µl MTG) administered i.p. 60 min before the toxicant. Others were pretreated i.p. with diethyl maleate (DEM) (250 µl/kg in 35 µl corn oil) both 60 and 30 min before metribuzin. PB inhibits cytochrome P-450-dependent oxidases (10) and DEM lowers the liver GSH content (11).

Serum Glutamic-Pyruvic Transaminase (GPT) Activity. Serum GPT activity serves as a sensitive indicator of hepatotoxicity (12). Twenty-four hr after the treatments described above the mice were anesthetized with ether, blood samples from the aorta were allowed to clot, and the serum was recovered by centrifigation. Twenty µl aliquots of serum or diluted serum were analyzed for GPT activity expressed such that one unit forms 4.82×10^{-4} µmol glutamate/min at pH 7.5 and 25°C (12,13).

Liver Histopathology. Twenty-four hr after the treatments described above the median lobe of each mouse liver was removed, cut into slices approximately 4 mm thick and fixed in 10% formal-saline for up to 24 hr. The liver slices were then processed by routine histopathological procedures and 5 µm thick paraffin embedded sections prepared. These were stained with haematoxylin and eosin and observed by light microscopy.

Liver GSH Content. Livers were removed from cervically-dislocated mice at 90 min posttreatment and were homogenized in 0.1 M phosphate buffer, pH 7.4, at 4.5 ml/g. After deproteinization of the homogenate with an equal volume of

4% sulfosalicylic acid, the supernatant was analyzed for GSH as acid-soluble thiols using 5,5'-dithiobis(2-nitrobenzoic acid) (14).

Protein Binding of [^{14}C]Metribuzin Metabolites. Livers from mice treated with [^{14}C]metribuzin (0.05 mCi/mmol) were homogenized as above. A portion (0.5-2 ml) of the homogenate or a centrifigal fraction thereof was mixed with an equal volume of aqueous 10% trichloroacetic acid (TCA). The protein precipitate was washed sequentially twice with 5% TCA and three times with 80% methanol. The precipitate was subjected to liquid scintillation counting (LSC) following solubilization with Soluene (Packard Instrument Co., Downers Grove, IL). Protein concentration on comparable liver homogenates or fractions was determined with bovine serum albumin as the standard (15).

Blood and brain (~ 50 mg samples) from mice treated with [^{14}C]metribuzin as above were analyzed for total ^{14}C content by combustion and LSC.

Metabolism of [^{14}C]Metribuzin in Rats and Mice. Male albino rats (180-200 g) were treated orally by stomach tube with [^{14}C]metribuzin (5 µCi/rat) and unlabeled metribuzin at 1 and 200 mg/kg with 200 µl MTG as the carrier vehicle. The rats were held five days in all-glass metabolism cages with ground rat chow and water ad libitum. The feces were extracted with methanol (10 ml/g) and the soluble radiocarbon was determined by LSC and the unextractable portion by combustion and then LSC. Urine was subjected to direct LSC and TLC analysis. Metribuzin mercapturate and deaminometribuzin mercapturate were isolated and identified by thin-layer chromatography (TLC), high performance liquid chromatography, ultraviolet spectroscopy, and gas chromatography-mass spectrometry as described earlier (2).

Male Swiss-Webster mice (20-25 g) were treated i.p. with [^{14}C]metribuzin (0.35 µCi/mouse) and unlabeled metribuzin at 75 mg/kg using 20 µl MTG as the

vehicle. The mice were held and the excreta analyzed for radioactivity as above. The urine was subjected to direct analysis by TLC.

RESULTS

Acute Toxicity in Mice. Metribuzin, deaminometribuzin, and metamitron differed in their toxicity and interactions with PB (Table 1). Deaminometribuzin was most toxic and rapid acting, producing respiratory difficulties and frequent spasms. Metribuzin was of intermediate toxicity and as with metamitron gave primarily sedation with death between 1 and 24 hr. PB increased the toxicity of deaminometribuzin and metamitron (Table 1), without changing the speed of action or poisoning signs. In contrast, the toxicity of metribuzin was decreased 3-fold on PB pretreatment (Table 1), and the poisoning signs were changed from sedation without PB to convulsions at metribuzin doses of > 400 mg/kg with PB.

Serum GPT Activity. Metribuzin at 200 mg/kg or greater induced a large increase in serum GPT activity within 24 hr (Fig. 1A). PB ameliorated this effect by about 2-fold.

Liver Pathology. Metribuzin caused changes in the appearance of the liver within 24 hr. A 200 mg/kg dose produced small white spots on portions of the liver and a slightly-pitted surface and a 250 mg/kg dose spotting and surface depressions uniformly distributed over the entire liver. In addition, at 200-250 mg/kg the color of the intestines was darker than normal and the blood appeared to be hemolyzed.

Histopathological examination revealed that the injury was confined to the centrilobular region of the liver [illegible] was apparent as a necrotic lesion only after a dose of 250 mg/kg (Fig. 2). Lower doses (100-200 mg/kg) caused progressively greater cloudy swelling and fatty change. A dose of 50 mg/kg had no readily observable effect. PB prevented the formation of the necrotic lesion after a dose of 400 mg/kg, but its protective effect was overcome at a higher dose

of 600 mg/kg (Fig. 2). Interestingly PB completely protected the centrilobular region from the toxic effects of a 400 mg/kg dose, but some fatty change was now evident in the periportal region (Fig. 2C), reflecting a differential sensitivity of the two regions.

Liver GSH Content. The GSH content of the liver decreased progressively with increasing doses of metribuzin to < 5% of the control value above 200 mg/kg (Fig. 1B). PB protected against GSH depletion so that even at 600 mg/kg the GSH content was 50% of normal. Metamitron caused much less GSH depletion than metribuzin, similar only to that resulting from metribuzin plus PB.

Protein Binding of [^{14}C]Metribuzin Metabolites. Radiocarbon from [^{14}C]metribuzin bound to liver proteins to an appreciable extent at doses of 150 mg/kg or higher (Fig. 1C), when GSH levels are low (Fig. 1B). PB blocked this protein binding with less effect on the overall liver radiocarbon (Fig. 1C, Tables 2 and 3). The protein-bound material was almost uniformly distributed among various liver fractions with a similar PB effect relative to each fraction (Table 2). The blood radiocarbon level (Fig. 1D) followed the same pattern as the bound liver radiocarbon (Fig. 1C) in its large increase at doses above 150 mg/kg and in the suppressing effect of PB. In contrast, the brain showed a dose-dependent increase of radiocarbon affected little, if at all, by PB (Table 3).

Metabolism of [^{14}C]Metribuzin in Rats and Mice. About 90% of the radiocarbon of [^{14}C]metribuzin was excreted within the first five days after oral administration to rats at 1 or 200 mg/kg. At both doses about 80% of the recovered radioactivity was excreted in the first day and 95% by the second day, with almost equal amounts in the urine and feces (Fig. 3). The major urinary metabolite was deaminometribuzin mercapturate (2), accounting for 16-21% of the recovered radiocarbon. No attempt was made to identify other urinary and fecal metabolites.

