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

MAR 30 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Acetochlor

FROM: Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769c)

TO: Robert Taylor
Product Manager (25)
Registration Division (TS-767c)

The Toxicology Branch Peer Review Committee met on Sept. 12, 1985 to discuss and evaluate the weight-of-the-evidence on Acetochlor, with particular reference to its oncogenic potential.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber

William L. Burnam

Reto Engler

Donald Barnes

Louis Kasza

for Bertram Litt (DER not read)

John A. Quest

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Stephen Saunders

Laurence Chitlik

for Winnie Teeters

02/15/90

PEER REVIEW FILES

007697

CHEMICAL NAME: Acetochlor
CASWELL NO.: 003B
CAS NO.: 34256-82-1
REVIEWER: Dapson

CURRENT AGENCY DECISION

B2; 1.0 x 10-2

TUMOR TYPE / SPECIES

Nasal turbinate papillary adenomas in Albino rats (M & F) (HED); Liver tumors, nose/turbinates, & thyroid in rats; Tumors at multiple sites in CD-1 mice (SAP).

REVIEWER PEER REVIEW PACKAGE	PEER REVIEW MEETING DATE	PEER REVIEW DOCUMENTS	PEER REVIEW CLASSIFICATION
5. / /	5. / /	5. / /	5.
4. / /	4. / /	4. / /	4.
3. / /	3. / /	3. / /	3.
2. 02/02/89	2. 02/08/89	2. 05/31/89	2. B2; 1.0 x 10-2
1. 08/29/85	1. 09/12/85	1. 03/30/87	1. B2

SAP MEETING	SAP CLASSIFICATION
2. / /	2.
1. 09/28/89	1. B2

QUALITATIVE/QUANTITATIVE RISK ASSESSMENT DOCUMENT	GENETIC TOXICITY ASSESSMENT DOCUMENT
2. 07/21/89	1. / /
1. 09/09/88	

MISCELLANEOUS:

Stamped 2/1/90; #PR-007697; about 300 p.; nha.

1 of 500

Peer Review Documents
(Memo dates)

A. 3. Peer review members in absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Anne Barton

Stephen Johnson

Robert Beliles

Diane Beal

Judith Hauswirth

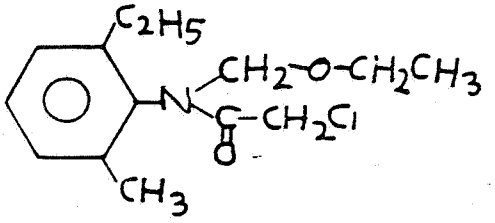
Esther Rinde

B. Material Reviewed:

The material available for review of acetochlor (Harness®) consisted of a chronic feeding study in the rat (DER) and an oncogenicity study in mice (DER), a metabolism study, and 4-mutagenicity studies.

C. Background Information:

Acetochlor, 2 chloro-N-ethoxymethyl-N-(2-ethyl-6-methylphenyl acetamide), is structurally related to alachlor, butachlor and metolachlor which have all been shown to be oncogenic in rats (alachlor was also oncogenic in mice).



ACETOCHLOR

D. Evaluation of Oncogenicity Evidence for Acetochlor

1. Chronic Feeding Toxicity and Oncogenicity Study in the Sprague-Dawley Rat.

Groups of 70 male and 70 female rats were fed 0, 500, 1500 or 5000 ppm acetochlor in their diet for 24/27 months (by month 24, there were still sufficient males in each group to allow the study to proceed to month 27).

Summary of the Incidence of the Most Frequently Observed Neoplastic Lesions in Rats Fed Diets Containing MON-097 (Acetochlor) for 24/27 Months

Organ/Lesion		0	500	1500	5000 PPM
Liver/					
Hepatocellular Adenoma	M	6/70	2/70	5/70	7/70
	F	0/70	2/70	2/70	2/70
Hepatocellular Carcinoma	M	0/70	2/70	3/70	6*[a]/70
	F	1/70	1/70	1/70	5[a]/70
Combined	M	6/70	4/70	8/70	13/70
	F	1/70	3/70	3/70	7/70
Testes/					
Interstitial cell tumor	M	2/70	4/70	4/70	7/70
Thyroid/					
C-cell adenoma	M	7/69	2/69	4/70	4/70
	F	4/69	1/69	1/69	0/69
Follicular cell adenoma	M	0/69	0/69	3/70	5*[a]/70
	F	2/69	0/69	0/69	3/69
Uterus/					
Adenocarcinoma	F	1/70	0/70	1/70	4/70

[a]Significant linear trend using the Cochran-Armitage test ($p<0.05$).
*Statistically different from control values ($p<0.05$) using the Fisher Exact test.

The incidences of hepatocellular carcinoma and follicular cell adenoma were significantly ($p<0.05$) increased at the high dose in male rats; a positive trend ($p<0.05$) was also noted for these tumors in males, as well as for the incidence of hepatocellular carcinoma in females.

The LOEL for chronic effects was determined to be 500 ppm, based on body and organ weight data; a NOEL for non-neoplastic effects was not established.

The MTD was exceeded at the high dose in both sexes, based on increased mortality.

D. Evaluation of Oncogenicity (continued)

2. Oncogenicity study of random-bred Swiss Albino CD-1 Mice

Groups of 60 male and 60 female mice were fed acetochlor (MON-097) in the diet for up to 23 months at dose levels of 0, 500, 1500, and 5000 ppm.

Incidence of Frequently Occurring Neoplastic Lesions in Mice Fed MON-097 for 23 Months[a]

Organ/Lesion		0	500	1500	5000 PPM
Harderian gland Adenoma	M	8/60	7/60	7/60	9/60
	F	3/60	1/60	5/60	4/59
Kidneys/	M	0/60	0/60	2/60	1/60
Adenocarcinoma	F	0/60	0/60	0/60	0/59
Adenoma	M	2/60	1/60	1/60	2/60
	F	0/60	0/60	0/60	3[b]/59
Sarcoma	M	0/60	0/60	0/60	0/60
	F	0/60	0/60	0/60	2/59
-Total Malignant	M	0/60	0/60	2/60	1/60
Kidney tumors	F	0/60	0/60	0/60	2/59
Liver/	M	8/60	4/59	9/60	7/59
Adenoma	F	2/60	0/60	0/60	4/58
Carcinoma	M	6/60	7/59	10/60	22[b,c]/59
	F	1/60	0/60	0/60	4[b]/58
Combined	M	14/60	11/59	19/60	29/59
	F	3/60	0/60	0/60	8/58
Lungs/	M	6/60	10/60	12/60	5/6
Adenoma	F	2/60	6/60	8[d]/60	4/59
Carcinoma	M	7/60	3/60	4/60	3/60
	F	0/60	5[d]/60	3/60	7[b,d]/59
Histiocytic sarcoma	M	0/60	0/60	0/60	0/60
	F	0/60	0/60	1/60	0/59
- Total Lung Tumors	M	13/60	13/60	16/60	8/60
	F	2/60	11[c]/60	12[c]/60	11[b,c]/59

(continued)

[a]Neoplastic lesions that occurred at a frequency of no more than one per dose group were excluded from this table unless significance was noted for total tumors of a given organ.

[b]Statistically significant linear trend ($p \leq 0.01$) using the Peto analysis.

[c]Statistically significant increase compared to control ($p \leq 0.01$) using the Chi-square test (uncorrected for continuity).

Incidence of Frequently Occurring Neoplastic Lesions in Mice Fed MON-097 for 23 Months[a] (continued)

Organ/Lesion		0	500	1500	5000 PPM
Lymphatic System Lymphoma	M	4/60	2/60	2/60	4/60
	F	6/60	7/60	12/60	1/59
Ovaries/					
Adenoma	F	0/59	0/60	1/60	0/58
Granulosa cell tumor	F	0/59	0/60	3/60	2/58
Luteoma	F	0/59	0/60	1/60	1/58
- Total benign ovarian tumors	F	0/59	0/60	5[d]/60	3[b]/58
Pituitary gland/					
Adenoma	M	0/58	0/49	0/58	1/54
	F	2/58	2/57	0/55	0/51
Uterus/					
Endometrial stromal polyp	F	1/59	2/57	2/60	2/59
Histiocytic sarcoma[1]	F	0/59	6[c]/57	6[c]/60	5[d]/59
Leiomyosarcoma	F	3/59	0/57	2/60	0/59

[a]Neoplastic lesions that occurred at a frequency of no more than one per dose group were excluded from this table unless significance was noted for total tumors of a given organ.

[b]Statistically significant linear trend ($p \leq 0.01$) using the Peto analysis (see note in DER).

[c]Statistically significant increase compared to control ($p \leq 0.01$) using the Chi-square test (uncorrected for continuity).

[d]Significantly different from control by Fisher's exact test ($p < 0.05$).

The MTD was exceeded in both sexes at the high dose, based on increased mortality and decreased body weights.

! It was noted that histiocytic sarcomas should be combined for all organ sites, however the incidence in other organs was not available.

D. 2. Oncogenicity Study in Mice, contd.

There was a statistically significant increase in the incidence of the following tumor types:

Liver carcinomas in males at the high level ($p<0.01$),
Total lung tumors in females at all levels ($p<0.01$),
Carcinomas of the lungs in low and high level females ($p<0.05$),
Uterine histiocytic sarcomas in females at all levels ($p<0.01$ at 500 & 1500 ppm; $p< 0.05$ at 5000 ppm)
Total benign ovarian tumors in mid-level females ($p<0.05$).

There were positive linear trends for: liver carcinomas in both sexes, and pulmonary carcinomas, total lung tumors, total ovarian benign tumors and kidney adenomas in females.

§ §

The preceding statistical analyses and tumor incidences were reported in the DER provided by Dynamac. In a Lab/Data Audit Memo (memo not dated-Audit performed Oct. 1985 and provided to HED on Nov. 4, 1986) Dr. Adrian Gross indicates that there are discrepancies in the statistical analyses of the mouse tumors obtained by him, and those obtained by Dynamac.

Dr. Gross also states in his memo that "the significance as estimated by Dynamac for..." the liver carcinomas in male and female rats "...was understated". (Dynamac found for these tumors, $p<0.05$, whereas Dr. Gross found $p<<0.01$.)

The "discrepancies" described by Dr. Gross are small differences in the p value and/or in the expression of the p value; nevertheless, the overall conclusions are the same.

In this same memo, Dr. Gross also indicates an apparently very different incidence for "kidney" tumors in the mouse from that reported in the DER (cf: pg. 4), as follows:

		0	500	1500	5000 PPM
Carcinoma	M	6/60	7/59	10/60	22/59
	F	1/60	0/60	0/60	4/58
Adenoma	F	2/60	0/60	0/60	4/58

However, the above data are actually for the liver tumors; it appears that on pages 4, and 5 of the Gross memo the word "kidney" was inadvertently substituted for liver. Thus, there is no discrepancy in the tumor incidence as reported by Dynamac and by Dr. Gross.

The above information was not available to the Peer Review Committee at the time of the meeting on acetochlor.

5. Historical Control Information: Not presented to the Committee.

E. Additional Toxicology Data on Acetochlor:

1. Metabolism: In a rat study, acetochlor was extensively metabolized with less than 1% parent compound found in the feces and none detectible in the urine. The early (<24 hour) metabolites were mostly mercapturates; later ones mostly sulfoxides, sulfones and sulfates; 20 metabolites were identified (Figures 1, 2, 3). Early conjugation with glutathione is assumed. The only structure retaining significant amounts of labeled acetochlor (about 2.5%) was the erythrocyte; the turnover rate of erythrocytes in the rat correlates well with the slow phase of acetochlor elimination. (Acetochlor was rapidly excreted with the urinary route accounting for about twice the percentage of the fecal route; pulmonary excretion was insignificant. Elimination was biphasic, with a rapid and slow phase.) There did not appear to be any significant sex differences in the metabolism of acetochlor.

2. Non-Oncogenic Toxicological Effects

In a one-year feeding study in dogs, the high dosed group (40 mg/kg) showed testicular atrophy (6/6) accompanied by decreased absolute and relative (to body weight) testicular weight, decreased body weight gain of males an decreased terminal body weight of females. There was also suggestive evidence at the high level for anemia and hepatotoxicity, but a NOEL and LOEL for these effects could not be be conclusively determined. There was also suggestive evidence for effects on adrenal weights.

In a two generation reproduction study in rats, a slight decrease (about 20%) in litter size was noted at the high dose (5000 ppm) in all matings. The high dose also caused decreased pup body weight gain during lactation for both generations; this effect was also seen in male F_{2b} pups at the mid level (1500 ppm).

Acetochlor was negative at a dose of 400 mg/kg in a rat teratology study.

MON 097 Technical, and an "E.C" formulation were positive dermal sensitizers in the guinea pig.

3. Mutagenicity:

Acetochlor was weakly positive in the CHO/HGPRT gene mutation assay at near toxic doses, but the vehicle used (alcohol) had some activity. It was also positive (with activation only) in the mouse lymphoma test. Negative results were obtained in the Ames salmonella test, in the hepatocyte DNA repair test, and in an unacceptable bone marrow chromosome aberration assay.

ACETOCHLOR Tox review 007697

Page_____ is not included in this copy.

Pages 10 through 12 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

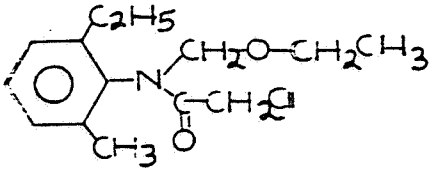
____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

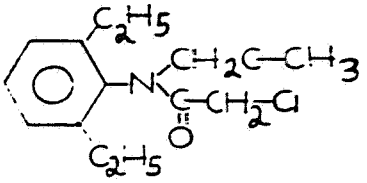
The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

4. Structure-Activity Correlations:

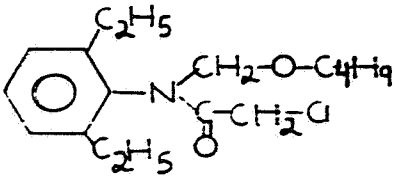
Acetochlor is structurally related to Alachlor, Butachlor, Metolachlor, and Propachlor.



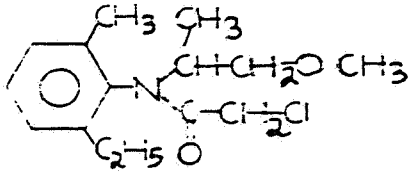
Acetochlor



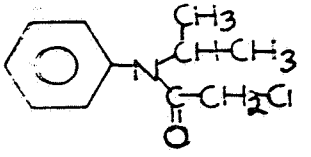
Alachlor



Butachlor



Metolachlor



Propachlor

Alachlor is oncogenic in both rats and mice. In rats, it caused nasal turbinate (42 mg/kg) and stomach tumors (126 mg/kg) in both sexes, and thyroid follicular adenomas in males (146 mg/kg). In mice, there was an increased incidence of lung tumors in females (260 mg/kg). Alachlor was evaluated by the Peer Review Committee as a Category B2 oncogen, and is presently undergoing Special Review.

Butachlor (Machete™) causes stomach tumors (defined as masses at necropsy) in female rats (3000 ppm). Butachlor has not been evaluated by the Peer Review Committee.

Metolachlor caused a significantly elevated incidence of proliferative liver lesions (neoplastic nodules and carcinomas, combined) at the highest dose level tested (3000 ppm) in female rats. The mouse oncogenicity study was negative for proliferative lesions. Metolachlor was evaluated by the Peer Review Committee as a tentative Category C oncogen (due in part to the borderline quality of the data).

Propachlor (Ramrod™) was tested by Industrial Bio-Test Laboratories and this study must be repeated.

F. Weight of Evidence Considerations:

The committee considered the following facts regarding toxicology data on acetochlor to be of importance in a weight of evidence determination of oncogenic potential.

Rats:

The incidences of hepatocellular carcinoma and follicular cell adenoma of the thyroid were significantly ($p<0.05$) increased at the high dose in male rats; a positive trend ($p<0.05$) was also noted for these tumors in males, as well as for the incidence of hepatocellular carcinoma in females.

Mice:

There was a statistically significant increase in the incidence of: liver carcinomas in males at the high level ($p<0.01$); total lung tumors in females at all levels ($p<0.01$); carcinomas of the lungs in low and high level females ($p<0.05$); in uterine histiocytic sarcomas at all dose levels ($p<0.01$ low and mid dose; $p<0.05$ high dose) and total benign ovarian tumors in mid-level females ($p<0.05$). There were positive linear trends ($p<0.01$) for: liver carcinomas in both sexes, and for pulmonary carcinomas, total lung tumors, ovarian benign tumors and kidney adenomas in females.

(See also page 6 and attached memo from Adrian Gross.)

* * * * *

Acetochlor was weakly positive in the CHO/HGPRT gene mutation assay at near toxic doses, but the vehicle used (alcohol) had some activity. It was also positive (with activation only) in the mouse lymphoma test. Negative results were obtained in the Ames salmonella test, hepatocyte DNA repair tests and in an unacceptable bone marrow chromosome aberration assay.

Acetochlor is structurally related to Alachlor, Butachlor, Metolachlor, and Propachlor:

- Alachlor is oncogenic in both rats and mice. In rats, it caused nasal turbinate (42 mg/kg) and stomach tumors (126 mg/kg) in both sexes, and thyroid follicular adenomas in males (146 mg/kg). In mice, there was an increased incidence of lung tumors in females (260 mg/kg).

- Butachlor (Machete™) causes stomach tumors (defined as masses at necropsy) in female rats (3000 ppm).

- Metolachlor caused a significantly elevated incidence of proliferative liver lesions (neoplastic nodules and carcinomas, combined) at the highest dose level tested (3000 ppm) in female rats. The mouse oncogenicity study was negative for proliferative lesions.

- Propachlor (Ramrod™) was tested by Industrial Bio-Test Laboratories and this study must be repeated.

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR 51: 33992-34003, 1986] for classifying a carcinogen were considered.

The weight of evidence for acetochlor is summarized as follows:

°Acetochlor was oncogenic in the rat (hepatocellular carcinoma in both sexes and thyroid follicular cell adenoma in males).

°Acetochlor was oncogenic in the mouse (hepatocellular carcinoma in both sexes, lung carcinoma in females, uterine histiocytic sarcomas and benign ovarian tumors in females, kidney adenomas in females).

°Acetochlor is structurally related to known or suspected oncogens (alachlor, butachlor, metolachlor.

°Acetochlor was mutagenic in mammalian cell culture tests: CHO/HGPRT (weakly positive) and in the mouse lymphoma test.

Based on the above evidence, acetochlor meets the criteria for Group B2 - Probable Human Carcinogen (causes an "increased incidence of malignant or combined malignant and benign tumors in multiple species"). Additional evidence for this classification was provided by SAR and mutagenicity.

5/31/89

007697

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

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MAY 31 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Second Peer Review of Acetochlor: Nasal Tumors

TO: Robert Taylor
Product Manager (25)
Registration Division (TS-767c)

FROM: William F. Sette, Ph.D. William F. Sette 4/28/89
Executive Secretary I, Peer Review Committee
Health Effects Division (TS-769c)

The Health Effects Division Peer Review Committee met on February 8, 1989 to discuss and evaluate the weight of the evidence on Acetochlor with special reference to its oncogenic potential for causing nasal tumors.

We reaffirmed the classification of the weight of evidence as category B2, probable human oncogen, and recommended that the quantitative risk assessment (Q_1*) be based on the data on nasal turbinate papillary adenomas in male and female Albino rats given 1000 ppm in their diet.

A. Individuals in Attendance

1. Peer Review Committee (Signature indicates concurrence with the peer review unless otherwise stated.)

William Burnam
Marion Copley
Kerry Dearfield
Reto Engler
Bernice Fisher
George Ghali
Richard Levy
Judith W. Hauswirth
John A. Quest
Esther Rinde

16

Robert Beliles [signature]

Lynnard Slaughter [signature]

Marcia Van Gemert [signature]

William Sette [signature]

2. Scientific Reviewers (People responsible for presentation of data; signature indicates technical accuracy of panel report.)

Stephen C. Dapson [signature]

3. Peer Review Members in Absentia (Those unable to attend the discussions; signature indicates concurrence with overall conclusions of the Committee.)

Diane Beal [signature]

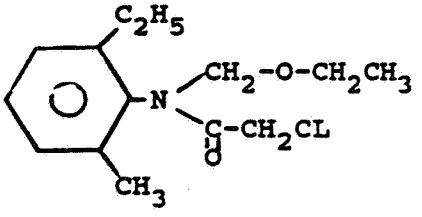
Richard Hill —

B. Material Received

We received an 11 page overview from Dr. Dapson dated 1/27/89 summarizing the issues and available data, and a set of attachments (A-J) including the first peer review of this material, data evaluation records of 3 oncogenicity studies, 2 mutagenicity studies, a qualitative risk assessment of one study, and related memoranda.

C. Background Information

Acetochlor, 2 chloro-N-ethoxymethyl-N-(2-ethyl-6-methylphenyl acetamide), is a herbicide used for control of annual grasses and certain broadleaf weeds on a variety of food crops.



In the first Peer Review Committee meeting (9/12/85), the weight of evidence for oncogenicity was classified as B2-Probable Human Oncogen based on:

an increased incidence in rats of hepatocellular carcinomas in both sexes, and thyroid follicular cell adenomas in males;

an increased incidence in mice of hepatocellular carcinomas in both sexes, lung carcinomas, uterine histiocytic sarcomas, benign ovarian tumors, and kidney adenomas in females;

positive mutagenicity data in the CHO/HGPRT and mouse lymphoma assays;

and positive oncogenicity data on structural analogues, alachlor, butachlor, and metolachlor.

Since that review, a newer rat study has been reviewed and slides from the original rat study re-evaluated. Further, a qualitative risk assessment has been performed on the newer rat study. The present meeting focused on these studies and primarily concerned the nasal turbinate adenomas observed.

D. Evaluation of Oncogenicity Evidence

1. Chronic Feeding Study of MON 097 in Albino Rats.
EPA Accession No. 400770601.

Groups of 70 rats/sex were fed 0, 40, 200, or 1000 ppm of Acetochlor in their diet for 2 years.

Statistical analysis was performed by C.J. Nelson of this division. There were no differences in survival in either sex.

There was a statistically significant ($p<0.05$) increase in both sexes in papillary adenomas of the nose/turbinates at 1000 ppm (50 mg/Kg). There also was a significant dose related trend.

There was a significant linear trend for combined thyroid carcinomas and adenomas for females, but not males.

A NOEL of 200 ppm (10 mg/Kg) for systemic toxicity was identified.

Based on the effects seen in the high dose group, decreased body weight gain, clinical chemistry changes, and non-neoplastic findings, it is apparent that the MTD was achieved in this study.

TABLE 4. ACETOCHLOR ALBINO RAT Study-- FEMALE Thyroid Follicular Tumor Rates* and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	1/39 a (2.6)	2/44 a (4.5)	2/36 (5.6)	4/46 (8.7)
	p= 0.1126	p= 0.4015	p= 0.3639	p= 0.1948
CARCINOMA	0/30 (0.0)	0/35 (0.0)	0/28 (0.0)	1/36 b (2.8)
	p= 0.0537	p= 1.0000	p= 1.0000	p= 0.5455
ADENOMA CARCINOMA	1/39 (2.6)	2/44 (4.5)	2/36 (5.6)	5/46 (10.9)
	p= 0.0457 *	p= 0.4015	p= 0.3639	p= 0.1222

a First Adenoma observed at 90 weeks in dose 0 and 40 ppm.
b First Carcinoma observed at 100 weeks in dose 1000 ppm.

TABLE 5. ACETOCHLOR ALBINO RAT Study-- FEMALE Nose Papillary Tumor Rates* and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	0/69 (0.0)	0/69 (0.0)	0/67 (0.0)	19/68 c (27.9)
	p< 0.0001 **	p= 1.0000	p= 1.0000	p< 0.0001 **

* Number of tumor bearing animals/Number of animals at risk. (Excluding animals that died before the observation of the first tumor or animals not examined).
( ) Per cent

c First Adenoma observed at 54 weeks in dose 1000 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

TABLE 6. ACETOCHLOR, ALBINO RAT Study-- MALE Thyroid Follicular Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	1/56 (1.8)	1/53 (1.9)	1/54 a (1.9)	2/54 (3.7)
	P= 0.2208	p= 0.5042	p= 0.5044	p= 0.3713

a First Adenoma observed at 75 weeks in dose 1000 ppm..
No Carcinomas occurred in male rats.

TABLE 7. ACETOCHLOR, ALBINO RAT Study-- MALE Nose Papillary Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	1/58 (1.7)	0/54 (0.0)	0/58 (0.0)	12/59 (20.3)
	P< 0.0001 **	p= 0.5179	p= 0.5000	p= 0.0010 **

+ Number of tumor bearing animals/Number of animals at risk. (Excluding animals that died before the observation of the first tumor or animals not examined).
( ) Per cent

a First Adenoma observed at 67 weeks in dose 1000 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

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2. Reexamination (EPA Accession No. 40484801) of Preserved Tissue from the earlier rat study (EPA Accession Nos. 071962-5).

Since treatment related nasal papillary adenomas were not seen in the earlier rat oncogenicity study (reviewed in the first peer review), the sponsor reexamined the preserved tissues of the rats from that study, focusing on the posterior portion of the nasal cavity, which were not previously analyzed and in which the probability of inhalation of the diet was minimized.

A. Summary from first Peer Review

Groups of 70 Sprague-Dawley rats/sex were fed 0, 500, 1500, and 5,000 ppm of acetochlor in their diet for 24-27 months. (Males only for 27 months).

The MTD was exceeded at the high dose for both sexes, based on body weight decreases and non-neoplastic lesions. In addition, there was increased mortality in females. The LOEL for chronic effects was 500 ppm, based on body weight and organ weight data; a systemic NOEL was not established.

The incidence of hepatocellular carcinomas and thyroid follicular cell adenomas were significantly increased at the high dose in male rats; a positive trend ($p<0.05$) was noted for these tumors in males as well as for the incidence of hepatocellular carcinomas in females.

The study was rated CORE Minimum, despite the need to repeat the study to establish a systemic NOEL.

B. Results of Re-examination

There was a dose-related increase in nasal papillary adenomas in male rats with statistically significant differences at the mid (1500 ppm) and high (5000 ppm) dose levels. Papillary adenocarcinomas were present in two high dose males who did not have adenomas. There were significant trends for these adenomas, carcinomas, and all nasal malignancies combined. There was an apparent increase in all treated males of inflammation of the nasal mucosa, which was statistically significant ($p<0.01$) at the high dose. However, for all but three high dose males, there was no association between adenomas and inflammation. There were no statistically significant differences in survival between treated and control males.

In females, there was a borderline significant ($p=0.055$) trend in the incidence of papillary adenomas. However, there was also significantly lower survival times for low and high dose females, as well as the overall treated group.

Since the statistical analyses presented by the Company and

used here were not completely annotated, it is not clear how well the reported trend analysis accounted for the survival differences. Similarly, the Bonferroni Inequality Multiple Comparison adjustment of Fisher's exact test on this data would not have been performed by us, although for this data set, the significance of the results are still apparent. For the male data, where there were no survival differences, a Cochran-Armitage trend test would have been preferred.

