



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

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JUL 25 1991

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Revised Section F Petition and additional data for
teratology study in rats with Metribuzin.
EPA Identifying No's.: 2F2677, 3125-314, 3125-325, EPA
MRID No: 415518-01, EPA Record Nos.: 268092, 268100,
268102, HED Project No. 0-1693, Caswell No. 033D.

TO: Robert Taylor/Vickie Walters (PM 25)
Herbicide-Fungicide Branch
Registration Division (H7505C)

FROM: Stephen C. Dapson, Ph.D. *Stephen C. Dapson*
Senior Pharmacologist, Review Section I *7/11/91*
Toxicology Branch II/HED (H7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. *Y.M. Ioannou 7/16/91*
Section Head, Review Section I
and
Marcia van Gemert, Ph.D. *M van Gemert 7/23/91*
Chief, Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Mobay Corporation
Agricultural Chemicals Division
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Action Requested: Review revised Section F Petition and
additional data submitted for a rat teratology study with
Metribuzin.

Recommendations: The Toxicology Branch II has no objection to the
proposed increase in the established tolerance for residues of
metribuzin and its triazinone metabolites in soybeans. Further,
the teratology study in rats with metribuzin (A Teratology Study
with SENCOR Technical (Metribuzin) in the Rat, Mobay Ag Chem
#91330, October 3, 1986, EPA MRID (Accession) No. 265336) can be
upgraded to Core-Minimum Data as a result of the additional data
submitted by the registrant in support of the study (see
discussion).

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I. Background**A. From the Memo dated 7/13/89 (S.C.Dapson to R.Taylor)**

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.

The following is from the Discussion/Conclusions section from the Data Evaluation Record dated 7/13/89 (A Teratology Study with SENCOR Technical (Metribuzin) in the Rat, Mobay Ag Chem #91330, October 3, 1986, EPA MRID (Accession) No. 265336).

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.

c. 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

d. Core Classification: Core-Supplementary Data.

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

The registrant submitted the following comments and information for the rat teratology study (referenced as Supplemental Submission to EPA Accession No. 265366, MOBAY No. 91330-1, MRID No. 418818-01):

COMPANY RESPONSE

1. Gravid uterine weights (individual animal and group means) have been provided in Table one... . The weights were analyzed by Dunnett's test and were not found to be significantly different from control.

The gravid uterine weights were used to calculate the actual body weight (final body weights minus the weight of the intact uterus). This parameter eliminates uterine weight as a confounding factor in assessing test article effects on maternal weight. Group means for this parameter were presented on Table I of the original report.

EPA COMMENT

The following table presents the mean gravid uterine weights:

Dose:	Control	25 mg/kg	70 mg/kg	200 mg/kg
(assume grams)	77.1	74.4	71.3	66.9
% of control		96.5	92.5	86.5
% difference from control		3.5	7.5	13.2

There is a dose response decrease in mean gravid uterine weights with no difference in litter size and this observation parallels the decrease in fetal body weights. This further supports maternal toxicity in all treatment groups.

COMPANY RESPONSE

2. Group mean fetal weights, including male, female, and combined weights and analysis of the means are provided in Table 2... . We have also included an expanded historical control (comprised of 27 studies) to support our position that fetal weights for the low- and mid-dose level were well within the historical control range for our laboratory. The means and analysis of the means presented... (Table 2) mimic the medians and analysis of the medians in the original report (Table IV).

There is some controversy among developmental toxicologists regarding which method, median (eg. Kruskal-Wallis), or means (eg. Dunnett's), is the most appropriate method of analyzing fetal weights, based on the dam as the primary experimental unit. Our statisticians feel that analysis of the median fetal weight is highly efficient in terms of power, relative to analysis of means, under the assumptions for a parametric test and more efficient in other situations.

