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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

PC 101101

GUIDELINE: 83-4

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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	93.8	345.8	127
	±10.2	±4.3	±8.1	±5.5
30	319.7	91.3	345.1	118.6
	±9.4	±4.8	±8.7	±3.9
150	344.0	86.9	353.9	117.1
	±7.8	±3.5	±11.0	±4.7
750	301.5*	61.1**	313.8*	98.7**
	±9.0	±3.6	±9.7	±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		
0 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		
0 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		
0 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		
0 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 dose 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

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Table XX

F_0 Dam Reproductive Efficiency and F_1 Neonatal Data

		Control	30 ppm	150 ppm	750 ppm
Copulation Index[a]		100	100	96.7	100
Fertility Index[b]		96.7	96.7	93.1	100
Gestation Index[c]		100	100	100	100
Gestation Length (Days):	Mean	21.8	21.9	21.9	21.7
	Median	22	22	22	22
	(Range)	(21-22)	(21-23)	(21-23)	(21-22)
No. of Litters		29	29	27	30
No. of Deaths Among Dams		0	0	0	0
Total No. of Pups Born		371	358	329	370
Litter Size:	Mean	12.8	12.3	12.2	12.3
	Median	13	13	13	12
	(Range)	(5-17)	(5-17)	(1-16)	(7-16)
Stillborn Pups:	Number (%)	8 (2.2)	6 (1.7)	13 (4.0)	6 (1.6)
	Mean	0.3	0.2	0.5	0.2
	Median	0	0	0	0
	(Range)	(0-3)	(0-3)	(0-5)	(0-3)
Total No. Dead Pups:	Number (%)	16 (4.3)	20 (5.6)	23 (7.0)	+29 (7.8)
(Stillborns + Deaths)	Mean	0.6	0.7	0.9	1.0
	Median	0	0	0	0
	(Range)	(0-4)	(0-3)	(0-5)	(0-10)
Pup Deaths After Livebirth	Days 0-4	8	14	10	23
Viability Index[d]:	Mean	98.0	96.2	97.0	92.4
	Median	100	100	100	100
	(Range)	(84.6-100)	(81.8-100)	(76.9-100)	(0-100)
Pup Deaths	Days 5-21	0	0	0	0
Weaning Index[e]:	Mean	100	100	100	100
	Median	100	100	100	100
	(Range)	(100-100)	(100-100)	(100-100)	(100-100)
Total No. of Implantations		393	381	363	395
	Mean	13.6	13.1	13.4	13.2
	Median	14	14	14	13
	(Range)	(6-17)	(5-17)	(1-17)	(8-16)
Birth Index[f]:	Mean	92.1	91.8	87.4	92.1
	Median	92.3	92.3	92.9	93.1
	(Range)	(78.6-100)	(66.7-100)	(50.0-100)	(58.3-100)
Median Wt. Viable Pups (gm):	Birth	6.2	6.0	6.0	5.0
	Day 4	9.2	9.7	9.6	9.2
	Day 7	15.4	15.8	15.3	14.6
	Day 14	32.4	31.4	31.3	30.7
	Day 21	52.6	51.5	51.6	48.5**
	Gain	46.8	45.3	45.8	42.5**
Percent Male Fetuses	Median	42.9	45.5	53.8	50.0
	Mean	45.5	47.4	53.1	48.4

+ Includes 10 deaths in one litter
** Significantly different from control at the 0.01 level using Kruskal-Wallis test

[a]No. of animals with successful copulation/No. of mated animals x 100
[b]No. of pregnant animals/No. of animals with successful copulation x 100
[c]No. of dams with liveborn/No. of pregnant dams x 100
[d]No. of neonates viable on Day 4/No. of viable neonates at birth x 100
[e]No. of viable neonates on Day 21/ No. of viable neonates on Day 4 following culling x 100
[f]No. of liveborn/No. of implantations x 100