Dose(ppm)		0	500	1500	5000
Observation:					
Nose/Turbinates:					
papillary adenoma					
	M	0/69	1/70	6*/69	18*/69
	F	0/69	0/68	2/70	1/69
papillary adenocarcinoma					
	M	0/69	0/70	0/69	2/69
squamous cell carcinoma					
	M	0/69	1/70	0/69	1/69
	F	1/69	2/68	1/70	0/69
squamous papilloma					
	M	0/69	0/70	1/69	0/69
carcinoma in-situ					
	F	0/69	0/68	1/70	0/69
esthesioneuroma (benign)					
	M	0/69	0/70	0/69	1/69
epithelial inflammatory squamous metaplasia					
	F	0/69	0/68	1/70	0/69
submucosal glandular hyperplasia					
	F	0/69	0/68	0/70	2/69
inflammatory epithelial hyperplasia					
	M	1/69	0/70	3/69	2/69
	F	1/69	0/68	2/70	0/69
inflammation:					
nasolacrimal duct					
	M	1/69	8/70	5/69	6/69
	F	5/69	1/68	2/70	2/69
nasal mucosa					
	M	3/69	9/70	7/69	16**/69
	F	2/69	8/68	6/70	8/69

* = p < 0.05 using Fisher's Exact Test w/ Bonferroni Inequality

** = p < 0.01 using Fisher's Exact Test w/ Bonferroni Inequality

Peto test for trend found the following "p" values:

nasal papillary adenoma, males	0.000
nasal papillary adenoma, females	0.055
nasal papillary adenoma, both sexes	0.000
papillary adenocarcinoma, males	0.027
esthesioneuroma, males	0.062
all nasal malignancies, males	0.031

E. Additional Toxicity Data

1. Mutagenicity The studies reviewed in the first peer review indicated that:

Acetochlor was weakly positive in the CHO/HGPRT gene mutation assay with and without activation. The alcohol vehicle appeared to have a higher baseline frequency under activated conditions.

It was also positive, with activation only, in the mouse lymphoma assay.

A DNA damage/repair assay in rat hepatocytes was negative.

An acceptable Salmonella assay was negative, but K. Dearfield notes that TA100 data were suggestive.

Two in-vivo chromosomal aberration studies were negative.

A new dominant lethal study could not be adequately evaluated with the provided data; however, a new study was not requested since all mutagenicity requirements were fulfilled.

2. Structure Activity Relationships

As the first peer review indicated, Acetochlor is structurally related to Alachlor, Butachlor, and Metolachlor.

Alachlor is oncogenic in rats and mice and was classified as a B2, probable human oncogen. It produces nasal turbinate tumors in both sexes of rats at 15 mg/Kg, as well as stomach tumors in both sexes at 126 mg/Kg, and thyroid follicular cell adenomas in males at 126 mg/Kg. In female mice, lung tumors were seen at 260 mg/Kg.Gavage exposure of rats to Alachlor lead to labelled material in the nasal turbinates, indicating its systemic distribution and discounting the view that tumors arose from breathing of food dust.

Butachlor produced stomach tumors in female rats at 150 mg/Kg. The Peer Review Committee has not reviewed this data.

Metolachlor is classified as a Category C oncogen based on liver tumors in female rats given 150 mg/Kg. More noteworthy here, however, were nasal turbinate tumors, adenocarcinomas and a fibrosarcoma in male rats. There was a significant trend for adenocarcinomas (0/67 controls, and 0/59, 0/53, and 2/69 for 30, 300, and 3000 ppm groups, respectively).

F. Weight of Evidence Considerations

The Committee considered the following facts regarding the toxicity of Acetochlor to be important in weighing the evidence of its oncogenic potential with respect to these nasal tumors.

In the "repeat" rat study, there was a statistically significant ($p<0.05$) increase in both sexes in papillary adenomas of the nose/turbinates at 1000 ppm (50 mg/Kg). There also were significant dose related trends.

There was a significant linear trend for combined thyroid carcinomas and adenomas for females, but not males.

In the first rat study, there was a dose-related increase in nasal papillary adenomas in male rats with statistically significant differences at the mid (1500 ppm) and high (5000 ppm) dose levels. Papillary adenocarcinomas were present in two high dose males who did not have adenomas. For males, there were significant trends for these adenomas, carcinomas, and all nasal malignancies combined.There was an apparent increase in all treated males of inflammation of the nasal mucosa, which was statistical significant ($p<0.01$) at the high dose. However, for all but three high dose males, there was no association between adenomas and inflammation.

In females, there was a borderline significant ($p=0.055$) trend in the incidence of nasal papillary adenomas.

There were positive mutagenicity data in the CHO/HGPRT and mouse lymphoma assays.

There were positive oncogenicity data on structural analogues, alachlor, butachlor, and metolachlor. Of particular note were the nasal tumors in rats given Alachlor, the thyroid tumors in Alachlor males, and the nasal tumors (not statistically significant) in Metolachlor rats.

G. Classification of Oncogenic Potential

The previous Peer Review Committee meeting classified the evidence as best fitting Group B2, Probable Human Oncogen based on an "increased incidence of malignant or combined malignant and benign tumors in multiple species", with additional evidence from mutagenicity studies and SAR.

Based on the data examined in the current meeting, we can now additionally cite an increased incidence of nasal adenomas in Sprague-Dawley rats in 2 studies, and stronger analogy to Alachlor, which also causes these nasal tumors.

We reaffirmed the classification of the weight of evidence as category B2, probable human oncogen, and recommended that the quantitative risk assessment (Q_1*) be based on the data on nasal turbinate papillary adenomas in male and female Albino rats given 1000 ppm (50mg/Kg) in the repeat study.

SAP Executive Summary

007697

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

OCT 16 1989

MEMORANDUM

SUBJECT: Transmittal of the Final FIFRA Scientific Advisory Panel Report on the September 28-29, 1989 Meeting

FROM: R. Bruce Jaeger
Executive Secretary
FIFRA Scientific Advisory Panel

TO: Douglas D. Campt, Director
Office of Pesticide Programs

The above mentioned meeting of the FIFRA Scientific Advisory Panel (SAP) was an open meeting held in Arlington, Virginia to review the following topics:

1. A set of Scientific Issues Being Considered by the Agency in Connection with the Proposed Guidelines for Neurotoxicity Testing Under FIFRA.

2. A set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Acetochlor as a Class B2 Oncogen.

3. A set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of DDVP as a Class C Oncogen.

4. A set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Simazine as a Class C Oncogen.

5. A set of Scientific Issues Being Considered by the Agency in Connection with the Proposed Guidelines for Mutagenicity Testing Under FIFRA.

Please find attached the Panel's final report on the agenda items discussed at the meeting.

Attachments

cc: Panel Members
Linda J. Fisher
Victor J. Kimm
Jim Roelofs
Susan H. Wayland
Anne Barton
Edwin Tinsworth
Penny Fenner-Crisp
Al Heier
Mary Beatty
EPA Participants
Don Barnes

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FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Acetochlor as a Class B2 Oncogen

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed review of a set of scientific issues being considered by the Environmental Protection Agency in connection with the peer review of Acetochlor. The review was conducted in an open meeting held in Arlington, Virginia, on September 28, 1989. Panel members present for the review were Dr. James Tiedje, Dr. Robert Anthony and Dr. Edward Bresnick. In addition, Dr. Ernest E. McConnell of Raleigh, NC served as a Special Government Employee on the Panel.

Public notice of the meeting was published in the Federal Register on August 25, 1989.

Oral statements were received from Dr. Dennis P. Ward of Monsanto.

In consideration of all matters brought out during the meeting and careful review of all documents presented by the Agency, the Panel unanimously submits the following report.

REPORT OF PANEL RECOMMENDATIONS

Acetochlor

The SAP agrees with the assessment of the EPA on the weight of evidence which has led to the classification of Acetochlor as a Class B2 Oncogen. This evidence includes: the incidence of tumors (benign and malignant) in liver, nose/turbinates, and thyroid in the rat; the increased incidence of both benign and malignant tumors at multiple sites in the mouse; the structural relationship of Acetochlor to analogues that are positive in oncogenicity bioassays; and the very limited positive mutagenicity data. We also wish to affirm that oncogenicity data obtained from animals receiving an agent at greater than the maximum tolerated dose (MTD) can be considered relevant if the data support observations (albeit nonsignificant) obtained at dose levels below the MTD.

In regard to the quantitative risk assessment determined from the combined male and female data as opposed to separate male and female incidences, i.e., q_1^* of 0.004 vs 0.01, the SAP considers these results as being indistinguishable in a practical sense. In other situations where a significant difference could result, the SAP would encourage the Agency to consider whether results from the two sexes should be considered collectively or individually.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

Robert B. Jaeger
Executive Secretary
FIFRA Scientific Advisory Panel

Date: October 16, 1989

Qualitative/Quantitative Risk Assessment

7/21/89

007697

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

FILE COPY

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Acetochlor - Quantitative Risk Assessment, Two Year Chronic/Oncogenicity Sprague-Dawley Rat Study

caswell no. 3B

From: Bernice Fisher, Biostatistician Bernice Fisher 7/21/89
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

To: Stephen C. Dapson Ph.D., Pharmacologist
Review Section II
Toxicology Branch I - Insecticide/Rodenticide Support
Health Effects Division (H7509C)

Thru: John A. Quest, Ph.D., Section Head John A. Quest 8/1/89
Science Support Section
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

Summary

The unit risk, Q_1^*, of acetochlor is 10^{-2} (mg/kg/day)$^{-1}$ in human equivalents. This estimate of Q_1^* is based upon papillary adenomas (nasal turbinates) in both male and female Sprague-Dawley rats fed 0, 40, 200, and 1000 ppm.

Significant differential mortality with increasing doses of acetochlor did not occur in either sex.

Both sexes had a significant increasing trend in papillary adenomas (nasal turbinates) with dose increments of acetochlor and both sexes had a significant difference in the pair-wise comparison of controls and the highest dose group. See the memorandum on Acetochlor - Qualitative Risk Assessment from a Rat 2-Year Chronic/Oncogenicity Study, C.J. Nelson 9/7/88 for details.

Background

The Peer Review Committee meeting on acetochlor on 2/8/89 concluded that the chemical compound should be classified as a [B_2] carcinogen. In addition they recommended that the unit risk, Q_1^*, should be estimated from both male and female rat nasal turbinate papillary adenoma tumor rates.

Dose-Response Analysis

As a result of the Peer Review Committee's recommendation of the use of rat papillary adenomas for the estimation of Q_1^* and since there was no significant dose related mortality in either sex the calculation of the unit risk was made by the use of the Global86, Multi-Stage Model computer program of K. Crump. The unit risk calculated from the rat data in ppm doses was converted to rat mg/kg/day by the use of Lehman's Tables and then to human equivalents by the use of interspecies surface area adjustments as recommended by EPA Cancer Guidelines (1986).

The resultant estimate of Q_1^* is as follows:

	Rat, Q_1^* $(mg/kg/day)^{-1}$	In Human Equivalents
Tumor - nasal turbinate papillary adenomas		
male	2.04×10^{-3}	1.08×10^{-2}
female	1.73×10^{-3}	9.20×10^{-3}

It is to be noted that Q_1^* is an estimate of the upper (95%) bound on risk and that (as stated in the EPA Guidelines) the "true value of the risk is unknown and that the lower limit of the risk may be as low as zero".

References

Howe, R.B., Crump, K.S. and Vam Landingham, C. (1986)
A Computer Program to Extrapolate Quantal Animal
Toxicity Data to Low Doses (unpublished report), 25 pgs.

9/9/88

007697

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

SEP - 9 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: [redacted] Qualitative Risk assessment from a Rat 2-Year Chronic/Oncogenicity Study. Caswell No. -3B

FROM: C.J. Nelson, Statistician C J Nelson 9/7/88
Science Support Section
Science Analysis and Coordination Branch, HED (TS-769C)

TO: Stephen C. Dapson, Ph.D. Pharmacologist
Review Section I
Toxicology Branch II, HED (TS-769C)

THRU: Richard Levy, M.P.H.,Leader-Biostatistics Team 9-7-88
Science Support Section
Science Analysis and Coordination Branch, HED (TS-769C)

and

John A Quest, Ph.D., Chief J.A. Quest 9/9/88
Science Support Section
Science Analysis and Coordination Branch, HED (TS-769C)

SUMMARY:

Acetochlor was fed to male and female Sprague-Dawley rats at doses of 0, 40, 200, and 1000 ppm in a 107 week chronic toxicity/oncogenicity study.

For the female rat there were no survival problems. The incidence of nose papillary adenomas was significantly increased in the 1000 ppm dose group compared to controls and there was a significant dose-related trend. There was a significant dose related trend for thyroid follicular cell adenomas and/or carcinomas combined.

For the male rat there were no survival problems. The incidence of nose papillary adenomas was significantly increased in the 1000 ppm dose group compared to controls and there was a significant dose-related trend.

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BACKGROUND:

This is a repeat of a previous chronic/oncogenicity study in the rat. Acetochlor (96.1 % purity) was fed to male and female Sprague-Dawley rats at doses of 0, 40, 200, and 1000 ppm in a 107 week chronic toxicity/oncogenicity study. Approximately 10 animals of each sex were sacrificed after 52 weeks of continuous dosing in each dose group. The study was conducted by the Monsanto Environmental Health Lab for Monsanto Company. The report number was MSL-6119, EPA Accesion Number 400770601. Data was extracted from a final report dated September 25, 1986. Test animals were assigned randomly to the following groups:

Table 1. Experimental Design for Rat Chronic Study

	Time of Sacrifice (weeks)			
Dose	Total Number		52	
(ppm)	Male	Female	Male	Female
Control	70	70	10	10
40	70	70	10	10
200	70	70	10	10
1000	70	70	10	10

SURVIVAL ANALYSIS:

For the female rat there were no statistically significant results detected in mortality for either pair-wise comparisons of differences between the treated and the control or a linear trend with dose (Table 2).

For the male rat there were no statistically significant results detected in mortality for either pair-wise comparisons of differences between the treated and the control, or a linear trend with dose (Table 3).

Test for mortality were made using the Thomas, Breslow, and Gart procedure.

TABLE 2. ACETOCHLOR, ALBINO RAT Study-- FEMALE Mortality Rates+ and Cox or Generalized K/W Test Results

	WEEK					
DOSE(PPM)	1-26	27-54	54a	55-78	79-107a	TOTAL
0.000	1/70 (1)	0/69 (0)	10/10	10/59 (17)	25/49 (51)	36/60 (60)
40.000	0/70 (0)	1/70 (1)	10/10	9/59 (15)	24/50 (48)	34/60 (57)
200.000	2/70 (3)	1/68 (1)	10/10	12/57 (21)	19/45 (42)	34/60 (57)
1000.000	0/70 (0)	3/70 (4)	10/10	5/57 (9)	22/52 (42)	30/60 (50)

TABLE 3. ACETOCHLOR, ALBINO RAT Study-- MALE Mortality Rates+ and Cox or Generalized K/W Test Results

	WEEK					
DOSE(PPM)	1-26	27-54	54a	55-78	79-107a	TOTAL
0.000	0/70 (0)	1/70 (1)	10/10	4/59 (7)	27/55 (49)	32/60 (53)
40.000	2/70 (3)	2/68 (3)	10/10	3/56 (5)	29/53 (55)	36/60 (60)
200.000	0/70 (0)	1/70 (1)	10/10	11/59 (19)	25/48 (52)	37/60 (62)
1000.000	1/70 (1)	0/69 (0)	10/10	7/59 (12)	27/52 (52)	35/60 (58)

\+ Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
( ) Per cent

a Interim sacrifice was conducted at 54 weeks. Final sacrifice occurred at week 107.

Note: Time intervals were selected for display purposes only. Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

TUMOR ANALYSIS:

In the absence of any mortality differences for either sex of rats, Fisher's exact test was used to test for pair-wise differences between control and treated rats. The Cochran-Armitage trend was used to test for increasing incidence with increasing dose levels.

In the female rats, there was a significant linear trend for combined thyroid carcinomas and adenomas ($p = .0457$, Table 6). There were no other significant pair-wise differences or linear trends for thyroid adenomas or thyroid carcinomas. The incidence of papillary adenomas of the nose in the 1000 ppm group was significantly increased ($p < .0001$, Table 7) compared to controls and there was a significant dose-related trend ($p < .0001$).

In the male rats, there were no significant pair-wise differences or linear trends for thyroid adenomas (Table 8). There were no thyroid carcinomas. The incidence of papillary adenomas of the nose in the 1000 ppm group was significantly increased ($p = .0010$, Table 9) compared to controls and there was a significant dose-related trend ($p < .0001$).

TABLE 4. ACETOCHLOR ALBINO RAT Study-- FEMALE Thyroid Follicular Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	1/39 a (2.6)	2/44 a (4.5)	2/36 (5.6)	4/46 (8.7)
	p= 0.1124	p= 0.4015	p= 0.3639	p= 0.1940
CARCINOMA	0/30 (0.0)	0/35 (0.0)	0/28 (0.0)	1/36 b (2.8)
	p= 0.0537	p= 1.0000	p= 1.0000	p= 0.5455
ADENOMA CARCINOMA	1/39 (2.6)	2/44 (4.5)	2/36 (5.6)	5/46 (10.9)
	p= 0.0457 *	p= 0.4015	p= 0.3639	p= 0.1222

a First Adenoma observed at 90 weeks in dose 0 and 40 ppm.
b First Carcinoma observed at 100 weeks in dose 1000 ppm.

TABLE 5. ACETOCHLOR ALBINO RAT Study-- FEMALE Nose Papillary Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	0/69 (0.0)	0/69 (0.0)	0/67 (0.0)	19/68 c (27.9)
	p< 0.0001 **	p= 1.0000	p= 1.0000	p< 0.0001 **

\+ Number of tumor bearing animals/Number of animals at risk. (Excluding animals that died before the observation of the first tumor or animals not examined).
( ) Per cent

c First Adenoma observed at 54 weeks in dose 1000 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

TABLE 6. ACETOCHLOR, ALBINO RAT Study-- MALE Thyroid Follicular Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	1/56 (1.8)	1/53 (1.9)	1/54 a (1.9)	2/54 (3.7)
	P= 0.2208	p= 0.5042	p= 0.5044	p= 0.3713

a First Adenoma observed at 75 weeks in dose 1000 ppm..
No Carcinomas occurred in male rats.

TABLE 7. ACETOCHLOR, ALBINO RAT Study-- MALE Nose Papillary Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	1/58 (1.7)	0/54 (0.0)	0/58 (0.0)	12/59 (20.3)
	P< 0.0001 **	p= 0.5179	p= 0.5000	p= 0.0010 **

\+ Number of tumor bearing animals/Number of animals at risk. (Excluding animals that died before the observation of the first tumor or animals not examined).
( ) Per cent

a First Adenoma observed at 67 weeks in dose 1000 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

REFERENCES:

Thomas, D G, N Breslow, and J J Gart, Trend and Homogeneity Analyses of Proportions and Life Table Data. Computers and Biomedical Research 10, 373-381, 1977.

Cochran, W.G. Some Methods for Strengthening the Common X^2 Test. Biometrics 10, 417-451, 1954.

Armitage, P. Tests for Linear Trends in Proportions and Frequencies. Biometrics 11, 375-386, 1955.

increasing linear trend with dose. The incidence of malignant mammary tumors and all mammary tumors combined was significantly increased in the 1000 ppm dose group compared to controls and there was a significant increasing dose-related trend for both analyses. The 3 ppm dose group was significantly increased compared to control for malignant mammary tumors.

007697

Reviewer's Peer Review Package for 2nd Meeting

.2/2/89

007697

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

FILE COPY
OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review on Acetochlor

FROM: Esther Rinde, Ph.D.
Manager, ONCO Peer Review
Health Effects Division (TS-769c)

TO: Addressees

Attached for your review is a package on Acetochlor, prepared by Dr. Stephen Dapson .

A meeting to consider the classification of Acetochlor. is scheduled for 2/08/89 at 10:00 in Room 821, CM2.

Addressees

W. Burnam
R. Engler
R. Hill
B. Beliles
D. Beal
J. Hauswirth
M. Van Gemert
M. Copley
J. Quest
L. Slaughter
K. Dearfield
R. Levy
W. Sette
G. Ghali
B. Fisher
S. Dapson
J. Rowe

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007697

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

TO: Reto Engler, Ph.D., Chief
Scientific Analysis And Coordination Branch
Health Effects Division (TS-769C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 1/27/89
Pharmacologist, Review Section I
Toxicology Branch - Herbicide, Fungicide, Antimicrobial
Support/Health Effects Division (TS-769C)

THRU: James N. Rowe, Ph.D. James N. Rowe 1/27/89
Acting Section Head, Review Section I
TB-HFAS/HED (TS-769C)

SUBJECT: Issues addressed to the Peer Review Committee in Connection with the Classification of Acetochlor as an Oncogen.

Attached is the overview of the oncogenic potential of Acetochlor including additional data received subsequent to the Peer Review Committee meeting of September 12, 1985. Please schedule a new meeting to discuss the additional data.

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I. Scientific Issues Considered by the Toxicology Branch-Herbicide, Fungicide, Antimicrobial Support in Connection with the Classification of Acetochlor as an Oncogen.

II. Background Documents

A: MEMORANDUM, March 30, 1987, Peer Review of Acetochlor.

B: DATA EVALUATION RECORD, July 29, 1985, Acetochlor, Chronic Feeding Toxicity and Oncogenicity Study in the Rat, study prepared by Pharmacopathics Research Laboratory, Inc. for Monsanto Company, Study No. PR-80-006, May 20, 1983.

C: DATA EVALUATION RECORD, in MEMORANDUM, January 29, 1988, Chronic Feeding Study of MON 097 in Albino Rats, study prepared by Monsanto Environmental Health Laboratory for Monsanto Company, September 25, 1986.

D: DATA EVALUATION RECORD, in MEMORANDUM, June 30, 1988, Histopathology Findings in Noses of Rats Administered MON 097 in a Lifetime Feeding Study, study prepared by Tegeris Laboratories and Monsanto Environmental Health Laboratory for Monsanto company, Study No. ML-86-44/EHL 86027, June 30, 1986.

E: MEMORANDUM, September 9, 1988, Acetochlor - Qualitiative Risk Assessment from a Rat 2-Year Chronic/Oncogenicity Study.

F: DATA EVALUATION RECORD, August 5, 1985, Acetochlor (Harness) Oncogenicity Study in Mice, prepared by Pharmacopathics Research Laboratories, Inc. for Monsanto Company, Study No. PR-80-007, May 4, 1983.

G: MEMORANDUM, February 3, 1987, data summary.

H: DATA EVALUATION RECORD, August 2, 1985, Rat Hepatocyte Primary Culture/DNA Repair Test, study prepared by Pharmakon Research International, Inc. for Monsanto Company, Study No. PK 82-151, February 17, 1983.

I: DATA EVALUATION RECORD, in MEMORANDUM, June 30, 1988, Dominant Lethal/Fertility Study of MON 097 in Sprague-Dawley Rats, study prepared by Monsanto Environmental Health Laboratory for Monsanto Company, Study No. EHL-86008, October 11, 1987.

J: MEMORANDUM, August 23, 1985, Structural Similarity of Acetochlor to Other Positive Oncogens.

I. Scientific Issues Considered by the Toxicology Branch-Herbicide, Fungicide, Antimicrobial Support in Connection with the Classification of Acetochlor as an Oncogen.

A. Introduction

Acetochlor (2 Chloro-N-Ethoxymethyl-N-[2 Ethyl-6-Methylphenyl]Acetamide), a herbicide effective for the control of annual grasses and certain broadleaf weeds in crops such as corn, soybeans, sorghum and peanuts grown on high organic matter soils, has been initially classified by the Toxicology Branch Peer Review Committee (PRC), meeting of September 12, 1985, as a Group B2-Probable Human Oncogen based upon (Attachment A):

a. increased incidence of malignant or combined malignant and benign tumors in multiple species,

b. positive mutagenic effects and

c. structurally related known oncogens.

B. **Assessment of Oncogenicity**

1. In a chronic/oncogenicity study (Pharmacopathics Research Laboratories, Inc., Study No. PR-80-006, May 20, 1983; Attachment B), rats were exposed to Acetochlor at 500 (25 mg/kg), 1500 (75 mg/kg) and 5000 ppm (250 mg/kg) in the diet for 2 years. There were statistically significant increases ($p<0.05$) in liver carcinomas and thyroid adenomas in the 5000 ppm (250 mg/kg) males. Further, there was a compound related positive trend ($p<0.05$) for the incidences of liver carcinomas in males and females and thyroid follicular cell adenomas in males.

Dose(ppm)		0	500	1500	5000
Observation:					
Liver:					
carcinoma	M	0/70	2/70	3/70	$6^{ab}/70$
	F	1/70	1/70	1/70	$5^{a}/70$
Thyroid:					
follicular cell adenoma	M	0/69	0/69	3/70	$5^{ab}/70$
	F	2/69	0/69	0/69	3/69

[a] = $p < 0.05$ using Fisher's Exact test

[b] = $p < 0.05$ for linear trend using Cochran-Armitage test

This study failed to demonstrate a No Observed Effect Level (NOEL) for systemic toxicity and the MTD (Maximum Tolerated Dose) was exceeded at the high dose in both sexes, based on increased mortality. This study was repeated by the sponsor. The repeat study is discussed next.

2. A repeat rat chronic/oncogenicity study (Monsanto Environmental Health Laboratory, Report No. MASL-6119, September 25, 1986; Attachment C) to establish a NOEL for systemic toxicity was conducted at the request of the Agency. Under the conditions of this repeat study, a NOEL for systemic toxicity could be tentatively indentified at 200 ppm (10 mg/kg) pending the submission of additional data. The neoplastic findings in this study were noted in the form of tumors of the liver (not statistically significant), thyroid (not statistically significant) and mucosa of the nose/turbinates in the 1000 ppm (50 mg/kg) animals ($p<0.05$). It should be emphasized that the tumor of the mucosa of the nose/turbinates was not reported in the first study (Pharmacopathics Research Laboratories, Inc., Study No. PR-80-006, May 20, 1983; Attachment B).

Dose(ppm)		0	40	200	1000
Observation:					
Liver:					
neoplastic nodule	M	1/70	2/69	1/70	1/70
	F	2/70	2/70	5/70	6/70
hepatocellular carcinoma	M	1/70	2/69	1/70	1/70
	F	2/70	1/70	0/70	1/70
Thyroid:					
follicular adenoma/cystadenoma					
	M	1/70	1/67	1/70	2/69
	F	1/70	2/69	2/70	4/70
Nose/Turbinates:					
adenoma	M	1/70	0/70	0/70	12*/70
	F	0/70	0/70	0/70	19*/70

* = $p < 0.05$ using Fisher's Exact test with Bonferroni Inequality

Based on the observations of decreased body weight gain, clinical chemistry observations, non-neoplastic findings and the neoplastic finding of an increase in papillary adenoma of the mucosa of the nose/turbinates in the males and females of the high dose group, it is apparent that the MTD was achieved in this study.

