



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

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MAY 29 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Metribuzin - Review of a teratology study in rabbits and an in vitro cytogenetic assay in CHO cells.
EPA ID # 101101, EPA Barcode D166515, MRID No. 412492-01 and 415551-02, EPA Pesticide Chemical Code 101101, Toxicology Chemical Code 033D, HED Project No. 1-1752.

TO: Walter Waldrop/Eric Feris, PM 71
SRRD (H7508W)

FROM: Stephen C. Dapson, Ph.D. *Stephen C. Dapson 5/22/92*
Senior Pharmacologist, Review Section I
Toxicology Branch II/HED (H7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. *Y. M. Ioannou 5/26/92*
Section Head, Review Section I
and
Marcia van Gemert, Ph.D. *M. van Gemert 5/26/92*
Branch Chief, Toxicology Branch II
Health Effects Division (H7509C)

Action Requested: Review teratology study in rabbits and in vitro cytogenetic assay in CHO cells with metribuzin.

Recommendations: The teratology study in rabbits with metribuzin (MRID # 412492-01, Teratology Study in the Rabbit with SENCOR Technical [Metribuzin], Lab Project ID 99654, August 2, 1989) has been reviewed previously by Toxicology Branch II (attached). The mutagenicity study "In an in vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells" (MRID # 415551-02, HLA Study No. 10857-0-437, March 30, 1990) has been reviewed and found to be acceptable (DER is attached). The registrant has fulfilled the requirements for the mutagenicity battery with previously submitted studies.

**I. Toxicology Profile for Metribuzin (SENCOR, CFR
180.332, 185.250, 186.250)**

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Technical: Metribuzin, SENCOR, LEXONE
Use Pattern: food
Action Type: reregistration

This compound is a registered active ingredient. The following data are required for Metribuzin technical. **THIS INFORMATION DOES NOT NECESSARILY REFLECT THE DATA REQUIREMENTS FOR REREGISTRATION.**

	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 ¹
§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
§83-3(a) Teratology - rat	Yes	Yes
§83-3(b) Teratology - rabbit	Yes	Yes ⁴
§83-4 Multigeneration reproduction-rat	Yes	Yes
§84-2(a) Mutagenicity-Gene Mutation	Yes	Yes
§84-2(b) Mutagenicity-Struct. Chrom. Aberr.	Yes	Yes ⁴
§84-4 Mutagenicity-Other Genotox. Effects	Yes	Yes
§85-1 General metabolism - rat	Yes	Yes

¹ = requirement met by adequate studies with a formulation

² = study submitted, additional data required

³ = requirement met by adequate chronic feeding study

⁴ = see discussion in this document

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 ¹
§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

II. Data Gaps

The following are the data gaps for Metribuzin Technical:

§82-2 21 day dermal - rat

§81-6 Dermal sensitization in guinea pigs - additional data
required

III. Actions Being Taken to Obtain Additional Information or Clarification

None at this time.

IV. 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.

V. 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

The mutagenicity study "In an *in vitro* Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells" (MRID # 415551-02, HLA Study No. 10857-0-437, March 30, 1990) has been reviewed and found to be acceptable, DER is attached to this action.

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 in response to a request from California.

C. Toxicology One-liners

Ammended one-liners are attached.



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CASWELL FILE

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SUBSTANCES

MEMORANDUM

SUBJECT: 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one (SENCOR Technical): Review of additional information submitted by the registrant.

Caswell No: 033D
HED Project No: 1-1094
MRID No: 418355-01

FROM: Timothy F. McMahon, Ph.D., Toxicologist *Tim F. McMahon 5/2/91*
Review Section I, Toxicology Branch II
Health Effects Division (H7509C)

TO: Lois Ross/PM Team 74
Registration Division (H7505C)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head *Y. M. Ioannou 5/9/91*
Review Section I, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia Van Gemert, Ph.D., Branch Chief *M. Van Gemert 5/10/91*
Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Mobay Corporation

Action Requested: Review of the Registrant's response to Agency review of a rabbit teratology study with 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one (SENCOR Technical), required for California registration.



Discussion and Conclusions:

Initial review of the registrant's report, "Teratology Study in the Rabbit with SENCOR Technical (Metribuzin)" (MRID#412492-01), revealed a number of deficiencies which resulted in a core supplementary classification of this study. In order to properly evaluate the original study, the Agency requested the following information from the registrant:

- 1) litter incidence data for fetal skeletal abnormalities listed in Table VI of report
- 2) historical control data for the skeletal abnormalities listed in Table VI of report
- 3) necropsy findings on does not listed in Appendix D
- 4) results of histological examination of maternal tissues, if any
- 5) times and dates of sacrifice for all maternal rabbits

The registrant's response to these concerns are addressed below.

- 1) litter incidence data for fetal skeletal abnormalities listed in Table VI of report

These data were requested as the litter is considered the experimental unit for developmental toxicity studies, and was not provided for assessment of skeletal abnormalities observed in the developmental toxicity study. The registrant supplied information on litter incidence for all values presented in Table VI of the original report. Evaluation of these data as submitted showed that the litter incidence for the skeletal abnormalities listed in Table 6 of the initial review (attached) were not significantly different between dose groups, with the exception of the litter incidence for irregular spinous process of the scapula, in which the litter incidence increased from 0 in controls to 5 in the 85mg/kg/day dose group.

- 2) historical control data for the skeletal abnormalities listed in Table VI of report

These data were requested as there was not proper historical control data with which to evaluate the significance of the skeletal abnormalities observed in the developmental toxicity study. The registrant responded to this request by supplying historical control data from 19 studies with the American Dutch rabbit on the skeletal abnormalities flagged in Table VI of the original report. Evaluation of these data showed that the historical incidence of the skeletal abnormalities listed in Table 6 of the review was adequate to explain the incidence of these abnormalities, with the exception of the irregular spinous process, which fell outside the historical control range (0-5 fetal incidence).

- 3) necropsy findings on does not listed in Appendix D

These data were requested in order to properly evaluate potential maternal toxicity of test article administration. The registrant supplied necropsy data for those does not listed in Appendix D of the original report. Evaluation of these data did not alter the original conclusions of test article toxicity to maternal rabbits in this study, as the additional data submitted were for does in which no findings were present at necropsy.

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4) results of histological examination of maternal tissues, if any.

These data were requested in order that maternal toxicity might be more carefully evaluated. However, the registrant stated that these data were not generated in this study. As this is not a required aspect for a developmental toxicity study, this response is considered adequate.

5) times and dates of sacrifice for all maternal rabbits

These data were requested as a significant difference in mean fetal body weight was observed between fetuses of control rabbits and those in the 30 mg/kg/day dose group which could have been due to differences in sacrifice times for maternal rabbits or differences in insemination times, i.e. differences in gestation time. However, data submitted by the registrant showed that the times of insemination and sacrifice were equivalent between dose groups of maternal rabbits. In addition, it was stated on page 10 of the review that the lower mean fetal body weight observed in the 30 mg/kg/day dose group as compared to control was within historical control range as supplied by the registrant.