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Table XXI

F_1 Dam Reproductive Efficiency and F_2 Neonatal Data

		Control	30 ppm	150 ppm	750 ppm
Copulation Index[a]		100	100	100	100
Fertility Index[b]		83.3	96.7	96.7	93.3
Gestation Index[c]		100	100	100	100
Gestation Length (Days):	Mean	21.7	21.6	21.9	21.6
	Median	22	22	22	22
	(Range)	(21-22)	(21-23)	(21-23)	(21-22)
No. of Litters		25	29	29	28
No. of Deaths Among Dams		0	0	0	0
Total No. of Pups Born		353	386	347	345
Litter Size:	Mean	14.1	13.3	12.0	12.3
	Median	14	14	13**	12**
	(Range)	(11-17)	(6-16)	(3-18)	(4-16)
Stillborn Pups:	Number (%)	5 (1.4)	4 (1.0)	3 (0.9)	3 (0.9)
	Mean	0.2	0.1	0.1	0.1
	Median	0	0	0	0
	(Range)	(0-2)	(0-1)	(0-1)	(0-1)
Total No. Dead Pups:	Number (%)	11 (3.1)	17 (4.4)	11 (3.2)	6 (1.7)
(Stillborns + Deaths)	Mean	0.4	0.6	0.4	0.2
	Median	0	0	0	0
	(Range)	(0-3)	(0-3)	(0-3)	(0-1)
Pup Deaths After Livebirth	Days 0-4	6	12	8	3
Viability Index[d]:	Mean	98.3	96.9	97.9	99.2
	Median	100	100	100	100
	(Range)	(92.9-100)	(85.7-100)	(83.3-100)	(91.7-100)
Pup Deaths	Days 5-21	0	1	0	0
Weaning Index[e]:	Mean	100	99.6	100	100
	Median	100	100	100	100
	(Range)	(100-100)	(87.5-100)	(100-100)	(100-100)
Total No. of Implantations		375	407	378	379
	Mean	15.0	14.0	13.0	13.5
	Median	15	14	14*	14*
	(Range)	(12-18)	(7-17)	(3-18)	(6-16)
Birth Index[f]:	Mean	93.1	93.7	90.5	89.7
	Median	93.3	93.3	92.3	92.3
	(Range)	(81.2-100)	(66.7-100)	(42.9-100)	(66.7-100)
Median Wt. Viable Pups (gm):	Birth	5.9	5.9	5.9	6.0
	Day 4	9.2	9.2	9.5	9.2
	Day 7	15.2	15.0	14.9	15.2
	Day 14	31.7	30.5	30.2*	30.0**
	Day 21	52.3	49.1*	49.2*	46.8**
	Gain	46.5	43.1*	43.0*	41.0**
Percent Male Fetuses	Median	50.0	50.0	42.9	42.3
	Mean	48.6	52.1	45.7	44.7

* Significantly different from control at the 0.05 level using Kruskal-Wallis test
** Significantly different from control at the 0.01 level using Kruskal-Wallis test

[a] No. of animals with successful copulation/No. of mated animals x 100

[b] No. of pregnant animals/No. of animals with successful copulation x 100

[c] No. of dams with liveborn/No. of pregnant dams x 100

[d] No. of neonates viable on Day 4/No. of viable neonates at birth x 100

[e] No. of viable neonates on Day 21/ No. of viable neonates on Day 4 following culling x 100

[f] No. of liveborn/No. of implantations x 100

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SENCOR Technical (Metribuzin), 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one, is a triazinone herbicide. This report describes a 2-generation reproduction study conducted in rats to determine the potential for metribuzin, administered orally via the diet, to affect gonadal function and mating behavior in treated F_0 and F_1 males; or estrous cycling, mating behavior, conception rate, and reproductive performance in treated F_0 and F_1 females exposed to those males. The study was also intended to monitor growth and development of the F_1 and F_2 generations which were also exposed to the test article in diet. This study was sponsored by Mobay Corporation, Kansas City, MO and was conducted in the Miles Corporate Toxicology Department, Elkhart, Indiana, in accordance with EPA Good Laboratory Practice Standards (40 CFR, Part 160). Animals used in the study were maintained in the Miles Corporate Animal Facility, which is registered by the USDA and fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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MATERIALS AND METHODS