3. Since treatment related papillary adenomas of the nose/turbinates were noted in the repeat study (Monsanto Environmental Health Laboratory, Report No. MASL-6119, September 25, 1986; Attachment C), the sponsor reexamined the preserved tissues of the animals of the first study (Pharmacopathics Research Laboratories, Inc., Study No. PR-80-006, May 20, 1983; Attachment B) focusing on the posterior portion of the nasal cavity (not examined initially) and the following information was provided (Attachment D):

Dose(ppm)		0	500	1500	5000
Observation:					
Nose/Turbinates:					
papillary adenoma					
	M	0/69	1/70	6*/69	18*/69
	F	0/69	0/68	2/70	1/69
papillary adenocarcinoma					
	M	0/69	0/70	0/69	2/69
squamous cell carcinoma					
	M	0/69	1/70	0/69	1/69
	F	1/69	2/68	1/70	0/69
squamous papilloma					
	M	0/69	0/70	1/69	0/69
carcinoma in-situ					
	F	0/69	0/68	1/70	0/69
esthesioneuroma (benign)					
	M	0/69	0/70	0/69	1/69
epithelial inflammatory squamous metaplasia					
	F	0/69	0/68	1/70	0/69
submucosal glandular hyperplasia					
	F	0/69	0/68	0/70	2/69
inflammatory epithelial hyperplasia					
	M	1/69	0/70	3/69	2/69
	F	1/69	0/68	2/70	0/69
inflammation:					
nasolacrimal duct					
	M	1/69	8/70	5/69	6/69
	F	5/69	1/68	2/70	2/69
nasal mucosa					
	M	3/69	9/70	7/69	16**/69
	F	2/69	8/68	6/70	8/69

* = p < 0.05 using Fisher's Exact Test w/ Bonferroni Inequality

** = p < 0.01 using Fisher's Exact Test w/ Bonferroni Inequality

Peto test for trend found the following "p" values:

nasal papillary adenoma, males	0.000
nasal papillary adenoma, females	0.055
nasal papillary adenoma, both sexes	0.000
papillary adenocarcinoma, males	0.027
esthesioneuroma, males	0.062
all nasal malignancies, males	0.031

4. A statistical review of the data on the repeat 2-year chronic rat was conducted by the Science Support Section of the Science Analysis and Coordination Branch of HED (Attachment E). They found no statistically significant differences in survival in either the males or females. Tumor analysis found a linear trend in females rats for combined thyroid carcinomas and adenomas. No linear trend for thyroid tumors were found in the male rats. The incidence of papillary adenomas of the nose was statistically significantly increased in high dose males and females and there was a significant dose related trend.

5. In a mouse oncogenicity study (Pharmacopathics Research Laboratories, Inc., Report No. PR-80-007, May 4, 1983; Attachment F) doses of Acetochlor at 500 (75 mg/kg), 1500 (225 mg/kg) and 5000 ppm (750 mg/kg) in the diet for 23 months were used. An increase in tumor incidences of the liver (high dose males), lung (total lung tumors, all dosed females) and uterus (all dosed females) was noted along with a positive trend increase in tumors of the ovaries (benign tumors) and kidneys (adenomas, females).

Dose(ppm)		0	500	1500	5000
Observation:					
Liver:					
carcinoma	M	6/60	7/59	10/60	22[bc]/59
	F	1/60	0/60	0/60	4[c]/58
Lung:					
total tumors	M	13/60	13/60	16/60	8/60
	F	2/60	11[b]/60	12[b]/60	11[bc]/59
carcinoma	M	7/60	3/60	4/60	3/60
	F	0/60	5[a]/60	3/60	7[ac]/59
Uterus:					
sarcoma		0/59	6[b]/60	6[b]/60	5[a]/59
Ovaries:					
total benign tumors		0/59	0/60	5[a]/60	3[c]/58
Kidney:					
adenoma	M	2/60	1/60	1/60	2/60
	F	0/60	0/60	0/60	3[c]/59
adenocarcinoma	M	0/60	0/60	2/60	1/60
	F	0/60	0/60	0/60	0/60
sarcoma	M	0/60	0/60	0/60	0/60
	F	0/60	0/60	0/60	2/59

[a] = $p < 0.05$ using Fisher's Exact test

[b] = $p < 0.01$ using Chi Square test

[c] = $p < 0.01$ for linear trend using Peto analysis

C. Assessment of Mutagenicity

Several mutagenicity studies were conducted with Acetochlor:

a. Acetochlor was weakly mutagenic in the CHO/HGPRT assay in the presence and absence of S-9 metabolic activation (available on request).

b. Acetochlor was a mutagen in the presence of S-9 metabolic activation in the reverse mutation assay using L5178Y mouse lymphoma cells (available on request).

c. There was no evidence of mutagenicity in the mouse micronucleus assay. The high dose exhibited mortality and signs of clinical toxicity (available on request).

d. An AMES assay with Acetochlor was negative (Attachment G).

e. An in vivo cytogenic assay was negative for chromosomal aberration. The high dose exhibited evidence of a statistically significant body weight loss in both males and females (available on request).

f. A DNA-Damage-Repair assay in primary rat hepatocytes was negative for unscheduled DNA synthesis/repair at the highest dose tested (Attachment H).

g. The dominant lethal study could not be adequately evaluated with the provided data; a new study was requested (Attachment I).

D. **Assessment of Structure-Activity Relationship**

Acetochlor is structurally related to Alachlor, Butachlor and Metolachlor (Attachment J).

Alachlor is oncogenic in 2 species (rats and mice). In a dietary administration study in rats, nasal turbinate tumors were found at 42 mg/kg, stomach tumors at 126 mg/kg in both sexes and thyroid follicular adenomas at 146 mg/kg in males. In a dietary administration study in mice there was an increased incidence of liver tumors at 260 mg/kg in females. The PRC has classified Alachlor as a Category B2 oncogen and Alachlor has undergone Special Review (PD4 has been completed).

Butachlor is oncogenic in the rats in the form of stomach tumors at 3000 ppm (150 mg/kg) in females (dietary administration study). The Peer Review Committee has not evaluated this chemical.

Metolachlor in a dietary administration study in rats was found to cause a significantly elevated incidence of proliferative liver lesions (neoplastic nodules and carcinomas, combined) at 150 mg/kg in females. Further, examinations of the nasal turbinates found nasal malignancies (adenocarcinoma and fibrosarcoma) in 3/59 in the 150 mg/kg/day dose group vs. 0/67 in control. The mouse study (dietary administration) was negative for proliferative lesions. The PRC has tentatively classified Metolachlor as a Category C oncogen.

E. Previous Peer Review Committee Assessment

The Peer Review Committee (PRC) considered the following toxiciology data on Acetochlor to be of importance in a weight of evidence determination of oncogenic potential.

Rat: From the first study, increased incidence (statistically significant) of hepatocellular carcinoma and follicular cell adenoma of the thyroid of high dose males along with a positive trend for these tumor in males and a positive trend for incidence of hepatocellular carcinoma in females.

Mouse: An increase in incidence (statistically significant) of liver carcinomas in high dose males, total lung tumors in all dosed females, carcinomas in the low and high dose females, and total benign ovarian tumors in mid dose females. Further, there were positive trends for liver carcinoma of both sexes, pulmonary carcinoma, total lung tumors, ovarian benign tumors and kidney adenomas in females.

Mutagenicity: Studies found that Acetochlor was weakly positive in the CHO/HGPRT gene mutation assay at near toxic doses (vehicle also had some activity). It was also positive, under metabolic activation only, in the mouse lymphoma assay.

Structure-Activity: Acetochlor is structurally related to known or suspected oncogens (Alachlor, Butachlor, Metolachlor).

F. Issues addressed to the Peer Review Committee (PRC)

The Toxicology Branch-Herbicide, Fungicide, Antimicrobial Support requests the PRC reassess Acetochlor in light of the additional data provided by the registrant and to determine if the weight of evidence allows Acetochlor to remain classified as a Class B2 (probable human) oncogen and if this is in accord with the Agency's Guidelines for Carcinogen Risk Assessment.

ATTACHMENT A

007697

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

MAR 30 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Acetochlor

FROM: Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769c)

TO: Robert Taylor
Product Manager (25)
Registration Division (TS-767c)

The Toxicology Branch Peer Review Committee met on Sept. 12, 1985 to discuss and evaluate the weight-of-the-evidence on Acetochlor, with particular reference to its oncogenic potential.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with peer review unless otherwise stated).

Theodore M. Farber

William L. Burnam

Reto Engler

Donald Barnes

Louis Kasza

for Bertram Litt (OER not read)

John A. Quest

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Stephen Saunders

Laurence Chitlik

for Winnie Teeters

56

A. 3. Peer review members in absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Anne Barton

Stephen Johnson

Robert Beliles

Diane Beal

Judith Hauswirth

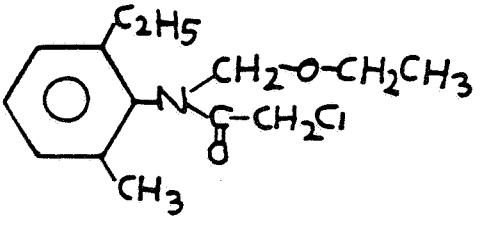
Esther Rinde

B. Material Reviewed:

The material available for review of acetochlor (Harness™) consisted of a chronic feeding study in the rat (DER) and an oncogenicity study in mice (DER), a metabolism study, and 4-mutagenicity studies.

C. Background Information:

Acetochlor, 2 chloro-N-ethoxymethyl-N-(2-ethyl-6-methylphenyl acetamide), is structurally related to alachlor, butachlor and metolachlor which have all been shown to be oncogenic in rats (alachlor was also oncogenic in mice).



ACETOCHLOR

D. Evaluation of Oncogenicity Evidence for Acetochlor

1. Chronic Feeding Toxicity and Oncogenicity Study in the Sprague-Dawley Rat.

Groups of 70 male and 70 female rats were fed 0, 500, 1500 or 5000 ppm acetochlor in their diet for 24/27 months (by month 24, there were still sufficient males in each group to allow the study to proceed to month 27).

Summary of the Incidence of the Most Frequently Observed Neoplastic Lesions in Rats Fed Diets Containing MON-097 (Acetochlor) for 24/27 Months

Organ/Lesion		0	500	1500	5000 PPM
Liver/					
Hepatocellular Adenoma	M	6/70	2/70	5/70	7/70
	F	0/70	2/70	2/70	2/70
Hepatocellular Carcinoma	M	0/70	2/70	3/70	6*[a]/70
	F	1/70	1/70	1/70	5[a]/70
Combined	M	6/70	4/70	8/70	13/70
	F	1/70	3/70	3/70	7/70
Testes/					
Interstitial cell tumor	M	2/70	4/70	4/70	7/70
Thyroid/					
C-cell adenoma	M	7/69	2/69	4/70	4/70
	F	4/69	1/69	1/69	0/69
Follicular cell adenoma	M	0/69	0/69	3/70	5*[a]/70
	F	2/69	0/69	0/69	3/69
Uterus/					
Adenocarcinoma	F	1/70	0/70	1/70	4/70

[a]Significant linear trend using the Cochran-Armitage test ($p<0.05$).
*Statistically different from control values ($p<0.05$) using the Fisher Exact test.

The incidences of hepatocellular carcinoma and follicular cell adenoma were significantly ($p<0.05$) increased at the high dose in male rats; a positive trend ($p<0.05$) was also noted for these tumors in males, as well as for the incidence of hepatocellular carcinoma in females.

The LOEL for chronic effects was determined to be 500 ppm, based on body and organ weight data; a NOEL for non-neoplastic effects was not established.

The MTD was exceeded at the high dose in both sexes, based on increased mortality.

D. Evaluation of Oncogenicity (continued)

2. Oncogenicity study of random-bred Swiss Albino CD-1 Mice

Groups of 60 male and 60 female mice were fed acetochlor (MON-097) in the diet for up to 23 months at dose levels of 0, 500, 1500, and 5000 ppm.

Incidence of Frequently Occurring Neoplastic Lesions in Mice Fed MON-097 for 23 Months[a]

Organ/Lesion		0	500	1500	5000 PPM
Harderian gland Adenoma	M	8/60	7/60	7/60	9/60
	F	3/60	1/60	5/60	4/59
Kidneys/	M	0/60	0/60	2/60	1/60
Adenocarcinoma	F	0/60	0/60	0/60	0/59
Adenoma	M	2/60	1/60	1/60	2/60
	F	0/60	0/60	0/60	3[b]/59
Sarcoma	M	0/60	0/60	0/60	0/60
	F	0/60	0/60	0/60	2/59
Total Malignant	M	0/60	0/60	2/60	1/60
Kidney tumors	F	0/60	0/60	0/60	2/59
Liver/	M	8/60	4/59	9/60	7/59
Adenoma	F	2/60	0/60	0/60	4/58
Carcinoma	M	6/60	7/59	10/60	22[b,c]/59
	F	1/60	0/60	0/60	4[b]/58
Combined	M	14/60	11/59	19/60	29/59
	F	3/60	0/60	0/60	8/58
Lungs/	M	6/60	10/60	12/60	5/6
Adenoma	F	2/60	6/60	8[d]/60	4/59
Carcinoma	M	7/60	3/60	4/60	3/60
	F	0/60	5[d]/60	3/60	7[b,d]/59
Histiocytic sarcoma	M	0/60	0/60	0/60	0/60
	F	0/60	0/60	1/60	0/59
Total Lung Tumors	M	13/60	13/60	16/60	8/60
	F	2/60	11[c]/60	12[c]/60	11[b,c]/59

(continued)

[a]Neoplastic lesions that occurred at a frequency of no more than one per dose group were excluded from this table unless significance was noted for total tumors of a given organ.

[b]Statistically significant linear trend ($p \leq 0.01$) using the Peto analysis.

[c]Statistically significant increase compared to control ($p \leq 0.01$) using the Chi-square test (uncorrected for continuity).

[d]Significantly different form control by Fisher's exact test ($p < 0.05$).

Incidence of Frequently Occurring Neoplastic Lesions in Mice Fed MON-097 for 23 Months[a] (continued)

Organ/Lesion		0	500	1500	5000 PPM
Lymphatic System Lymphoma	M	4/60	2/60	2/60	4/60
	F	6/60	7/60	12/60	1/59
Ovaries/					
Adenoma	F	0/59	0/60	1/60	0/58
Granulosa cell tumor	F	0/59	0/60	3/60	2/58
Luteoma	F	0/59	0/60	1/60	1/58
- Total benign ovarian tumors	F	0/59	0/60	5[d]/60	3[b]/58
Pituitary gland/					
Adenoma	M	0/58	0/49	0/58	1/54
	F	2/58	2/57	0/55	0/51
Uterus/					
Endometrial stromal polyp	F	1/59	2/57	2/60	2/59
Histiocytic sarcoma[1]	F	0/59	6[c]/57	6[c]/60	5[d]/59
Leiomyosarcoma	F	3/59	0/57	2/60	0/59

[a]Neoplastic lesions that occurred at a frequency of no more than one per dose group were excluded from this table unless significance was noted for total tumors of a given organ.

[b]Statistically significant linear trend ($p \leq 0.01$) using the Peto analysis (see note in DER).

[c]Statistically significant increase compared to control ($p \leq 0.01$) using the Chi-square test (uncorrected for continuity).

[d]Significantly different from control by Fisher's exact test ($p < 0.05$).

The MTD was exceeded in both sexes at the high dose, based on increased mortality and decreased body weights.

! It was noted that histiocytic sarcomas should be combined for all organ sites, however the incidence in other organs was not available.

D. 2. Oncogenicity Study in Mice, contd.

There was a statistically significant increase in the incidence of the following tumor types:

Liver carcinomas in males at the high level ($p<0.01$),
Total lung tumors in females at all levels ($p\leq 0.01$),
Carcinomas of the lungs in low and high level females ($p<0.05$),
Uterine histiocytic sarcomas in females at all levels ($p\leq 0.01$ at 500 & 1500 ppm; $p< 0.05$ at 5000 ppm)
Total benign ovarian tumors in mid-level females ($p<0.05$).

There were positive linear trends for: liver carcinomas in both sexes, and pulmonary carcinomas, total lung tumors, total ovarian benign tumors and kidney adenomas in females.

§ §

The preceding statistical analyses and tumor incidences were reported in the DER provided by Dynamac. In a Lab/Data Audit Memo (memo not dated-Audit performed Oct. 1985 and provided to HED on Nov. 4, 1986) Dr. Adrian Gross indicates that there are discrepancies in the statistical analyses of the mouse tumors obtained by him, and those obtained by Dynamac.

Dr. Gross also states in his memo that "the significance as estimated by Dynamac for..." the liver carcinomas in male and female rats "...was understated". (Dynamac found for these tumors, $p<0.05$, whereas Dr. Gross found $p<<0.01$.)

The "discrepancies" described by Dr. Gross are small differences in the p value and/or in the expression of the p value; nevertheless, the overall conclusions are the same.

In this same memo, Dr. Gross also indicates an apparently very different incidence for "kidney" tumors in the mouse from that reported in the DER (cf: pg. 4), as follows:

		0	500	1500	5000 PPM
Carcinoma	M	6/60	7/59	10/60	22/59
	F	1/60	0/60	0/60	4/58
Adenoma	F	2/60	0/60	0/60	4/58

However, the above data are actually for the liver tumors; it appears that on pages 4, and 5 of the Gross memo the word "kidney" was inadvertently substituted for liver. Thus, there is no discrepancy in the tumor incidence as reported by Dynamac and by Dr. Gross.

The above information was not available to the Peer Review Committee at the time of the meeting on acetochlor.

5. Historical Control Information: Not presented to the Committee.

E. Additional Toxicology Data on Acetochlor:

1. Metabolism: In a rat study, acetochlor was extensively metabolized with less than 1% parent compound found in the feces and none detectible in the urine. The early (<24 hour) metabolites were mostly mercapturates; later ones mostly sulfoxides, sulfones and sulfates; 20 metabolites were identified (Figures 1, 2, 3). Early conjugation with glutathione is assumed. The only structure retaining significant amounts of labeled acetochlor (about 2.5%) was the erythrocyte; the turnover rate of erythrocytes in the rat correlates well with the slow phase of acetochlor elimination. (Acetochlor was rapidly excreted with the urinary route accounting for about twice the percentage of the fecal route; pulmonary excretion was insignificant. Elimination was biphasic, with a rapid and slow phase.) There did not appear to be any significant sex differences in the metabolism of acetochlor.

2. Non-Oncogenic Toxicological Effects

In a one-year feeding study in dogs, the high dosed group (40 mg/kg) showed testicular atrophy (6/6) accompanied by decreased absolute and relative (to body weight) testicular weight, decreased body weight gain of males an decreased terminal body weight of females. There was also suggestive evidence at the high level for anemia and hepatotoxicity, but a NOEL and LOEL for these effects could not be be conclusively determined. There was also suggestive evidence for effects on adrenal weights.

In a two generation reproduction study in rats, a slight decrease (about 20%) in litter size was noted at the high dose (5000 ppm) in all matings. The high dose also caused decreased pup body weight gain during lactation for both generations; this effect was also seen in male F_{2b} pups at the mid level (1500 ppm).

Acetochlor was negative at a dose of 400 mg/kg in a rat teratology study.

MON 097 Technical, and an "E.C" formulation were positive dermal sensitizers in the guinea pig.

3. Mutagenicity:

Acetochlor was weakly positive in the CHO/HGPRT gene mutation assay at near toxic doses, but the vehicle used (alcohol) had some activity. It was also positive (with activation only) in the mouse lymphoma test. Negative results were obtained in the Ames salmonella test, in the hepatocyte DNA repair test, and in an unacceptable bone marrow chromosome aberration assay.

ACETOCHLOR Tox review 007697

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Pages 63 through 65 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

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____ Description of quality control procedures.

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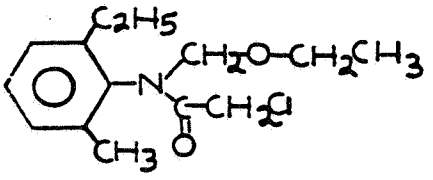
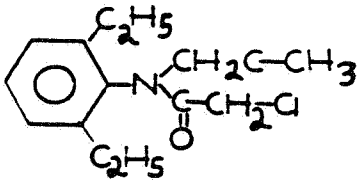
The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

4. Structure-Activity Correlations:

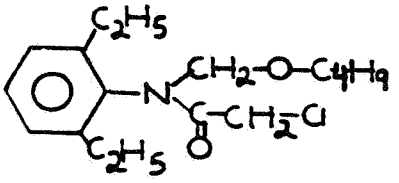
Acetochlor is structurally related to Alachlor, Butachlor, Metolachlor, and Propachlor.



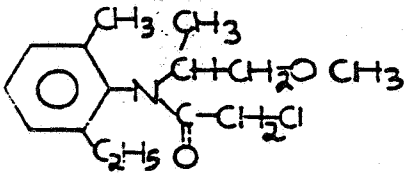
Acetochlor



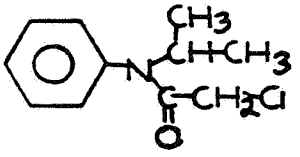
Alachlor



Butachlor



Metolachlor



Propachlor

Alachlor is oncogenic in both rats and mice. In rats, it caused nasal turbinate (42 mg/kg) and stomach tumors (126 mg/kg) in both sexes, and thyroid follicular adenomas in males (146 mg/kg). In mice, there was an increased incidence of lung tumors in females (260 mg/kg). Alachlor was evaluated by the Peer Review Committee as a Category B2 oncogen, and is presently undergoing Special Review.

Butachlor (Machete®) causes stomach tumors (defined as masses at necropsy) in female rats (3000 ppm). Butachlor has not been evaluated by the Peer Review Committee.

Metolachlor caused a significantly elevated incidence of proliferative liver lesions (neoplastic nodules and carcinomas, combined) at the highest dose level tested (3000 ppm) in female rats. The mouse oncogenicity study was negative for proliferative lesions. Metolachlor was evaluated by the Peer Review Committee as a tentative Category C oncogen (due in part to the borderline quality of the data).

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Propachlor (Ramrod®) was tested by Industrial Bio-Test Laboratories and this study must be repeated.

F. Weight of Evidence Considerations:

The committee considered the following facts regarding toxicology data on acetochlor to be of importance in a weight of evidence determination of oncogenic potential.

Rats:

The incidences of hepatocellular carcinoma and follicular cell adenoma of the thyroid were significantly ($p<0.05$) increased at the high dose in male rats; a positive trend ($p<0.05$) was also noted for these tumors in males, as well as for the incidence of hepatocellular carcinoma in females.

Mice:

There was a statistically significant increase in the incidence of: liver carcinomas in males at the high level ($p<0.01$); total lung tumors in females at all levels ($p<0.01$); carcinomas of the lungs in low and high level females ($p<0.05$); in uterine histiocytic sarcomas at all dose levels ($p<0.01$ low and mid dose; $p<0.05$ high dose) and total benign ovarian tumors in mid-level females ($p<0.05$). There were positive linear trends ($p<0.01$) for: liver carcinomas in both sexes, and for pulmonary carcinomas, total lung tumors, ovarian benign tumors and kidney adenomas in females.

(See also page 6 and attached memo from Adrian Gross.)

* * * * *

Acetochlor was weakly positive in the CHO/HGPRT gene mutation assay at near toxic doses, but the vehicle used (alcohol) had some activity. It was also positive (with activation only) in the mouse lymphoma test. Negative results were obtained in the Ames salmonella test, hepatocyte DNA repair tests and in an unacceptable bone marrow chromosome aberration assay.

Acetochlor is structurally related to Alachlor, Butachlor, Metolachlor, and Propachlor:

- Alachlor is oncogenic in both rats and mice. In rats, it caused nasal turbinate (42 mg/kg) and stomach tumors (126 mg/kg) in both sexes, and thyroid follicular adenomas in males (146 mg/kg). In mice, there was an increased incidence of lung tumors in females (260 mg/kg).

- Butachlor (Machete™) causes stomach tumors (defined as masses at necropsy) in female rats (3000 ppm).

- Metolachlor caused a significantly elevated incidence of proliferative liver lesions (neoplastic nodules and carcinomas, combined) at the highest dose level tested (3000 ppm) in female rats. The mouse oncogenicity study was negative for proliferative lesions.

- Propachlor (Ramrod™) was tested by Industrial Bio-Test Laboratories and this study must be repeated.

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR 51: 33992-34003, 1986] for classifying a carcinogen were considered.

The weight of evidence for acetochlor is summarized as follows:

°Acetochlor was oncogenic in the rat (hepatocellular carcinoma in both sexes and thyroid follicular cell adenoma in males).

°Acetochlor was oncogenic in the mouse (hepatocellular carcinoma in both sexes, lung carcinoma in females, uterine histiocytic sarcomas and benign ovarian tumors in females, kidney adenomas in females).

°Acetochlor is structurally related to known or suspected oncogens (alachlor, butachlor, metolachlor.

°Acetochlor was mutagenic in mammalian cell culture tests: CHO/HGPRT (weakly positive) and in the mouse lymphoma test.

Based on the above evidence, acetochlor meets the criteria for Group B2 - Probable Human Carcinogen (causes an "increased incidence of malignant or combined malignant and benign tumors in multiple species"). Additional evidence for this classification was provided by SAR and mutagenicity.

ATTACHMENT B 14

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-01-6561
TASK: 109
July 29, 1985

DATA EVALUATION RECORD

ACETOCHLOR

Chronic Feeding Toxicity and Oncogenicity Study in the Rat

STUDY IDENTIFICATION: Ahmed, F. E., Seely, J. C. MON 097: Chronic toxicity and oncogenicity study in the rat. (Unpublished study No. PR-80-006, prepared by Pharmacopathics Research Laboratories, Inc., Laurel, MD, for Monsanto Company, St. Louis, MO; dated May 20, 1983.) Accession Nos. 071962 - 071965.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: Irvin Cwande/fn

Date: 7/26/85

1. CHEMICAL: MON 097, 2-chloro-N(ethoxymethyl)-6'ethyl-ortho-aceto-toluidine, a herbicide (acetochlor).

2. TEST MATERIAL: The test material was from Lot # NBP 1737874 with 94.5% purity. The compound was described as a maroon liquid with a characteristic odor.

3. STUDY/ACTION TYPE: Chronic feeding toxicity and oncogenicity study in the rat.

4. STUDY IDENTIFICATION: Ahmed, F. E., Seely, J. C. MON 097: Chronic toxicity and oncogenicity study in the rat. (Unpublished study No. PR-80-006, prepared by Pharmacopathics Research Laboratories, Inc., Laurel, MD, for Monsanto Company, St. Louis, MO; dated May 20, 1983.) Accession Nos. 071962 - 071965.

5. REVIEWED BY:

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Date: 7-30-85

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Signature: Laurence D. Chitlik
Date: 8/5/85

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7. CONCLUSIONS:

A. Under the conditions of this chronic/oncogenicity feeding study with Sprague-Dawley rats, there was increased mortality in females receiving the high dose (5000 ppm). There was a significant ($p < 0.05$) dose-related decrease in the mean body weights of males and females receiving the mid (1500 ppm) and high doses, and a significant ($p < 0.05$) decrease in food consumption by males and females receiving the high dose. A decrease in the mean body weight of males receiving the low dose (500 ppm) also reached a significant ($p < 0.05$) level at the end of the study (weeks 103 to 115). Histopathologic examination of the tissues indicated increased incidences of polyarteritis of the testis and arteries of males and liver necrosis and alveolar histiocytosis in females receiving the high dose ($p < 0.05$). There was also a statistically significant increase in the incidences of liver carcinomas and thyroid adenomas in males receiving the high dose ($p < 0.05$). In addition, a compound-related positive trend ($p < 0.05$) was noted for the incidences of liver carcinomas in males and females and thyroid follicular cell adenomas in males.