Based on the additional information supplied by the registrant in support of the developmental toxicity study with SENCOR technical, this study is upgraded from core supplementary to core minimum data. In addition, the Developmental toxicity NOEL and LEL are stated as follows:

Developmental toxicity NOEL= 30 mg/kg/day

Developmental toxicity LEL= 85 mg/kg/day (increase in irregular spinous process)

TABLE 6
Developmental Toxicity of SENCOR Technical: Skeletal Examination^a

Dose group (mg/kg/day)	<u>0</u>	<u>10</u>	<u>30</u>	<u>85</u>
Observations^a				
#pups(litters) examined	85 (15)	84 (14)	86 (14)	77 (14)
skull:				
incomplete ossification	17(10)	31 ^b (11)	38 ^c (11)	16(7)
enlarged fontanelle	13(10)	30 ^c (11)	36 ^c (11)	16(7)
sternbrae				
unossified 5th sternebra	6(3)	5(2)	19 ^b (8)	6(5)
scapula				
irregular spinous process	- (0)	5(3)	1(1)	6 ^b (5)
pubis				
incomplete ossification	8(5)	10(7)	27 ^c (6)	3(2)
appendages				
posterior-IO Talus	3(3)	9(4)	23 ^c (6)	6(2)

^a Data are taken from Table VI, pages 23-24 of registrant report., and represent number of fetuses affected. ()=litter incidence

^bsignificantly different vs control by pair-wise comparison (p < 0.05).

^csignificantly different vs control by pair-wise comparison (p < 0.01)



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CASWELL FILE

~~REVIEW~~

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SUBSTANCES

MEMORANDUM

SUBJECT: 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one (SENCOR Technical): Review of a Rabbit Teratology Study Submitted by the Registrant.

Caswell No: 33D
HED Project No: 9-2295
MRID No: 412492-01

FROM: Timothy F. McMahon, Ph.D., Toxicologist *Timothy F. McMahon 1/24/91*
Review Section I, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

TO: PM Team 74
Registration Division (H7505C)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head *Y. Ioannou 1/22/91*
Review Section I, Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

and

Marcia Van Gemert, Ph.D., Branch Chief *M. Van Gemert 1/29/91*
Toxicology Branch II (HFAS)
Health Effects Division (H7509C)

Registrant: Mobay Corporation

Action Requested: Review of the following Toxicology study with 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one (SENCOR Technical), required for California registration:

Rabbit Teratology Study

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Conclusions:

Administration of SENCOR Technical to pregnant female American Dutch rabbits resulted in maternal toxicity at 30 and 85 mg/kg/day, as evidenced by decreased body weight gain at the 30 mg/kg/day dose level on gestation days 18-28, and by decreased body weight gain, food consumption, and food efficiency on gestation days 7-19 at the 85 mg/kg/day dose level. Evidence was presented suggesting developmental toxicity at 10 and 30 mg/kg/day in the form of skeletal abnormalities, but was insufficient for proper interpretation.

Classification:

core supplementary

This study does not satisfy the guideline requirements (83-3) for a teratology study in rabbits.

Reviewed by: Timothy F. McMahon, Ph.D. *J.C.M. 11/24/91*

Section I, Toxicology Branch II (HFAS) (H7509C)

Secondary Reviewer: Stephen C. Dapson, Ph.D. *Stephen C. Dapson 1/24/91*

Section I, Toxicology Branch II (HFAS) (H7509C)

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Data Evaluation Report

Study type: Developmental Toxicity- Teratology
Species: rabbit
Guideline: 83-3

EPA ID Numbers: MRID number: 412492-01
EPA ID No: 101101-4
EPA Record No: 253329
Caswell No: 33D
HED Project No: 9-2295

Test material: 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one

Synonyms: SENCOR technical; Metribuzin

Study number(s): MTD 0100

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

Sponsor: Mobay Corporation, Kansas City, Missouri

Title of report: Teratology Study in the Rabbit with SENCOR Technical (Metribuzin)

Author(s): G.R. Clemens and R.E. Hartnagel Jr.

Study Completed: August 2, 1989

Conclusions: Administration of SENCOR technical to pregnant female American Dutch rabbits resulted in maternal toxicity at 30 and 85 mg/kg/day. Evidence was presented suggesting developmental toxicity at 10 and 30 mg/kg/day in the form of skeletal abnormalities, but was insufficient for proper interpretation.

Maternal NOEL= 10 mg/kg/day

Maternal LOEL= 30 mg/kg/day (decreased body weight gain on days 18-28).

Developmental toxicity NOEL and LEL could not be determined due to a lack of information

IV. CLASSIFICATION Core supplementary

This study does not satisfy the guideline requirements (83-3) for a teratogenicity study in rabbits. The following materials are requested in order to upgrade the study to core minimum:

litter incidence data for fetal skeletal abnormalities listed in Table VI of report

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historical control data for the skeletal abnormalities listed in Table VI of report

necropsy findings on does not listed in Appendix D

results of histological examination of maternal tissues, if any

times and dates of sacrifice for all maternal rabbits

I. MATERIALS, METHODS, AND RESULTS

A. Test Material: 4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one
purity: 92.7% a.i. (information supplied to Miles Inc. by Mobay Corporation)
description: white crystalline solid
batch #: 77-297-50

B. Vehicle: aqueous carboxymethylcellulose, composed of:

0.5% w/v carboxymethylcellulose (CMC)
0.4% w/v Tween 80
distilled water

C. Compound Stability and Homogeneity: Stability and homogeneity data on SENCOR technical dosing solutions were provided by the registrant as summary data in Appendix "B" (pages 44-48 of primary study). For homogeneity analysis, one ml aliquots of each batch were taken from the top, middle, and bottom of each batch suspension immediately after batch preparation, diluted appropriately with acetonitrile, and analyzed by HPLC. Results of homogeneity analysis are summarized in Table 1:

TABLE 1
Homogeneity of SENCOR Technical Dose Solutions^a

		<u>Desired Concentration (mg/ml)</u>			
Batch # 8831-1					
	<u>0</u>	<u>2.0</u>	<u>6.0</u>	<u>17.0</u>	
Found conc. (mg/ml)					
Top	0.0	2.1	6.3	17.8	
Middle	-	2.0	6.1	18.4	
Bottom	-	2.3	6.3	18.3	

TABLE 1 (cont.)

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Mean	NA	2.18±0.16 (109)	6.30±0.10 (105)	18.2±0.28 (107)
range:	-	(100-115%)	(101-105%)	(104-108%)

Batch #
8831-2Desired Concentration (mg/ml)

	<u>0</u>	<u>2.0</u>	<u>6.0</u>	<u>17.0</u>
Found conc. (mg/ml)				
Top	0.0	2.1	6.3	16.8
Middle	-	1.9	6.1	17.5
Bottom	-	2.1	6.3	17.2

Mean	NA	2.06±0.07 (103)	6.28±0.11 (104)	17.1±0.34 (100)
range:	-	(99-105%)	(101-105%)	(98-102%)

^aData taken from report Appendix B, page 44 of registrant report. Numbers in () indicate percent mean nominal concentration.

As shown in Table 1, the concentration of test material in batch suspensions was in the overall range of 98-115% of nominal for all concentrations tested. Concentration of test material in samples from top, middle, and bottom did not differ from one another by > 10%, with the exception of the 2.0 mg/ml batch suspension #8831-1, where one sample from the middle (2.0 mg/ml) differed from the bottom sample (2.3 mg/ml) by 13%. However, this difference is not considered significant, as the mean concentration in this batch is within 10% of nominal.

For stability analysis, test suspensions of 0.2% and 1.7% were prepared and stored covered at a mean temperature of 45 °C. On days of analysis (days 0, 10, 21, and 28 of the study), one ml aliquots were removed after allowing suspensions to reach room temperature and stirring for 20 minutes. On non-analysis days, suspensions were removed from storage for 30 minutes, stirred for 20 minutes, and then returned to storage.

At a concentration of 2.0 mg/ml (0.2%), concentration of test suspension ranged from 2.0-2.1 mg/ml over the 28 day test period. At a concentration of 17.0 mg/ml (1.7%), concentration of test suspension ranged from 17.4-18.6% over the 28 day test period. These results demonstrate the stability of test material in the dosing solution over a 28 day period (Appendix B, pages 47-48 of registrant report).