Test Article

Batch number 77-297-50 (92.6% active ingredient) of metribuzin[1] was used in this study. Fresh diets were formulated by mixing[2] the test article with pulverized commercial dry basal laboratory diet[3] on a weight/weight basis at concentrations of 0, 30, 150, and 750 parts per million (ppm), or 0, 0.003, 0.015, and 0.075% of the total diet (Table I - page 19). The doses were selected based on the results of various previously conducted studies; namely, 3-month dietary studies (Mobay Report Nos. 66649 and 27908) where significant toxic effects were seen at 1500 ppm with some effect seen at 500 ppm, and a special study (Mobay Report No. 8256) where significant effects were seen at 900 ppm. The results of the rat chronic/oncogenicity (Mobay Report No. 41816) and the 3-generation rat reproduction study (Mobay Report No. 41818) were also taken into consideration. Based on the results of the studies, the highest dose of 750 ppm was considered a realistic estimate of maternally toxic dose. The low dose of 30 ppm was selected based on the NOEL in the rat chronic/onco study.

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[1]White crystalline solid supplied by Mobay Corporation, Agricultural Chemicals Division, Kansas City, MO
[2]Hobart Model A-200-DT Mixer, Hobart Manufacturing Company, Troy, OH; Toxicology Department Method (TDM) Nos. 43 and 96
[3]Purina Certified Rodent Chow #5002, Ralston Purina Company, St. Louis, MO

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The middle dose was then selected to be 150 ppm. These doses were discussed with the EPA and were acceptable. For each diet concentration, metribuzin was combined with a small portion of the basal diet as a premix. The premix was then ground using a mortar and pestle, combined with the remaining basal diet, and mixed. Test diets were appropriately labelled and stored in a freezer[4]. The diets were prepared at 1 to 4 week intervals. At the time each new batch was prepared, empty clean feed cups were filled with the appropriate freshly prepared test and control diets. Feed cups were refilled each day as necessary. Test and control diets were provided ad libitum throughout the multigeneration study.

Stability of Metribuzin Diets

A stability study on the high and low concentrations of metribuzin in the diet demonstrated stability while stored in a freezer over a 6-week period with less than 10% deviation from the initial concentration (Helms, 1987). Results of the analyses for the stability study are included in Appendix B.

Analysis of Metribuzin Diets

Verification of the homogeneity and concentration of metribuzin in test diets was accomplished by gas chroma-

[4]TDM No. 200

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tography[5] done prior to usage on the first batch and every fourth batch thereafter. Samples of test and control diets not analyzed were frozen for possible future analysis. Results of the analyses for the test diets are included in Appendix B[6] (Helms, 1988). Each test diet was homogeneous and the concentration of metribuzin was within ±8% of the desired concentration.

Control Article/Vehicle

The basal laboratory diet[3] alone (0 ppm) was used in this study and served as the control. The diet was labelled, stored, analyzed, and fed as described above for the test diets.

Study Design

A schematic and calendar outlining the course of events in this study are provided on pages 20 and 21.

Selection of F_0 and F_1 Parents. The F_0 generation was comprised of 120 male and 120 female, naive, healthy, 56-day old Charles River Crl:CD®BR rats[7] (ranging in body weight from 186 to 239 g for males and from 150 to 205 g for females) that were placed on test diets following a 1-week

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[5]Pharmacokinetics Method No. 83

[6]Information regarding preparation, stability, and analysis of the test article is contained in Notebook 1096 stored in the Toxicology Department Archive.