Mice treated i.p. with [^{14}C]metribuzin at 75 mg/kg showed a similar pattern for radiocarbon excretion and TLC of urinary products to that obtained with rats. About 20% of the dose was recovered as metribuzin mercapturate plus deaminometribuzin mercapturate in urine, 20% as other products in the urine, and 60% as metabolites in the feces.

DISCUSSION

Dietary metribuzin does not elevate serum GPT activity or alter other indices of liver function in rats at subchronic doses causing liver enlargement (9). Liver or other tissue damage was not reported in extensive acute toxicity studies with many mammalian species (9) but it is evident in the present investigation. Metribuzin at i.p. doses of 200 mg/kg or higher causes hepatotoxicity in mice evident by necrotic lesions and greatly elevated serum GPT activity. It also depletes the liver GSH content and binds to liver proteins. Partial depletion of liver GSH by DEM pretreatment increases liver damage from metribuzin, evident by histopathological changes (results not illustrated here) and mortality studies. The cytochrome P-450 inhibitor PB decreases the toxicity of metribuzin by 3-fold and at least partially protects mice from the liver lesions. High levels of metribuzin plus metabolites in the blood of mice treated at 200 and 300 mg/kg and the ability of PB to suppress these levels indicate that in the blood, as in the liver, persisting fragments may originate from an oxidatively-activated intermediate, e.g., metribuzin sulfoxide. Analogous situations are found on treating rats with cyanatryn or molinate (3-8).

The poisoning signs in mice from i.p.-administered metribuzin change from sedation without PB to convulsions with PB. The brain levels of metribuzin plus metabolites increase linearly with administered i.p. dose in the range of 200 to 600 mg/kg regardless of PB pretreatment. PB may act by inhibiting oxidation so more of the parent compound enters the brain (17). On this basis metribuzin

per se may be the causal agent for the convulsions. It therefore appears that metribuzin poisoning involves two mechanisms or sites of action, i. e., the liver or blood following oxidative bioactivation and the brain when oxidase activity is inhibited by PB.

In contrast to metribuzin, deaminometribuzin and metamitron which lack the N-amino and S-methyl substituents, respectively, become more toxic with PB. These compounds also differ from metribuzin in other ways, i. e., deamino-metribuzin is more toxic and acts faster with different poisoning signs and metamitron causes only a small decrease in liver GSH content.

Liver damage from metribuzin probably involves the reactions shown in Fig. 4. The MFO system forms metribuzin sulfoxide and/or deaminometribuzin sulfoxide which reacts with GSH until its level is depleted and then with proteins or other macromolecules. The mercapturates are the major urinary metabolites. Tissue binding at high metribuzin levels is extensive, nonspecific as to liver fractions, and probably covalent, leading to the suggested formation of triazinonyl proteins. The acute and subchronic toxicity of metribuzin is apparently due at least in part to metabolic activation to the electrophilic sulfoxide. Metribuzin therefore joins an expanding list of compounds that require activation to an electrophilic intermediate both for their toxic action in mammals and their detoxification (18,19).

ACKNOWLEDGMENTS

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REFERENCES

1. M. C. Saeman and J. E. Casida, Chemical and metabolic oxidation of metribuzin, paper 38, Division of Pesticide Chemistry, 184th Amer. Chem. Soc. Nat'l. Meeting, Kansas City, MO, Sept. 1982.

2. M. C. Bleeke and J. E. Casida, Metribuzin metabolites in mammals and liver microsomal oxidase systems: identification, synthesis and reactions. J. Agric. Food Chem., accepted (1984).

3. C. T. Bedford, M. J. Crawford, and D. H. Hutson, Sulphoxidation of cyanatryn, a 2-mercapto-sym-triazine herbicide, by rat liver microsomes, Chemosphere 5, 311 (1975).

4. M. J. Crawford, D. H. Hutson, and G. Stoydin, The metabolic fate of a herbicidal methylmercapto-s-triazine (cyanatryn) in the rat, Xenobiotica 10, 169 (1980).

5. D. H. Hutson, S-Oxygenation in herbicide metabolism in mammals, in "Sulfur in Pesticide Action and Metabolism", ACS Symposium Series No. 42 (J. D. Rosen, P. S. Magee and J. E. Casida, Eds.), p. 53, American Chemical Society, Washington, D.C., 1981.

6. J. P. Hubbell and J. E. Casida, Metabolic fate of the N,N-dialkylcarbamoyl moiety of thiocarbamate herbicides in rats and corn, J. Agric. Food Chem. 25, 404 (1977).

7. J. R. DeBaun, D. L. Bova, K. A. Finley, and J. J. Menn, Metabolism of [ring-^{14}C]Ordram (molinate) in the rat. 1. Balance and tissue residue study, J. Agric. Food Chem. 26, 1096 (1978).

8. J. R. DeBaun, D. L. Bova, C. K. Tseng, and J. J. Menn, Metabolism of [ring-^{14}C]Ordram (molinate) in the rat. 2. Urinary metabolite identification, J. Agric. Food Chem. 26, 1098 (1978).

9. E. Löser and G. Kimmerle, Acute and subchronic toxicity of ®Sencor active ingredient, Pflanzenschutz-Nachr. 25, 186 (1972).

10. B. Testa and P. Jenner, Inhibitors of cytochrome P-450s and their mechanism of action, Drug. Metab. Rev. 12, 1 (1981).

11. J. L. Plummer, B. R. Smith, H. Sies, and J. R. Bend, Chemical depletion of glutathione in vivo, Methods in Enzymol. 77, 50 (1981).

12. T. Balazs, J. M. Airth, and H. C. Grice, The use of the serum glutamic pyruvic transaminase test for the evaluation of hepatic necrotropic compounds in rats, Canad. J. Biochem. Physiol. 40, 1 (1962).

13. Sigma Chemical Co., The quantitative colorimetric determination of glutamic-oxalacetic and glutamic-pyruvic transaminases at 490-520 nm in serum, plasma or cerebrospinal fluid, Sigma Technical Bulletin No. 505, 15 pp. 1977.

14. J. R. Mitchell, D. J. Jollow, W. Z. Potter, J. R. Gillette, and B. B. Brodie, Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione, J. Pharmacol. Exp. Ther. 187, 211 (1973).

15. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193, 265 (1951).

16. J. T. Litchfield, Jr. and F. Wilcoxon, A simplified method of evaluating dose-effect experiments, J. Pharmacol. Exp. Ther. 96, 99 (1949).

17. A. E.-S. M. Marei, L. O. Ruzo, and J. E. Casida, Analysis and persistence of permethrin, cypermethrin, deltamethrin, and fenvalerate in the fat and brain of treated rats, J. Agric. Food Chem. 30, 558 (1982).

18. L. F. Chasseaud, Conjugation with glutathione and mercapturic acid excretion, in "Glutathione: Metabolism and Function" (I. M. Arias and W. B. Jakoby, Eds.), p. 77, Raven Press, New York, 1976.

19. J. R. Mitchell, W. R. Snodgrass, and J. R. Gillette, The role of biotransformation in chemical-induced liver injury, Environ. Health Perspec. 15, 27 (1976).