D. Test Animals:

Species: American Dutch Rabbit, male and female (nulliparous)
Source: Langshaw Farms, Augusta, MI
Age: males, approximately 4.5 months prior to breeding; females, approximately 4.5 months prior to randomization.
Weight: males, 2.4-3.5 kg; females, 2.2-2.9 kg

E. Animal Husbandry:

A total of 16 male and 68 female rabbits were used in this study. Rabbits were housed individually in stainless steel cages in the same climate controlled room (page 10 of registrant report), and were individually identified by metal ear tag. Food (Certified Laboratory Rabbit Chow #5322, Ralston Purina Co.) and tap water were available ad libitum. Food was provided as approximately 130 g/ rabbit/day until study initiation, when exactly 130 g of food was given to each rabbit. Rabbits were acclimated at least 28 days prior to study initiation. No significant deviations were reported in environmental conditions during the study.

F. Experimental Design and Dosing:

SENCOR Technical was administered as a suspension in 0.5% CMC vehicle by gavage to female rabbits on gestation days 6 through 18 inclusive in order to assess developmental toxicity of this chemical. Prior to dose selection for the main study, a preliminary range finding study was conducted to determine a dose range for the main study.

In the preliminary range-finding study (page 102 of registrant report), doses of 0, 25, 65, 105, 145, and 185 mg/kg were administered to groups of 3 artificially inseminated does from gestation days 6 through 18. Doses of 105 mg/kg and higher resulted in significant maternal mortality and adverse reproductive effects. A slight loss in body weight gain was observed at 65 mg/kg in all treated rabbits, but no other toxicity was reported at this dose. Thus, doses below 105 mg/kg were selected for use in the main study.

Based on the results of the range finding study, doses of 0, 10, 30, and 85 mg/kg were recommended for the main teratology study. Control rabbits in the main study received 0.5% CMC vehicle. Information on assignment of rabbits to treatment groups was not provided. Dose volume was 5ml/kg. Dose volumes were based upon body weight obtained on day 6 of gestation.

Doses and numbers of rabbits tested at each dose level are as follows:

0 mg/kg/day:	17 rabbits
10 mg/kg/day:	17 rabbits
30 mg/kg/day:	17 rabbits
85 mg/kg/day:	17 rabbits

G. Mating

Four groups of 17 female rabbits (previously primed by intravenous injection of approximately 50 I.U. HCG) were artificially inseminated using semen (diluted in 0.9% saline) collected from proven bucks. A second intravenous injection of 100 I.U. HCG was given concomitantly at insemination.

H. Statistical Analysis:

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A copy of the statistical tests used in this study and the purposes for which they were employed is appended to this report.

I. Compliance:

A signed statement of Compliance with Good Laboratory Practice Standards was provided.

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

A signed statement of Quality Assurance was provided.

A signed statement of Flagging of Studies for Potential Adverse Effects was provided.

J. Observations:**1. Maternal Toxicity****a. Mortality**

All animals were observed daily for signs of mortality.

During the dosing period, one rabbit each from the 10, 30, and 85 mg/kg dose groups died on days 27, 17, and 16, respectively. The deaths did not appear to be related to treatment with test article, but were apparently the result of either dosing trauma or systemic infection.

b. Clinical Toxicity

Animals were observed daily for signs of clinical toxicity.

A dose-related increase in the number of rabbits with stool changes (soft stool and/or reduced quantity) was observed during treatment with test article. No other clinical signs were reported in any dose group for the duration of the study. The apparent effect of test article on stool changes does not appear to be toxicologically significant.

c. Body Weight:

Body weights were recorded on day 0 of gestation (day of insemination), and on days 6, 10, 14, 18, 21, and 28 of gestation. Group mean body weights, group mean body weight gain, and individual body weight data were provided. Group mean body weight gain is shown in Table 2.

TABLE 2
Group Mean Body Weight Gains (kg) in SENCOR Technical-Treated Rabbits^a

Study Interval (days)	Dose groups (mg/kg/day)			
	control	10	30	85
0-6	0.08	0.04	0.07	0.08
6-18	0.19	0.16	0.18	0.08*
18-28	0.15	0.11	0.05 ^b	0.15
0-28	0.42	0.31 ^c	0.33	0.31 ^c

* = $p < 0.05$ vs. control by Dunnett's test. Animals which were non-pregnant were excluded from analysis.

^aData from Table 1, page 19 of registrant report.

^brecalculated from Appendix C, pages 50 and 52

^c $p < 0.05$ vs control by Student's t-test

The only treatment related effect on maternal body weight gain reported by the registrant appeared to be in rabbits in the 85 mg/kg/day dose group, where weight gain was significantly decreased relative to control during the dosing period (days 6-18). This was apparently the result of a significant difference in group mean body weight between control and high dose rabbits (3.07 vs 2.89 kg, respectively) on day 18 of the study.

According to data presented in Table 1, body weight gain over the entire gestation period did not appear to be significantly different between dose groups. However, analysis of body weight gain data between the control and the 10 and 85 mg/kg/day dose groups showed a significant ($p < 0.05$) difference in body weight gain between these groups over the entire dosing period (comparison of mean body weight gain of 0.42 kg in control vs 0.31 in the 10 and 85 mg/kg/day dose groups). The significant difference in overall weight gain between the high dose group and control is the result of the difference in weight gain between days 6-18 in these groups, while weight gain in the 10 mg/kg/day dose group was decreased vs control over the entire study period.

The post dosing weight gain for the 30 mg/kg/day dose group, while decreased from control, was not significant, due to variability of weight gain and loss within this group during this time period.

d. Food consumption

Food consumption was monitored on days 1, 6, 7, 12, 15, 19, 23, and 28. When food consumption was monitored, each rabbit received exactly 130g of food. Data on group mean food consumption

and individual food consumption were provided by the registrant. Food consumption data are summarized in the following Table (Table 3):

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TABLE 3
Food Consumption (grams) in SENCOR Technical-Treated Rabbits^a

Study day	Dose Group (mg/kg/day)			
	control	10	30	85
1	126.9	123.4	121.1	117.2
6	128.1	123.4	123.1	124.6
7	127.9	122.9	116.9	79.3*
12	127.3	111.9	123.8	99.3*
15	127.6	91.3	113.8	62.3*
19	127.4	120.4	124.4	111.1*
23	126.5	100.3*	109.6	114.9
28	112.8	99.8	86.4	108.1

^adata taken from Table II, page 19 of registrant report. *p < 0.05 vs control (Dunnett's test).

As shown in Table 3, rabbits treated with 85 mg/kg/day SENCOR technical consumed significantly less food during the period of treatment, as shown by the values for food consumption on days 7 through 19. This observation concurs with the earlier mentioned observation of a decreased body weight gain during this period in the high dose group. As test article was given by oral gavage, the results of food consumption in the high dose group indicate a decreased desire to consume food following dosing. However, no clinical toxicity was reported in this dose group.