Based on body and organ weight data, the LOEL for chronic effects is 500 ppm (LDT).

B. The study is classified as core minimum, although a NOEL for non-neoplastic effects was not established; one must be established in a new chronic study.

8. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

A photocopy of the detailed materials and methods used in this study are presented in Appendix A; the following is a brief description:

1. The test material, MON-097, was mixed with the basic diet at specified amounts and provided to the rats ad libitum. Diets were prepared fresh weekly to give dietary levels of 0, 500, 1500, and 5000 ppm.

2. The rats were random-bred Sprague Dawley, Cesarean-derived weanlings purchased from Charles River Breeding Laboratories, Wilmington, MA. Of the 640 rats purchased, 20 were used for baseline studies, 560 were used for the lifetime phase of the study, and the remaining 60 rats were sacrificed on day zero. Animals were acclimatized to laboratory conditions and randomly assigned into four groups, based on body weight; each consisted of 70 males and 70 females.

3. Test diets were analyzed at various intervals throughout the study (weeks 1, 2, 3, 4, 6, 8, 12, 18, 24, 30, 36, 42, 48, 52, 60, 78, 90, and 104) for MON-097 concentrations, homogeneity and 7-day stability.

4. Clinical observations were performed twice daily, and body weight and food consumption were determined weekly during the first 13 weeks and biweekly thereafter. Clinical chemistry, hematology and urinalysis determinations were performed on 10 males and 10 females per group one week prior to study initiation and at 6, 12, 18, and 24/27 months.

5. All rats that died or that were sacrificed when moribund, sacrificed at month 12 (interim, 10/group/sex), or sacrificed at termination were necropsied and tissues were examined histologically. Organ weights were determined for animals sacrificed at month 12 and at termination.

6. Food consumption and body weight data were statistically analyzed by one-way analysis of variance (ANOVA) using F-test for variance comparison, and significant differences were further analyzed by Dunnett's test. Clinical chemistry, hematology and organ-weight data were analyzed by the independent, two-sided t-test. The histopathology data were analyzed for statistical significance by the sponsor using the Cochran-Armitage test for linear trend, the chi-square test and the Peto method which utilizes survival and time to tumor information; these analyses utilized a p value of < 0.01 for significance.

9. REPORTED RESULTS:

A. Clinical Observation and Mortality: The most frequently observed clinical signs in both sexes in all groups were opaque eyes and alopecia. Opaque eyes were observed much earlier, i.e., by week 9, and occurred at a higher incidence in males than females; whereas alopecia was observed much earlier and at a higher incidence in females than in males. However, these effects were apparently not compound-related. Skin lesions and tumors were also evident by month 7 for both males and females. The incidences were progressively higher in females than in males, but were similar among dosed and control animals.

Ophthalmology examinations during the study did not reveal any compound-related effects.

Increased mortality was noted in females receiving the high (5000 ppm) dose by month 12 when compared to control animals (Table 1). Due to increased mortality in the high-dose group, all females were sacrificed by week 103 of the study when survival was

TABLE 1. Percent Survival of Rats Fed Diets Containing MON-097 for 24/27 Months

Dietary Level (ppm)	Percent Survival (No. of Dead Animals) at Month			
	12[a]	18	24[b]	27
Males				
Control	98.6(1)	86.7(8)	53.3(28)	31.7(41)
500	100.0(0)	90.0(6)	55.0(27)	33.3(40)
1500	97.1(2)	86.7(8)	65.0(21)	45.0(33)
5000	94.3(4)	86.7(8)	58.3(25)	25.0(45)
Females				
Control	98.6(1)	91.7(5)	41.7(35)	—
500	98.6(1)	71.7(17)	31.7(41)	—
1500	97.1(2)	83.3(10)	43.3(34)	—
5000	91.4(6)	68.3(19)	18.3(49)	—

[a] Interim sacrifice animals (10/sex/group) included in percent survival calculations.

[b] Females were sacrificed during week 103 of the study.

18.3% in the high-dose group. There were no compound-related mortalities noted throughout the study in males when compared to controls. By month 24, there were still sufficient males in each group to allow the study to proceed to month 27.

B. Diet Analyses: The concentrations of MON-097 in freshly prepared diets throughout the study were within acceptable limits of the theoretical. Mixing efficiency values ranged between 85 - 113 percent with a few exceptions. Analyses conducted 7 days after diet preparation throughout the study to determine the compound's stability were variable (range 83-118) and no specific pattern, i.e., decrease or increase, compared to day 1 could be detected.

C. Body Weight Determinations: A significant compound-related decrease in mean body weights of males receiving the mid and high doses was noted throughout the study when compared to controls (Table 2). Similarly a significant compound-related decrease in mean body weights of females receiving the high dose was noted during the study when compared to controls. Females receiving the mid-dose (1500 ppm) showed decreased mean body weight between weeks 31 and 103 of the study, but not all intervals were statistically different from control values. There were no significant compound-related effects on body weight in animals receiving the low dose, except in males at study termination. Mean body weights of low-dose males decreased gradually after week 103 of the study and were approximately 14% lower than the control group by termination

D. Food Consumption: Compound-related decreases in mean food consumption were noted at a few time intervals during the study in males and females receiving the high dose. A few isolated incidences of reduced food consumption were also noted for animals receiving the mid dose. There were no other changes noted (Table 3). Feed efficiency data for the first 13 weeks of the study indicated that high-dose animals of both sexes did not utilize feed as efficiently as the other groups. This was in agreement with reduced body weight data. Compound intake data indicated that the amount of compound consumed at the early weeks of the study was higher, as expected, because of the fast rate of growth. The time-weighted average was 22, 69, and 250 mg/kg body weight for males and 30, 93, and 343 mg/kg/body weight for females receiving the low- (25 mg/kg), mid- (75 mg/kg), and high- (250 mg/kg) doses, respectively.

E. Hematology: Females receiving the high dose showed a slight but significant decrease in hemoglobin and hematocrit values for months 6, 12 and 18, but not for month 24 of the study (Table 4). There were no other compound-related changes noted in the parameters investigated for males and females at months 6, 12, 18, or 24/27 of the study.

TABLE 2. Selected Mean Body Weights of Rats Fed Diets Containing MON-097 for 24/27 Months

Dietary Level (ppm)	Group Mean Body Weight (g) at Week[a]						
	0	13	27	53	79	103[b]	115
Males							
Control	174.5 18.4	511.5 50.5	584.0 67.4	693.1 88.2	752.4 102.4	751.8 130.2	745.4 104.1
500	170.6 17.5	495.7 47.8	568.7 62.6	677.8 93.9	731.5 110.9	721.0 117.5	640.9* 131.4
1500	170.4 17.1	472.1* 46.3	538.0* 49.6	631.0* 77.3	678.6* 91.0	664.7* 111.6	618.7* 126.1
5000	172.1 16.6	418.9* 49.1	479.5* 49.2	534.5* 62.9	545.9* 69.6	529.2* 67.3	479.8* 65.1
Females							
Control	147.0 14.1	315.1 32.6	358.0 43.0	450.6 77.4	483.6[c] 93.5	449.5 92.3	—
500	144.6 12.0	318.7 27.7	354.9 41.7	445.4 75.3	491.2 85.1	503.3 75.4	—
1500	147.9 10.3	307.8 33.2	342.3 45.4	416.6* 75.9	437.4* 93.9	431.4 101.4	—
5000	146.5 11.1	269.4* 22.8	284.0* 32.7	302.8* 48.8	308.2* 60.5	308.1* 42.3	—

[a] Mean value and standard deviation.

[b] Females were sacrificed during week 103 of the study.

[c] Value corrected by reviewers (original value being 486.5).

* Statistically different from control value ($p < 0.05$).

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TABLE 3. Selected Mean Food Consumption of Rats Fed Diets containing MON-097 for 24/27 Months

Dietary Level (ppm)	Mean Food Consumption (g/rat/week) at Week						
	0	13	27	53	79	103[a]	115
Males							
Control	154.1	171.5	170.4	160.4	175.0	180.4	165.6
500	153.6	170.7	163.0	170.6*	181.4	176.0	151.8
1500	156.4	165.6	158.9	166.1	181.0	171.7	166.1
5000	124.2*	164.4	162.4	145.8*	159.4*	155.1*	161.1
Females							
Control	131.4	131.2	149.9	160.9	178.4	153.6	--
500	135.1	140.4	147.0	158.7	186.6	151.4	--
1500	133.8	135.4	146.4	155.1	170.2	161.7	--
5000	110.4*	130.1	141.6	119.6*	137.4*	138.9	--

[a] Females were sacrificed during week 103 of the study.

* Significantly different from control value ($p < 0.05$)

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TABLE 4. Mean Hemoglobin and Hematocrit Values of Female Rats Fed Diets Containing MON-097 for 24 Months

Dietary Level (ppm)	Mean Hemoglobin (g %) and Hematocrit (pc %) values on months 0		6		12		18		24	
	Hgb	Hct	Hgb	Hct	Hgb	Hct	Hgb	Hct	Hgb	Hct
Control	12.2	39.0	15.0	44.3	14.1	45.0	13.9	41.2	12.9	39.6
500	-	-	14.8	42.7*	14.3	43.5	14.1	41.0	13.3	40.2
1500	-	-	13.9*	43.9	14.3	42.1	13.3	40.5	13.1	40.2
5000	-	-	14.2*	41.6*	12.5*	40.1*	11.4*	34.8*	12.7	39.5

*Statistically different from control value ($p < 0.05$).

F. Clinical Chemistry: There were some isolated significant differences noted in blood chemistry parameters among control and dosed groups. These differences were not consistent over time and were apparently not compound-related.

G. Urinalysis: There were no compound-related changes noted in urine chemistry values and microscopic examination of urine sediments of males and females throughout the study.

H. Gross Examination: Gross pathology findings were summarized for each organ system instead of specific tissues and lesions were not specified, except on individual animal pathology sheets. However, individual animal data indicate that all gross lesions were examined histologically. There was a slight increase in urinary lesions noted in animals receiving the high dose at the 12-month interim sacrifice. For high-dose animals that died or were sacrificed moribund during the second year of the study, an increase in the number of lesions in the following systems was noted: the cardiovascular system of males and females, the endocrine system of males, and urinary and reproductive systems of females. In addition, an increase in the number of lesions of the urinary system of all dosed male groups was observed. At terminal sacrifice, increased lesions were noted in the urinary system of the mid- and high-dose males and high-dose females.

I. Organ Weights: There were no significant differences in organ weights and organ-to-body weight ratios among control and dosed males at the one-year interim sacrifice. In females, lower mean adrenal weights in mid- (0.13 g) and high-dose (0.10 g) animals (and lower mean adrenal-to-body weight ratios in high-dose animals) were observed when compared to control (0.19 g). However, the authors stated that the mean adrenal weight of the corresponding female control at month 12 was almost twice as high as the value for historical control rats (0.08 - 0.11 g) of that age.

At final sacrifice, the mean brain and heart weights of mid- and high-dose males and the mean brain weight of mid- and high-dose females were lower than the control values. These decreases were accompanied by corresponding increases in organ-to-body weight ratios (Table 5) The mean pituitary, heart, and adrenal weights of high-dose females were lower than the control values, but the organ-to-body weight ratios were similar among control and dosed animals. The mean thyroid/parathyroid weights and organ-to-body weight ratios in all dosed females were significantly higher when compared to control values (Table 5). In addition, the mean relative weights of thyroid in mid- and high-dose males, and the relative weights of liver, adrenals, kidneys, and testis in high-dose males and liver and kidneys in high-dose females were significantly higher than control values.

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TABLE 5. Organ Weight Data for Rats Fed Diets containing MON-097 for 24/27 Months

Dietary Level (ppm)	Organ									
	Brain		Thyroid		Heart		Adrenals		Gonads	
	W[a] (g)	RW (%)	W (g)	RW (%)	W (g)	RW (%)	W (g)	RW (%)	W (g)	RW (%)
Males										
Control	2.23	3.03	0.05	0.07	2.12	2.83	0.10	0.14	3.48	4.72
	0.097	0.472	0.020	0.026	0.368	0.371	0.037	0.049	0.522	0.969
500	2.19	3.45*	0.06	0.09	2.01	3.14	0.12	0.19	3.52	5.44
	0.125	0.774	0.034	0.05	0.453	0.898	0.069	0.125	0.761	1.272
1500	2.15*	3.65*	0.05	0.09*	1.87*	3.13	0.20	0.36	3.26	5.41
	0.109	0.835	0.010	0.022	0.324	0.766	0.408	0.788	0.751	1.441
5000	2.11*	4.56*	0.07	0.15*	1.61*	3.42*	0.09	0.20*	4.45	9.85*
	0.115	0.888	0.024	0.053	0.266	0.663	0.018	0.067	3.180	8.522
Females										
Control	2.19	5.01	0.03	0.06	1.66	3.75	0.21	0.48	0.25	0.55
	0.131	1.286	0.007	0.013	0.285	0.912	0.163	0.362	0.407	0.809
500	2.13	4.39	0.04*	0.08*	1.68	3.47	0.17	0.36	0.17	0.35
	0.119	0.690	0.008	0.018	0.324	0.583	0.097	0.220	0.177	0.415
1500	2.09*	5.11	0.04*	0.09*	1.60	3.84	0.17	0.42	0.33	0.68
	0.084	1.058	0.012	0.030	0.441	1.118	0.101	0.249	0.927	1.718
5000	1.99*	6.59*	0.04*	0.12*	1.20*	3.91	0.10*	0.34	0.13	0.45
	0.097	1.150	0.013	0.044	0.231	0.748	0.021	0.100	0.114	0.390

[a] W - weight.
RW - organ-to-body weight ratio.

* Statistically different from control value ($p < 0.05$).

J. Histopathology: At interim sacrifice, an increase in the incidence of prostatitis was noted in males and hemosiderosis of the spleen in females receiving the high dose. There were no other effects noted. A summary of the most frequently observed non-neoplastic lesions at 24/27 months is presented in Table 6. There was a significant increase in the incidence of liver necrosis ($p < 0.05$) and alveolar histiocytosis ($p < 0.05$) in females receiving the high dose. There was also a significant linear trend ($p < 0.05$) in the incidences of peripheral nerve neuropathy, heart thrombosis, and stomach fibrosis. In males, a significant increase in the incidences of polyarteritis of the testes and in polyarteritis of the arteries ($p < 0.05$) was noted in animals receiving the high dose. A significant linear trend ($p < 0.05$) was also noted for these lesions.

A summary of the most frequently observed neoplastic lesions is presented in Table 7. The incidence of hepatocellular carcinomas was significantly higher (Fisher Exact test) in males receiving 5000 ppm when compared to control and there was a significant dose-related trend. The incidence was also higher in females receiving 5000 ppm, and although there was a significant dose-related trend ($p < 0.05$), the incidence was not significantly different from control using the Fisher Exact test. The data also indicated an increase in the incidence of liver adenomas in the concurrent control (and dosed) males when compared to historical controls from the testing laboratory. The latter were reported to be 2 of 401 (0.5%) at final sacrifice, whereas in concurrent controls the incidence was 3 of 19 (15.8%) males. There was also an increase in the incidence of follicular cell adenoma of the thyroid in males receiving the high dose (Fisher Exact test, $p < 0.05$) and a dose-related trend (Cochran-Armitage test, $p < 0.05$). In addition, an increase in the incidence of interstitial cell tumors of the testes was noted in males receiving the high dose, but the increase was not statistically significant and did not show a linear trend.

10. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that MON-097 fed to this strain of rat caused a statistically significant decrease in food consumption in both high-dose groups, and a decrease in body weights of mid- and high-dose males and females. In addition, dose-related increases in thyroid follicular cell adenomas in mid- and high-dose males, hepatic carcinomas in low-, mid-, and high-dose males and high-dose females, and testicular interstitial cell tumors in all dosed males were observed. These neoplastic changes were considered by the authors to be compound related due to the increased incidences noted when compared to the concurrent and historical control data (see Histopathology Section). Statistical analysis of the histopathology data was not performed by the study authors but was conducted by the sponsor; these analyses are discussed below.

TABLE 6. Summary of Most Frequently Observed Nonneoplastic Lesions in Rats Fed Diets Containing MON-097 for 24/27 Months (continued)

Organ/Lesion		Males				Females			
		0	500	1500	5000	0	500	1500	5000
Peripheral nerve	N[a]	69	67	70	67	66	70	67	63
Neuropathy		1	0	1	1	0	0	0	4[d]
Pituitary	N	68	70	70	70	70	70	70	67
Hyperplasia		3	5	3	3	4	6	6	4
Prostate	N	70	70	70	69	-	-	-	-
Prostatitis		19	18	18	15				
Skin	N	70	69	70	70	70	69	70	70
Granuloma foot-pad		15	17	22	11	19	10	10	0
Spleen	N	70	70	70	70	70	70	70	70
Hemosiderosis		18	13	10	10	20	15	20	27
Stomach	N	70	70	70	70	70	70	70	70
Fibrosis		13	10	13	14	4	5	7	12[d]
Testes	N	70	70	70	70	-	-	-	-
Polyarteritis		7	11	12	17*[b,d]				
Thyroid	N	69	69	70	70	69	69	69	69
C-cell hyperplasia		2	3	2	3	0	0	1	0
Uterus		-	-	-	-	70	70	70	70
Endometritis						10	13	11	5

[a]Number of tissues examined, including interim sacrifice animals.

[b]Statistical analysis conducted by our reviewers, using the Fisher Exact test.

[c]Statistical analysis conducted by the sponsor.

[d]Significant linear trend using the Cochran-Armitage test ($p < 0.05$).

*Statistically different than control values ($p < 0.05$).

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TABLE 7. Summary of Most Frequently Observed Neoplastic Lesions in Rats Fed Diets Containing MON-097 for 24/27 Months

Organ/Lesion		Males				Females			
		0	500	1500	5000	0	500	1500	5000
Adrenals	N[a]	70	70	70	70	70	70	70	70
Pheochromocytoma (benign)		4	5	4	1	0	0	0	1
Liver	N	70	70	70	70	70	70	70	70
Hepatocellular adenoma		6	2	5	7	0	2	2	2
Hepatocellular carcinoma		0	2	3	6*[bd]	1	1	1	5[bd]
Hemangiosarcoma		0	0	0	0	1	0	0	1
Mammary gland	N	12	18	10	11	67	69	67	55
Adenoma		0	0	0	0	7	12	7	2
Fibroadenoma		1	0	0	0	50	61	64	39
Adenocarcinoma		0	0	0	0	13	13	13	7
Pancreas	N	69	70	70	70	70	70	70	70
Islet cell adenoma		10	11	10	8	2	1	0	1
Pituitary	N	68	70	70	70	70	70	70	67
Adenoma		23	18	23	19	35	41	34	24
Carcinoma		13	9	5	4	17	6	13	4
Testes	N	70	70	70	70	-	-	-	-
Interstitial cell tumor		2	4	4	7				
Thyroid	N	69	69	70	70	69	69	69	69
C-cell adenoma		7	2	4	4	4	1	1	0
Follicular cell adenoma		0	0	3	5*[bd]	2	0	0	3
Uterus		-	-	-	-	70	70	70	70
Adenocarcinoma						1	0	1	4

[a]Number of tissues examined, including interim sacrifice animals.

[b]Statistical analysis conducted by our reviewers, using the Fisher Exact test.

[c]Statistical analysis conducted by the sponsor.

[d]Significant linear trend using the Cochran-Armitage test ($p < 0.05$).

*Statistically different than control values ($p < 0.05$) using the Fisher Exact test.

For other parameters investigated in this study, the sponsor was in agreement with the conclusions of the authors except for some hematology results. The study authors considered that only the statistically significant decrease at month 18 in the mean hemoglobin count in females receiving the high-dose was compound-related, whereas, the sponsor indicated that the decrease in both hemoglobin and hematocrit values in this group at months 6, 12, and 18 was compound-related.

B. Quality assurance inspections were performed periodically throughout the study.

11. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Our evaluation of the results of this chronic toxicity/oncogenicity study with MON-097 in rats indicates that it was adequately conducted and reported yet there were some deficiencies noted e.g., summarizing gross examination data by systems, rather than by organ or tissues, weighing organs after fixation and use of a $p < 0.01$ rather than 0.05 for statistical significance of histopathologic data. The conclusions of the authors are supported by the data.

The results indicate increased mortality in females receiving 5000 ppm of test material in the diet (high dose). However, adequate numbers of animals were alive after month 18 to allow for evaluation of late-developing tumors. There was also a compound-related decrease in the mean body weights of males and females receiving 1500 and 5000 ppm of test material, although the decrease in mid-dose females was less pronounced. In addition a decrease in the mean body weights of males receiving the low dose by about week 103 was noted; this effect appears to be biologically significant and compound-related. Decreased food consumption and food efficiency was also observed in males and females receiving the high dose throughout the study. There were no compound-related effects in clinical signs and eye examinations, hematology, blood chemistry, and urinalysis noted in dosed animals, except for lower hemoglobin and hematocrit values in females receiving the high dose at months 6, 12, and 18, but not month 24. Changes in organ weights and organ-to-body weight ratios were usually associated with lower body weights of dosed animals. However, it should also be noted that the organs were weighed after fixation in 10% buffered formalin. Consequently, these changes could not be definitively related to compound administration, except for the liver, thyroid, and testis, where the animals exhibited histopathologic changes.

Individual animal data indicate that all gross lesions were further examined histologically. There were some compound-related effects

noted during histologic examination of the tissues. Based on statistical analysis conducted by these reviewers, nonneoplastic lesions included increased incidences of polyarteritis of the testes and arteries of males and liver necrosis and alveolar histiocytosis of females receiving the high dose ($p < 0.01$). In addition, the statistical analyses of the data by the sponsor indicated significant linear trends for these lesions ($p < 0.01$) as well as significant increases in the incidences of liver necrosis and alveolar histiocytosis and inflammation of the tongue in females receiving the high dose.

Neoplastic lesions were also noted and the data were analyzed statistically by our reviewers. A significant increase ($p < 0.05$) in liver carcinomas and thyroid follicular cell adenomas was noted in males receiving 5000 ppm using the Fisher Exact test. A statistically significant increase in the incidences of liver carcinomas in the females receiving the high dose was not observed by our reviewers using either the Fisher Exact test or the Peto method at a $p < 0.05$. However, a significant positive trend (Cochran-Armitage test) in the incidences of liver carcinomas in females ($p < 0.05$) as well as in the incidence of liver carcinomas and thyroid follicular cell adenomas in males ($p < 0.05$) was noted.

The statistical analyses of neoplastic lesions conducted by the sponsor indicated only a linear trend in the incidence of liver carcinomas for both sexes combined ($p < 0.01$) and a significant increase in the incidence of liver carcinomas for both sexes combined using the Peto method ($p < 0.01$). In addition, a positive trend was noted for the thyroid follicular cell adenomas. It should be noted, however, that the sponsor utilized a p value of 0.01 instead of 0.05 which is more commonly accepted, and is an EPA policy.

The following deficiencies were noted: organs were weighed following fixation in 10% buffered formalin and necropsy data were reported as the number of lesions per organ system.

Based on body weight data the LOEL for chronic effects is 500 ppm (LDT) of test material in the diet, and a NOEL cannot be established.

12. CBI APPENDIX: Appendix A, Materials and Methods, CBI, pp. 9-22.

ATTACHMENT C

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

006571

JAN 29 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a chronic/oncogencity study in rats with MON 097 (Acetochlor, Harness® and Top-Hand® Herbicides). EPA ID #'s 524-GUI & 3F2966; EPA Record #'s 195381 & 195383; EPA Accession # 400770601; Caswell #3B; Tox Branch Project #7-0702.

TO: Robert Taylor/Vickie Walters (PM #25)
Fungicide/Herbicide Branch
Registration Division (TS-767C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 1/29/88
Pharmacologist, Review Section V
Toxicology Branch/HED (TS-769C)

THRU: Quang Q. Bui, Ph.D., D.A.B.T. Quang Q. Bui 1/20/88
Acting Section Head, Review Section V
and
Theodore M. Farber, Ph.D., D.A.B.T.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Monsanto Agricultural Products Company
800 N. Lindbergh Boulevard
St. Louis, Missouri 63167

Action Requested: Review a repeat chronic/oncogemcity study in rats with MON 097.

Recommendations: Under the conditions of this repeat study, there was evidence of systemic toxicity in the high dose groups (1000 ppm) expressed as decreased body weights and body weight gains in both males and females accompanied by increases in serum gamma glutamyl transpeptidase activity and cholesterol levels in high dose males, increased total bilirubin in high dose females, increased absolute and relative kidney and liver weights in high dose males and increased testicular weights in high dose males (at final sacrifice). There were increases in several non-neoplastic histopathological findings in high dose males and females. Neoplastic histopathological findings were noted in the form of neoplastic nodules of the liver, follicular adenoma/cystadenoma of the thyroids and papillary adenoma of the mucosa of the nose/turbinates in high dose animals.

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From the evidence presented in this study, MON 097 is an oncogen in male and female rats at doses of 1000 ppm as evidenced by the findings of neoplastic nodules of the liver, follicular adenoma/cystadenoma of the thyroids and papillary adenoma of the mucosa of the nose/turbinates in high dose animals.

NOEL for Systemic Toxicity = 200 ppm
LOEL for Systemic Toxicity = 1000 ppm

This study is classified as Core-Supplementary Data for chronic toxicity and oncogenicity. The registrant should be directed to supply the data requested in the DER (see Conclusions section). Submission and acceptance of these requested data may permit upgrading of this study.

UU/69/32

Reviewed by: Stephen C. Dapson, Ph.D.
Pharmacologist, Review Section V, Toxicology Branch/HED (TS-769C)
Secondary Review by: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, Review Section V, Toxicology Branch/HED (TS-769C)

006571

DATA EVALUATION RECORD

STUDY TYPE: Chronic Feeding/Oncogenicity Rodent
Guideline §83-1 and 83-2

EPA IDENTIFICATION NUMBERS:
EPA ID NO.: 3F2966 and 524-GUI
EPA ACCESION NUMBER: 400770601
EPA RECORD NO.: 195381 and 195383
SHAUGHNESSY NO.: 121601
CASWELL NO.: 3B
TOX BRANCH PROJECT NO.: 7-0702
DOCUMENT NO.:

006571

TEST MATERIAL: Acetochlor
EHL Substance Identification Code: T830072
Lot No. Dayton RDNT 08001

SYNONYMS: MON 097

STUDY NUMBER(S): Laboratory Project ID: EHL-83107
Report No.: MSL-6119
Study (DMEH Project No.) No.: ML-83-200; EHL #83107

SPONSOR: Monsanto Company
1101 17th Street, N.W.
Washington, D.C. 20036

TESTING FACILITY: Monsanto Environmental Health Laboratory
St. Louis, Missouri

TITLE OF REPORT: Chronic Feeding Study of MON 097 IN Albino Rats

AUTHOR(S): M.W. Naylor
W.E. Ribelin

REPORT ISSUED: September 25, 1986 (date study completed)

BACKGROUND INFORMATION:

The study reviewed in this DER (Laboratory Project ID: EHL-83107) is a repeat of a previous chronic/oncogenicity study in the rat (Study #PR-80-006, 5/20/83) which was classified as minimum data. A NOEL for systemic effects was not established and a repeat study was requested by the Agency.