EPA COMMENT

The following table presents the mean and median fetal weights (assume in grams):

Dose:	Control	25 mg/kg	70 mg/kg	200 mg/kg
Male Median	3.9	3.7	3.7*	3.3**
Mean	3.9	3.7	3.6*	3.3*
HC range*	3.5-4.1			
Female Median	3.7	3.5*	3.5*	3.1**
Mean	3.7	3.5*	3.4*	3.1*
HC range	3.3-3.9			
Combined Median	3.8	3.6**	3.6**	3.1**
Mean	3.8	3.6*	3.5*	3.2*
HC range	3.4-4.0			
% difference from control		5.3	7.9	15.2
% of control		94.7	92.1	84.8
* Historical control median or weighted mean (gm) range				

As noted in the table above, no major differences were noted between the mean or median value in this case; however, in future submissions, if median data are reported, mean data must accompany the values to allow for a complete statistical evaluation. Further, although fetal body weights may be within historical control data range for the laboratory, comparison with concurrent control is always necessary, in this case the biological relevance of the 5.3 and 7.9 % reduction in the low and mid dose group, respectively, is unclear. The effect in the high dose group appears to be biologically relevant and it is below the historical control range.

COMPANY RESPONSE

3. The number of fetuses and litters examined for external, visceral, and skeletal anomalies are presented in Table 3...

All individual external and visceral findings observed in the study appear in Table VI of the original report (this table included common variations as well as malformations), individual skeletal findings appear in Appendix J, summary of skeletal findings appear in Table VII, incidence summary of skeletal malformations is presented in Table VIII, and a listing of these skeletal findings considered to be malformations is presented in Table IX.

At the time this report was submitted, a summary table listings incidence of external and visceral malformations and a table defining these malformations was not presented; however, a table (Table VIII) listing incidence of skeletal malformations and selected variations and defining the skeletal malformations (Table IX) were presented. We have compiled an incidence of external, visceral, skeletal, and combined malformations. This information appears in Table 4... . A listing of all findings (external, visceral, and skeletal) considered to be malformations are presented in Table 5 Furthermore, we have identified a typographical error on Table IX of the report. The skeletal malformation (fetus No. 149 from dam RS5076) should be placed in the 70 mg/kg group. Table VIII listing one skeletal malformation for the 25 mg/kg group and two skeletal malformations for the 70 mg/kg group is accurate.

EPA COMMENT

The following table presents the submitted data on the number of fetuses and litters examined:

Dose:	Total	Control	25 mg/kg	75 mg/kg	200 mg/kg
		fetuses/litters			
External	1412/106	367/27	352/26	351/27	341/26
Visceral	680/106	176/27	169/26	171/27	163/26
Skeletal	732/106	191/27	183/26	180/27	178/26

From the data presented relative to observations considered as malformations, no treatment related effects were noted.

COMPANY RESPONSE

4. A summary of the fetal incidence of skeletal findings (a composite study of degrees of skeletal ossification, skeletal variations and malformation) and statistical analysis of these incidences can be found in Table VII (pp. 21-22) of the SENCOR report. Table 6... presents the litter incidence of skeletal findings and statistical analysis of these incidences.

It is clear to us, on the basis of both the fetal and litter incidence of skeletal observation, that growth and development of the fetal skeleton, based on degrees of ossification, incidence of skeletal variation, and/or malformations, were unaffected at the low- and mid-dose levels. However, as concluded in the report, there was a delay in ossification of several skeletal elements for the high-dose group. This was statistically significant for both the fetal and litter incidence. The delay in ossification corresponds to the reduction in fetal weight for the high-dose group and should be considered evidence of developmental toxicity.

EPA COMMENT

The table on the following page presents the skeletal examination data. It is apparent from the presented data that developmental toxicity occurred in the high dose in the form of reduced or unossified skull bones, ribs, vertebrae, sternbrae, pelvic bones, and appendages. No other observations appear to be biologically relevant.