A significant decrease in food consumption was also reported for the rabbits in the 10 mg/kg/day dose group on day 23 of the study. However, this decrease appears to be the result of variability in food consumption, as well as one rabbit that was apparently sick during the latter phase of the study and died on day 27.

e. Gross Pathology

Any rabbits which died, appeared moribund or showed signs of early termination of pregnancy were submitted for complete necropsy. On day 28 of gestation, all surviving does were terminated by intravenous barbiturate overdose. The abdomen was opened, ovaries were excised, corpora lutea counted, and pregnancy determined. The uterus was removed intact and weighed. The uterus was then opened, fetuses and resorptions removed, and each implant noted. Abdominal and thoracic viscera were examined in maternal rabbits and any gross anatomical changes recorded. Tissues and/or organs showing signs of gross pathology were removed and fixed in 10% modified Millonigs buffered formalin for histologic evaluation, if necessary. Maternal organ weights were not provided.

i) Gross Observations

Necropsy findings on individual female rabbits were provided in Appendix D, page 61 of the registrant's report. There were no apparent test article related gross pathological changes in maternal rabbits at any dose level. Gross pathological changes that were observed were related primarily to those rabbits which died during the study, and included purulent peritonitis (1 rabbit from the 10 mg/kg/day dose group), dosing trauma of the lungs (1 rabbit from the 30 mg/kg/day dose group), and hemorrhagic enteritis (1 rabbit from the 85 mg/kg/day dose group). One additional rabbit from the 85 mg/kg/day dose group aborted on day 25 and was found to have pneumonia.

Note: On page 14 of the registrant's report, the following statement regarding gross pathological changes in treated rabbits is made: "These changes, along with others also considered spontaneous in nature and commonly observed in this species, were observed in all groups *including control...*" (italics added). There is no evidence presented in this report that control rabbits displayed similar gross pathologic changes as treated rabbits. Infections seem to be associated only with treated rabbits.

ii) Histopathologic Observations

No histological data were provided on maternal tissues in this report.

iii) Organ Weights

No data on maternal organ weights were provided in this report.

iv) Cesarean Section Observations

Table 4: Cesarean Section Observations^a

<u>Dose (mg/kg/day):</u>	<u>0</u>	<u>10</u>	<u>30</u>	<u>85</u>
#Animals Assigned	17	17	17	17
#Animals Mated/ Inseminated	17	17	17	17
Pregnancy Rate (%)	88	88	88	94
Maternal Wastage				
#Died	0	1	1	1
#Died/pregnant	0	1	1	1

Table 4 (cont.)

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<u>Dose (mg/kg/day):</u>	<u>0</u>	<u>10</u>	<u>30</u>	<u>85</u>
#Non pregnant	2	2	2	1
#Aborted	0	0	0	1
#Premature Delivery	0	0	0	0
Whole Litter Resorptions	0	0	0	0
Total # of litters	15	14	14	14
Total Corpora Lutea	94	101	97	94
Corpora Lutea/dam	6.3	7.2	6.4	5.8
Total Implantations ^b	88	91	90	81
Implantations/Dam	5.9	6.5	6.4	5.8
Total Live Fetuses	85	84	86	77
Live Fetuses/Dam	5.7	6.0	6.1	5.5
Total Resorptions	3	7	4	4
Early	[data on early and late resorptions not provided]			
Late				
Resorptions/Dam	0.2	0.5	0.3	0.3
Total Dead Fetuses	[no dead fetuses were reported]			
Dead Fetuses/Dam				
Mean Fetal Weight (gm)	39.9	38.6	35.0 ^c	39.4
% Preimplantation Loss (mean)	11.5	15.7	14.4	7.3
% Postimplantation Loss (mean)	3.6	9.9	4.1	4.6

Table 4 (cont.)

Sex Ratio (mean M/F)	2.8/2.8	3.0/2.9	2.9/3.2	2.5/3.0
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^aData taken from Table III, page 20 of report and from Appendix E, page 62 of report.

^bImplantations from rabbits that died or aborted were excluded from the total.

^csignificantly different from control ($p < 0.01$) using Healy's test.

There were no apparent treatment related alterations in any of the above measured parameters suggestive of maternal toxicity. Possible developmental toxicity was evident as shown by the 11% decrease in mean fetal body weight at the 30 mg/kg/day dose level. However, this value falls within the range of historical control data on fetal body weight (page 108 of registrant report; data provided from 19 studies).

2. Developmental Toxicity

Each fetus was removed from its amniotic sac, the umbilical cord severed close to its attachment to the fetus, and viability of the fetus determined. Each fetus was then blotted dry, weighed, and subjected to a complete external examination. Following external examination, fetuses were individually identified and subjected to a complete internal examination of the abdominal and thoracic viscera, using a stereomicroscope. Skin was removed from the head for purposes of examining the eyes, and a transverse section was made through the skull posterior to the coronal suture through the cerebrum.

Following internal examination, fetuses were eviscerated and fixed in 70% ethanol. Fetuses were skinned and processed for skeletal examination using the KOH Alizarin Red-S method.

TABLE 5
Developmental Toxicity of SENCOR Technical^a

Dose group (mg/kg/day)	0	10	30	85
<u>Observations^a</u>				
#pups(litters) examined	85 (15)	84 (14)	86 (14)	77 (14)
#pups(litters) affected (includes external, visceral and skeletal alterations)	3 (3)	2 (2)	2 (2)	3 (3)

^a Data taken from Table VIII, page 25 of registrant report.

^a Data taken from Table VIII, page 25 of registrant report.

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a. External Malformations

Nonspecific external changes unrelated to treatment were observed in all fetuses with external abnormalities, including control fetuses. These included umbilical hernia (1 control fetus), and missing phalanges (2 fetuses from 85 mg/kg/day dose group)

b. Visceral Malformations

No treatment related visceral abnormalities were observed in any fetus from any dose group. Dilated brain ventricles were noted in 1 fetus from the 10 mg/kg/day dose group, as was a displaced vena cava in another fetus from a different litter in the same dose group.

c. Skeletal Malformations

Note: Data on the litter incidence of specific skeletal abnormalities were not provided.

TABLE 6
Developmental Toxicity of SENCOR Technical: Skeletal Examination^a

Dose group (mg/kg/day)	<u>0</u>	<u>10</u>	<u>30</u>	<u>85</u>
<u>Observations^a</u>				
#pups(litters) examined	85 (15)	84 (14)	86 (14)	77 (14)
skull:				
incomplete ossification	17 ¹²	31 ^{b 11}	38 ^{c 11}	16 ⁷
enlarged fontanelle	13 ¹⁰	30 ^{c 11}	36 ^c	16 ⁷
sternbrae				
unossified	6 ³	5 ²	19 ^{b 8}	6 ⁵
5th sternbra				
scapula				
irregular spinous				
process	0	5 ³	1 ¹	6 ^{b 5}

for litter incidence

Table 6 (cont.)

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Dose group (mg/kg/day)	<u>0</u>	<u>10</u>	<u>30</u>	<u>85</u>
pubis				
incomplete ossification	8 ³	10 ¹	27 ^c	3
appendages				
posterior-IG Talus	3 ²	9 ⁴	23 ^c	6

^a Data are taken from Table VI, pages 23-24 of registrant report., and represent number of fetuses affected

^b significantly different vs control by pair-wise comparison ($p < 0.05$).

^c significantly different vs control by pair-wise comparison ($p < 0.01$)

Statistically significant increases in delayed ossification of the bones of the skull were observed in fetuses from rabbits at the 10 and 30 mg/kg/day dose level. The percent incidence rose from 20% in concurrent controls to 37 and 44% fetal incidence in the 10 and 30 mg/kg/day dose groups, respectively. This anomaly in skeletal development may be related to the increased incidence of enlarged fontanelles observed in these same dose groups, which increased from a fetal incidence of 15% in concurrent controls to 36 and 42% in the 10 and 30 mg/kg/day dose groups, respectively.