The dose levels tested in the initial study were 500, 1500 and 5000 ppm. MON 097 was found to be carcinogenic in the rat (classified as B_2). At the highest dose level there was increased incidence of liver carcinomas and thyroid follicular cell adenomas in males.

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Positive trends were noted for hepatic carcinomas in females and thyroid follicular cell adenomas in males (see the Peer Review Document, dated 3/30/87 from R. Engler to R. Taylor).

At the highest dose level there were also increased incidences of polyarteritis of the testes and arteries in the males and liver necrosis and alveolar histiocytosis in females. Further, at the high dose there was increased mortality in females and decreased food consumption in both sexes. A dose-related decrease in body weights were noted in both sexes at the mid and high dose levels and in males at the low dose level. There were systemic effects at the low dose level in the form of organ weight effects and decreased body weights in males, therefore, a systemic NOEL could not be determined.

CONCLUSIONS:

Under the conditions of this repeat study, there was evidence of systemic toxicity in the high dose groups (1000 ppm) expressed as decreased body weights and body weight gains in both males and females accompanied by increases in serum gamma glutamyl transpeptidase activity and cholesterol levels in high dose males, increased total bilirubin in high dose females, increased absolute and relative kidney and liver weights in high dose males and increased testicular weights in high dose males (at final sacrifice). There were increases in several non-neoplastic histopathological findings in high dose males and females. Neoplastic histopathological findings were noted in the form of neoplastic nodules of the liver, follicular adenoma/cystadenoma of the thyroids and papillary adenoma of the mucosa of the nose/turbinates in high dose animals. From evidence presented in this study, MON 097 is a oncogen in male and female rats.

NOEL for Systemic Toxicity = 200 ppm
LOEL for Systemic Toxicity = 1000 ppm

The registrant is directed to supply the following data. Submission and acceptance of this data may permit upgrading of this study.

1. Summary tables of all reported clinical observations.

2. Tables with actual numbers of tissues examined for each organ/dose level used for histopathological examination.

Classification: Core-Supplementary Data for chronic toxicity and oncogenicity. This study may be upgraded if information requested is submitted and accepted by the Agency.

Special Review Criteria (40 CFR 154.7)

Based on evidence examined by the Toxicology Branch Peer Review Committee (meeting of September 12, 1985, MEMO of March 30, 1987), Acetochlor meets the criteria for Group B2 - Probable Human Carcinogen. Acetochlor is oncogenic in the rat (first study) with evidence of hepatocellular carcinoma in both sexes and thyroid folicular cell adenoma in males. Acetochlor is oncogenic in the mouse with evidence of hepatocellular carcinoma in both sexes, lung carcinoma in females, uterine histiocytic sarcomas, benign ovarian tumors and kidney adenomas in females. Acetochlor is structurally related to known carcinogens and has been shown to be mutagenic.

It should be noted that in this repeat study, papillary adenomas of the mucosa of the nose/turbinates (a neoplastic finding not previously observed) were statistically significantly increased at the 1000 ppm dose level.

According to the investigators "The homogeneity of the diet mixture was determined to be adequate for study use." They determined the homogeneity on the low and high concentrations prior to study initiation and at week 89. The data provided indicated an adequate mixing of the diet.

Stability analysis of the "test material/diet mixture" was determined for the low and high dose mixtures at room temperature for 14 days and when refrigerated for 42 days. Provided data indicate that the diet mixtures were stable under both storage conditions.

The investigators also performed weekly analysis of diet concentrations during the first 6 weeks for all the dose levels, one dose level per week after the initial 6 weeks, and for all 3 dose groups at week 89 due to change in batch size. All dose levels tested were slightly less than the target dose but within 10% of the planned level.

3. Animals received food (Ralston Purina rodent Chow No. 5002) and water (St. Louis Public Water Supply) ad libitum.

4. Statistics - According to the investigators' report:

> "The following statistical procedures were used to detect statistically significant differences between treated animals and their respective controls":
>
> "Dunnett's Multiple Comparison Test (two-tailed): body weights, food consumption, noncategorical clinical pathology data, absolute organ weights".
>
> "Mann-Whitney Test with Bonferroni Inequality Procedure: Organ weight/body weight ratios".
>
> "Fishers's Exact Test with Bonferroni Inequality Procedure: Incidence of microscopic lesions".
>
> "Generalized Wilcoxin, Generalized Savage Statistics, and life table analysis: Mortality".
>
> "Peto Analysis (one-tailed): Selected microscopic lesions and combinations thereof".
>
> Other statistical procedures used were: "Bartlett's Test to evaluate homogeneity of variances, Analysis of Variance to determine if the sample (group) means could be considered as an estimate of a common population, and Grubb's Test to detect outliers".

5. A Signed "Statement of No Data Confidentiality Claims" was included (no claim of confidentiality made).

A signed "Statement of Compliance" with USEPA-GLP's was included.

A signed "DMEH Quality Assurance Audit Statement [redacted]s included.

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C. METHODS AND RESULTS:

1. Observations

Animals were inspected twice daily for signs of mortality and moribundity. They were further inspected once weekly for signs of toxicity.

Toxicity/Mortality (survival)

The investigators' provided group mean and individual animal data for mortality. The following Table (1) presents the survival data:

Table 1. Survival Data†

	Sex	Dose Group Control	Low	Mid	High
% Survival at termination[a]	M	47	40	37	38
	F	40	43	43	48
Mean survival time (days)	M	681	663	651	670
	F	654	667	634	674

[a] - denominator excludes 10 animals/sex/group sacrificed at 12 months
† = Table appended from the investigators' report (MSL-6119).

No statistically significant differences were noted in the presented data. Inspection of individual data showed that nearly all deaths, either "spontaneous" or sacrifices "in extremis," occurred during the second year of the study. No specific time-to-death pattern was apparent. Gross and non-neoplastic microscopic necropsy observations occurred in similar incidence in all study groups, therefore, no treatment related cause of death could be determined. Neoplastic findings will be discussed later.

Clinical Observations:

The investigators provided a description of clinical signs, however, no effort was made to distinguish between dose groups, except for "Infrequent observations of head tilt, circling movements, somersaulting and dilation of conjunctival blood vessels....primarily in the last one-third of the study and appeared to effect [sic] T-2 and T-3 level rats more than controls (particularly females)." Individual animal data were provided. Inspection of these data reveals a possible dose-response effect on certain observations such as periorbital encrustation and soft stool. The investigators are directed to provide summary tables of all clinical observations.

2. Body weight

Animals were weighed once weekly for 13 weeks, then once every four weeks (following the initial 13 weeks) thereafter.

Table 2 and Figures 1 and 2 for males and females (appended from the investigators' report, only for the first 13 weeks) present body weights and body weight gains at selected intervals. High dose males had lower body weights and body weight gains from day 8 on, statistically significantly lower from days 455 to 678. High dose females also tended to have lower body weights and body weight gains, although values did not obtain statistical significance.

Table 2: Body Weights and Body Weight Gains at Selected Intervals (gm)[a]

	Males											
Day:	0	8	43	91	175	371	399	455	539	623	678	735
Dose (ppm)												
Control	222.7	264.3	443.5	535.0	612.3	752.7	766.7	813.1	813.7	840.4	820.2	744.9
		(41.6)†	(220.8)	(312.3)	(389.6)	(530.0)	(544.0)	(590.4)	(609.0)	(617.7)	(597.5)	(522.2)
40	222.6	272.8	449.1	542.1	615.9	750.5	750.4	795.4	797.4	791.5	780.9	710.1
		(50.2)	(226.5)	(319.5)	(393.3)	(527.9)	(527.8)	(572.8)	(574.8)	(568.9)	(558.3)	(487.5)
200	222.6	273.9*	449.2	536.2	608.9	751.7	762.6	798.8	827.0	814.4	787.5	759.2
		(51.3)	(226.6)	(313.6)	(386.3)	(529.1)	(540.0)	(576.2)	(604.4)	(591.8)	(564.9)	(536.6)
1000	222.6	269.5	439.3	527.1	599.3	719.7	725.9	747.8**	760.8*	732.6**	700.6**	681.7
		(46.9)	(216.7)	(304.5)	(376.7)	(497.1)	(503.3)	(525.2)	(538.2)	(510.0)	(478.0)	(459.1
	Females											
Day:	0	9	44	92	176	372	400	456	540	624	679	736
Control	157.2	176.8	241.5	279.2	318.2	425.7	436.3	455.5	467.3	486.0	505.8	465.1
		(19.6)	(84.3)	(122.0)	(161.0)	(268.5)	(279.1)	(298.3)	(310.1)	(328.8)	(348.6)	(307.9)
40	157.2	177.1	244.4	284.8	326.1	433.2	445.9	467.7	499.0	519.4	522.1	487.6
		(19.9)	(87.2)	(127.6)	(168.9)	(276.0)	(288.7)	(310.5)	(341.8)	(362.2)	(364.9)	(330.4)
200	157.2	177.5	246.3	285.3	326.1	435.2	455.7	475.5	481.7	512.4	527.5	525.7
		(20.3)	(89.1)	(128.1)	(168.9)	(278.0)	(298.5)	(318.3)	(324.5)	(355.2)	(370.3)	(368.5)
1000	157.3	176.1	241.6	281.8	316.9	405.3	412.4	435.2	455.5	469.3	469.6	450.4
		(18.8)	(84.3)	(124.5)	(159.6)	(248.0)	(255.1)	(277.9)	(298.2)	(312.0)	(312.3)	(293.1)

* = $P < 0.05$; ** = $P < 0.01$

† = Body weight gains

[a] = Data extracted from Report MSL-6119, Appendix II, Table 2.

ACETOCHLOR Tox review 007697

Page_____ is not included in this copy.

Pages 94 through 95 are not included.

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____ Identity of the source of product ingredients.

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____ A draft product label.

____ The product confidential statement of formula.

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3. Food consumption and compound intake

Food consumption was determined weekly for 13 weeks and then every 4 weeks and mean daily diet consumption was calculated. Food efficiency was calculated from the food consumption and body weight gain data for the first 13 weeks. The investigators supplied group summary and individual animal data for food consumption. They did not calculate compound intake.

Food consumption/Food Efficiency/Compound Intake

Table 3 presents the food consumption data at selected intervals (similar intervals to body weight data). A slight dose-related increase in food consumption in both sexes (high dose) was noted especially during the period when a decrease in body weight and body weight gain was noted. This is indicative of reduced food efficiency.

Table 4 presents the food efficiency data for the first 13 weeks. Reduced food efficiency was observed in both males and females of the high dose during the first 13 weeks of the study.

Table 3: Food Consumption (mean gm/kg body weight/day)[a]

	Males										
Days	1-8	35-43	84-91	168-175	364-371	391-399	448-455	532-539	616-623	672-678	728-735
Dose (ppm)											
Control	80.7	58.4	52.1	42.5	34.6	34.1	33.5	31.3	30.8	33.4	29.2
40	84.9*	58.2	51.4	42.0	35.7*	33.6	33.7	31.3	30.6	34.0	32.8
200	86.1**	59.2	52.6	42.6	36.0**	34.1	33.3	31.9	32.5	32.9	35.8*
1000	85.4**	59.6*	52.6	42.7	36.2**	35.4	34.0	34.3*	33.0	33.3	34.1
	Females										
Days	2-9	36-44	85-92	169-176	365-372	392-400	449-456	533-540	617-624	673-679	729-736
Dose (ppm)											
Control	85.7	69.9	71.8	59.6	46.5	45.3	41.1	40.9	38.3	40.0	36.9
40	86.6	71.1	72.6	60.6	47.1	42.8	41.4	41.5	37.8	37.6	36.2
200	88.7	72.2	71.5	60.8	47.2	43.8	40.0	42.6	37.1	40.4	40.0
1000	89.6**	73.2**	71.6	61.1	47.5	45.8	43.7	41.9	40.6	40.9	37.9

* = P < 0.05 ; ** = P < 0.01

[a] = Data extracted from Report MSL-6119, Appendix II, Table 4.

Table 4: Food Efficiency (mean %†)[a]

	Males												
Days	1-8	8-14	14-22	22-29	29-35	25-43	43-49	49-56	56-62	62-70	70-77	77-84	84-91
Dose (ppm)													
Control	11.8	30.9	21.6	18.4	15.4	13.5	12.5	9.5	9.1	4.1	5.5	6.0	4.3
40	30.9**	30.2	21.3	17.9	15.7	13.2	12.4	7.3*	11.2	4.5	5.3	6.3	4.1
200	31.0**	28.7	21.3	18.4	14.9	13.3	11.8	9.6	6.2*	5.2	5.9	5.0	4.5
1000	29.1*	29.9	21.2	17.6	13.0**	13.9	11.3	10.6	6.1**	5.8**	6.1	4.0**	5.1
	Females												
Days	2-9	9-15	15-23	23-30	30-36	36-44	44-50	50-57	57-63	63-71	71-78	78-85	85-92
Dose (ppm)													
Control	18.3	12.9	14.0	10.5	6.5	10.3	3.4	8.5	3.0	1.3	5.2	4.5	4.1
40	18.3	12.2	14.7	12.1	5.4	10.6	1.2	9.5	2.2	2.4	5.0	4.3	5.3
200	18.3	15.5*	13.6	11.9	4.5	9.7	1.0*	9.8	2.1	4.0**	5.4	3.2	5.1
1000	16.9	16.9**	13.2	9.8	5.6	8.0*	2.6	8.9	-0.4**	6.0**	4.0	3.5	4.8

* = P < 0.05 ; ** = P < 0.01 (by Dunnett's Test [Two-Tailed])

† = mean %, calculated from food consumption and body weight data for the first 13 weeks.

[a] = Data extracted from Report MSL-6119, Appendix II, Table 5.

4. Ophthalmological examinations

Ophthalmic examinations were conducted prior to study initiation and at 6, 12, 18 and 24 months on all animals of the high dose and control (all animals were screened prior to study initiation). The following Table (5) presents the observations.

Table 5: Observed Ophthalmic Lesions[a]

Months	Control Males	High Dose Males	Control Females	High Dose Females
6	2(N=70)	1(N=69)	1(N=70)	2(N=70)
12	4	1	9	9
18	6	9	10	9
24	12(N=30)	13(N=27)	9(N=24)	9(N=30)

[a] = Data extracted from Report MSL-6119, Appendix II, Table 26.

Data for 6, 12 and 18 months may have been provided under clinical observations, but only as individual animal data. At study termination (24 months) the investigators described "senile lens changes and the presence of ocular discharge" as the predominant findings occurring in the control and high dose groups.

5. Blood was collected at approximately six months intervals for hematology and clinical analysis from 10 animals per sex, per dose group. The following parameters were examined.

a. Hematology

Hematocrit (HCT)*
Hemoglobin (HGB)*
Leukocyte count (WBC)*
Erythrocyte count (RBC)*
Platelet count*
Leukocyte differential count*
Mean corpuscular HGB (MCH)
Mean corpuscular HGB conc.(MCHC)
Mean corpuscular volume (MCV)
Reticulocyte count

* Required for chronic studies

Blood clotting measurements were not conducted. The investigators provided group summary and individual animal data. Blood was collected from fasted animals (food withheld 24 hours prior to sampling), from the retroorbital sinus for month 6 and 18 and from the posterior vena cava (under anesthesia) for months 12 and 24. Only occasional differences were noted. There were statistically significant decreases in white blood cell counts in low and mid dose males at 1 year and low dose females at 18 months; a decrease in MCH in low dose females at 1 year and high dose males at 2 years; a decrease in MCHC in high dose males at 1 and 2 years, low dose females at 1 year and mid dose females at 18 months; platelets were increased in mid dose females at 1 year; reticulocyte counts were decreased in low dose females at 1 year; absolute lymphocyte counts were decreased in all male treated groups and mid dose females at 1 year. None of the differences appear biologically relevant as they were not sustained with no dose response apparent and no related pathological changes noted.

b. Clinical Chemistry

Electrolytes:
- Calcium*
- Chloride*
- Phosphorous*
- Potassium*
- Sodium*

Enzymes
- Alkaline phosphatase
- Lactic acid dehydrogenase
- Serum alanine aminotransferase (also SGPT)*
- Serum aspartate aminotransferase (also SGOT)*
- gamma glutamyl transferase

Other:
- Albumin*
- Blood creatinine*
- Blood urea nitrogen*
- Cholesterol*
- Glucose*
- Total Bilirubin*
- Total Serum Protein*
- Direct Bilirubin

* Required for chronic studies

The investigators did not measure magnesium and creatinine phosphokinase, which are required for chronic toxicity studies. The investigators provided group mean and individual animal data. Several measurements achieved statistical significance: decreased glucose levels in low and high dose females at 2 years; decrease BUN levels in mid dose females at 1 year; slightly decreased total protein levels in all dosed males at 1 year; decreased alkaline phosphorus levels in high dose males and females at 6 months; decreased LDH levels in high dose males at 6 months, mid dose males at 1 year, all 3 treated male groups at 18 months, mid and high dose females at 6 months, high dose females at 1 year and low dose female at 18 months; slightly decreased creatinine in high dose females at 6 months; slightly decreased sodium levels in mid dose males at 1 year. None of these differences appear to be related to treatment also, no dose response was apparent. However, several differences were attributable to treatment, (according to the investigators): statistically significant increase in gamma glutamyl transpeptidase in high dose males at 18 months and 2 years (also nonstatistically significant increase in mid and high dose males at 1 year and mid dose males at 2 years); increased cholesterol in high dose males at 2 years (also non-statistically significant increase at 18 months); increased total bilirubin in high dose females at 2 years.

6. Urinalysis

Urine was collected from fasted and "water-withheld" animals at 6, 12, 18 and 24 months. The following parameters were examined.

- Specific gravity*
- pH†
- Bilirubin*†
- Blood*†
- Sediment (microscopic)*
- Protein*†
- Glucose*†
- Ketones*†
- Urobilinogen

* Required for chronic studies

† = Assay with MULTISTIX reagent strips and CLINI-TEK reader.

Urine was collected for 6 hours using "metabolism trays". The investigators did not report appearance or volume of the urine as

required for chronic studies. The only observation of note was a slight increase in specific gravity of the urine of females in all 3 dose groups at 6 months (low dose was statistically significant) and 1 year. However, this observation had no dose response and was not observed in subsequent examinations.

7. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
X	.Salivary glands*	X	.Aorta*	XX	.Brain*†
X	.Esophagus*	XX	.Heart*	X	Periph. nerve*
X	.Stomach*	X	.Bone marrow*	X	Spinal cord(3 levels)*
X	.Duodenum*	X	.Lymph nodes*	X	.Pituitary*
X	.Jejunum*	X	.Spleen*	X	Eyes(optic n.)*
X	.Ileum*	X	.Thymus*		Glandular
X	.Cecum*		Urogenital	XX	.Adrenals*
X	.Colon*	XX	.Kidneys*†	X	Mammary gland*
X	.Rectum*	X	.Urinary bladder*	XX	.Parathyroids*
XX	.Liver*†	XX	.Testes*†(w/epidid)	XX	.Thyroids*(w/parathyroid)
X	.Pancreas*	XX	Epididymides		Other
	Respiratory	X	Prostate	X	Bone*(with marrow)
X	.Trachea*	X	Seminal vesicle	X	Skeletal muscle*
X	.Lung with bronchi*	X	Ovaries*†	X	Skin*
X	Nasal turbinates	X	.Uterus*	X	All gross lesions/masses*
X	Penis			X	Middle ear

* Required for chronic studies
† Organ weights required in chronic studies

a. Organ weight

The investigators supplied group mean and individual animal data for absolute organ weights and organ weights relative to body weights for interim and final sacrifice. Organ weight to brain weight ratios were not calculated. Table 6 presents the mean absolute and relative organ weight data for interim sacrifice and at study termination.

At the interim sacrifice, slight increases in absolute and relative kidney weights were noted in the high dose males along with a slight dose-related increase in absolute and relative liver weights in all treated males. At terminal sacrifice the high dose males had slightly increased absolute and relative kidney weights, a slight increase in absolute and relative (statistically significant in the high dose) liver weights and slightly increased absolute and relative (statistically significant in the high dose) testes weight.

TABLE 6: Absolute and Relative Organ Weights[a]

Organ	Dose (ppm):	Males - Interim Sacrifice Control	40	200	1000	Females - Interim Sacrifice Control	40	200	1000
Adrenals	A[†]	0.055[††]	0.067	0.070	0.060	0.078	0.081	0.079	0.081
	R[†]	0.008	0.009	0.010	0.009	0.019	0.021	0.021	0.022
Brain	A	2.188	2.174	2.171	2.182	2.094	2.048	1.995	1.941*
	R	0.308	0.291	0.305	0.315	0.502	0.534	0.530	0.503
Heart	A	1.908	1.916	1.855	1.861	1.295	1.272	1.261	1.220
	R	0.268	0.254	0.259	0.268	0.307	0.323	0.332	0.311
Kidneys	A	3.796	4.330	3.775	4.327	2.533	2.405	2.578	2.651
	R	0.532	0.575	0.535	0.720	0.606	0.618	0.686	0.675
Liver	A	19.430	19.787	21.184	21.328	11.841	11.113	10.863	11.332
	R	2.721	2.605	2.958	3.050	2.817	2.795	2.827	2.879
Testes	A	5.913	6.602	6.533	6.520	-	-	-	-
	R	0.829	0.867	0.898	0.931	-	-	-	-
Thyroids	A	0.036	0.043	0.040	0.043	0.036	0.040	0.038	0.038
	R	0.005	0.006	0.006	0.006	0.009	0.010	0.010	0.010

* = P<0.05 using Dunnett's Test

[†] = A = Absolute; R = Relative (mean %)

[††] = grams

[a] = Data extracted from Report MSL-6119, Appendix II, Table 10, 11, 12 and 13.

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TABLE 6 continued: Absolute and Relative Organ Weights[a]

Organ	Dose (ppm):	Males - Terminal Sacrifice Control	40	200	1000	Females - Terminal Sacrifice Control	40	200	1000
Adrenals	A[†]	0.099[††]	0.100	0.098	0.123	0.113	0.113	0.140	0.109
	R[†]	0.014	0.015	0.014	0.019	0.028	0 024	0.031	0.027
Brain	A	2.358	2.325	2.348	2.304	2.053	2.037	2.031	2.036
	R	0.349	0.352	0.335	0.361	0.502	0.441	0.434	0.515
Heart	A	2.199	2.321	2.238	2.291	1.596	1.521	1.608	1.462
	R	0.321	0.351	0.316	0.354	0.387	0.320	0.343	0.366
Kidneys	A	5.656	5.948	5.441	6.271	3.311	3.201	3.425	3.182
	R	0.839	0.922	0.774	0.967	0.804	0.690	0.708	0.797
Liver	A	20.320	20.842	20.925	22.331	13.910	13.765	14.331	12.652
	R	2.941	3.117	2.908	3.479*	3.260	2.903	2.975	3.096
Testes	A	5.576	5.571	5.756	5.893	-	-	-	-
	R	0.807	0.820	0.790	0.903*	-	-	-	-
Thyroid	A	0.059	0.060	0.056	0.062	0.047	0.051	0.051	0.049
		0.009	0.009	0.008	0.010	0.011	0.011	0.011	0.012

* = P<0.05 using Dunnett's Test
† = A = Absolute; R = Relative (mean %)
†† = grams
[a] = Data extracted from Report MSL-6119, Appendix II, Table 10, 11, 12 and 13.

2b. Gross pathology

Gross pathological observations during the 1 year interim sacrifice were infrequent and apparently not treatment related. Observations can be seen on Table 7. Observations at 2 years were not significantly different between dose groups (Table 8 presents selected observations). Table 9 presents selected observations of animals dying on study. No biologically relevant differences were noted. Table 10 presents a selected summary of all gross necropsy observations, again no biologically relevant differences were noted.

TABLE 7: Selected Gross Necropsy Observations (1 year)[a]

Dose (ppm):	Control	40	200	1000
#examined m/f	10/10	10/10	10/10	10/10
Observation:				
Adrenals:				
enlarged	0/1	0/1	1/0	0/0
Heart:				
enlarged	3/0	2/0	2/0	1/1
abnormal color	3/0	3/0	2/2	1/1
Kidneys:				
hydronephrosis	0/1	1/0	0/2	0/1
Liver:				
abnormal color	2/2	0/0	1/0	0/0
foci/spots	0/0	0/1	0/0	0/0
Lymph Node				
enlarged	0/0	0/0	1/0	0/0
Nose/Turbinates:				
mass/nodule	0/1	0/0	0/0	0/0
Pitutary:				
enlarged	0/0	0/0	0/1	0/0
hemorrhage	0/0	0/0	0/1	1/0
focus/spots	0/0	0/3	0/2	0/3
Spleen:				
enlarged	0/0	0/0	1/0	0/0
Testes:				
atrophic	0	1	1	1
Thyroids:				
atrophic	0/0	0/0	0/1	0/0
Urinary Bladder:				
urolithiasis	0/0	0/0	0/0	0/1
Uterus:				
thickened walls	0	1	0	0
hydrometra	0	0	1	0

[a] = Data extracted from Report MSL-6119, Appendix II, Table 14.

TABLE 8: Selected Gross Necropsy Observations (2 years)[a]

Dose (ppm):	Control	40	200	1000
#examined m/f	28/24	24/26	22/26	23/30
Observation:				
Adrenals:				
enlarged	7/9	0/6	0/10	2/9
atrophic	0/2	1/0	0/0	0/0
focus(i)	6/11	4/14	5/13	4/13
Brain:				
compressed by pituit.	3/6	1/6	1/9	2/11
Eyes:				
corneal opacity	1/2	0/0	2/4	2/0
Kidneys:				
hydronephrosis	2/0	3/2	0/6	4/0
cyst(s)	1/1	4/1	1/0	3/0
abnormal color	9/3	8/0	4/2	7/0
granular/pitted	6/2	6/1	0/2	6/0
Liver:				
abnormal color	1/5	3/2	4/1	2/2
foci/spots	13/10	4/6	6/6	12/10
mass/nodule	0/2	4/0	1/0	3/4
cyst(s)	0/1	2/2	0/1	2/0
Lymph Node:				
enlarged	3/2	2/0	1/1	3/3
congested	0/3	0/1	0/2	1/0
Nose/Turbinates:				
mass/nodule	0/0	1/0	0/1	0/0
Ovaries:				
cyst(s) [within]	1	1	1	1
paraovarian cysts(s)	3	2	0	0
Pancreas:				
nodule	1/2	2/1	2/1	0/1
Pituitary:				
enlarged	6/11	3/13	6/12	5/13
hemorrhagic	6/3	4/9	1/8	4/7
focus/spots	3/4	3/6	2/4	2/5
mass/nodule	2/4	2/2	3/2	2/5
Spleen:				
enlarged	2/1	2/0	2/0	3/0
mass/nodule	0/0	0/0	1/0	1/1
Testes:				
atrophic	3	6	4	3
growth(s)/mass(es)	0	0	1	0
Thyroids:				
enlarged	0/0	0/0	0/3	0/1
Urinary Bladder:				
urolithiasis	2/0	0/0	1/0	0/0
growths/masses	1/0	0/0	0/0	1/0
Uterus:				
thickened walls	1	1	3	1
hydrometra	0	1	2	2
endometrial polyp(s)	2	3	2	2
cyst(s)	1	0	0	2
Subcutis:				
growth/mass	8/29	5/26	3/27	2/22

[a] = Data extracted from Report MSL-6119, Appendix II, Table 15.