Observations^a	Control	Low Dose	Mid Dose	High Dose
# fetuses examined	191	183	180	178
# litters examined	27	26	27	26
Skull:				
Bone inc.oss.	106/25	102/24	109/25	160**/25
Sutures enlarged	17/7	5*/3	13/9	54**/18
Fontanelle enlarg.	28/11	12*/10	21/10	70**/20*
Hyoid-variations	41/18	32/15	23/10	63**/19
Ribs:				
Incompletely oss.	1/1	0/0	3/2	13**/6
Wavy or curved	2/1	0/0	4/3	12**/6
Vertebrae:				
incomplete ossification:				
Cervical arch	4/4	14*/9	6/5	41**/15**
Lumbar arch	0/0	0/0	3/3	6*/5
Sacral arch	82/20	110**/25	92/25	149**/25
unossified:				
Thoracic centra	0/0	1/1	0/0	7*/3
Sacral arch	2/1	1/1	2/2	17**/5
Caudal arch	8/5	30/2	18/4	34**/10
Caudal centra	0/0	0/0	0/0	4/3
Sternebrae:				
incomplete ossification:				
1st	12/9	22/10	7/5	33**/13
2nd	28/15	50**/17	58**/21	112**/25**
3rd	9/5	19/9	10/8	45**/16**
4th	137/25	141/26	149/26	149*/24
5th	150/27	136/21	138/25	98**/19
6th	138/26	145/25	145/25	136/24
1st unossified	0/0	0/0	0/0	3/3
2nd unossified	2/1	4/4	2/2	11*/2
3rd unossified	0/0	0/0	0/0	2/6
4th unossified	0/0	0/0	1/1	7*/4
5th unossified	41/17	43/20	38/16	76**/20
6th unossified	3/2	7/5	6/5	27**/9
Pelvis:				
Pubis incomp.oss.	7/3	4/3	2/2	18*/7
Appendages:				
incomplete ossification:				
Ant.-metacarpals	4/2	2/1	1/1	16*/7
Post.-metatarsals	2/1	1/1	0/0	11*/6
unossified:				
Ant.-metacarpals	0/0	0/0	0/0	11**/6*
Post.-metatarsals	0/0	2/1	0/0	15**/6*

* = $p < 0.05$ ** = $p < 0.01$ ^a = Some observations may be grouped together.^b = Fetal/Litter incidence.

COMPANY CONCLUSIONS RELATIVE TO THE STUDY

In conclusion, on the basis of the definitive study, a maternal NOEL (no observed effect level) could be established, but is <25 mg/kg. The developmental NOEL is considered to be 70 mg/kg.

EPA CONCLUSIONS RELATIVE TO THE STUDY

The Agency generally agrees with the investigators conclusions:

The study involved metribuzin (SENCOR technical) administered by gavage to pregnant rats from gestation days 6 through 18 inclusive (Mobay AG Chem No. 91330, October 3, 1986). Maternal toxicity was evidenced at all dose levels in the form of reduced body weight gain, reduced mean gravid uterine weights and decreased food consumption. Further, there was an effect on the thyroid gland in the mid and high dose groups with reduced T4 levels and in the high dose group with an increase in thyroid weight. Developmental toxicity was evidenced in the high dose by decreased fetal body weights and reduced or unossified skull bones, ribs, vertebrae, sternebrae, pelvic bones, and appendages. No other observations appear to be biologically relevant.

Maternal NOEL < 25 mg/kg/day

Maternal LOEL < 25 mg/kg/day

Developmental Toxicity NOEL = 70 mg/kg/day

Developmental Toxicity LOEL = 200 mg/kg/day

This study can be upgraded to Core-Minimum Data and fulfills the guideline requirement (§83-3(a)) for a teratology study in rats.

Further, an earlier teratology study of SENCOR [metribuzin] in rats (EPA Accession No. 112892, Bayer AG Report No. 35073, Study No. 3678, 9/29/72) using dose levels of 5, 15, 50, and 100 mg/kg/day by gavage (in Cremophor EL, 1.5% aqueous solution) to pregnant rats from gestation days 6 through 15 inclusive found evidence of maternal toxicity at 100 mg/kg/day (evidenced by decreased body weight gain and corroborative clinical signs) with no evidence of developmental toxicity; however, the study had several deficiencies.

Additionally the registrant submitted the following information on the point mutations study:

EPA CONCLUSIONS

From Memo dated 7/13/89 (S.C.Dapson to R.Taylor): 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.

COMPANY RESPONSE

The Agency's reviewers also found a minor deficiency in the point mutation study on Saccharomyces cerevisiae [Mobay Report No. 94786 - MRID 40347701] as discussed on page 4 of your letter, and they concluded that this study is not fully acceptable but that it is upgradeable. We will obtain the analytical data to confirm concentrations of the test material from the testing laboratory, and when available, these additional data will be submitted in order to upgrade this study. In any event, we feel that the Agency already has adequate data to conclude that metribuzin is not mutagenic. The various mutagenicity studies we have submitted for metribuzin are fully referenced in the registration standard files and correspondence.