An increased incidence in incomplete ossification of the pubis as well as an unossified 5th sternebra was also observed in fetuses from SENCOR technical treated rabbits. Incomplete ossification of the pubis was significantly different from control at the 30 mg/kg/day dose level ($p < 0.01$), and the fetal incidence was 9% and 31% in concurrent controls and the 30 mg/kg/day dose group, respectively. A statistically significant increase in the incidence of unossified 5th sternebrae was also observed only in the 30 mg/kg/day dose group ($p < 0.05$). Fetal incidence of this anomaly was increased from 7% in concurrent controls to 22% in the 30 mg/kg/day dose group.

The only skeletal anomaly observed in the 85 mg/kg/day dose group which reached statistical significance was the finding of irregular spinous process at the scapular level. This anomaly was not observed to be significant at the lower dose levels.

It should be noted that on page 17 of the registrant's report, the statement is made that values for skeletal variations in the dose groups used in this study were not "outside this laboratory's historical control range and are, therefore, considered incidental." However, examination of historical control data for skeletal malformations (Appendix I, page 114 of report) shows no historical control data in support of the types of skeletal variations indicated in Table VI, pages 23-24 of the report.

II. DISCUSSION

In the present study, the developmental toxicity of SENCOR technical was assessed by oral administration of the chemical at doses of 0, 10, 30, and 85 mg/kg/day to pregnant female American Dutch Rabbits on days 6-18 of gestation inclusive. These doses were selected based upon the findings of a range-finding study conducted with SENCOR technical in this same strain of rabbit. Daily observations were made for maternal toxicity of SENCOR technical, while body weights were recorded on days 0, 6, 10, 14, 18, 21, and 28 of gestation. On day 28 of gestation, surviving rabbits were killed by intravenous barbiturate overdose and were subjected to cesarean section to assess developmental toxicity of SENCOR technical.

Minor mortality was observed in pregnant female rabbits during the study period, but was related to dosing trauma and/or infection, and not to test article administration. This is supported by the observation of no overt clinical toxicity in any rabbit at any dose level during the course of the study, with the exception of stool changes as a dose-related effect. The finding of stool changes, while dose-related, did not result in an increase in mortality with dose. This clinical effect may possibly be related to an effect on the gastrointestinal tract and/or autonomic nervous system which was not evident under the conditions of this study.

Changes in body weight gain of dosed rabbits were evident at various times during the study at all dose levels used in this study. In the 10 mg/kg/day dose group, body weight gain appeared to be depressed vs control from beginning to end of the study period. The cause of this decrement could not be determined, as there was no indication of any significant test article related toxicity in maternal or fetal rabbits at this dose. At the 30 mg/kg/day dose level, a decrease in body weight gain occurred from days 18-28, the period following dosing and prior to termination. The occurrence of this change in body weight gain during the post-dosing period would suggest some type of maternal toxicity from test article administration, although no apparent changes were observed in food consumption or clinical toxicity of maternal rabbits at this dose.

Maternal toxicity of SENCOR technical was most evident at the 85 mg/kg/day dose level. Significant reduction in maternal body weight gain occurred during the period of test article administration (days 7-19). In addition, decreased food consumption and food efficiency was also observed during this period. No other obvious signs of maternal toxicity were observed at this dose level.

Gross pathologic findings at necropsy were unremarkable in female rabbits which were examined, and did not appear test article related. However, necropsy observations were not provided for all rabbits dosed in this study (page 61 of registrant report). Furthermore, no statement regarding results of any histological examination was made. Organ weights from dosed rabbits were not provided. This dearth of information is considered a hindrance to proper evaluation of test article effects on maternal organ histology.

Most observations made at cesarean section were not significant between the control and dosed rabbits. However, a significant decrease in mean fetal body weight was observed at the 30 mg/kg/day dose level. At this dose, skeletal variations which were significantly increased in incidence from control were also observed. According to the registrant, the delay in ossification of the skeletal elements observed at this dose could be due to the decreased mean fetal body weight, i.e. delayed development. Significant increases in fetal skeletal anomalies were also observed at the 10 mg/kg/day dose level. The possibility that these increases might be due to test article administration is not supported by data from fetuses in the 85 mg/kg/day dose group, which do not follow this trend, but resemble control values. Thus, the possibility exists that the differences

observed in skeletal anomalies among fetuses from the various dose groups is due to differences in the times of sacrifice of maternal rabbits in these groups.

While evidence was provided for maternal toxicity of SENCOR technical at the 30 and 85 mg/kg/day dose levels, the fetal toxicity and teratogenicity of this compound could not be adequately evaluated in the absence of litter incidence data for fetal skeletal anomalies, times of sacrifice of the maternal rabbits in each dose group, and proper historical control data.

III. CONCLUSIONS

Administration of SENCOR technical to pregnant female American Dutch rabbits resulted in maternal toxicity at 30 and 85 mg/kg/day. Evidence was presented suggesting developmental toxicity at 10 and 30 mg/kg/day in the form of skeletal abnormalities, but was insufficient for proper interpretation.

Maternal NOEL = 10 mg/kg/day

Maternal LOEL = 30 mg/kg/day (decreased body weight gain on days 18-28).

Developmental toxicity NOEL and LE_L could not be properly ascertained due to a lack of information

IV. CLASSIFICATION Core supplementary

This study does not satisfy the guideline requirements (83-3) for a teratogenicity study in rabbits. The following materials are requested in order to upgrade the study to core minimum:

litter incidence data for fetal skeletal abnormalities listed in Table VI of report

historical control data for the skeletal abnormalities listed in Table VI of report

necropsy findings on does not listed in Appendix D

results of histological examination of maternal tissues, if any

times of sacrifice for all maternal rabbits

<u>Variable</u>	<u>Statistical Method Used to Compare Sencor Treated Groups to the Control Group</u>
1. Doe Body WWeight - weights on Days 0, 6, 10, 14 18, 21, and 28, % weight gained (6-18 and 0-28), actual weight, and % actual weight gained	Dunnnett's test
2. Food Consumption - food consumed on Days 1, 6, 7, 12, 15, 19, 23, and 28	Dunnnett's test
3. Doe Reproductive Parameters - fertility index - gestation index - % viable fetuses - % non-viable fetuses - litter size - number of resorption sites - number viable fetuses - number corpora lutea - % male fetuses - number implantations - pre-implantation loss - post-implantation loss - average placental weight	Fisher's exact test Kruskal-Wallis & Dunn's tests
4. Fetal Weight Analysis - average combined fetal weights - average male fetal weight - average female fetal weight	Healy's test
5. Fetal Skeletal Analysis - all fetal skeletal structures with any changes were compared - fetal and litter incidence of malformation and select variations	Chi-square test, Fisher's exact test, Pair-wise Fisher's exact test

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DATA EVALUATION REPORT

METRIBUZIN

Study Type: Mutagenicity: In Vitro Chromosome Aberrations in
Chinese Hamster Ovary (CHO) Cells

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
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Prepared by:

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QA/QC Manager Sharon A. Segal Date 5/7/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-56
Clement Number: 91-175
Project Officer: James Scott

GUIDELINE SERIES 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE CYTOGENETICS

MUTAGENICITY STUDIES

009520

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Signature: Stephen C. Dapson
Date: 5/13/92
Signature: Y. Ioannou
Date: 5/18/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro chromosome aberration in Chinese hamster ovary (CHO) cells.

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 033D

MRID Number: 425551-02

TEST MATERIAL: Sencor

SYNONYMS: Metribuzin

SPONSOR: Mobay Corporation, Stilwell, Kansas

STUDY NUMBER: 10857-0-437

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD

TITLE OF REPORT: Mutagenicity Test on Sencor Technical in an In Vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells

AUTHOR: Murli, H.