TABLE 9: Selected Gross Necropsy Observations (early deaths)[a]

Dose (ppm):	Control	40	200	1000
#examined m/f	32/36	35/34	38/34	37/30
Observation:				
Adrenals:				
enlarged	4/10	5/10	6/8	2/6
atrophic	0/0	0/4	0/2	0/0
focus(i)	8/15	6/10	5/9	8/10
Brain:				
compressed by pituitary	16/22	12/23	20/15	9/18
Eyes:				
corneal opacity	5/3	6/1	8/2	5/3
encrustation	3/5	2/4	1/7	3/5
dicharge	2/11	9/10	4/6	6/5
Heart:				
enlarged	3/0	2/0	2/0	1/1
abnormal color	2/0	3/0	2/1	1/1
Kidneys:				
enlarged	4/1	7/0	5/0	7/1
hydronephrosis	10/4	7/4	7/7	7/3
calculus(i)	1/1	3/2	1/2	0/1
cyst(s)	2/2	6/1	2/0	6/1
abnormal color	12/4	11/3	13/6	12/3
atrophy	0/0	1/0	0/0	0/0
granular pitted	11/1	16/4	17/5	17/3
Liver:				
abnormal color	9/10	20/6	12/10	10/6
foci/sports	9/6	9/11	12/5	8/10
enlarged	0/0	0/0	2/0	1/1
abnormal texture	1/0	0/0	1/2	0/1
pitted/nodular/ granular surface	0/0	0/0	2/0	2/0
mass/nodule	2/1	2/0	2/2	0/1
cyst(s)	1/0	2/1	0/2	0/2
Lymph Node:				
enlarged	0/3	4/3	4/0	1/0
congested	3/0	5/0	3/1	4/1
Lung:				
foci/spots	6/0	3/1	1/3	2/1
congested	7/6	4/5	8/2	6/4
abnormal	3/2	2/3	3/5	2/1
Mammary Gland:				
growth(s)/mass(es)/ nodule(s)	0/1	0/2	1/2	0/0
Nose/Turbinates:				
discharge	5/10	7/7	6/6	6/8
mass/nodule	0/0	1/0	0/0	0/0
Ovaries:				
cyst(s)[within]	0	0	2	1
paraovarian cyst(s)	0	0	1	6
Pancreas:				
nodule	5/0	7/3	4/1	5/2

continued

TABLE 9 continued: Selected Gross Necropsy Observations (early deaths)[a]

Dose (ppm):	Control	40	200	1000
#examined m/f	32/36	35/34	38/34	37/30
Observation:				
Pituitary:				
enlarged	22/31	18/30	24/25	15/24
hemorrhagic	10/15	10/20	16/11	5/10
focus/spots	1/2	11/1	2/2	2/0
mass/nodule	0/1	1/0	0/1	3/0
Prostate:				
atrophy	5	6	7	5
Parathyroids:				
enlarged	4/0	4/0	3/2	5/0
Skin:				
growth(s)/mass(es)	2/1	4/0	3/1	1/0
Spleen:				
enlarged	0/3	2/0	2/3	2/0
atrophic	0/0	0/0	0/2	0/1
mass/nodule	1/0	0/0	1/0	0/0
Seminal Vesicles:				
atrophy	10	9	11	11
enlarged	3	1	1	1
Testes:				
atrophic	10	17	14	13
growth(s)/mass(es)	1	1	0	0
enlarged	0	0	0	1
Thyroids:				
enlarged	2/1	1/0	3/0	6/1
focus	0/0	0/0	1/0	0/0
Urinary Bladder:				
dilated	2/1	6/2	4/1	1/3
urolithiasis	1/0	2/0	1/0	1/1
growths/masses	0/0	0/0	0/0	0/0
thickened walls	0/0	0/0	0/0	0/0
Uterus:				
thickened walls	0	2	2	2
hydrometra	1	0	1	0
endometrial polyp(s)	4	5	5	2
cyst(s)	1	0	0	1
Subcutis:				
growth/mass	4/25	10/30	6/27	7/19

[a] = Data extracted from Report MSL-6119, Appendix II, Table 17.

TABLE 10: Selected Gross Necropsy Observations (all deaths)[a]

Dose (ppm):	Control	40	200	1000
#examined m/f	70/70	69/70	70/70	70/70
Observation:				
Adrenals:				
enlarged	11/20	5/17	7/18	4/15
atrophic	0/2	1/4	0/2	0/0
focus(i)	14/26	10/24	10/22	12/23
Brain:				
compressed by pituitary	19/28	13/29	21/24	11/29
Eyes:				
corneal opacity	6/7	6/1	10/7	7/3
encrustation	4/6	2/4	1/7	3/8
discharge	2/11	9/10	4/6	6/5
Heart:				
enlarged	3/0	2/0	2/0	1/1
abnormal color	3/0	3/0	2/2	1/1
Kidneys:				
enlarged	4/1	7/0	5/0	9/1
hydronephrosis	12/5	11/6	7/15	11/4
calculus(i)	1/1	3/3	1/3	0/1
cyst(s)	3/3	11/3	3/1	9/1
abnormal color	21/7	19/3	17/8	19/3
atrophy	0/0	1/0	0/0	0/0
granular pitted	17/3	22/5	17/7	23/3
Liver:				
abnormal color	12/17	23/8	17/11	12/8
foci/spots	22/16	13/18	18/11	20/20
enlarged	0/0	0/0	2/0	1/1
abnormal texture	1/1	0/0	1/2	1/1
pitted/nodular/ granular surface	0/0	1/0	2/0	2/1
mass/nodule	2/3	6/0	3/2	3/5
cyst(s)	1/1	4/3	0/3	2/2
Lymph Node:				
enlarged	3/5	6/3	6/4	4/3
congested	3/3	5/1	3/3	5/1
Lung:				
foci/spots	6/0	3/2	1/4	2/2
congested	7/6	4/5	8/2	6/4
abnormal color	3/4	2/3	3/5	3/1
nodule(s)	0/0	0/0	2/2	1/0
Mammary Gland				
growth(s)/mass(es)/ nodules(s)	0/1	0/2	1/2	0/1
Nose/Turbinates:				
discharge	5/10	7/7	6/6	6/8
mass/nodule	0/1	2/0	0/1	0/0
Ovaries:				
cyst(s)[within]	1	1	3	1
paraovarian cyst(s)	3	2	1	6
Pancreas:	6/2	9/4	6/2	5/3
nodule				

continued

TABLE 10 continued: Selected Gross Necropsy Observations (all deaths)[a]

Dose (ppm):	Control	40	200	1000
#examined m/f	70/70	69/70	70/70	70/70
Observation:				
Pituitary				
enlarged	28/42	21/43	30/38	20/37
hemorrhagic	16/18	14/29	17/20	10/17
focus/spots	4/6	4/10	4/8	4/8
mass/nodule	2/5	3/2	3/3	5/5
Prostate:				
atrophy	5	7	7	5
Parathyroids:				
enlarged	4/1	4/0	3/2	5/0
Skin:				
growth(s)/mass(es)	4/1	10/2	5/1	3/1
Spleen:				
enlarged	2/4	4/0	5/3	5/0
atrophic	0/0	0/0	0/2	0/1
mass/nodule	1/0	0/0	2/0	1/1
Seminal Vesicles:				
atrophy	10	10	12	11
enlarged	3	2	1	1
Testes:				
atrophic	13	24	19	17
growth(s)/mass(es)	1	1	1	0
enlarged	0	0	1	1
Thyroids:				
enlarged	2/1	1/0	3/3	6/2
atrophic	0/0	0/0	0/1	0/0
Urinary Bladder:				
dilated	2/2	6/2	4/1	1/4
urolithiasis	3/0	2/0	2/0	1/2
growths/masses	1/0	0/0	0/0	1/0
thickened walls	0/0	0/1	0/1	0/1
Uterus:				
thickened walls	1	4	5	3
hydrometra	1	1	4	2
endometrial polyp(s)	6	8	7	4
cyst(s)	2	0	0	3
Subcutis:				
growth/mass	12/55	15/56	9/54	9/41

[a] = Data extracted from Report MSL-6119, Appendix II, Table 17.

c. Microscopic pathology

1) Non-neoplastic

The investigators provided group summary and individual animal data for interim sacrifices, early deaths and final sacrifices. Table 11 presents selected observations from the 1 year interim sacrifice. The major observations were an increase hepatocyte cellular alterations and bile duct hyperplasia in the high dose males and an increase in inflammation of the nasal mucosa in the high dose males and females. Table 12 presents selected observations from animals dying prior to study termination. There was an indication of an increase in hepatocyte cellular alteration, liver bile duct hyperplasia, hepatocyte necrosis and "nodular or diffuse" hyperplasia in the parathyroids of the high dose males. However, the summary data provided did not indicate if the observation listed as "autolysis" changed the number of tissues available for examination. Inspection of the individual data indicates that many of these tissues were not available for microscopic evaluation. Therefore, a thorough evaluation of all animals on study was not possible with the provided summary tables. The investigators' are directed to supply tables indicating the actual number of tissues examined for each organ/dose level used for histopathological examination. Table 13 presents selected observations from the animals at terminal sacrifice (2 years). An interesting observation in these animals and from animals that died prior to the end of the study (Table 12) was that of the brain being compressed by an enlarged pituitary. This occured in roughly equal incidence in all groups, however, an accurate description of the finding was not provided. It is possible that a pituitary tumor could be causing this compression of the brain, this will be discussed later. For the animals sacrificed at study termination, there was an increase in mid and high dose males and high dose females with plasma cell hyperplasia in the lymph node. Also, an increase in high dose males with papillary hyperplasia of the nasal epithelium and "c" cell hyperplasia of the thyroids (statistically significantly different). Table 14 presents a summary of selected observations for all animals on study (again some tissues were autolyzed).

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TABLE 11: Selected Microscopic Observations (1 year)[a]

Dose (ppm):	Control	40	200	1000
#animals m/f	10/10	10/10	10/10	10/10
Observation:				
Adrenals:				
hyperplasia/hypertrophy-				
medullary	0/0	0/0	0/0	1/1
cortical nodular	0/0	0/0	0/2	0/1
Brain:				
Compressed by pituitary	0/1	0/0	0/1	0/0
Epididymides:				
epithelial degenerative changes	0	1	1	2
Heart:				
myocarditis	3/0	2/1	2/0	2/0
myocardiolysis	2/0	1/1	3/0	0/0
proliferation of endomysial/ myocyte nuclei	1/0	1/1	1/0	1/0
Kidneys:				
glomerular/periglomerular sclerosis	1/0	1/0	0/0	0/0
chronic nephritis	8/0	8/0	9/0	9/0
hydronephrosis -				
bilateral	0/1	0/0	0/1	0/0
unilateral	0/0	1/0	0/2	0/2
pyelitis	0/1	0/0	1/0	0/1
pelvic epithelium and hyperplasia-				
non-papilliform	0/0	1/0	0/0	0/1
papilliform	0/0	0/0	0/2	0/0
Liver:				
cellular alteration	2/2	2/0	1/0	4/3
hyperplasia-bile duct	1/1	2/2	2/1	4/2
telangiectasis	0/0	0/3	1/0	1/0
nodular hypertrophy/ hyperplasia	0/0	0/1	0/0	0/0
Lymph Node:				
hyperplasia-plasma cell	0/0	0/0	0/2	0/3
mononuclear cell leukemia	0/0	0/0	1/0	0/0
Lung:				
pneumonia	1/0	3/1	2/2	1/2
Nose/Turbinates:				
mucosal lymphoid hyperplasia	6/1	1/0	1/1	1/0
inflammation-				
nasal sinus	1/0	1/0	1/1	0/1
nasal mucosa	1/0	3/1	2/1	4/2
papillary hyperplasia of nasal epithelium	0/0	0/0	2/0	1/0
Pituitary:				
hyperplasia-chromophobe	1/4	2/1	3/4	0/1
Thyroids:				
hyperplasia-"c" cell	0/1	0/0	0/0	0/0
Urinary Bladder:				
hyperplasia-epithelial	0/0	0/0	1/0	0/1

a = Data extracted from Report MSL-6119, Appendix II, Table 18

TABLE 12: Selected Microscopic Observations (early deaths)†

Dose (ppm):	Control	40	200	1000
#animal m/f	32/36	36/34	38/34	37/30
Observation:				
Adrenals:				
hyperplasia/hypertrophy-				
medullary	5/0	8[a]/1	5/3	7/0
cortical nodular	1/5	2[a]/2	3/3	4/3
Bone Marrow:				
hyperplasia-				
myelocyte/granulocyte	5/10[a]	8[b]/5	5[c]/5	4/6[h]
pancytic	2/4[a]	1[b]/3	0/4	0/2[h]
Brain:				
compressed by pituitary	17/25	20[a]/29	21/23	13/24
Bone:				
fibrotic replacement	4/0	7[b]/0	4[c]/1	9/0
osteolysis	3/0	5[b]/0	2[c]/0	9/0
Epididymides:				
epithelial degenerative changes	3	5[a]	4	5
Eyes				
keratitis	8/3	4/0	6/5	4/2
Heart:				
myocarditis	3/3	4[b]/1	4/2[g]	7/4
myocardiolysis	12/5	10[b]/4	12/3[g]	15/4
proliferation of endomysial/ myocyte nuclei	18/8	22[b]/7	25/9[g]	23/12
myocardial fibrosis	20/8	21[b]/9	22/7[g]	23/11
Kidneys:				
glomerular/periglomerular sclerosis	7/3	10[a]/1	14/1	14/1
chronic nephritis	23/15	30[a]/13	34/15	32/18
hydronephrosis -				
bilateral	1/2	2[a]/1	0/2	5/2
unilateral	3/2	4[a]/3	5/6	1/2
pyelitis	2/1	1[a]/2	2/5	2/2
pylonephritis	2/2	2/1	3/1	6/3
pelvic epithelium hyperplasia-				
non-papilliform	1/0	1/2	2/4	2/1
papilliform	0/0	0/1	1/2	2/4
Liver:				
cellular alteration	2/7	3[a]/9	3/2	6/4
hyperplasia-bile duct	7/1	4/3	6/5	10/5
telangiectasis hepatocyte necrosis	6/2	7/0	6/1	12/2
nodular hypertrophy/ hyperplasia	0/3	1/1	2/1	2/1
Lymph Node:				
hyperplasia-plasma cell	1/7	2[b]/9	2/2	0/4
mononuclear cell leuk.	0/0	1/0	0/0	0/0
Lung:				
pneumonia	6/6	8[a]/3	8/11	6/6
edema	2/2	3[a]/1	2/0	6/5
emphysema	3/0	2[a]/2	2/1	1/1
leukemia-				
myclogenous	0/0	0/0	1/0	0/0
mononuclear	0/0	1/0	0/0	0/0

continued

TABLE 12 continued: Selected Microscopic Observations (early deaths)†

Dose (ppm):	Control	40	200	1000
#animals m/f	32/36	36/34	38/34	37/30
Observation:				
Nose/Turbinates:				
mucosal lymphoid hyperplasia	0/0	0/0	0/1	0/0
inflammation				
nasal sinus	0/0	0/0	0/0	0/0
nasal mucosa	2/4	6[a]/4	6/1	6/6
papillary hyperplasia of nasal epithelium	0/0	0/3	0/1	0/3
Pancreas:				
islet cell hyperplasia	1[d]/1	0/2	2[c]/1	0/1
Pituitary:				
hyperplasia-chromophobe	1/1[a]	2[b]/0	1/1	4[e]/1
pars intermedia	0/0	0/0	2/0	1[e]/0
Parathyroids:				
hyperplasia-nodular(or diffuse)	7[d]/10	14[f]/1[f]	7/3[g]	15[f]/0
Spleen:				
hyperplasia-plasma cell	1/0[b]	0/0	1[c]/0	2/2
Testes:				
hyperplasia-interstitial cell	0	1[a]	0	0
Urinary Bladder:				
hyperplasia-epithelial	2[d]/0	4[g]/3[g]	4/1	2/2[h]
Uterus:				
mucosal polyp	2	3	2	1

[a] = 35(#animals); [b]=34; [c]=37; [d]=31; [e]=36; [f]=32; [g]=33; [h]=29.
† = Data extracted from Report MSL-6119, Appendix II, Table 20.

TABLE 13: Selected Microscopic Observations (2 years)†

Dose (ppm):	Control	40	200	1000
#animals m/f	28/24	24/26	22/26	23/30
Observation:				
Adrenals:				
hyperplasia/hypertrophy-				
medullary	1/2	5/2	4/1	3/2
cortical nodular	3/1	4/2	3/5	1/6
Bone Marrow:				
hyperplasia-				
myelocyte/granulocyte	5/4	2/6	5/3	4/5
pancytic	2/1	0/3	0/2	1/1
Brain:				
compressed by pituitary	11/13	4/11	8/15	8/19
Epididymides:				
epithelial degenerative changes	3	1	3	0
Eyes:				
keratitis	2/2	4/3	1/0	2/0
Heart:				
myocarditis	1/3	1/6	4/5	3/3
myocardiolysis	7/6	5/1	1/2	9/3
proliferation of endomysial/ myocyte nuclei	16/11	16/14	12/16	15/10
myocardial fibrosis	18/11	14/12	12/10	15/11
Kidneys:				
glomerular/periglomerular sclerosis	9/3	12/3	6/4	2/1
chronic nephritis	26/16	24/19	22/18	23/21
hydronephrosis-				
bilateral	0/0	0/1	0/3	2/0
unilateral	2/0	0/2	2/3	4/1
pyelitis	3/2	1/2	1/1	2/2
pyelonephritis	2/3	3/2	1/0	1/0
pelvic epithelium/hyperplasia-				
non-papilliform	0/1	1/3	0/1	1/3
papilliform	2/0	1/6	0/2	2/2
Liver:				
cellular alteration	13/10	9/9	9/12	15/15
hyperplasia-				
bile duct	9/9	7/9	6/5	8/9
telangiectasis	17/2	10/1	11/4	16/3
hepatocyte necrosis	1/4	0/1	0/2	1/1
nodular hypertrophy/ hyperplasia	0/1	4/0	2/2	1/3
Lymph Node:				
hyperplasia-				
plasma cell	0/2	0/0	3/1	4/5
Lung:				
pneumonia	0/4	2/3	0/0	0/3
edema	0/0	0/1	0/1	0/0
emphysema	0/0	2/1	1/1	3/2

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TABLE 13 continued: Selected Microscopic Observations (2 years)†

Dose (ppm):	Control	40	200	1000
#animals m/f	28/24	24/26	22/26	23/30
Observation:				
Nose/Turbinates:				
inflammation-				
nasal mucosa	10/4	9/5	2/5	9/4
papillary hyperplasia of				
nasal epithelium	1/1	1/0	2/0	4/1
Pancreas:				
islet cell hyperplasia	1/0	2/1	0/0	0/2
Pituitary:				
hyperplasia-				
chromophobe	5/4	6/2	5/4	2/4
pars intermedia	2/0	2/0	0/0	2/0
Parathyroids:				
hyperplasia-				
nodular (or diffuse)	6[a]/1[c]	8[b]/0	2/1	4[b]/0
Spleen:				
hyperplasia-				
plasma cell	0/0	1/0	0/0	1/1
Testes:				
hyperplasia-				
interstitial cell	1	0	0	2
Thyroids:				
hyperplasia-				
"c" cell	0/2	3/4[d]	1/2	8**[c]/4
Urinary Bladder:				
hyperplasia-				
epithelial	3/0	1/1	1/1	1/1
Uterus:				
mucosa polyp	1	2	0	4

[a] = (#animals) = 27; [b]=21; [c]=22; [d]=25

** = $p<0.01$ by Fisher's Exact Test with Bonferroni inequality.

† = Data extracted from Report MSL-6119, Appendix II, Table 19.

TABLE 14: Selected Microscopic Observations (all deaths)†

Dose (ppm):	Control	40	200	1000
#animals m/f	70/70	70/70	70/70	70/70
Observation:				
Adrenals:				
hyperplasia/hypertrophy-				
medullary	6/2	13[a]/3	9/4	11/3
cortical nodular	4/6	6[a]/4	6/10	5/10
Bone Marrow:				
hyperplasia-				
myelocyte/granulocyte	10/14[b]	11[b]/12	10[a]/9	6/12[a]
pancytic	4/5[b]	1[b]/8	0/6	1/4[a]
Brain:				
compressed by pituitary	28/39	24[a]/40	29/39	21/43
Bone:				
fibrotic replacement	5/0	8[b]/0	5[a]/1	10/1[a]
osteolysis	4/0	6[b]/0	3[a]/0	10/0
Epididymides:				
epithelial degenerative changes	6	7[a]	8	7
Eyes:				
keratitis	11/5	8[a]/3	7/5	6/2
Heart:				
myocarditis	7/6	7[b]/8	10/7[a]	12/7
myocardiolysis	21/11	16[b]/6	16/5[a]	24/7
proliferation of endomysial/ myocyte nuclei	35/19	39[b]/22	38/25[a]	39/22
myocardial fibrosis	38/19	35[b]/21	34/17[a]	38/22
Kidneys:				
glomerular/periglomerular sclerosis	17/6	23[b]/4	20/5	16/2
chronic nephritis	57/33	62[b]/35	65/40	64/45
hydronephrosis-				
bilateral	1/3	2[b]/2	0/6	7/2
unilateral	5/2	5[b]/5	7/11	5/5
pyelitis	5/4	2[b]/4	4/6	4/5
pyelonephritis	4/5	5[b]/3	4/1	7/3
pelvic epithelium/hyperplasia-				
non-papilliform	1/1	3[b]/5	2/5	3/5
papilliform	2/0	1[b]/7	1/6	4/6
Liver:				
cellular alteration	17/19	14[b]/18	13/14	25/22
hyperplasia-				
bile duct	17/11	13[b]/14	14/11	22/16
telangiectasis	23/4	17[b]/4	18/5	29/5
hepatocyte necrosis	4/5	4[b]/4	5/10	7/4
nodular hypertrophy/ hyperplasia	0/4	5[b]/2	4/3	3/4
Lymph Node:				
hyperplasia-				
plasma cell	1/4	2[b]/4	5/6	2[a]/11
mononuclear cell leukemia	0/0	1[b]/0	1/0	0/0

continued

TABLE 14 continued: Selected Microscopic Observations (all deaths)†

Dose (ppm):	Control	40	200	1000
#animals m/f	70/70	70/70	70/70	70/70
Observation:				
Lung:				
pneumonia	7/10	13[b]/7	10/13	7/11
edema	2/2	3[b]/2	2/1	6/5
emphysema	3/0	4[b]/3	3/2	4/3
leukemia-myelogenous	0/0	0/0	1/0	0/0
Nose/Turbinates:				
mucosal lymphoid hyperplasia	6/1	1[b]/0	1/2	1/0
inflammation-				
nasal sinus	1/0	1[b]/0	1/1	0/1
nasal mucosa	13/8	18[b]/10	10/7	19/12
papillary hyperplasia of nasal epithelium	1/1	1[b]/3	4/1	5/4
Pancreas:				
islet cell hyperplasia	2[a]/1	2[b]/3	2[a]/1	2[b]/3
Pituitary:				
hyperplasia-				
chromophobe	7/9[a]	10[b]/3	9/9	6[a]/6
pars intermedia	2/1	2[b]/0	2/0	3[a]/0
Parathyroids				
hyperplasia-				
nodular(or diffuse)	13[b]/2[d]	22[c]/19	9[d]/4[b]	19[e]/0
Spleen:				
hyperplasia-				
plasma cell	1/0	1[a]/0	1[a]/0	3/3
Testes:				
hyperplasia-				
interstitial cell	2	1[a]	0	2
Thyroids:				
hyperplasia-				
"c" cell	3/3	5[f]/4[a]	4/3	8[a]/4
Urinary Bladder:				
hyperplasia-				
epithelial	5[a]/0	5[f]/4[a]	6/2	3/4[a]
Uterus				
mucosa polyp	3	5	2	5

[a] = 69 (#animals); [b] = 68; [c] = 62; [d] = 66; [e] = 63; [f] = 67; [g] = 64
† = Data extracted from Report MSL-6119, Appendix II, Table 21.

2) Neoplastic

The investigators provided group summary and individual animal data for all reported lesions. Table 15 presents selected observations from the 1 year interim sacrifice. Most lesions were infrequent, however, a lesion of note was the papillary adenoma of mucosa in the nose/turbinates in one female of the high dose. Table 16 presents selected observations at the final sacrifice. Again, most lesions were infrequent and scattered throughout the study groups with the exception of an increase in neoplastic nodules of the liver in the mid and high dose females, follicular adenoma/cystadenoma of the thyroids in high dose males and females and papillary adenoma of muscosa of the nose/turbinates in high dose animals (statistically significantly greater in high dose females). Papillary adenoma of nasal mucosa was also noted in the high dose animals dying prior to the end of the study (statistically significant in both sexes), Table 17. Other lesions present in animals that died early were infrequent and did not reveal any dose-response relationship. Combining observations time of all animals (Table 18) shows an increase neoplastic nodules of the liver in mid and high dose females and a statistically significant increase in the number of papillary adenomas of mucosa in the nose/turbinates in high dose males and females. The high incidence of pituitary adenoma in both sexes of all dose groups may be the reason for the high incidence of the observation "brain compressed by pituitary" noted in gross observations. There were 4 cases of malignant astrocytoma of the brain, 3 in control males and 1 in a high dose female and 2 cases of oligodendroglioma of the brain, 1 each in a control male and a high dose female.

TABLE 15: Selected Neoplastic Observations (1 year)[a]

Dose (ppm):	Control	40	200	1000
#animal m/f	10/10	10/10	10/10	10/10
Observation:				
Pituitary:				
adenocarcinoma	1/0	0/0	0/1	0/0
adenoma	1/4	0/4	5/3	4/3
Mammary Gland:				
adenoma/adenofibroma/ fibroma	0/1	0/0	0/0	0/0
adenocarcinoma	0/0	0/0	0/0	0/1
Nose/Turbinates:				
papillary adenoma of mucosa	0/0	0/0	0/0	0/1

[a] = Data extracted from Report MSL-6119, Appendix II, Table 22.