[7]Animals were obtained from Charles River Breeding Laboratories, Portage, Michigan and were maintained in the Miles Corporate Animal Facility, which is registered by the USDA and fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC)

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acclimation period[8]. Animals were selected for the study based on their general physical condition upon arrival in the Miles Corporate Animal Facility. Only animals that appeared healthy were used. F_0 animals were randomly[9] assigned (stratified based on body weight) to 4 test groups of 30 males and 30 females each. A total of 30 male and 30 female F_1 neonates per group (at least 1 male and 1 female from every litter where possible) were randomly[9] selected (using numbered cards) for the F_1 growth and development segment of the study.

Exposure and Sacrifice Schedule. Groups received the ground control diet[3] or a test diet containing either 30, 150, or 750 ppm of metribuzin. Animals from the F_0 generation were exposed to test or control diet for 10 weeks prior to initial mating and continued on diet until the end of the 21-day lactation period when they were sacrificed. F_0 and F_1 females that failed to become pregnant were sacrificed 21 days following their last exposure to a male. Dams that delivered but lost their entire litter prior to lactation day 21 were sacrificed as soon as all pups were dead. Culled F_1 pups were sacrificed at 4 days of age and nonselected F_1 pups were sacrificed at 21 days of age (F_1 alternates slightly older when sacrificed). Selected F_1 males and females were assigned to a 10-week growth and

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[8]TDM Nos. 36 and 58
[9]TDM No. 25

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development period during which they received the test or control diet and at the end of which they were mated to obtain the F_2 offspring. The F_1 males were terminated following delivery of the F_2 litters while F_1 females remained on test or control diets and were permitted to rear the F_2 generation until the end of a 21-day lactation period when they were sacrificed. F_2 culled pups were sacrificed at 4 days of age and remaining pups at 21 days of age.

Identification, Housing, and Clinical Observations for Adults. Adults were individually identified[10] by metal ear tags, housed individually in suspended cages[11] in a climate controlled room[12], and permitted the appropriate diet and water[13] ad libitum. A 12-hour photoperiod was maintained and desired temperature and relative humidity ranges were 65-76°F and 40-70% RH[14], respectively. Temperature was low (64°F) on 3 occasions and high (77-78°F) on 4 occasions. Humidity was below the stated range on 7 occasions and above the range 20 times. Humidity remained between 31 and 80% except on 3 occasions recorded as 25, 85, and 89%. No known impurities, contaminants, or interfering substances that could compromise the health of the animals were present in

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[10] TDM No. 64
[11] Unifab Corporation, Kalamazoo, MI
[12] TDM No. 127
[13] TDM No. 106
[14] Deviation from original Protocol 8721 because SOP for humidity range was revised after issue of protocol, see Amendment 2.

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food, water, or litter. Litter under suspended cages was changed at least twice a week and all cages were changed at least every other week. During the study all animals were observed[15] daily for overt changes in appearance and behavior. Food consumption was recorded twice weekly (during 24 hours) on all F_0 and F_1 animals except during cohabitation for both sexes and during lactation for females. Body weights were obtained twice weekly for F_0 and F_1 animals and for females on Days 0, 7, 14, and 20 of gestation and Days 0, 7, 14, and 21 of lactation.

Breeding, Gestation, Lactation, and Neonatal Observations. Estrous cycling was monitored for F_0 and F_1 females for 10 days prior to cohabitation with males. Random pairing of F_0 and F_1 animals for mating is presented in Appendix C. Breeding for both the F_0 and F_1 generations was accomplished over 28-day periods. Each female was assigned to a male from the same treatment group, except that sibling matings were not permitted. The assigned pairs cohabited overnight during mating periods. If insemination did not occur after 21 days, the females were housed with a different proven male from the same treatment group for an additional 7 days. On the morning following overnight exposure, the pairs were separated and all males were

[15]TDM No. 91

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returned to individual cages. Vaginal smears[16] were obtained on all exposed females to determine if copulation had occurred. The day spermatozoa were identified microscopically in a vaginal smear was designated as Day 0 of gestation[17] for the female.