FOOTNOTES

[1]Study supported in part by National Institute of Environmental Health Sciences Grant P01 ES00049 (J.E.C.) and Northern California Occupational Health Center (M.T.S.) Presented as paper 13, Division of Pesticide Chemistry, at the 187th National Meeting of the American Chemical Society, St. Louis, MO, April, 1984.

[2]To whom correspondence should be addressed.

[3]Abbreviations used: DEM, diethyl maleate; DMSO, dimethylsulfoxide; GPT, glutamic-pyruvic transaminase; GSH, glutathione; i.p., intraperitoneal; LD_{50}, median lethal dose; LSC, liquid scintillation counting; MFO, mixed-function oxidase; MTG, methoxytriglycol; PB, piperonyl butoxide; TCA, trichloroacetic acid; TLC, thin-layer chromatography.

TABLE 1

Mouse Intraperitoneal 24-Hr LD_{50} Values for Metribuzin, Deaminometribuzin and Metamitron Alone or Following Piperonyl Butoxide Pretreatment

Compound	Time of death, min	LD_{50}, mg/kg		
		alone	+PB[a]	ratio, PB/alone
metribuzin	>60	210(85-270)[bcd]	620(490-770)[b]	3.0
deaminometribuzin	10-60	130(95-160)[b]	100(85-120)[b]	0.8
metamitron	>60	>600	480	<0.8

[a]PB administered at 150 mg/kg 60 min prior to the triazinone.

[b]Determined by log-dose probit mortality analysis (16) with 15-30 mice for each determination. The 90% confidence limits are given in parentheses.

[c]Reported 247 mg/kg (9).

[d]LD_{50} following DEM pretreatment is 104 (88-123) mg/kg.

TABLE 2

Distribution of Protein-Bound Metabolites in Liver Fractions of Mice Treated With [^{14}C]Metribuzin Alone or Following Piperonyl Butoxide Pretreatment

Fraction[a]	Bound ^{14}C, nmol metribuzin equiv/mg protein[b]			
	Metribuzin, mg/kg		Metribuzin, mg/kg following PB[c]	
	200	300	400	600
Whole liver	3.4	7.4	0.2	0.3
Nuclei and cell debris	2.6	6.0	0.1	0.3
Mitochondria	3.1	5.8	0.2	0.5
Microsomes	4.4	6.9	0.4	0.5
Soluble	4.2	8.8	0.3	0.5

[a]Sedimentation at 3000 x g for nuclei and cell debris, 9,000 x g for mitochondria, and 100,000 x g for microsomes. Soluble is the 100,000 x g supernatant.

[b]Mice sacrificed 90 min after treatment with [^{14}C]metribuzin with one animal at each dose.

[c]PB administered at 150 mg/kg 60 min prior to [^{14}C]metribuzin.

TABLE 3

Radiocarbon Levels in Liver and Brain of Mice Treated with [^{14}C]Metribuzin Alone or Following Piperonyl Butoxide Pretreatment

Metribuzin administered, mg/kg	Ppm [^{14}C]metribuzin equivalents[a]	
	Liver	Brain
	Metribuzin alone	
50	20,70	1
100	70,90	3
200	550,200	10,30
300	570,640	100
400	800,1260	90
	Metribuzin + PB[b]	
400	380,690	155
600	800,730	340

[a]When two values are given they were determined with different animals.

[b]PB administered at 150 mg/kg 60 min prior to [^{14}C]metribuzin with sacrifice 90 min after treatment with the labeled compound.

FIGURE LEGENDS

FIG. 1. Effect of increasing metribuzin doses alone or following piperonyl butoxide pretreatment on mouse serum GPT activity (A), liver GSH concentration (B), liver protein bound residues (C) and total blood levels (D) and of metamitron on mouse liver GSH concentration (B). PB administered at 150 mg/kg 60 min prior to the triazinone with determination 24 hr later for A and sacrifice 90 min later for B-D.

FIG. 2. Histological appearance of mouse liver 24 hr after the administration of (A) methoxytriglycol only (control); (B) 250 mg/kg metribuzin; (C) 400 mg/kg metribuzin + 150 mg/kg PB; (D) 600 mg/kg metribuzin + 150 mg/kg PB. Centrilobular necrosis is evident with regimens B and D.

FIG. 3. [^{14}C]Mercapturic acids and other labeled products in the urine and feces of rats up to five days after oral administration of [^{14}C]metribuzin. The figure shown is for 200 mg/kg but essentially identical curves are obtained at 1 mg/kg except for more rapid excretion during the first 12 hr after treatment.

FIG. 4. Metabolic reactions of metribuzin in rats and mice.

Serum GPT Activity, Units
10,000
1,000
100
10
A
metribuzin alone
metribuzin + PB
Liver GSH, mM
9
6
4
2
0
B
metamitron
metribuzin + PB
metribuzin alone
nMol Metribuzin Equivalents per Mg Liver Protein
12
8
4
0
C
metribuzin alone
metribuzin + PB
ppm Metribuzin Equivalents in Blood
1200
800
400
0
D
metribuzin alone
metribuzin + PB
0 100 200 300 400 600
mg/kg

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Fig. 2
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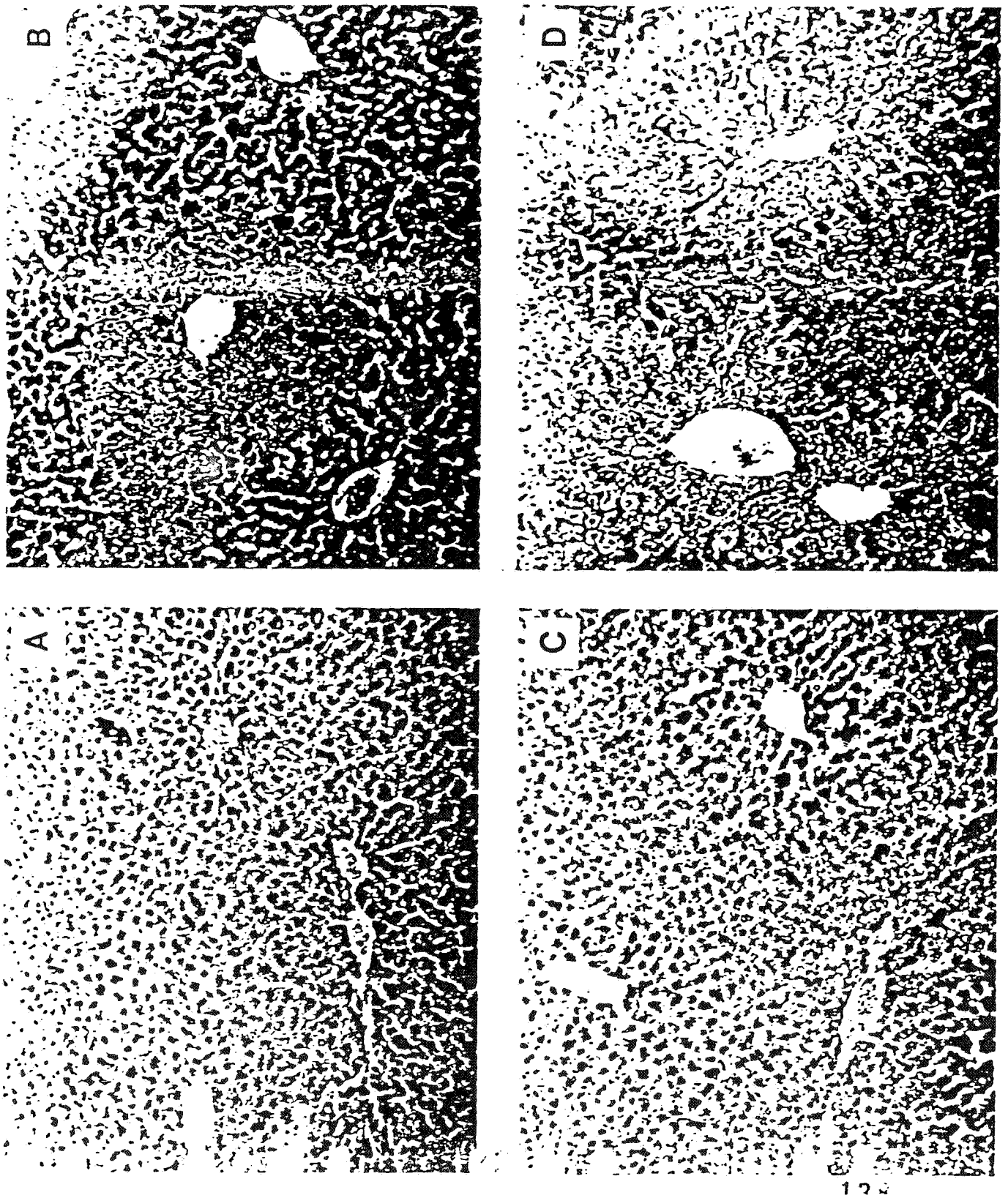