REPORT ISSUED: March 30, 1990

CONCLUSIONS--EXECUTIVE SUMMARY: Five nonactivated and four S9-activated doses of Sencor were assayed for the potential to cause structural chromosome aberrations in Chinese hamster ovary (CHO) cells. Severe cytotoxicity was observed at doses ≥ 1750 $\mu\text{g/mL}$ -S9 and ≥ 584 $\mu\text{g/mL}$ +S9 activation. Less severe cytotoxicity was observed at 58.4-584 $\mu\text{g/mL}$ -S9 and 58.4-175 $\mu\text{g/mL}$ +S9. Cell-cycle delay occurred at nonactivated levels ≥ 175 $\mu\text{g/mL}$ and S9-activated levels ≥ 58.4 $\mu\text{g/mL}$. Accordingly, a 20-hour harvest was used to evaluate cultures exposed to doses ranging from 199 to 598 $\mu\text{g/mL}$ -S9 and 50.1 to 150 $\mu\text{g/mL}$ +S9.

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A normal 10-hour harvest was also used for cultures treated with 37.5 and 50 µg/mL S9-activated Sencor.

No statistically significant or dose-related increases in aberration frequencies were observed with nonactivated Sencor. A ring chromosome was seen, however, at 299 µg/mL -S9. While dicentrics were observed at 199, 299, and 399 µg/mL, their biological significance was minimized by the finding of comparable frequencies of dicentrics in the negative and solvent control cultures.

In the presence of S9 activation, a significant increase in the aberration frequency was observed with 150 µg/mL of the test material (20-hour harvest). The most common types of scored aberrations were chromosome and chromatid breaks, triradials, and quadriradials. Although statistically significant clastogenic effects were not observed at lower S9-activated levels, rare complex aberrations were noted: two triradials at 100 µg/mL (20-hour harvest) and one quadriradial at 50.0 µg/mL (10-hour harvest). We, therefore, conclude that Sencor required exogenous metabolic activation to induce a clastogenic effect in this cell line. This study satisfies Guideline requirements for genetic effects, Category II, Structural Chromosomal Aberrations.

STUDY CLASSIFICATION: The study is acceptable; S9-activated Sencor is clastogenic in this test system.

A. MATERIALS:

1. Test Material: Sencor technical

Description: White powder

Identification Number: Not listed

Purity: 93.0% active ingredient (a.i.)

Receipt date: May 8, 1989

Stability: Not reported

Contaminants: None listed

Solvent used: Ethanol

Other provided information: The test material was stored in the freezer. Dose solutions were prepared immediately before use. Analytical determination was performed on the stock solution for the highest and lowest doses used with S9 activation, and on the stock solution for the highest nonactivated dose.

2. Control Materials:

Negative: Untreated cells grown in McCoy's 5a culture medium supplemented with 10% fetal calf serum (FCS), 1% L-glutamine, and antibiotics.

Solvent/concentration: Ethanol/10 µL/mL

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Positive:

- (a) Nonactivation (concentrations, solvent): Mitomycin C (Mit C) was prepared in water to yield final concentrations of 0.25 and 0.50 $\mu\text{g/mL}$ for the range-finding assay; only the 0.25- $\mu\text{g/mL}$ dose was analyzed. Mit C was used at 0.04 and 0.08 $\mu\text{g/mL}$ for the chromosomal aberrations assay; only the latter dose was analyzed.
- (b) Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in water to yield final concentrations of 12.5 and 20 $\mu\text{g/mL}$ for the range-finding assay; only the 20- $\mu\text{g/mL}$ dose was analyzed. For the 10-hour harvest, CP was used at 25.0 and 50.0 $\mu\text{g/mL}$ and analyzed at 50 $\mu\text{g/mL}$. CP was used at 10.0 and 12.5 $\mu\text{g/mL}$ for the 20-hour harvest, and analyzed at 10.0 $\mu\text{g/mL}$.

3. Activation: S9 derived from male Sprague-Dawley

☒ Aroclor 1254	☒ induced	☒ rat	☒ liver
☐ phenobarbital	☐ noninduced	☐ mouse	☐ lung
☐ none		☐ hamster	☐ other
☐ other		☐ other	

The rat S9 liver homogenate was purchased from commercial suppliers and was characterized prior to use for its ability to metabolize selected promutagens to forms that cause sister chromatid exchanges (SCEs) in CHO cells.

S9 mix composition:

<u>Component</u>	<u>Amount/mL of Culture Medium</u>
Isocitric acid	2.7 mg (10.5 mM)
NADP (sodium salt)	1.5 mg (1.8 mM)
S9	15.0 μL

4. Test Compound Concentration Used:

- (a) Preliminary cytotoxicity assay: Half-log dilutions of ten doses ranging from 0.0584 to 1750 $\mu\text{g/mL}$ were assayed with and without S9 activation. One hundred metaphase cells from each of four nonactivated doses (17.5, 58.4, 175, and 584 $\mu\text{g/mL}$) and three S9-activated doses (17.5, 58.4, and 175 $\mu\text{g/mL}$) were evaluated for cell-cycle kinetics.
- (b) Cytogenetic assay:
- (1) Nonactivated conditions: Six doses (99.7, 199, 299, 399, 499, and 598 $\mu\text{g/mL}$) were assayed using a 20-hour harvest; cells exposed to the five highest doses were analyzed.

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- (2) S9-activated conditions: For the 10-hour harvest, three doses (25.0, 37.5, and 50.0 $\mu\text{g/mL}$) were assayed; cells exposed to 37.5 and 50.0 $\mu\text{g/mL}$ were analyzed. Cells were analyzed from all four doses (50.1, 100, 150, and 200 $\mu\text{g/mL}$) assayed for the 20-hour harvest.
5. Test Cells: Chinese hamster ovary (CHO-WBL) were obtained from Dr. S. Wolff, University of California, San Francisco. Prior to use, the CHO cells were grown for one day in McCoy's 5a medium supplemented with 10% FCS, 2 mM L-glutamine, and antibiotics.

Properly maintained? Yes.

Cell line or strain periodically checked for mycoplasma contamination? Yes.

Cell line or strain periodically check for karyotype stability? Yes.

B. TEST PERFORMANCE:

1. Cell Treatments:

- (a) Cells exposed to test compound for:
17.25 hours (nonactivated) 2 hours (activated)
- (b) Cells exposed to positive controls for:
17.25 hours (nonactivated) 2 hours (activated)
- (c) Cells exposed to negative and/or solvent controls for:
17.25 hours (nonactivated) 2 hours (activated)

2. Preliminary Cytotoxicity Assay:

Cell cultures, seeded at 0.3×10^6 cells/flask, were exposed to half-log dilutions of the test material ranging from 0.0584 to 1750 $\mu\text{g/mL}$, the solvent control (ethanol), the negative control (culture medium), or the positive controls (Mit C -S9, CP +S9).

In the nonactivated system, cells were exposed for about 2 hours to the test material; BrdU was added at a final concentration of 10 μM , and incubation continued for an additional 23 hours. Approximately 2.75 hours before the cell harvest, monolayers were washed and fed fresh complete medium containing BrdU and 0.1 $\mu\text{g/mL}$ Colcemid. In the S9-activated system, cultures were exposed for 2 hours without FCS. The cells were then washed, fed complete medium containing 10 μM BrdU, and incubated for 23 hours. Colcemid was added, and cultures were incubated for an additional 2.5 hours.

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After incubation, monolayers were visually evaluated for confluency and mitotic or dead cells. Cells were harvested by mitotic shakeoff, swollen in hypotonic 0.075 M KCl, fixed in absolute methanol:glacial acid (3:1), and stained using a modified fluorescence-plus Giemsa technique. One hundred metaphase cells per culture were examined for the percentage of first (M_1), between first and second (M_{1+}), and second or beyond second ($\geq M_2$) division metaphases. Based on these findings, doses and harvest times were selected for the cytogenetic assay.