EPA COMMENT

The Agency reserves comment on the study until the additional data are submitted; however, we agree with the registrant that the requirements for a mutagenicity battery have been met for metribuzin (see mutagenicity testing section on page 11).

B. 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 terrestrial crops, ornamentals and noncrops. SENCOR is chemically 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one.

The proposed tolerance (Section F) is as follows:
Raw Agricultural Commodities - 40 CFR 180.332

<u>Commodity</u>	<u>Proposed Tolerance (PPM)</u>
Soybeans	0.3 (increase from 0.1)

The preemergence and preplant application preharvest interval is 40 days and the preemergence and post emergence applications pre-harvest interval is 70 days.

III. Data Requirements (CFR 180.332, 185.250, 186.250)

Technical: Metribuzin

Use Pattern: food

Action Type: Section F for tolerances in soybeans

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

	Required	Satisfied
§81-1 Acute oral toxicity in rats	Yes	Yes
§81-2 Acute dermal toxicity in rats	Yes	No ¹
§81-3 Acute inhalation toxicity in rats	Yes	Yes
§81-4 Primary eye irritation in rabbits	Yes	No ¹
§81-5 Primary dermal irritation in rabbits	Yes	No ²
§81-6 Dermal sensitization in guinea pigs	Yes	No ²
§82-1(a) Subchronic oral (rodent)	Yes	No ³
§82-1(b) Subchronic oral (nonrodent)	Yes	No ³
§82-2 21 day dermal - rat	Yes	No
§83-1(a) Chronic toxicity (rodent)	Yes	Yes
§83-1(b) Chronic Toxicity (nonrodent)	Yes	Yes
§83-2(a) Carcinogenicity - rat	Yes	Yes
§83-2(b) Carcinogenicity - mouse	Yes	Yes ⁴
§83-3(a) Teratology - rat	Yes	Yes
§83-3(b) Teratology - rabbit	Yes	Yes
§83-4 Multigeneration reproduction-rat	Yes	Yes

¹ requirement met by adequate studies with a formulation

² not reviewed

³ requirement met by adequate chronic feeding study

⁴ see discussion in this document

	Required	Satisfied
§84-2(a) Mutagenicity-Gene Mutation	Yes	Yes
§84-2(b) Mutagenicity-Struct. Chrom. Aberr.	Yes	Yes
§84-4 Mutagenicity-Other Genotox. Effects	Yes	Yes
§85-1 General metabolism - rat	Yes	Yes

Formulation: Sencor/Lexone DF 75% Dry Flowable Herbicide
(both 75 and 84 % a.i.)

	Required	Satisfied
§81-1 Acute oral toxicity in rats	Yes	Yes
§81-2 Acute dermal toxicity in rats	Yes	Yes
§81-3 Acute inhalation toxicity in rats	Yes	No
§81-4 Primary eye irritation in rabbits	Yes	Yes
§81-5 Primary dermal irritation in rabbits	Yes	Yes
§81-6 Dermal sensitization in guinea pigs	Yes	Yes

¹ = requirement met by an adequate study with the technical

IV. Data Gaps

For Metribuzin Technical:

§82-2 21 day dermal - rat

V. Actions Being Taken to Obtain Additional Information or Clarification

None at this time.

VI. Reference Dose

The RfD is 0.025 mg/kg/day based on a 2 year feeding study in the dog with a systemic NOEL of 2.5 mg/kg and a safety factor of 100 (PADI). The additional data reported here will be submitted to the RfD workgroup for consideration of the RfD.

VII. Pending Regulatory Actions:

None at this time.

VIII: Toxicological Issues Pertinent to this Request:

This chemical was a registration standard in 1985.

A. New toxicology Data on Metribuzin**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 thyroid 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 were submitted and allowed an assessment of the maternal and developmental toxicity. **SEE DISCUSSION ABOVE.**

B. Carcinogenicity

There is no evidence of carcinogenicity in the rat and mouse study submitted. A new 2 year rat feeding/carcinogenicity study is being conducted by the registrant at this time.

C. Toxicology One-liners

Attached.