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Fig 3
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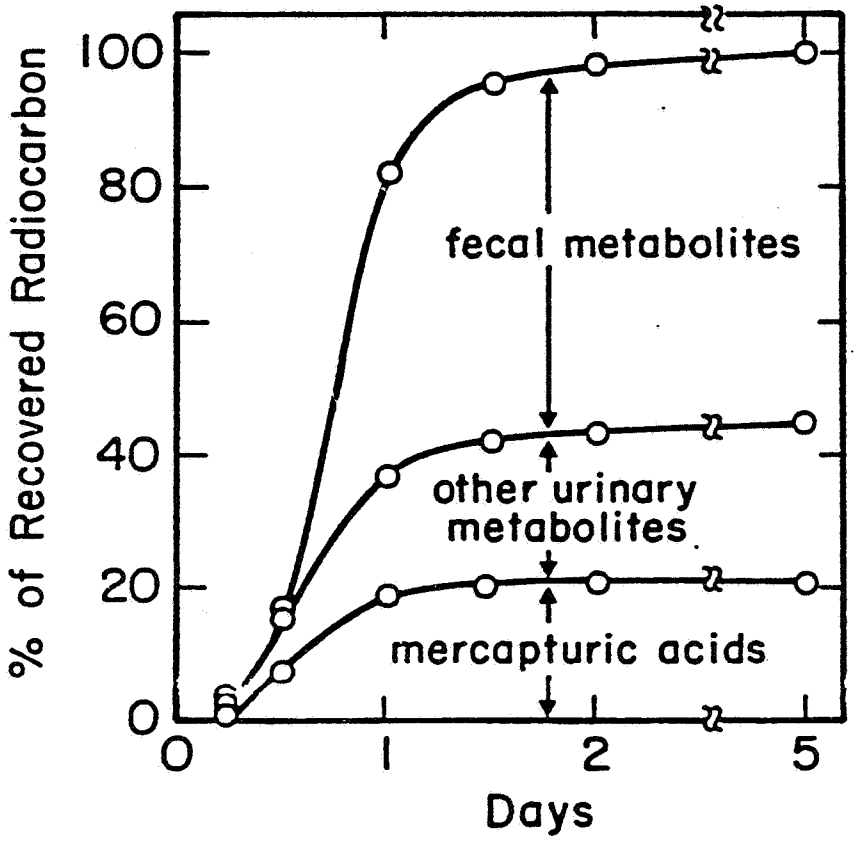

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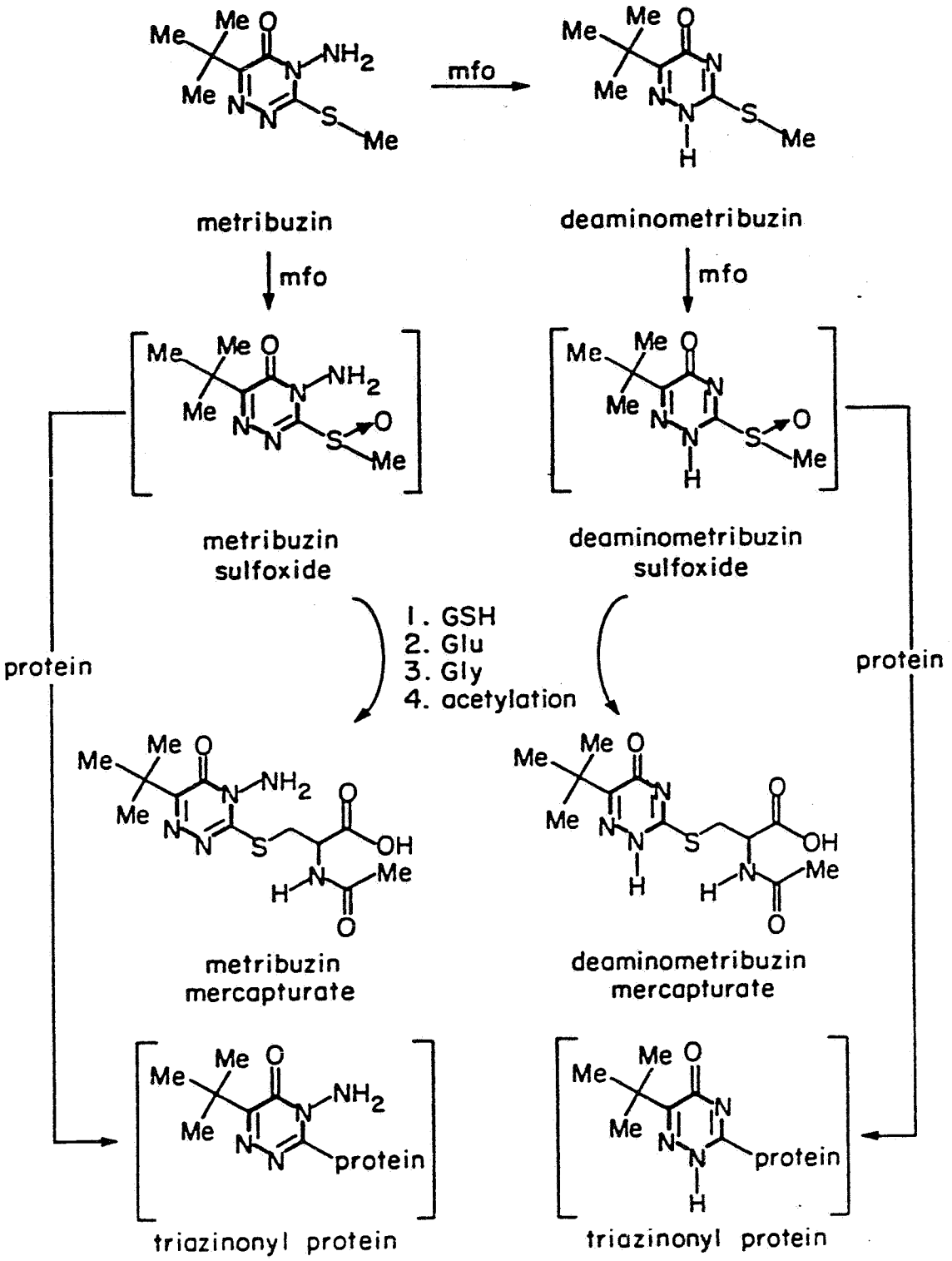
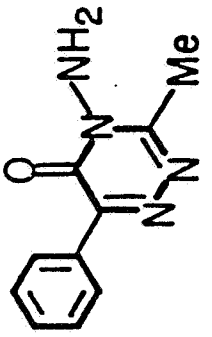
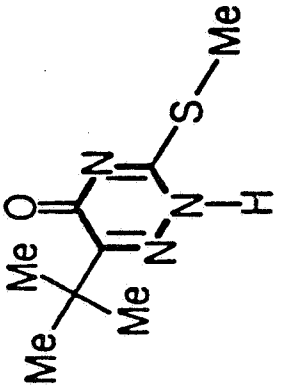
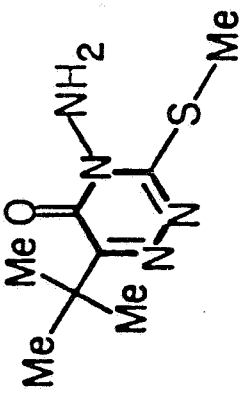