3. Cytogenetic Assay:

- (a) Treatment: Duplicate cultures, seeded at 1.2×10^6 (+/-S9, 20-hour harvest) or 1.5×10^6 (+S9, 10-hour harvest), were exposed to the selected test material doses, the solvent control, or the positive controls (Mit C-S9; CP+S9).

In the nonactivated assay, cells were dosed for 17.25 hours, washed, fed complete medium containing 0.1 $\mu\text{g/mL}$ Colcemid, and incubated an additional 2.5 hours. Under S9-activated conditions, cells were exposed for 2 hours, washed, fed complete culture medium, and incubated for an additional 7.75 hours (10-hour harvest) or 17.75 hours (20-hour harvest). Colcemid was added 2.5 hours before the cultures were harvested.

Metaphase cells were collected and fixed as described for the preliminary cytotoxicity assay, and stained with pH 6.8 buffered 5% Giemsa. With the exception of the positive controls, slides were coded prior to analysis.

- (b) Metaphase analysis: If possible, one hundred morphologically normal cells (containing 19-23 centromeres) per test material, negative control, or solvent control culture were scored for chromosome aberrations. Twenty-five cells from one culture of each positive control chemical were scored. Chromatid and chromosome gaps were counted, but not included in the statistical analysis.
- (c) Statistical analysis: The data were evaluated for statistical significance at $p < 0.01$ by the Fisher's exact test with an adjustment for multiple comparisons. The negative and solvent controls were pooled if no statistical differences were found.
- (d) Evaluation criteria:
- (1) Assay validity: The assay was not considered valid if (1) the negative and solvent controls were higher than the upper limit ($\approx 5\%$ of cells with aberrations) of the historical control values; or (2) the positive control was not signifi-

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cantly ($p < 0.01$) higher than the pooled negative and solvent controls.

- (2) Positive result: The biological significance of the results was evaluated relative to the overall chromosome aberration frequencies, percentage of cells with aberrations, percentage of cells with >1 aberration, dose response, and the types of aberrations observed.

4. Protocol: See Appendix B.

C. REPORTED RESULTS:

1. Solubility: Solubility was evaluated in dimethyl sulfoxide (DMSO) and in ethanol. At concentrations ≥ 219 mg/mL, the test material was unevenly suspended in ethanol. Repeated pipetting produced clear solutions at 164 and 109 mg/mL. Sham dosing of these solutions at 1% into culture medium produced a white precipitate that coated the dilution tubes at 3280 and 2190 $\mu\text{g/mL}$; final concentrations of 1640 and 1090 $\mu\text{g/mL}$ in culture medium formed a white precipitate that went into solution.

Attempts to dissolve the test material in DMSO resulted in a grey viscous solution at 477 mg/mL, a clear blue solution at 358 mg/mL, and light blue solutions at 238 and 159 mg/mL. Sham dosing of these solutions at 1% into culture medium produced a globular oily precipitate at 4770 $\mu\text{g/mL}$, a white waxy precipitate at 3580 and 2380 $\mu\text{g/mL}$, and an initial precipitate that later dissolved at 1590 $\mu\text{g/mL}$. Since solubility was not improved with DMSO, and the color change observed with DMSO was of concern to the study author, ethanol was selected as the solvent.

2. Analytical Determination: Analytical determinations were performed by the sponsor on aliquots of the highest and lowest dosing solutions used for both harvests of the S9-activated assay. The highest dosing solution in the nonactivated portion of the study was also analyzed. No undissolved material was observed. The concentration of all dosing solutions ranged from 93.6 to 97.7% of their respective nominal values.
3. Preliminary Cytotoxicity Tests: The cytotoxicity assessment was conducted with ten test material levels ranging from 0.0584 to 1750 $\mu\text{g/mL}$ +/- S9. Severe cytotoxicity was observed at 1750 $\mu\text{g/mL}$ +/- S9. Marked cytotoxicity was also observed at the nonactivated dose of 584 $\mu\text{g/mL}$, as evidenced by severe cell-cycle delay (100% metaphase cells in M_1 , compared with 1% for the solvent control), floating dead cells, and reduced visible mitotic cells and monolayer confluence. Numerous dead cells and cell-cycle delay (35% M_1) were also observed at 175

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TABLE 1. Representative Results from the Preliminary Cytotoxicity Assay with Sencor Technical

Substance	Dose/mL	S9 Activation	Average % ^a Metaphases in			Monolayer Confluence % Solvent Control ^b
			M ₁	M ₁ +	≥M ₂	
<u>Negative Control</u>						
Untreated cells	--	-	--	10	90	100
	--	+	--	12	88	100
<u>Solvent Control</u>						
Ethanol	10 µL	-	1	11	88	100
	10 µL	+	1	6	93	100
<u>Positive Control</u>						
Mitomycin C	0.250 µg	-	99	1	--	100
Cyclophosphamide	20.0 µg	+	76	24	--	100
<u>Test Material</u>						
Sencor technical	17.5 µg ^c	-	--	18	82	100
	58.4 µg	-	--	29	71	100
	175 µg	-	35	57	8	100
	584 µg ^d	-	100	--	--	57
	17.5 µg ^c	+	--	25	75	100
	58.4 µg	+	1	58	41	86
	175 µg ^{d,e}	+	100	--	--	29
		-				

^aPercent cells at first (M₁), between first and second (M₁+), and at or beyond second (≥M₂) division; based on 100 metaphases per culture.

^bBased on visual observation.

^cCultures exposed to lower levels (0.0584, 0.175, 0.584, 1.75, and 5.84 µg/mL) with or without S9 activation were not evaluated.

^dHigher doses (1750 µg/mL -S9, 584 and 1750 µg/mL +S9) were severely cytotoxic.

^eCytotoxic dose; only 22 cells were available for analysis.

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µg/mL. There was no conclusive evidence of an adverse effect on cell cycle kinetics at 17.5 or 58.4 µg/mL (Table 1). S9-activated doses of 175 and 584 µg/mL caused marked reductions in monolayer confluence and in the number of mitotic cells; no metaphase cells were found at 584 µg/mL, and all of those found at 175 µg/mL were in M₁. Cell cycle delay and cytotoxic effects on the monolayer of cells were also apparent at 58.4 µg/mL. Based on these findings, the nonactivated cytogenetic assay was conducted with doses ranging from 99.7 to 598 µg/mL (20-hour harvest); the S9-activated phase of testing was performed with 25.0-50.0 µg/mL (10-hour harvest) and 50.1-200 µg/mL (20-hour harvest).

2. Cytogenetic Assays:

Nonactivated conditions: Severe cytotoxicity was observed at non-activated concentrations of 499 and 598 µg/mL; less than 200 cells were available for analysis from these slides. Floating dead cells were further observed at 399 µg/mL, and monolayer confluence was reduced ~15% at all tested levels ≤399 µg/mL. Although an elevated number of aberrations per cell and percent of cells with >1 aberration was scored at 598 µg/mL, the increases were not significant (Table 2). Dicentrics were observed at 199, 299, and 399 µg/mL (1 each at 199 and 399 µg/mL and 3 at 299 µg/mL); however, three dicentrics were also scored in the solvent and negative control cultures. The biological relevance of this finding in the test material cultures is, therefore, questionable. A ring chromosome was also found at 299 µg/mL.