Following mating, F_0 and F_1 females were placed into littering boxes on Day 18 of gestation and permitted to deliver. Evidence of premature or prolonged labor, dystocia, or atypical nesting habits were recorded. The day of delivery was recorded and designated as Day 0 of lactation[20]. The litters were inspected following parturition for live and/or stillborns[18], and the offspring were examined[19] for gross malformations, sexed, and weighed. All viable progeny were individually identified[20]. Stillborns and neonates which died were necropsied[21] to ascertain, when possible, cause of death. Evidence of cannibalization and neonatal survival (viability and weaning indices)[22] were also noted.

On Day 4 of lactation, each litter having greater than 8 viable offspring was randomly[9] (numbered cards) culled to 8 (4 males and 4 females, where possible). All culled pups were sacrificed and necropsied. Neonates were weighed on

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[16]TDM No. 70
[17]TDM No. 68
[18]TDM No. 196
[19]TDM No. 75
[20]TDM No. 78
[21]TDM No. 77
[22]TDM No. 180

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Days 0, 4, 7, 14, and 21 postpartum and observed daily for general appearance and behavior. Photoperiod and temperature and humidity ranges for neonates were as stated earlier for adults.

Clinical Pathology and Histopathology. Blood samples were obtained at sacrifice via open-chest cardiac puncture from 10 males and 10 females randomly selected from both the F_0 and F_1 generations. The samples were used for determination of alanine aminotransferase (ALT)[23], aspartate aminotransferase (AST)[24], and gamma glutamyl transferase (GGT)[25]. All adults and pups were necropsied at death or sacrifice. Implantation sites were counted and any gross pathologic conditions that may have prevented or compromised pregnancy were noted. Vagina, uterus, cervix, ovaries, testes, epididymides, seminal vesicles, prostate, pituitary, liver, and any tissue exhibiting remarkable gross pathologic changes were retained for F_0 and F_1 parents. All tissues retained were examined microscopically for high-dose and control animals along with tissues exhibiting gross lesions for mid- and low-dose rats. Additionally, livers were examined for all mid- and low-dose animals, and prostate glands were examined for mid- and low-dose males in the F_0 generation.

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[23]TDM No. 137
[24]TDM No. 138
[25]TDM No. 150

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Statistical Analysis

Statistical analysis of the data was accomplished by application of one or more of the following tests: Dunnett's (1955; 1964), Fisher's exact (Pagano and Halvorsen, 1981), Kruskal-Wallis (1952), and Dunn (1964). A listing summarizing the statistical analyses used is presented in Appendix D. All statistical comparisons of reproductive efficiency were done on median values using the dam as the unit of comparison.

Schematic
Two-Generation Reproduction Study

F_0 males and females (about 8 weeks old at initiation of dosing) on test or control diet for a minimum of 10 weeks prior to first mating. All F_0 rats remained on diet until sacrifice

F_0

30 F_0 males and 30 F_0 females/group. F_0 males necropsied following weaning of F_1 litters. F_0 females necropsied after they deliver and rear litter for 21 days. Litters culled to 8 pups/litter on Day 4 of lactation.

F_0 Breeding

F_1 Delivery
Cull (Day 4), Necropsy
Select*
Sacrifice (Day 21), Necropsy

Selected F_1 neonates on test or control diet for 10 weeks after last F_1 litter was weaned.

F_1 Breeding
F_1 mating initiated after the 10 weeks.

F_2 Delivery
Sacrifice (Day 21), Necropsy

* 30 male and 30 female F_1 neonates/group (from as many different litters as possible) were randomly selected for a 10 week growth and development period. At the end of this time, the males and females were mated 1:1 (mating between siblings was avoided). F_1 males were necropsied following delivery of F_2 litters. F_1 females were permitted to deliver and rear litters through 21-day lactation period. F_2 litters were culled to 8 pups/litter on Day 4 of lactation and culled pups were necropsied. F_1 dams and F_2 pups necropsied on Day 21 of lactation.

Note: Selected tissues taken on all F_0 and selected F_1 males and females.

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