metamitron



deaminometribuzin



metribuzin

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCE

MEMORANDUM

SUBJECT: Review of additional data on a chronic feeding study in rats (Bayer Report No. 4888, Mobay Report No. 41816) submitted by Mobay in support of the Registration Standard on Metribuzin (Sencor) EPA ID #3125-270; EPA Accession #258756 & 258757; EPA Record #156177; Shaugnessy #101101-4; Caswell #330, Tox Branch Project No. 208.

TO: Robert Taylor, PM #25
Registration Division (TS-767C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 11/14/85
Pharmacologist, Review Section V
Toxicology Branch/HED (TS-769C)

THRU: Laurence D. Chitlik, D.A.B.T. W. Testerso for L. Chitlik 11/14/85
Section Head, Review Section V
Toxicology Branch/HED (TS-769C)
and
Gary J. Burin, M.P.H.
Toxicologist
Science Integration Staff/HED (TS-769C)
and
Theodore M. Farber, Ph.D., D.A.B.T.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

11 15 85

Registrant: Mobay Chemical Corporation
Agricultural Chemicals Division
Kansas City, Missouri 64120

Action Requested: Review additional data for the rat oncogenicity study with Metribuzin (Sencor), submitted in support of the Registration Standard for the chemical.

Recommendations:

The sponsor has now satisfactorily responded to all requests made in the review conducted for the Registration Standard of the chronic rat study and the study can now be upgraded to Core-Minimum Data. Assessment of the chronic toxicity/oncogencity potential for metribuzin in the rat has included the additional data and clarifying information provided by the registrant.

Metribuzin is not oncogenic to the rat in dietary levels up to 300 ppm. The No Observed Effect Level (NOEL) for systemic effects is 100 ppm. The Lowest Observed Effect Level (LOEL) for systemic effects is 300 ppm, based on the decreased weight gain, along with the pathological changes in the liver, kidneys, uterus and mammary glands.

The additional data reviewed here were for the study identified as follows:

Study Title: BAY 94 337 Chronic Toxicity Studies on Rats (2-year feeding experiment)

EPA Identification Numbers: EPA Accession No. 112891

Sponsor: Mobay Chemical Corporation
Chemagro Agricultural Division
Kansas City, Missouri 64120

Testing Laboratory: BAYER AG
Institut fur Toxikologie
Wuppertal-Elberfeld

Report Numbers: Bayer AG Report No.4888 & Mobay Ag Chem No.41816

Date of Study: September 25, 1974

Study Director: Dr. rer. nat. Eckhard Loser

Histopathological Examination: Prof. Dr. med. U. Mohr

Test Compound: BAY 94 337 (Metribuzin) Technical (also called SENCOR)
Purity: 99.5%
Batch No.: 1603/71

Dosage: 25, 35, 100 and 300 ppm mixed with pulverized Altromin R feed (from Altrogge, Lage/Lippe).

Test Animal: SPF Rats (Wistar Strain) bred by Winkelmann, Kirchborchen, Kreis Paderborn. At start of experiment rats were about 28 to 32 days old with males having a mean body weight of 51.4 gm. and females with a 52.1 gm mean body weight.

Materials and Methods: A copy of the materials and methods section from the investigators report is appended.

The relevant item in this section of the review of the study which pertains to the additional data reviewed here is as follows:

The investigators examined all tissues that are required by CORE, however histopathology was performed on all animals only in control and the high dose group. In the other three dose groups only selected tissues in selected animals (10 per group) were examined.

Background:

During preparation of the Registration Standard for Metribuzin, the two year chronic feeding study in rats (Bayer AG Report No.4888, Mobay Report. No.41816, dated 9/25/74) was re-reviewed and classified as Core-Supplementary Data since the oncogenic potential of metribuzin in this species could not be fully assessed without consideration of additional data and clarification of certain pathological terminology. The re-review found a statistically significant increase in liver bile duct adenomas ($p < 0.01$) and pituitary adenomas ($p < 0.05$), as well as an increase in ovarian adenoma in females of the 300 ppm test group. There were statistically significant increases in the non-neoplastic finding of liver "changes in the nucleus" in the females of the 300 ppm test group ($p < 0.01$, males also showed a slight increase). However, all the animals of the 25, 35 and 100 ppm test groups had not been examined and a complete evaluation of the oncogenic potential could not be accomplished without these additional data.

It was concluded that there was no evidence of a compound related effect on hematological, clinical-chemical, urinalysis, kidney function, liver function and thyroid function test parameters.

The following are the data and/or clarifications requested:

a. Histopathological examination of the liver and pituitary gland of the animals not previously examined in the 23, 35 and 100 ppm test groups. The registrant was directed to provide individual as well as summarized data.

b. Historical control data on the incidence of histopathological findings in the rat strain used.

c. Explanation of the finding called "tumor".

d. Explanation of the finding called "changes in the nucleus".

e. A table of weekly body weight data divided by sex for each study group.