S9-activated conditions: No cytotoxicity was observed at any dose harvested 10 hours posttreatment. Similarly, no significant increase in the percentage of cells with aberrations or the percentage of cells with >1 aberration was seen in the 37.5- and 50.0-µg/mL cultures from the 10-hour harvest (Table 3). Our reviewers noted, however, that single dicentrics were scored at both levels and a quadriradial was seen at 50.0 µg/mL. There also appeared to be a slight increase in simple aberrations at 50.0 µg/mL. As previously stated for the nonactivated test, the relevance of dicentric chromosomes at both doses could not be determined because they occurred at a similar frequency in the negative control cultures.

The initial 20-hour harvest was aborted due to ambiguous cytotoxicity data. No cells survived exposure to 200 µg/mL in the successfully completed 20-hour harvest with S9 activation. Severe cytotoxicity, as evidenced by floating dead cells, reduced monolayer confluence, and marked reductions in the number of mitotic cells, were observed at 150 µg/mL. Slight reductions in the number of mitotic cells and monolayer confluence were also reported at 100 µg/mL.

As shown in Table 3, a low frequency of simple and complex aberrations was scored in the untreated culture at the 20-hour harvest; none were

TABLE 2. Results of the Chinese Hamster Ovary (CHO) Cell In Vitro Cytogenetic Assay with Nonactivated Sencor Technical Following a 20-Hour Cell Harvest

Substance	Dose/mL	No. of Cells Scored	Harvest Time (Hours)	Total Number of Aberrations ^a	Cells with Aberrations ^a (%)	Cells with >1 Aberration ^a (%)	Aberrations per Cell	Biologically Significant Aberrations (No/Type)
Negative Control								
Untreated cells	--	100	20	1	1.0	0.0	0.01	1D
Solvent Control								
Ethanol	10 µL	100	20	3	3.0	0.0	0.03	1SB; 2D
Positive Control								
Mitomycin C	0.08 µg	25	20	9	24.0*	12.0*	0.36	5SB; 3TR; 1CI;
Test Material								
Sencor technical	199 µg	200	20	4	2.0	0.0	0.02	3SB; 1D
	299 µg	200	20	8	3.0	1.0	0.04	1TR; 3SB; 3D; 1R
	399 µg	200	20	3	1.5	0.0	0.02	1TR; 1SB; 1D
	499 µg ^b	150	20	3	2.0	0.0	0.02	2TR; 1SB
	598 µg ^b	39	20	4	5.1	2.6	0.10	4SB

^aExcluding gaps.

^bCytotoxic dose; all available cells were analyzed.

Abbreviations used:

TR = Chromatid break SB = Chromosome break D = Dicentric
 CI = Chromosome Interchange TR = Triradial
 R = Ring

*Significantly higher than the pooled negative and solvent controls (p<0.01) by Fisher's exact test.

TABLE 3. Results of the Chinese Hamster Ovary (CHO) Cell *In Vitro* Cytogenetic Assay with S9-Activated Sencor Technical Following a 10- and 20-Hour Cell Harvest

Substance	Dose/mL	No. of Cells Scored	Harvest Time (Hours)	Total Number of Aberrations ^a	Cells with Aberrations ^a (%)	Cells with >1 Aberration ^a (%)	Aberrations per Cell	Biologically Significant Aberrations (No/Type)
Negative Control								
Untreated cells	--	100	10	2	2.0	0.0	0.02	1SB; 1D
		100	20	6	3.0	2.0	0.06	17B; 2SB; 1QR; 1D; 1R
Solvent Control								
Ethanol	10 μ L	100	10	0	0.0	0.0	0.00	--
	10 μ L	100	20	0	0.0	0.0	0.00	--
Positive Control								
Cyclophosphamide	50 μ B	25	10	10	28.0*	8.0*	0.40	3SB; 5TR; 1QR; 1D
	10 μ B	25	20	30	76.0*	44.0*	>2.28	4TB; 10SB; 11D; 4TR; 5QR; 1QR; 2CI; 3OT
Test Material								
Sencor technical	37.5 μ B	200	10	3	1.5	0.0	0.02	1TB; 1SB; 1D
	50.0 μ B	200	10	5	2.5	0.0	0.03	2TB; 1SB; 1QR; 1D
	50.1 μ B	200	20	4	2.0	0.0	0.02	2TB; 1SB; 1D
	100 μ B	200	20	6	2.5	0.5	0.03	3TB; 1SB; 2TR
	150 μ B	150	20	100	40.0*	18.0*	>0.65	34TB; 17SB; 11D; 15TR; 19QR; 7CR; 1D; 3R; 3OT

^aExcluding gaps.

^bSlight cytotoxicity observed prior to harvest.

^cSeverely cytotoxic dose; no cells were available for analysis from the highest dose (200 μ B/mL) tested.

Abbreviations used:

TB = Chromatid break SB = Chromosome break D = Dicentric
 QR = Quadridial R = Ring TR = Triradial
 ID = Interstitial deletion CR = Complex rearrangement CI = Chromosome interchange
 OT = Greater than 10 aberrations; counted as one aberration

*Significantly higher than the pooled negative and solvent controls ($p < 0.01$) by Fisher's exact test.

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seen in the solvent control group. The occurrence of complex aberrations in the untreated culture should have prompted the termination of the assay. Nevertheless, significant chromosome damage ($p < 0.01$), compared to the pooled negative and solvent control cultures was obtained at the highest dose (150 $\mu\text{g/mL}$). At this concentration, the test material induced high yields of both simple (34 chromatid breaks, 17 chromosome breaks) and complex aberrations. Complex aberrations included 15 triradials, 19 quadriradials, and 7 complex rearrangements. A slight but not significant clastogenic effect was also seen at 100 $\mu\text{g/mL}$. The evidence of clastogenesis was further supported by the slight increase in simple aberrations and the occurrence of two triradials at this dose. Results for the low dose (50.1 $\mu\text{g/mL}$) were negative.

The study author concluded from the overall results that Sencor technical was clastogenic at the severely cytotoxic concentration of 150 $\mu\text{g/mL}$ +S9 activation.

- D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that Sencor was tested over an appropriate range of concentrations in the presence and absence of S9 activation. Although complex aberrations (dicentrics at 199, 299, and 399 $\mu\text{g/mL}$ and a ring chromosome at 299 $\mu\text{g/mL}$) were observed in the presence of nonactivated Sencor, there was no definitive evidence that the nonactivated test compound was clastogenic. The response was not dose-related and dicentrics were found at comparable or higher frequencies in the solvent and negative controls. In contrast, a marked and statistically significant increase in the number of aberrations per cell and the percent cells with aberrations was observed with 150 $\mu\text{g/mL}$ S9-activated Sencor (20-hour harvest). The most frequent types of scored aberrations were chromosome and chromatid breaks, triradials, and quadriradials. The study author claimed that clastogenic activity of Sencor was confined to a severely cytotoxic level. However, the presence of rare complex aberrations at lower levels (two triradials at 100 $\mu\text{g/mL}$ --20-hour harvest and one quadriradial at 50.0 $\mu\text{g/mL}$ --10-hour harvest) suggests that the positive response could be strengthened by testing several doses in the narrowed concentration range of 50-150 $\mu\text{g/mL}$, and possibly by increasing the cell recovery time beyond 20 hours. Nonetheless, the study provided sufficient evidence to conclude that S9-activated Sencor is a clastogen in this cultured mammalian cell assay.
- E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated April 2, 1990).
- F. CBI APPENDICES: Appendix A, Materials and Methods, CBI pp. 11-16; Appendix B, Protocol and Protocol Amendments, CBI pp. 35-46, and 52.

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APPENDIX A
MATERIALS AND METHODS
CBI pp. 11-16

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