TABLE 16: Selected Neoplastic Observations (2 years)†

Dose (ppm):	Control	40	200	1000
#animals m/f	28/24	24/26	22/26	23/30
Observations:				
Adrenals:				
cortical adenoma	1/0	0/1	0/0	0/0
pheochromocytoma	3/0	5/1	1/1	5/0
malignant pheochromocytoma	1/0	1/1	0/0	1/0
Brain:				
astrocytoma, malignant	1/0	0/0	0/0	0/1
granular cell tumor	0/0	1/0	0/0	0/0
oligodendroglioma	1/0	0/0	0/0	0/1
Liver:				
neoplastic nodule	1/0	1/1	0/4	1/5
hepatocellular carcinoma	1/1	2/1	1/0	1/1
Mammary Gland:				
adenoma/adenofibroma/ fibroma	0/12[a]	0/13[b]	0/10	0/10
adenocarcinoma	0/4[a]	0/2[b]	1[c]/3	0/1
Nose/Turbinates:				
papillary adenoma of mucosa	1/0	0/0	0/0	3/9*
Pancreas:				
islet cell adenoma	4/3	3/2	4/2	0/2
Pituitary:				
adenocarcinoma	0/0	0/0	0/0	0/3
adenoma	16/19	16/20	15/21	17/20
Testes:				
interstitial cell tumor	2	3	1	2
Thyroids:				
follicular adenoma/ cystadenoma	1/0	0/1[b]	0/1	2[d]/3
"c" cell adenoma	2/2	2/2	0/4	0/1
Subcutis:				
fibrosarcoma	0/-•	1[c]/2[f]	0/0	-/0
fibroma	1[f]/-	4[c]/2[f]	0/0	-/0
neurofibroma	1[f]/-	0/0	0/19	-/0

* = $p<0.05$ by Fisher's Exact Test with Bonferroni Inequality

[a] = (#animals) = 22; [b] = 25; [c] = 8; [d] = 22; [e] = 6; [f] = 4; [g] = 3.

• = - = no data provided.

† = Data extracted from Report MSL-6119, Appendix II, Table 23.

Table 17: Selected Neoplastic Observations (early deaths)†

Dose (ppm):	Control	40	200	1000
#animals m/f	32/36	36/34	38/34	37/30
Observation:				
Adrenals:				
cortical carcinoma	0/0	0/2	1/2	0/0
cortical adenoma	2/1	2[a]/0	5/1	3/0
pheochromocytoma	0/1	0/1	0/0	0/0
malignant pheochromocytoma	0/0	0/0	0/0	1/0
Brain:				
astrocytoma, malignant	2/0	0/0	0/0	0/0
granular cell tumor	0/0	0/0	0/1	0/0
Liver:				
neoplastic nodule	0/2	1[a]/1	1/1	0/1
hepatocellular carcinoma	0/1	0/0	0/0	0/0
Mammary Gland:				
adenoma/adenofibroma/ fibroma	0/14	0/10[c]	2[b]/13[d]	0/8[e]
adenocarcinoma	0/4	0/3[c]	0/3[d]	0/4[e]
Nose/Turbinates:				
papillary adenoma of mucosa	0/0	0/0	0/0	9*/9*
adenocarcinoma of submucosal gland	0/0	0/0	0/1	0/0
Pancreas:				
islet cell adenoma	0/0	5[f]/5	4[g]/1	1[a]/2
islet cell carcinoma	0/0	0/0	0/1	0/0
acinar cell adenoma	1[d]/0	0/0	1[g]/0	0/0
acinar cell carcinoma	0/0	1[f]/0	0/0	0/0
Pituitary:				
adenocarcinoma	0/2[a]	0/1	1/3	0/3
adenoma	27/28[a]	27[f]/32	27/24	22[h]/22
Testes:				
interstitial cell tumor	0	0	0	1
Thyroids:				
follicular adenoma/ cystadenoma	0/1	1[i]/1	1/1	0/1
"c" cell adenoma	1/5	3[i]/0	0/1	2/1
Subcutis:				
fibrosarcoma	2[j]/0	1[k]/0	0/2[l]	1[m]/1[m]
fibroma	1[j]/0	3[k]/1[n]	1[n]/0	0/0
neurofibroma	0/1[n]	0/0	0/1[l]	0/0

* = $p<0.05$ using Fisher's Exact Test with Bonferroni Inequality

[a] = (#animals) = 35; [b]=11; [c]=29; [d]=31; [e]=28; [f]=34; [g]=37; [h]=36; [i]=33; [j]=3; [k]=6; [l]=5; [m]=1; [n]=2.

† = Data extracted from Report MSL-6119, Appendix II, Table 24.

TABLE 18: Selected Neoplastic Observations (all deaths)†

Dose (ppm):	Control	40	200	1000
#animals m/f	70/70	70/70	70/70	70/70
Observation:				
Adrenals:				
cortical carcinoma	0/0	0/2	1/2	0/0
cortical adenoma	1/1	0/2	0/0	0/0
pheochromocytoma	5/1	7[a]/1	6/2	8/0
malignant pheochromocytoma	1/0	1[a]/1	0/0	2/0
Brain:				
astrocytoma, malignant	3/0	0/0	0/0	0/1
granular cell tumor	0/0	1[a]/0	0/1	0/0
oligodendroglioma	1/0	0/0	0/0	0/1
Liver:				
neoplastic nodule	1/2	2[a]/2	1/5	1/6
hepatocellular carcinoma	1/2	2[a]/1	1/0	1/1
Mammary Gland:				
adenoma/adenofibroma/ fibroma	0/27[b]	0/23[c]	2[d]/23[e]	0/18[b]
adenocarcinoma	0/8[b]	0/5[c]	1[d]/6[e]	0/6
Nose/Turbinates:				
papillary adenoma of mucosa	1/0	0/0	0/0	12*/19*
adenocarcinoma of submucosal gland	0/0	0/0	0/1	0/0
Pancreas:				
islet cell adenoma	4[a]/3	8[b]/7	8[a]/3	1[b]/4
islet cell carcinoma	0/0	0/0	0/1	0/0
acinar cell adenoma	1[a]/0	0/0	1[a]/0	0/0
acinar cell carcinoma	0/0	1[b]/0	0/0	0/0
Pituitary:				
adenocarcinoma	1/2[a]	0/1	1/4	0/6
adenoma	41/51[a]	43[b]/56	47/48	43[a]/45
Testes:				
interstitial cell tumor	2	3[a]	1	3
Thyroids:				
follicular adenoma/ cystadenoma	1/1	1[f]/2[a]	1/2	2[a]/4
"c" cell adenoma	3/7	5[f]/2[a]	0/5	2[a]/2
Subcutis:				
fibrosarcoma	2[g]/0	2[h]/2[i]	0/2[i]	1[k]/1[l]
fibroma	2[g]/0	7[h]/3[i]	1[m]/0	0/0
neurofibroma	1[g]/1[k]	0/0	0/2[i]	0/0

* = $p<0.05$ using Fisher's Exact Test with Bonferroni Inequality.
[a] = (#animals) = 69; [b]=68; [c]=64; [d]=21; [e]=65; [f]=67; [g]=7; [h]=12; [i]=6; [j]=8; [k]=1; [l]=2; [m]=3.
† = Data extracted from Report MSL-6119, Appendix II, Table 25.

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D. DISCUSSION:

Acetochlor administered in doses of 40, 200 and 1000 ppm did not appreciably affect mortality or time-to-death. The clinical observation data were not presented in an adequate form for evaluation. Inspection of the individual animal clinical signs data reveals a possible dose-response in some observations. Body weight and body weight gain data showed a decrease in high dose males from day 8 on (statistically significant from days 455 to 678). High dose females also had a slight, but not statistically significant decrease in body weight and body weight gain. Food consumption was slightly decreased in the high dose animals. Food efficiency was reduced in animals of the high dose group (data was only presented for the first 13 weeks). No treatment related opthalmic observations were noted.

No biologically relevant or dose-related observations were noted in hematological parameters at 6, 12, 18 or 24 months.

The investigators did not conduct several clinical chemistry analyses, especially magnesium determinations, which can reveal several defects. Of the parameters measured, those attributable to treatment were statistically significant increases in gamma glutamyl transpeptidase in high dose males at 18 months and 2 years (mid and high dose males at 1 year showed slight increases as did mid dose males at 2 years). Also, cholesterol levels were increased (statistically significant) in high dose males at 2 years (a slight increase was noted at 18 months) and total bilrubin was increased in high dose females at 2 years. The observations of increased levels of gamma glutamyl transpeptidase and cholesterol may be indicative of liver toxicity.

No biologically relevant observations were noted in urinalysis data.

Organ weights determined at the interim sacrifice showed a slight increase in absolute and relative kidney weights in high dose males and a slight, dose-related increase in absolute and relative liver weights in treated males. This continued to final sacrifice where similar observations were noted including a statistically significant increase in relative liver weight of high dose males and an increase in absolute and relative testicular weight (statistically significant) in high dose males. Females were not similarly affected.

The gross pathological observations revealed no biologically relevant differences.

Microscopic observations for non-neoplastic findings at one year consisted of an increase in hepatocyte cellular alterations and liver bile duct hyperplasia in high dose males (1000 ppm) and an increase in inflammation of the nasal mucosa in high dose males and females. Of those animals dying prior to the end of the study, there was an apparent increase in hepatocyte cellular alteration, liver bile duct hyperplasia, hepatocyte necrosis and "nodular or diffuse" hyperplasia in the parathyroids of high dose males. However, tissue availability was not presented and since many organs had the observation "autolysis" with no indication if the autolysis involved the whole organ or just a defined area, a thorough evaluation of microscopic observations was not possible. At terminal sacrifice there was an increase in mid and high dose males and high dose females with plasma cell hyperplasia of the lymph node along with an increase in high dose males with papillary hyperplasia of the nasal epithelium and "c" cell hyperplasia of the thyroids (statistically significant). In the previous study (Study #PR-80-006, 5/20/83) with MON 097, there were increased histopathological observations in the liver and kidney in the high dose group (5000 ppm), see following discussion on neoplastic findings.

Neoplastic findings at the 1 year interim sacrifice were minimal with one incidence of a papillary adenoma of the mucosa in the nose/turbinates of a female in the high dose (1000 ppm) A statistically significant increase in this observation was noted in high dose males and females that died prior to study termination. At final sacrifice this observation was also increased where a statistically significant increase in high dose female and increase in high dose males of the observation of papillary adenoma of the mucosa in the nose/turbinates was noted. Other observations consisted of liver neoplastic nodules in high dose males and females at final sacrifice and early deaths and follicular adenoma/cystadenoma of the thyroids in high dose animals. These latter observations are similar to those observed in the earlier study (Study #PR-80-006, 5/20/83). In the earlier study, the high dose level (5000 ppm) caused increased incidence of liver carcinomas and thyroid follicular cell adenomas in males along with positive trends of increased hepatic carcinomas in high dose females (5000 ppm) and thyroid follicular cell adenomas in high dose males.

Based on the observations of decreased body weight gain, clinical chemistry observations, non-neoplastic findings and the neoplastic finding of an increase in papillary adenoma of the mucosa of the nose/turbinates in the males and females of the high dose group, it is apparent that the MTD (Maximum Tolerated Dose) was achieved in this study. This study is therefore acceptable for the chronic/oncogencity data requirement for Acetochlor (MON 097), however, the study is classified as supplementary data which possibly can be upgraded if requested. data is submitted and accepted by the Agency.

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ATTACHMENT D

007697

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

006764

JUN 30 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of additional rat nasal histopathology data for Monsanto Study # PR 80-006; Record no. 223011/223012/223016/223018; EPA ID no. 3F2966/6G3345/524-GUI/524-EUP-AT; Accession No. 40484801; Proj. No. 8-0776; Caswell No. 3B

TO: Robert Taylor/V.K. Walters (PM 25)
Registration Division (TS-769C)

FROM: James N. Rowe, Ph.D.
Section V, Toxicology Branch
Hazard Evaluation Branch (TS-769C)

James N. Rowe 6/27/88

THRU: Quang Q. Bui, Ph.D.
Section Head
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

6/30/88

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ACTION: Expedited review of additional rat chronic histopathology data for Monsanto Study # PR 80-006, May 83; Record no. 223011/223012/223016/223018; EPA ID no. 3F2966/6G3345/524-GUI/524-EUP-AT; Accession No. 40484801; Proj. No. 8-0776; Caswell No. 3B

RECOMMENDATIONS:

There is a dose-related increase in nasal papillary adenomas in male rats with statistically significant differences at the mid (1500 ppm) and high dose (5000 ppm) levels. Papillary adenocarcinomas are present also in two high dose males. A small number of papillary adenomas are also present in the mid and high dose females. However, the lack of dose-related findings for the female adenomas may relate to the significantly lower survival rate observed in these groups.

This data is consistent with the findings of nasal papillary tumors in a second rat chronic feeding study performed for acetochlor (EHL-83107). Results of this report should be considered in the context of its oncogenic classification.

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Reviewed By: James N. Rowe, Ph.D. James N. Rowe 6/27/88
Section V, Toxicology Branch (TS-769C)
Secondary Reviewer: Quang Bui, Ph.D.
Section Head, Section V, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study type: Partial Chronic Rat Histopathology (nasal tissues)
Test system: Rats, Sprague-Dawley
Guideline: 83-1, 83-2

Study Title: Histopathology Findings in Noses of Rats Administered MON 097 in a Lifetime Feeding Study

EPA ID NOS.: EPA ID NO. 3F2966/6G3345/524-GUI/524-EUP-AT
EPA Accession No. 40484801
Caswell No. 3B
Project No. 8-0776

Sponsor: Monsanto Company
St. Louis, MO 63110

Testing Laboratory: Tegeris Laboratories
9705 N. Washington Boulevard
Laurel, MD 20707
and
Monsanto Environmental Health Laboratory
645 S. Newstead
St. Louis, MO 63110

Laboratory Project No.: ML-86-44/EHL 86027

Final Report Date: 11/4/87

Date of Study Completion: 6/30/86

Study Author: W.E. Ribelin, D.V.M., Study director

Quality Assurance: A statement of Quality Assurance is signed by Arthur F. Uelner, Manager, Quality Assurance at EHL (10/11/87)

Compound: MON-097; chemical name is acetochlor.

CONCLUSIONS:

Based upon the histopathological reexamination, there is evidence for a dose-related increase in nasal papillary adenomas in male rats with statistically significant differences at the mid (1500 ppm) and high dose (5000 ppm) levels. Papillary adenocarcinomas are also present in two high dose males. A small number of papillary adenomas are also present in the mid and high dose females. However, the lack of dose-related findings for female adenomas may relate to the significantly lower survival rate observed in these groups.

Recommendations:

This data is consistent with the findings of nasal papillary tumors in a second rat chronic feeding study performed for acetochlor(MSL-83-200; EHL-83107). Results of this report should be considered in the context of its oncogenic potential and classification.

Background:

In a rat chronic study (Monsanto Study No. ML-83-200; EHL Project No. 83107), treatment related papillary adenomas were noted in the nasal mucosa of the posterior regions of the nasal cavity at 1000 ppm (MTD), a dose considerably lower than the HDT (5000 ppm) in an original study (Monsanto PR 80-006). This prompted a re-analysis of the nasal tissues with histopathological examination focusing on the posterior portion of the nasal cavity which had not been systematically examined in the original study. This submission consists of histopathology data from a rat chronic feeding study performed by Pharmacopathics Research Laboratories at dosage levels of 0, 500, 1500 and 5000 ppm and reported in May, 1983 (Monsanto Study No. PR-80-006; Pharmacopathics Report No. 8004).

Methods:

The nasal tissues were trimmed, processed, sectioned and stained at Tegeris labs. Original blocks of paraffin-embedded tissue were re-sectioned, or wet tissue remnants processed and sectioned, depending on the amount of available tissue. Sections were requested from all three functioning areas of the nose--squamous, respiratory and olfactory--and were generally available. Seventy males and seventy females were used per dose group. Tissues from the following animals were not available: MN068(control male), M2014(1500 ppm male), M3057 (5000 ppm male), FN069 (control female), F1001, F1008 (500 ppm females), F3037 (5000 ppm female). Each animal was re-identified with an EHL number. Survival times for each animal were obtained and plotted as life span tables.

Statistical methods:

Mean survival times for each treatment group were compared and survival rates were analyzed using the method of Breslow (Generalized Wilcoxon Procedure) and the method of Mantel (Generalized Savage procedure). Frequencies of lesions and tumors were analyzed with Fisher's Exact test with the Bonferroni Inequality Procedure. The relationship of time and dose to tumor was analyzed using the Peto test for linear trend.

Results:

Mean survival times with their statistical significance are presented below (taken from p. 5 of report):

Dose group:	Survival (days)	Breslow p =	Mantel p =
Males			
0 ppm	699	----	----
500 ppm	720	0.580	0.719
1500 ppm	722	0.191	0.159
5000 ppm	706	0.963	0.882
All treated	---	0.501	0.349
Females			
0 ppm	660	----	-----
500 ppm	617	0.027	0.067
1500 ppm	645	0.574	0.768
5000 ppm	593	0.001	0.002
All treated	---	0.004	0.006

Mean survival time for compound-treated males were not different from control times. Female survival times were statistically significantly lower for the low and high dose groups as well as for all treated females as compared to the controls.

A summary of selected histopathology findings is presented below along with trend analysis data:

There was a dose-related increase in papillary adenomas in treated males with the mid and high dose groups being statistically significantly different from the control group (control/0, 500 ppm/1, 1500 ppm/6, 5000 ppm/18). Two males in the high dose group(not present in animals with papillary adenoma) also had papillary adenocarcinoma. There was an apparent increase in all compound-treated males of inflammation of the nasal mucosa which was statistical significant in the 5000 ppm dose group (control/3 vs 5000 ppm/16; $p<.01$). For all but three high dose animals, there was no association between nasal inflammation and the presence of papillary adenomas in the males. Statistically significant treatment-related trends in males were calculated for nasal papillary adenomas and adenocarcinomas as well as for all nasal malignancies.

There were papillary adenomas observed in the female mid and high dose groups (control/0, mid/2, high/1) which approached statistical significance ($p<0.055$) with the Peto trend analysis. The lack of a clear dose-related elevation in nasal papillary adenomas among treated females may relate to the significantly lower survival rate observed in the groups.

Selected Histopathology findings (from pp. 1 and 2 of pathology section)

No. tissues examined: Dose group:	(69) 0ppm	(70) 500ppm	(69) 1500ppm	(69) 5000ppm
(MALES)				
NOSE/TURBINATES				
-autolysis	2	0	0	0
-inflammation, nasolacrimal duct	1	8	5	6
-inflammation, nasal mucosa	3	9	7	16**
-inflammatory epithelial hyperpl.	1	0	3	2
-papillary adenomas	0	1	6*	18**
-Squamous cell carcinoma	0	1	0	1
-Squamous papilloma	0	0	1	0
-osteoma, maxillary (benign)				
-papillary adenocarcinoma	0	0	0	2
-Esthesioneuroma (benign tumor)	0	0	0	1
No. tissues examined:	(69)	(68)	(70)	(69)
(FEMALES)				
NOSE/TURBINATES				
-inflammation, nasolacrimal duct	5	1	2	2
-inflammation, nasal mucosa	2	8	6	8
-inflammatory epithelial hyperpl.	1	0	2	0
-Papillary adenomas	0	0	2	1
-Squamous cell carcinoma	1	2	1	0
-carcinoma in-situ	0	0	1	0
-epithel. inflamm. squamous metaplasia	0	0	1	0
-submucosal glandular hyperplasia	0	0	0	2

* significantly different ($p \leq 0.05$) from control using Fisher's Exact Test with Bonferroni Inequality
** significantly different ($p \leq 0.01$) from control using Fisher's Exact Test with Bonferroni Inequality

Peto test* for trend:	"p"
nasal papillary adenoma, males	0.000
nasal papillary adenoma, females	0.055
nasal papillary adenoma, both sexes	0.000
papillary adenocarcinoma, males	0.027
esthesioneuroma, males	0.062
all nasal malignancies, males	0.031

* taken from p. 5 of EHL 86027 report

Summary/Conclusions:

Reanalysis of all acceptable nasal tissues (squamous, respiratory, and olfactory portions of the nose) from a rat chronic feeding study (Monsanto PR 80-006) conducted at dietary levels of 0, 500, 1500 and 5000 ppm of acetochlor were performed. Based upon the histopath-ological examination, there is evidence for a dose-related increase in papillary adenomas in male rats which is statistically significant at the mid and high dose levels. A statistically significant increase in nasal inflammation was also observed in high dose males but is not generally present in males having the papillary adenomas. Papillary adenocarcinomas are present also in two high dose males. A small number of papillary adenomas are also present in the mid and high dose females. The lack of dose-related findings for female adenomas may relate to the significantly lower survival rate observed in females.

Compound-related findings of nasal papillary adenomas/carcinomas in this study (Monsanto PR 80-006) at 1500 and 5000 ppm in males, thus, corroborate the similar findings at 1000 ppm in Monsanto Study ML-83-200.

(DUPLICATE)

ATTACHMENT E
007697
80

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

SEP - 9 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: ACETOCHLOR - Qualitative Risk assessment from a Rat 2-Year Chronic/Oncogenicity Study. Caswell No. -3B

FROM: C.J. Nelson, Statistician C J Nelson 9/7/88
Science Support Section
Science Analysis and Coordination Branch, HED (TS-769C)

TO: Stephen C. Dapson, Ph.D. Pharmacologist
Review Section I
Toxicology Branch II, HED (TS-769C)

THRU: Richard Levy, M.P.H.,Leader-Biostatistics Team 9-7-88
Science Support Section
Science Analysis and Coordination Branch, HED (TS-769C)

and

John A Quest, Ph.D., Chief J.A. Quest 9/9/88
Science Support Section
Science Analysis and Coordination Branch, HED (TS-769C)

SUMMARY:

Acetochlor was fed to male and female Sprague-Dawley rats at doses of 0, 40, 200, and 1000 ppm in a 107 week chronic toxicity/oncogenicity study.

For the female rat there were no survival problems. The incidence of nose papillary adenomas was significantly increased in the 1000 ppm dose group compared to controls and there was a significant dose-related trend. There was a significant dose related trend for thyroid follicular cell adenomas and/or carcinomas combined.

For the male rat there were no survival problems. The incidence of nose papillary adenomas was significantly increased in the 1000 ppm dose group compared to controls and there was a significant dose-related trend.

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BACKGROUND:

This is a repeat of a previous chronic/oncogenicity study in the rat. Acetochlor (96.1 % purity) was fed to male and female Sprague-Dawley rats at doses of 0, 40, 200, and 1000 ppm in a 107 week chronic toxicity/oncogenicity study. Approximately 10 animals of each sex were sacrificed after 52 weeks of continuous dosing in each dose group. The study was conducted by the Monsanto Environmental Health Lab for Monsanto Company. The report number was MSL-6119, EPA Accesion Number 400770601. Data was extracted from a final report dated September 25, 1986. Test animals were assigned randomly to the following groups:

Table 1. Experimental Design for Rat Chronic Study

	Time of Sacrifice (weeks)			
Dose	Total Number		52	
(ppm)	Male	Female	Male	Female
Control	70	70	10	10
40	70	70	10	10
200	70	70	10	10
1000	70	70	10	10

SURVIVAL ANALYSIS:

For the female rat there were no statistically significant results detected in mortality for either pair-wise comparisons of differences between the treated and the control or a linear trend with dose (Table 2).

For the male rat there were no statistically significant results detected in mortality for either pair-wise comparisons of differences between the treated and the control, or a linear trend with dose (Table 3).

Test for mortality were made using the Thomas, Breslow, and Gart procedure.

TABLE 2. ACETOCHLOR, ALBINO RAT Study-- FEMALE Mortality Rates+ and Cox or Generalized K/W Test Results

	WEEK					
DOSE(PPM)	1-26	27-54	54a	55-78	79-107a	TOTAL
0.000	1/70 (1)	0/69 (0)	10/10	10/59 (17)	25/49 (51)	36/60 (60)
40.000	0/70 (0)	1/70 (1)	10/10	9/59 (15)	24/50 (48)	34/60 (57)
200.000	2/70 (3)	1/68 (1)	10/10	12/57 (21)	19/45 (42)	34/60 (57)
1000.000	0/70 (0)	3/70 (4)	10/10	5/57 (9)	22/52 (42)	30/60 (50)

TABLE 3. ACETOCHLOR, ALBINO RAT Study-- MALE Mortality Rates+ and Cox or Generalized K/W Test Results

	WEEK					
DOSE(PPM)	1-26	27-54	54a	55-78	79-107a	TOTAL
0.000	0/70 (0)	1/70 (1)	10/10	4/59 (7)	27/55 (49)	32/60 (53)
40.000	2/70 (3)	2/68 (3)	10/10	3/56 (5)	29/53 (55)	36/60 (60)
200.000	0/70 (0)	1/70 (1)	10/10	11/59 (19)	25/48 (52)	37/60 (62)
1000.000	1/70 (1)	0/69 (0)	10/10	7/59 (12)	27/52 (52)	35/60 (58)

\+ Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
( ) Per cent

a Interim sacrifice was conducted at 54 weeks. Final sacrifice occurred at week 107.

Note: Time intervals were selected for display purposes only. Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

TUMOR ANALYSIS:

In the absence of any mortality differences for either sex of rats, Fisher's exact test was used to test for pair-wise differences between control and treated rats. The Cochran-Armitage trend was used to test for increasing incidence with increasing dose levels.

In the female rats, there was a significant linear trend for combined thyroid carcinomas and adenomas ($p = .0457$, Table 6). There were no other significant pair-wise differences or linear trends for thyroid adenomas or thyroid carcinomas. The incidence of papillary adenomas of the nose in the 1000 ppm group was significantly increased ($p < .0001$, Table 7) compared to controls and there was a significant dose-related trend ($p < .0001$).

In the male rats, there were no significant pair-wise differences or linear trends for thyroid adenomas (Table 8). There were no thyroid carcinomas. The incidence of papillary adenomas of the nose in the 1000 ppm group was significantly increased ($p = .0010$, Table 9) compared to controls and there was a significant dose-related trend ($p < .0001$).

TABLE 4. ACETOCHLOR ALBINO RAT Study-- FEMALE Thyroid Follicular Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	1/39 a (2.6)	2/44 a (4.5)	2/36 (5.6)	4/46 (8.7)
	p= 0.1124	p= 0.4015	p= 0.3639	p= 0.1940
CARCINOMA	0/30 (0.0)	0/35 (0.0)	0/28 (0.0)	1/36 b (2.8)
	p= 0.0537	p= 1.0000	p= 1.0000	p= 0.5455
ADENOMA CARCINOMA	1/39 (2.6)	2/44 (4.5)	2/36 (5.6)	5/46 (10.9)
	p= 0.0457 *	p= 0.4015	p= 0.3639	p= 0.1222

a First Adenoma observed at 90 weeks in dose 0 and 40 ppm.
b First Carcinoma observed at 100 weeks in dose 1000 ppm.

TABLE 5. ACETOCHLOR ALBINO RAT Study-- FEMALE Nose Papillary Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	0/69 (0.0)	0/69 (0.0)	0/67 (0.0)	19/68 c (27.9)
	p< 0.0001 **	p= 1.0000	p= 1.0000	p< 0.0001 **

\+ Number of tumor bearing animals/Number of animals at risk. (Excluding animals that died before the observation of the first tumor or animals not examined).
( ) Per cent

c First Adenoma observed at 54 weeks in dose 1000 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

TABLE 6. ACETOCHLOR, ALBINO RAT Study-- MALE Thyroid Follicular Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	1/56 (1.8)	1/53 (1.9)	1/54 a (1.9)	2/54 (3.7)
	P= 0.2208	p= 0.5042	p= 0.5044	p= 0.3713

a First Adenoma observed at 75 weeks in dose 1000 ppm..
No Carcinomas occurred in male rats.