The registrant responded with the following data and/or clarifying information:

a. An addendum (Addendum 2) to Report No. 41816 which contained tables presenting histopathological findings from additional animals in the 25, 35 and 100 ppm groups along with histopathological data from all the animals tested. This included a re-evaluation of liver findings and of the observation called "tumor".

b. Legible copies of life tables were provided.

c. Legible copies of the body weight tables which had been requested in a telephone conversation with the registrant.

d. Histopathological historical control data (Mobay Report No. 90236) from chronic studies using the same strain as used in the metribuzin study and run at approximately the same time period.

e. During telephone conversations between the registrant's pathologist and Dr. Louis Kasza, pathologist for the Toxicology Branch, the following items were clarified:

1. Reclassification of liver bile duct proliferative changes.
2. Clarification of the term "changes in the cell nucleus" of the liver.
3. Definition of the term "tumor",as used in the original report.

Results:

1. Adequacy of dosage levels.

The dosage range selected for this study is consistent with the observations noted in 2 ninety day sub-chronic feeding studies that were conducted in rats. Data from the chronic study indicated that the high dose (300 ppm) produced minimally toxic effects in the form of slightly lower body weights of both sexes, increased mortality in the males and increased incidences of minor histopathological changes in the females This is consistent with the concept of the maximum tolerated dose (MTD) as discussed in the Standard Evaluation Procedure, Toxicity Potential: Guidance for Analysis and Evaluation of Subchronic and Chronic Exposure Studies, June 1985.

2. Histopathological Findings

The registrant provided additional histopathological data on the animals of the 25, 35 and 100 ppm test groups, as well as a re-evaluation of the neoplastic findings. Tables I and II below present a summary of the combined observed findings (tables prepared by the reviewer). The registrant also provided historical control data from rat chronic studies using the same strain and conducted at approximately the same time period as the metribuzin study (although not conducted in the same test facility). These data are shown in Table I under the heading "Historical".

A. Neoplastic Findings

The following Table I presents the summary of the neoplastic findings.

Table I: Neoplastic Findings for BAY 94 337 (Metribuzin)

: Male Rats

RGAN	DOSE(ppm):	Control	25	35	100	300	Historical
ituitary	#†	62	29	32	29	29	609
Adenoma		8(13%)	6(21%)	2(6%)	5(17%)	5(17%)	123(20%)
Adenocarcinoma		2(3%)	0	0	0	1(4%)	3(0.5%)
ıyroid	#	72	21	35	34	37	650
Adenoma		0	2(10%)	1(3%)	0	2(5%)	55(9%)
Adenocarcinoma		0	0	0	0	0	8(1%)
irenals	#	66	31	34	37	37	724
Medullary adenoma		6(9%)	2(7%)	1(3%)	0	1(3%)	-
Cortical adenoma		0	1(3%)	0	0	0	3(0.4%)
Pheochromocytoma		0	0	1(3%)	0	1(3%)	50(7%) ††
ɜstes	#	66	31	32	30	29	760
Leydig's cell tumor		3(5%)	2(7%)	1(3%)	0	0	24(3%)
ɔididymides	#	66	21	22	20	29	450
Papillary cystoma		0	0	1(5%)	0	0	1(0.2%)
ʻostate	#	65	31	32	30	29	381
Adenoma		0	1(3%)	0	0	0	0
.omach	#	66	31	32	30	29	413
Carcinoma(forestomach)		1(2%)	0	0	0	0	0
testines	#	66	31	32	30	29	405
Adenocarcinoma		1(2%)	0	0	0	0	0
dneys	#	73	39	38	38	37	765
Papillary adenocarcinoma		1(1%)	0	0	0	0	2(0.3%)
ncreas	#	65	22	23	30	29	435
Islet cell adenoma		0	0	0	1(3%)	0	9(2%)
Exocrine adenoma		0	1(5%)	0	0	0	-
Malignant schwannoma		0	0	0	1(3%)	0	-

continued

Table I continued:

RGAN	DOSE(ppm):	Control	25	35	100	300	Historical
. Female Rats							
ituitary	#	71	34	31	33	35	676
Adenoma		16(23%)	6(18%)	9(29%)	11(33%)	14(40%)	148(22%)
Adenocarcinoma		11(16%)	1(3%)	0	0	7(20%)	5(0.7%)
Myxomatous cranio-pharyngioma		1(1%)	0	0	0	0	-
yroid	#	73	37	31	36	36	665
Adenoma		3(4%)	0	2(7%)	3(8%)	1(3%)	68(10%)
Adenocarcinoma		0	1(3%)	0	0	0	6(0.9%)
renals	#	75	37	31	37	35	754
Medullary adenoma		0	1(3%)	0	0	0	-
Cavernous hemangioma		0	0	0	0	1(3%)	-
erus	#	72	34	31	32	35	718
Myoma		1(1%)	0	0	0	0	2(0.3%)
Adenomcarcinoma		1(1%)	1(3%)	1(3%)	1(3%)	1(3%)	44(6%)
Leiomyosarcoma		0	0	1(3%)	0	0	0
aries	#	73	37	32	36	39	710
Granulosa cell tumor		0	1(3%)	1(3%)	0	0	5(0.7%)
Myxoma		0	1(3%)	0	0	0	-
mmary Glands	#	72	30	25	19	35	-
Adenofibroma		4(6%)	5(17%)	2(8%)	2(11%)	1(3%)	-
Fibroadenoma		1(1%)	1(3%)	1(4%)	2(11%)	1(3%)	23 †††

† = Total examined - tissue samples
†† = includes malignant pheochromocytoma findings
††† = findings in 11 studies

Data extracted from Bayer Report No. 4888 Histopathology Addendum and Addendum 2.

Table I presents the "updated" (including the additional animals) neoplastic findings from the 2 year chronic rat study. As can be seen from this summary table (prepared by this reviewer) there were no biologically significant differences for neoplastic findings between any of the treated groups and both concurrent and historical controls for the males. Females fed levels of 35 ppm and above showed a dose-related increase in pituitary adenomas (23, 18, 29, 33 and 40% respectfully for control, 25, 35, 100 and 300 ppm study groups, statistically significant at the high dose, no differences noted in the grading of the lesions). The incidence for this tumor in males of the treated groups did not differ significantly from that of the control group. The historical control incidence for this particular observation averages 22% for 9 studies; however, the incidence in each of four of the individual historical control studies approached 40% (for females in 2 of the studies and for males in 2 different studies). There were no neoplastic findings in the liver of either sex (since "bile duct adenoma" was redefined as bile duct proliferation, see Table II); also, the incidence of ovarian adenomas was reassessed by the registrant and reduced (see section 3, below).

B. Non-neoplastic Findings

The following Table II presents the summary of the non-neoplastic findings.

Table II: Non-neoplastic Findings for BAY 94 337 (Metribuzin)

A: Male Rats

ORGAN	DOSE(ppm):	Control	25	35	100	300
Trachea	#	66	29	32	30	29
Hemorrhage		1(2%)	0	0	1(3%)	1(4%)
ICI ††		2(3%)	2(7%)	2(6%)	1(3%)	2(7%)
Heart	#	75	39	38	38	38
Hemorrhage		0	1(3%)	0	0	0
ICI		16(21%)	7(18%)	5(13%)	8(21%)	11(29%)
Scar		13(17%)	17(44%)	17(45%)	16(42%)	12(32%)
Edema		0	2(5%)	0	1(3%)	0
Lungs	#	74	39	38	37	38
Atelectasis		6(8%)	4(10%)	1(3%)	6(16%)	0
Hemorrhage		7(10%)	1(3%)	0	1(3%)	0
Hyperplasia - bronchial mucosa		0	0	1(3%)	0	1(3%)
Bronchitis		27(37%)	4(10%)	8(21%)	5(14%)	12(32%)
Emphysema		39(53%)	20(51%)	20(53%)	16(43%)	18(47%)
Parasitic cell granuloma		0	0	0	0	1(3%)
Edema		1(1%)	2(5%)	1(3%)	1(3%)	2(5%)
Peribronchial lymphocytic infiltration		35(47%)	33(85%)	29(76%)	22(60%)	15(40%)
ICI		0	4(10%)	0	0	0
Pneumonia		12(16%)	3(8%)	4(11%)	5(14%)	5(13%)
Foam cells in the alveoli		12(16%)	4(10%)	4(11%)	5(14%)	2(5%)
Liver	#	74	39	38	38	38
Dissociation		3(4%)	5(13%)	7(18%)	8(21%)	5(13%)
Fatty change (& fat)		65(88%)	12(31%)	11(29%)	10(26%)	29(76%)
Parasitic cell granuloma		4(5%)	2(5%)	2(5%)	9(24%)	6(16%)
Bile duct proliferation †††		19(26%)	9(23%)	7(18%)	10(26%)	9(23%)
Nuclear changes		5(7%)	10(26%)	6(16%)	3(8%)	4(11%)
Necrosis		4(5%)	2(5%)	0	2(5%)	2(5%)
Edema		4(5%)	3(8%)	1(3%)	2(5%)	1(3%)
Spleen	#	74	39	38	38	37
Giant cells		1(1%)	0	0	0	1(3%)
Kidneys	#	73	39	38	38	37
Cysts		0	0	1(3%)	1(3%)	3(8%)
ICI		7(10%)	0	0	2(5%)	0
Glomerular damage		0	0	1(3%)	0	0
Scar		1(1%)	3(8%)	3(8%)	2(5%)	0
Tubule proliferation		5(7%)	2(5%)	3(8%)	0	0
Renal pelvis proliferation		1(1%)	0	0	0	1(3%)
Cast		39(53%)	15(39%)	11(29%)	9(24%)	13(35%)

continued

Table II continued:

ORGAN	DOSE(ppm):	Control	25	35	100	300
Adrenals	#	66	31	34	37	37
Fatty change		0	0	2(6%)	4(11%)	1(3%)
Stomach	#	66	31	32	30	29
Hemorrhage		0	1(3%)	1(3%)	0	0
Cyst		0	1(3%)	0	0	0
ICI		0	3(10%)	1(3%)	2(7%)	0
Calcium concretions		1(2%)	3(10%)	0	0	0
Urinary bladder	#	65	21	25	20	28
Calcium concretions		0	0	3(12%)	1(5%)	0
Skeletal Muscle	#	66	29	32	29	29
Parasitic cell granuloma		1(2%)	0	0	0	0
Testes	#	66	31	32	30	29
Atrophy		5(8%)	7(23%)	0	2(7%)	0
Sperm detected		64(97%)	9(29%)	10(31%)	10(33%)	29(100%)
Epididymes	#	66	21	22	20	29
Atrophy		0	3(14%)	0	2(10%)	0
Thyroid	#	72	21	35	34	37
Cysts		0	1(5%)	0	1(3%)	0
B. Female Rats						
Trachea	#	71	33	28	32	35
Hemorrhage		0	1(3%)	3(11%)	0	0
ICI		2(3%)	3(9%)	1(4%)	1(3%)	1(3%)
Heart	#	77	37	36	40	39
Hemorrhage		2(3%)	2(5%)	0	0	1(3%)
ICI		23(30%)	1(3%)	1(3%)	5(13%)	5(13%)
Scar		16(21%)	13(35%)	16(44%)	20(50%)	8(21%)
Edema		0	0	0	0	2(5%)
Lungs	#	76	38	36	39	39
Atelectasis		9(12%)	2(5%)	2(6%)	7(18%)	4(10%)
Hemorrhage		4(5%)	1(3%)	0	0	0
Hyperplasia - bronchial mucosa		0	0	0	0	2(5%)
Bronchitis		12(16%)	4(11%)	6(17%)	3(8%)	2(5%)
Emphysema		56(74%)	15(40%)	16(44%)	15(39%)	11(28%)
Parasitic cell granuloma		1(1%)	0	0	0	0
Edema		1(1%)	1(3%)	0	0	1(3%)
Peribronchial lymphocytic infiltration		53(70%)	19(50%)	28(78%)	21(54%)	16(41%)
ICI		0	0	0	0	0
Pneumonia		3(4%)	4(11%)	2(6%)	2(5%)	2(5%)
Foam cells in the alveoli		3(4%)	4(11%)	5(14%)	1(3%)	2(5%)

continued

Table II continued:

ORGAN	DOSE(ppm):	Control	25	35	100	300
Liver	#	76	38	36	39	39
Dissociation		0	3(8%)	3(8%)	2(5%)	0
Fatty change (& fat)		71(93%)	10(26%)	10(28%)	10(26%)	35(90%)
Parasitic cell granuloma		0	0	1(3%)	0	0
Bile duct proliferation †††		13(17%)	6(16%)	9(25%)	5(13%)	19(49%)
Hypertrophy/hyperplasia		0	3(8%)	3(8%)	0	0
Nuclear changes		10(13%)	1(3%)	3(8%)	7(18%)	6(15%)
Necrosis		0	2(5%)	0	0	0
Edema		0	0	2(6%)	0	0
Spleen	#	77	38	36	39	39
Giant cells		0	0	0	0	2(5%)
Kidneys	#	77	38	36	39	39
Cysts		1(1%)	1(3%)	1(3%)	1(3%)	1(3%)
ICI		2(3%)	2(5%)	1(3%)	0	1(3%)
Glomerular damage		1(1%)	2(5%)	4(11%)	0	4(10%)
Scar		0	0	2(6%)	3(8%)	0
Tubule proliferation		0	2(5%)	0	5(13%)	0
Renal pelvis proliferation		3(4%)	1(3%)	3(8%)	5(13%)	10(26%)
Cast		10(13%)	6(16%)	10(28%)	4(10%)	2(5%)
Adrenals	#	75	37	31	37	35
Hemorrhage		4(5%)	4(11%)	0	0	0
Cysts		0	8(22%)	0	0	0
Fatty change		1(1%)	0	0	1(3%)	0
Uterus	#	72	34	31	32	35
ICI		0	0	1(3%)	1(3%)	1(3%)
Hypertrophy/hyperplasia		7(10%)	3(8%)	1(3%)	4(13%)	7(20%)
Polyps		6(8%)	4(12%)	1(3%)	0	3(9%)
Ovaries	#	73	37	32	36	39
Aschheim-Zondek		73(100%)	12(32%)	11(34%)	13(36%)	38(97%)
Cysts		2(3%)	3(8%)	1(3%)	1(3%)	0
Fatty change (& fat)		0	0	1(3%)	0	1(3%)
Mammary Glands	#	72	30	25	19	35
Cysts		9(13%)	3(10%)	3(12%)	1(5%)	9(26%)

* = Total examined, tissue samples
†† = inflammatory cellular infiltration
††† = previously diagnosed as bile duct adenoma

Data extracted from Bayer Report No. 4888 Histopathology Addendum and Addendum 2.

Table II presents the "updated" (including additional animals) non-neoplastic findings from the 2 year chronic rat feeding study. As can be seen from this summary table (prepared by this reviewer), no biologically significant differences in non-neoplastic findings were noted in the males, except for a statistically significant increase, $p < 0.01$, in the 100 ppm group for parasitic cell granuloma of the liver; however, this is considered to be an inflammatory response rather than a compound related effect. On the other hand, the high dose females presented with increases in bile duct proliferation, renal pelvis proliferation, hpertrophy/hyper plasia of the uterus and cysts in the mammary glands; only the renal pelvis proliferation showed a dose response relationship (statistically significant at the $p < 0.01$ level for the high dose group).

3. Clarification of nomenclature

The registrant's consultant pathologist clarified certain questions on nomenclature and definitions of histopathological findings with Dr. Louis Kasza of the Toxicology Branch. These items included reclassification of liver bile duct proliferative changes and definitions for liver "changes in the nucleus" and for "tumor" as used in the report. According to the investigators regarding bile duct lesions: "These findings were first described by Dr. Emminger many years ago. Nowadays, it is accepted that what was once termed 'bile duct adenoma' is better described as 'bile duct proliferation'". According to the investigators regarding the incidence of ovarian adenomas: "...a number of slides from the Sencor experiment was misinterpreted. Lesions such as those described as ovarian cytadenomas, represented follicular cysts. True neoplasms were found only in 2 instances - the recheck revealed granulosa cell tumours. In this respect, ovarian cystadenomas have also been overdiagnosed, and require changes. The new tumour tables have been changed accordingly".

I concur with the explanations and revisions by the registrant regarding pathological findings for this study and with the conclusions reached in this review.

Louis Kasza,D.V.M.,Ph.D.
Toxicology Branch Pathologist

4. Body and Organ Weight Data.

In response to the request for tables of mean weekly body weight data by sex, the registrant supplied only growth curves and individual animal body weight data; no summary tables were provided. However, legible copies of the individual animal body weight data were provided and mean weekly body weights (calculated by this reviewer) at selected intervals is shown in Tables III and IV.

Table III: Mean Animal Body Weights (gms) at Selected Time Points (values calculated by the reviewer)

Dose(ppm)	Week: 0	26	53	79	105
A: Males					
Control	51.4	390.8	426.7	445.5	401.1
25	51.5	393.1	421.5	443.9	430.1
35	51.5	387.0	409.1	443.7	407.1
100	51.5	394.0	411.0	451.6	420.9
300	51.5	381.3	414.0	439.8	403.4
B: Females					
Control	52.1	238.8	255.9	270.7	258.9
25	52.1	231.4	254.5	273.0	261.1
35	52.1	242.1	259.6	283.4	270.1
100	52.1	229.4	252.0	270.2	254.1
300	52.1	221.6	243.7	262.2	256.9

Data extracted from Bayer Report No. 4888 Addendum.

Table IV: Animal Body Weight Gain At Selected Intervals (gm)

	Weeks:							
Dose(ppm)	0-26 Males	0-53	0-79	0-105	0-26 Females	0-53	0-79	0-105
Control	339.4	375.3	394.1	349.7	186.7	203.8	218.6	206.8
25	341.4	370.0	392.4	378.6	179.3	202.4	220.9	209.0
35	335.5	357.6	392.2	355.6	190.0	207.5	231.3	218.0
100	342.5	360.0	400.1	369.4	177.3	199.9	218.1	202.0
300	329.8	362.5	388.3	351.9	169.5	191.6	210.1	204.8

Data extracted from Bayer Report No. 4888 Addendum.

These data substantiate the statement in the registration standard re-review that no significant differences were found between the control and the 25 to 100 ppm test groups through the 24 month test period. The males of the 300 ppm test group showed significant differences at weeks 70 to 80 and 90 to 100 while the females showed significant differences (for both sexes according to the investigators: $p < 0.05$) from weeks 20 to 100, but at the end of the test period there was only a slight difference from control for the females.

The absolute organ weights are presented on Table V and relative organ weights are presented on Table VI below:

Table V: Absolute Organ Weight (mg)

Dose(ppm)	Thyroid	Heart	Lung	Liver	Spleen	Kidney
Male Rats						
Control	24.7	1012	1902	10191	842	2602
25	26.2	1058*	1809	11547**	921*	2510
35	26.9*	1009	1863	10880*	804	2497
100	28.6**	1029	1913	10521	915	2491
300	27.4	979	1867	9711	781	2362**
Female Rats						
Control	21.9	772	1319	8610	669	1761
25	21.5	754	1332	8411	649	1676
35	24.5	766	1483	8156	725	1767
100	20.2	715**	1231	7605**	663	1656**
300	20.9	721**	1199**	7762	613*	1705

* = $p < 0.05$
** = $p < 0.01$
Data extracted from BAYER AG Report No. 4888 Table 16a.

The absolute weights of female rat heart (significant at 100 and 300 ppm) and lung (significant at 300 ppm) showed a dose related decrease. The absolute kidney weight in males showed a dose-related decrease with the 300 ppm level being statistically significant.

Table VI: Relative Organ Weights (mg/100 gm body weight)

Dose(ppm)	Thyroid	Heart	Lung	Liver	Spleen	Kidney
Male Rats						
Control	6.2	255	408	2553	211	654
25	6.0	246	424**	2683*	215	584**
35	6.8*	250	466	2702	198	620
100	6.8*	247	458	2511	217	595**
300	6.8	243	466	2416	193	588**
Female Rats						
Control	8.7	301	519	3336	260	685
25	8.2	290	514	3236	250	646
35	9.1	285	548	3031**	270	660
100	7.9	283*	483	2999**	264	656
300	8.3	283*	471*	3028**	238*	668

* = $p < 0.05$
** = $p < 0.01$
Data extracted from BAYER AG Report No. 4888 Table 16a.

The average relative organ weights show a similar pattern except that liver weight is reduced over control in the 35, 100 and 300 ppm dosage levels.

Although these above changes were noted, they were of uncertain biological significance since there were no corroborative histological or clinical chemistry findings.

Conclusions:

Metribuzin is not oncogenic to the rat in dietary levels up to 300 ppm. The No Observed Effect Level (NOEL) for systemic effects is 100 ppm. The Lowest Observed Effect Level (LOEL) for systemic effects is 300 ppm, based on the decreased weight gain, along with the pathological changes in the liver, kidneys, uterus and mammary glands.

The sponsor has now satisfactorily responded to all requests made in the re-review of the chronic rat study and the study is upgraded to Core-Minimum Data. Assessment of the chronic toxicity/ oncogencity potential for metribuzin in the rat has included the additional data and clarifying information provided by the registrant.