TABLE 7. ACETOCHLOR, ALBINO RAT Study-- MALE Nose Papillary Tumor Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

DOSE	0.000	40.000	200.000	1000.000
ADENOMA	1/58 (1.7)	0/56 (0.0)	0/58 (0.0)	12/59 (20.3)
	P< 0.0001 **	p= 0.5179	p= 0.5000	p= 0.0010 **

+ Number of tumor bearing animals/Number of animals at risk. (Excluding animals that died before the observation of the first tumor or animals not examined).
( ) Per cent

a First Adenoma observed at 67 weeks in dose 1000 ppm.

Note: Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. * denotes $p < 0.05$ and ** denotes $p < 0.01$

REFERENCES:

Thomas, D G, N Breslow, and J J Gart, Trend and Homogeneity Analyses of Proportions and Life Table Data. Computers and Biomedical Research 10, 373-381, 1977.

Cochran, W.G. Some Methods for Strengthening the Common X^2 Test. Biometrics 10, 417-451, 1954.

Armitage, P. Tests for Linear Trends in Proportions and Frequencies. Biometrics 11, 375-386, 1955.

increasing linear trend with dose. The incidence of malignant mammary tumors and all mammary tumors combined was significantly increased in the 1000 ppm dose group compared to controls and there was a significant increasing dose-related trend for both analyses. The 3 ppm dose group was significantly increased compared to control for malignant mammary tumors.

ATTACHMENT F
007697 C04586

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-01-6561
TASK: 109
August 5, 1985

DATA EVALUATION RECORD

ACETOCHLOR (Harness)

Oncogenicity Study in Mice

STUDY IDENTIFICATION: Ahmed, F. E., Tegeris, A. S., Seely, J. C. MON-097: 24-month oncogenicity study in the mouse. (Unpublished report No. PR-80-007 prepared by Pharmacopathics Research Laboratories, Inc., Laurel, MD, for Monsanto Agricultural Products Company, St. Louis, MO; dated May 4, 1983.) Accession Nos. 071966-071968.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: James R. Plant IV

Date: August 2, 1985

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1. CHEMICAL: Acetochlor: 2-chloro-N-(ethoxymethyl)-6'-ethyl-ortho-acetotoluidine.

2. TEST MATERIAL: MON-097, purity 94.5%; Lot No. NBP 1737874.

3. STUDY/ACTION TYPE: Oncogenicity study in mice.

4. STUDY IDENTIFICATION: Ahmed, F. E., Tegeris, A. S., Seely, J. C. MON-097: 24-month oncogenicity study in the mouse. (Unpublished report No. PR-80-007 prepared by Pharmacopathics Research Laboratories, Inc., Laurel, MD, for Monsanto Agricultural Products Company, St. Louis, MO; dated May 4, 1983.) Accession Nos. 071966-071968.

5. REVIEWED BY:

William L. Richards, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. Richards
Date: 8-7-85

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: Aug 2, 1985

6. APPROVED BY:

Norbert Page, D.V.M., D.A.B.T.
Oncogenicity & Chronic Effects
Technical Quality Control
Dynamac Corporation

Signature: Jim Lawrence for
Date: 8/2/85

Winnie Teeters, Ph.D.
EPA Reviewer

Signature: W. Teeters
Date: 8-3-85

Laurence Chitlik, D.A.B.T.
EPA Section Head

Signature: Laurence D Chitlik
Date: 8/3/85

7. CONCLUSIONS:

A. Under the conditions of this study, treatment of random-bred Swiss albino CD-1 mice with MON-097 resulted in a definite increase in tumors of the liver, lung, and uterus with suggestive increased tumors of the ovaries and kidneys:

1. Definite increases based on pairwise comparison using the chi square test or Fisher exact test.

a) liver carcinomas, high-dose males ($p \leq 0.01$)

b) total lung tumors, females at all doses ($p \leq 0.01$)

c) carcinomas of the lung in low and high dose females ($p \leq 0.05$)

d) uterine histiocytic sarcomas, low- and mid-dose females ($p \leq 0.01$) and high dose females ($p \leq 0.05$)

e) Total benign tumors of the ovaries in mid-dose females ($p \leq 0.05$)

2. Only suggestive increases based on linear trend analysis using the Peto method ($p < 0.01$)

a) liver carcinomas, females and males
b) lung carcinomas, females
c) total lung tumors in females
d) ovary benign tumors
e) kidney adenomas, females

Changes in other parameters that appeared to be related to dosing included: 1) an increased mortality in both high-dose males and females; 2) decreased mean body weights in high-dose males and females; 3) decreased red blood cell count, hematocrit, and hemoglobin in high-dose females at terminal sacrifice; 4) increased white blood cell count in high-dose males at terminal sacrifice; 5) increased platelet count in mid- and high-dose females at terminal sacrifice; 6) increased mean liver weights and liver-to body weight ratios at study termination in all dosed groups of males and in high-dose females as well as an increase in liver-to-body weight ratios in all dosed males and females at 12 months; an increase in absolute and relative kidney weights in all dosed groups of males at termination; and an increase in absolute and relative adrenal weights in all groups of males and in high-dose females at study termination; 7) an increase in interstitial nephritis in high-dose males and females.

A NOEL for chronic toxicity could not be established based on increased liver, and kidney, weights at the low-dose level. The LOEL for chronic toxicity of MON-097 in mice was 500 ppm (lowest dose tested).

Core Classification: Core Minimum.

8. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

For details of the author's Materials and Methods see Appendix A of this review.

The test material was MON-097 (CP 55097, NBP 1737874), a maroon liquid with a stated purity of 94.5%. The major componemt is 2-chloro-N-(ethoxymethyl)-6'-ethyl-ortho-aceto-toluidine. The basic experimental design consisted of the exposure of Swiss albino CD-1 mice to MON-097 in the diet for up to 23 months at dose levels of 0, 500, 1500, and 5000 ppm. Five hundred random-bred Swiss albino CD-1 weanling mice were inspected upon arrival, quarantined for 22-23 days, and randomized by weight into the experimental groups prior to dosing. Twenty mice were sacrificed before the start of dosing to determine baseline gross pathology and histopathology, with the remainder assigned to groups of 60 male and 60 female mice at each dose level. Ten of each group were sacrificed at 12 months so that the long-term study, in effect, consisted of 50 animals per group fed the indicated doses for up to 23 months. The diets were prepared weekly.

Animals were observed twice daily for mortality or other signs of toxicity. Body weights and food consumption were determined once pre-test, weekly during the first 13 weeks, and biweekly thereafter. Terminal body weights were those determined at necropsy or weights taken within 7 days before sacrifice. Organ weights were determined at the interim and the terminal sacrifices on fixed tissues.

Urinalysis, hematology, and blood chemistry values were determined in 10 mice/sex/dose at a 12-month interim sacrifice and at study termination. Blood was pooled from 3-4 mice for chemistry determinations.

Complete gross pathology examinations and histopathological evaluations were performed on each animal.

Body weight and food consumption data were analyzed statistically by one way analysis of variance using F test for comparison of variances and Dunnett's test was used to determine which means were significantly different from controls. Clinical laboratory data and organ weight data were analyzed by a two-sided Student's t-test. Neither the protocol nor materials and methods indicated

that the study author analyzed histopathology data. The study sponsor analyzed the incidence of tumor and nontumor lesions to detect statistically significant ($p \leq 0.01$) dose-related linear trends and differences between control and dosed animal values.

B. Protocol:

See Appendix B for Protocol details.

9. REPORTED RESULTS:

Analysis of Diets: The analytical procedure for MON-097 was validated prior to initiation of the study. The response was linear in the range to be used for the analysis, and diet analyses prior to the study were reasonably reproducible. For nominal values of 500, 1500, and 5000 ppm, the respective reproducibilities were 110.83%, 109.17%, and 88.89%; the respective standard deviations expressed as percent were 9.77, 12.69, and 12.80. Mixing was efficient and test compound was stable in the diet for 14 days. Diet analyses during the study indicated MON-097 was stable in the diets for at least one week (diets were prepared weekly) and was homogeneously mixed with the diets. MON-097 in the diet was analyzed at weeks 1, 2, 3, 4, 6, 8, 12, 18, 24, 30, 36, 42, 48, 52, 60, 78, and 90. The means and standard deviations for the study, as calculated by our reviewers, were:

Nominal (ppm)	Analytical (ppm)	Coefficient of Variation (%)	Range (ppm)
500	492.06± 37.14	7.5	454.92 - 529.2
1500	1468.71± 93.09	6.3	1375.62 - 1561.80
5000	4894.29±307.90	6.3	4586.39 - 5202.19

Clinical Observations: No unusual clinical signs were observed that were considered to be related to dosing. The most frequently observed signs were alopecia, skin lesions, and distended abdomens; these were random in occurrence.

Mortality: Mortality data at selected intervals are summarized in Table 1. A general increase in mortality began to appear after month 12 in dosed animals as compared with controls. Survival at 18 months ranged from 66-94% in males groups and 60-86% in female groups. The study was terminated at 23 months when survival was 26% in both males and females receiving 5000 ppm (high-dose).

TABLE 1. Mortality and Percent Survival at Selected Intervals in Mice Fed MON-097 for 23 Months[a]

Groups/Dose (ppm)	Mortality (and % Survival) at End of Month				
	1	6	12	18	23
Males					
0	0 (100%)	0 (100%)	0 (100%)	3 (94%)	20 (60%)
500	0 (100%)	3 (94%)	7 (86%)	16 (68%)	25 (50%)
1500	0 (100%)	0 (100%)	2 (96%)	6 (88%)	25 (50%)
5000	0 (100%)	3 (94%)	5 (90%)	17 (66%)	37 (26%)
Females					
0	1 (98%)	1 (98%)	3 (94%)	7 (86%)	19 (62%)
500	0 (100%)	0 (100%)	2 (96%)	9 (82%)	25 (50%)
1500	0 (100%)	0 (100%)	2 (96%)	11 (78%)	33 (34%)
5000	1 (98%)	2 (96%)	7 (86%)	20 (60%)	37 (26%)

[a]Fifty animals/sex/group; animals scheduled for sacrifice at 12 months were not included in mortality calculations.

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Body Weights: Body weights at selected intervals are summarized in Table 2. Significantly ($p \leq 0.05$) lower body weights in dosed animals as compared with controls were observed in the following groups: mid-dose males at 53 and 79 weeks; high-dose males and females at all selected time intervals. The mean body weights of high-dose males and females was approximately 80% of control at study termination. In mid-dose males mean body weights were decreased 6.5% at 18 months but only 3% at 23 months as compared to controls.

Food and Water Consumption: Water consumption was not measured. Food consumption data at selected intervals are summarized in Table 3. Although significant ($p \leq 0.05$) sporadic changes in food consumption were found in both sexes, they were not consistent and there were no changes that were related to dose level.

Food Efficiency: Mean food efficiencies during the first 13 weeks of study are summarized in Table 4. There were no changes in food efficiency that were related to dose level during this early phase of the study. Food efficiency was not studied beyond week 13.

Hematology: Except for a decrease in red cell parameters (RBC, Hmct, and Hb) in high-dose females at month 23, which the authors correlated with anemia, and an increase in white cell count in high-dose males at month 23, which the authors indicated to correlate with hepatocellular carcinoma, other changes were not consistent with time or dose and not considered compound related (authors). The following significant ($p \leq 0.05$) decreases in hematology parameters were observed (Table 5): red blood cell count (RBC) in high-dose females at months 12 and 23; hemoglobin (Hgb) in high-dose females at month 23; hematocrit (Hmct) in high-dose females at month 23 and in mid- and high-dose males at month 12. Significant increases in the following hematology parameters were also observed (Table 5): white blood cell count (WBC) in high-dose males at month 23; RBC in mid-dose females at month 12; platelet count (Plt Ct) in low- and mid-dose males at month 12 and in mid- and high-dose females at month 23.

Clinical Chemistry: For serum alkaline phosphatase (SAP), serum glutamic oxaloacetic transaminase (SGOT), and total bilirubin (TB), some significant increases were observed (Table 6) as follows: SAP in high-dose females at month 12; SGOT in high-dose males at month 12; TB in mid-dose females at month 23. The authors attributed changes in total protein to hemolysis of blood samples. There were no good correlations between SAP, SGOT, SGPT, and TB and histologic findings. Since all values were from pooled blood samples of 3-4 animals, direct animal correlations of chemistry and histologic changes could not be made (CBI pp 52-57).

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TABLE 2. Selected Mean Body Weights for Mice Fed MON-097 for 23 Months

Groups/Dose (ppm)	Body Weights (g) at Week:			
	27	53	79	99
Males				
0	35.683 ± 3.427[a]	37.017 ± 3.895	36.787 ± 3.526	35.500 ± 3.214
500	34.947 ± 2.649	36.547 ± 2.932	36.088 ± 2.843	35.880 ± 3.206
1500	34.950 ± 2.873	35.293[b] ± 3.195	34.386[b] ± 3.059	34.720 ± 3.156
5000	31.386[b] ± 2.527	31.018[b] ± 2.621	30.545[b] ±2.251	29.286[b] ± 2.054
Females				
0	29.441 ± 2.866[a]	30.368 ± 2.932	32.744 ± 2.945	31.545 ± 4.131
500	28.433 ± 2.936	31.069 ± 3.722	33.000 ± 3.413	32.040 ± 2.993
1500	29.267 ± 3.156	30.448 ± 3.039	31.974 ± 2.716	31.750 ± 2.826
5000	25.931[b] ± 2.183	26.830[b] ± 2.293	28.933[b] ± 2.753	28.267[b] ± 3.788

[a] Standard deviation.

[b] Significantly different from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

TABLE 3. Selected Mean Food Consumption in Mice Fed MON-097 for 23 Months

Group/Dose (ppm)	Grams of Food/Mouse/Week at Week 13	27	53	79	99
Males					
0	35.2 ±4.6[a]	38.0 ±3.8	37.4 ±4.5	39.0 ±4.4	38.1 ±4.6
500	34.6 ±4.9	38 0 ±3.0	36.7 ±3.6	36.9 ±3.4	35.5 ±3.9
1500	33.2* ±3.2	37.7 ±3.3	36.5 ±3.8	37.0 ±4.8	36.9 ±4.2
5000	35.7 ±4.7	39.2 ±5.4	36.5 ±4.8	36.1* ±4.4	37.0 ±4.6
Females					
0	35.1 ±4.5	40.3 ±5.0	39.4 ±6.0	39.2 ±4.0	36.5 ±5.9
500	32.9* ±3.8	39.7 ±4.4	40.6 ±6.4	40.4 ±3.1	38.7 ±3.2
1500	33.7 ±4.0	40.0 ±4.1	40.1 ±4.8	38.9 ±3.9	37.8 ±4.0
5000	37.9* ±5.1	45.4* ±7.4	41.9 ±8.5	37.4 ±4.6	35.5 ±8.1

[a] Standard deviation.

* Significantly different from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

TABLE 4. Mean Food Efficiency (Change in Body Weight/Food Consumed/Week) During the First 13 Weeks of a 23-Month Study of MON-097 Oncogenicity[a]

Dose (ppm)	Males	Females
0	0.015[b] ±0.025[c]	0.011 ±0.027
500	0.015 ±0.019	0.011 ±0.023
1500	0.013 ±0.025	0.014 ±0.023
5000	0.008 ±0.022	0.011 ±0.030

[a] Statistical analysis of these data by our reviewers indicated no significant differences in mean food efficiencies between control and dosed groups (ANOVA followed by Duncan's multiple range test) and no dose-related trends (regression analysis)

[b] g body weight/g food consumed/week, mean for first 13 weeks of study.

[c] Standard deviation

TABLE 5. Determination of Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Hemoglobin (Hgb), Hematocrit (Hmct), and Platelet Count (Plt Ct) in Mice Fed MON-097

	Males Dose (ppm)				Females Dose (ppm)			
	0	500	1500	5000	0	500	1500	5000
RBC ($\times 10^6/mm^3$)								
12 months	7.4 ±1.1[a]	7.0 ±0.8	7.3 ±1.0	6.7 ±1.1	7.3 ±0.7	7.7 ±0.6	8.0[b] ±0.5	6.5[b] ±0.9
23 months	5.3 ±1.3	5.0 ±0.6	5.4 ±1.2	4.5 ±0.6	4.8 ±0.6	5.4 ±0.8	5.4 ±1.0	3.8[b] ±0.7
WBC ($\times 10^3/mm^3$)								
12 months	6.9 ±1.7	9.1 ±4.1	7.8 ±2.8	7.8 ±2.8	8.7 ±2.5	9.8 ±4.9	9.3 ±3.1	10.0 ±4.5
23 months	9.7 ±2.4	13.6 ±5.9	12.0 ±3.8	14.5[b] ±3.5	15.2 ±4.4	14.2 ±8.8	13.9 ±4.8	26.0 ±30.0
Hgb (g/dL)								
12 months	12.7 ±0.9	12.7 ±1.0	11.8 ±1.3	11.6 ±1.5	11.9 ±1.9	12.7 ±0.8	12.7 ±1.2	11.7 ±0.7
23 months	12.0 ±2.2	12.0 ±1.2	12.7 ±1.7	10.9 ±1.7	12.0 ±1.5	13.0 ±1.8	12.5 ±2.0	9.3[b] ±2.0
Hmct (pc/dL)								
12 months	40.0 ±3.2	37.9 ±3.0	36.6[b] ±3.4	37.4[b] ±2.2	39.2 ±4.1	39.5 ±2.7	39.5 ±2.2	38.0 ±2.9
23 months	35.8 ±9.6	36.7 ±3.5	37.1 ±4.4	32.6 ±5.5	37.8 ±4.5	38.9 ±5.4	39.5 ±4.0	29.1[b] ±6.4
Plt Ct ($\times 10^3/mm^3$)								
12 months	437 ±262	710 [b] ±180	847 [b] ±153	565 ±242	513 ±303	579 ±261	454 ±187	662 ±168
23 months	456 ±281	408 ±187	547 ±170	478 ±179	302 ±129	309 ±99	484 [b] ±185	482 [b] ±212

[a]Standard deviation.

[b]Significantly different from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

TABLE 6. Serum Levels of Alkaline Phosphatase (SAP), Glutamic Oxaloacetic Transaminase (SGOT), and Total Bilirubin (TB) in Mice Fed MON-097

	Males Dose (ppm)				Females Dose (ppm)			
	0	500	1500	5000	0	500	1500	5000
SAP (IU/L)								
12 months	206 ±42 [a]	212 ±31	211 ±12	199 ±48	167 ±11	182 ±54	183 ±20	195 ±13 [b]
23 months	273 ±113 [c]	192 ±27	227 ±36	179 ±5	198 ±56	246 ±55	243 ±83	351 ±180 [c]
SGOT (IU/L)								
12 months	75 ±8	85 ±14	84 ±18	105 ±7 [b]	88 ±16	82 ±13	98 ±1	102 ±7
23 months	116 ±40 [c]	77 ±20	106 ±24	103 ±16	82 ±18	61 ±46	109 ±20	85 ±18 [c]
TB (mg/dL)								
12 months	0.4 ±0.0	0.4 ±0.1	0.4 0.0	0.4 0.0	0.4 ±0.0	0.5 ±0.2	0.4 ±0.1	0.4 ±0.1
23 months	0.4 ±0.1 [c]	0.4 ±0.0	0.6 ±0.1	0.6 ±0.2	0.4 ±0.1	0.5 ±0.3	0.6 ±0.1 [b]	0.6 ±0.1 [c]

[a] Standard deviation.

[b] Significantly different from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

[c] Only 2 pools were analyzed.

Urinalysis: There were no changes in urinary parameters related to dosing.

Organ Weights: At month 12, parallel increases in organ weights and organ-to-body weight ratios were reported for livers (Table 7), and adrenals in dosed females (Table 8). At month 23, parallel increases in organ weights and organ-to-body weight ratios were observed for male livers (Table 7), male and female adrenals (Table 8), male kidneys (Table 9), and female thyroids/parathyroids (Table 10). There were no treatment-related weight changes in the other organs that were examined (brain, pituitary, heart, and gonads).

Gross Pathology: Summary tabulation of gross pathology findings by the report authors (CBI pp II83-II86) did not include the number of animals per group with specific lesions in a particular tissue but tabulated the number of animals with neoplastic or nonneoplastic lesions in an organ system (e.g., digestive, endocrine, reproductive) by dose and sex. Individual pathology data records contained more specific data Gross observations at necropsy included: 1) an increase in urinary tract lesions in males (scheduled sacrifices for all dose groups; those that died or were sacrificed in moribund condition in the high-dose group) and in the females (scheduled sacrifices for mid- and high-dose groups; those that died or were sacrificed in moribund condition in the high-dose group); 2) an increase in digestive tract (primarily liver) masses in males (scheduled sacrifices for mid- and high-dose groups); 3) an increase in pulmonary masses in females (scheduled sacrifices and animals that died or were sacrificed in moribund condition for all dose groups); and 4) reproductive tract masses in females (scheduled sacrifices for high-dose group). The author stated that a variety of other lesions and masses were observed but were not considered treatment-related.

Histopathology: Table 11 presents a summary of the incidence of neoplastic lesions. If only one animal in any dose group had a tumor, it was not included in the table. The report authors did not indicate any statistical analysis of the data. However, they concluded that there was a dose-related increase in the incidence of the following neoplasms:

- o histiocytic sarcomas of the uterus in low-, mid-, and high-dose females
- o lung adenomas and carcinomas combined in all groups of dosed females
- o lung adenomas in low- and mid-dose groups of males and low-, mid-, and high-dose groups of females
- o hepatic carcinomas in low-, mid- and high-dose groups of males and in high-dose females

TABLE 7. Mean Liver Weights and Liver-to-Body Weight Ratios in Mice Fed MON-097[c]

Dietary Level (ppm)	Males		Females	
	Liver Weight (g)	g Liver / 1000 g body weight	Liver Weight (g)	g Liver / 1000 g body weight
12-Month Sacrifice				
0	1.49 ±0.265[a]	41.964 ±7.865	1.30 ±0.240	48.861 ±7.212
500	1.58 ±0.123	49.636[b] ±6.313	1.45 ±0.235	55.653[b] ±7.245
1500	1.44 ±0.262	52.166[b] ±6.536	1.62[b] ±0.316	60.340[b] ±8.056
5000	1.68 ±0.279	56.324[b] ±12.418	1.53[b] ±0.098	70.994[b] ±5.370
23-Month Sacrifice				
0	1.62 ±0.327[a]	45.507 ±8.169	1.76 ±1.303	54.357 ±30.808
500	2.10[b] ±1.031	58.464[b] ±27.742	1.72 ±0.260	53.886 ±7.989
1500	1.96[b] ±0.451	56.271[b] ±12.167	1.62 ±0.258	50.873 ±7.723
5000	2.52[b] ±0.852	87.088[b] ±32.926	1.92 ±0.311	65.595 ±7.695

[a] Standard deviation.

[b] Significantly different from control ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test; analysis by report authors.

[c] Performance of Bartlett's test by our reviewers indicated inhomogeneous variances for these data; transformation of data to achieve homogeneity of variance was performed by our reviewers prior to reanalysis by ANOVA followed by Duncan's multiple range test. The means and standard deviations are presented as the values before transformation.

TABLE 8. Mean Adrenal Weights and Adrenal-to-Body Weight Ratios in Mice Fed MON-097

Dietary Level (ppm)	Males		Females	
	Adrenal Weight (g)	g Adrenal / 1000 g body weight	Adrenal Weight (g)	g Adrenal / 1000 g body weight
12-Month Sacrifice				
0	0.01 ±0.004[a]	0.342 ±0.134	0.01 ±0.003	0.420 ±0.131
500	0.01 ±0.005	0.439 ±0.154	0.02[b] ±0.009	0.834[b] ±0.426
1500	0.01 ±0.003	0.411 ±0.167	0.02[b] ±0.005	0.606[b] ±0.219
5000	0.01 ±0.005	0.471 ±0.181	0.02[b] ±0.004	0.831[b] ±0.187
23-Month Sacrifice				
0	0.007 ±0.002[a]	0.191 ±0.068	0.013 ±0.003	0.431 ±0.106
500	0.009[b] ±0.003	0.246[b] ±0.077	0.014 ±0.004	0.443 ±0.106
1500	0.009[b] ±0.003	0.259[b] ±0.105	0.015 ±0.004	0.470 ±0.123
5000	0.010[b] ±0.003	0.360[b] ±0.122	0.016[b] ±0.003	0.556[b] ±0.127

[a] Standard deviation.

[b] Statistically significant from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

TABLE 9. Mean Kidney Weights and Kidney-to-Body Weight Ratios in Mice Fed MON-097

Dietary Level (ppm)	Males		Females	
	Kidney Wt. (g)	g kidney / 1000 g body wt.	Kidney Wt. (g)	g kidney / 1000 g body wt.
12-Month Sacrifice				
0	0.73 ±0.108[a]	20.705 ±2.869	0.46 ±0.064	17.403 ±2.485
500	0.89[b] ±0.143	27.762[b] ±4.999	0.54 ±0.105	20.695[b] ±3.483
1500	0.78 ±0.179	28.001[b] ±4.625	0.55[b] ±0.090	20.504[b] ±2.494
5000	0.81 ±0.180	26.983[b] ±5.667	0.41 ±0.047	19.172 ±2.401
23-Month Sacrifice				
0	0.76 ±0.127[a]	21.352 ±2.833	0.55 ±0.099	17.330 ±2.716
500	1.06[b] ±0.183	29.696[b] ±5.254	0.64[b] ±0.084	19.978 ±2.829
1500	1.08[b] ±0.270	31.028[b] ±6.423	0.52 ±0.050	16.283 ±1.397
5000	0.87[b] ±0.178	29.657[b] ±5.696	0.60 ±0.100	20.748[b] ±3.345

[a] Standard deviation.

[b] Significantly different from control ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

TABLE 10. Mean Thyroid/Parathyroid Weights and Thyroid/Parathyroid-to-Body Weight Ratios in Mice Fed MON-097

Dietary Level (ppm)	Males		Females	
	Thyroid/ Parathyroids Wt. (g)	g thyroid/parathyroids / 1000 g body wt.	Thyroid/ Parathyroids Wt. (g)	g thyroid/parathyroids / 1000 g body wt.
12-Month Sacrifice				
0	0.005 ±0.002[a]	0.133 ±0.047	0.005 ±0.003	0.196 ±0.100
500	0.005 ±0.002	0.170 ±0.071	0.005 ±0.002	0.205 ±0.079
1500	0.005 ±0.002	0.191[b] ±0.064	0.007 ±0.002	0.277[b] ±0.069
5000	0.005 ±0.002	0.156 ±0.074	0.006 ±0.002	0.384[b] ±0.083
23-Month Sacrifice				
0	0.007 ±0.002[a]	0.212 ±0.070	0.007 ±0.002	0.212 ±0.071
500	0.009[b] ±0.003	0.253 ±0.092	0.008[b] ±0.002	0.247 ±0.068
1500	0.007 ±0.002	0.206 ±0.065	0.009[b] ±0.002	0.296[b] ±0.089
5000	0.008 ±0.002	0.277[b] ±0.073	0.010[b] ±0.003	0.356[b] ±0.106

[a] Standard deviation.

[b] Significantly different from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test; analysis by report authors.

TABLE 11. Frequently Occurring Neoplastic Lesions in Mice Fed MON-097 for 23 Months[a]

Organ/Lesion	Males/Dose (ppm) 0	500	1500	5000	Females/Dose (ppm) 0	500	1500	5000
No. of animals examined microscopically	60	60	60	60	60	60	60	59
No. of animals with tumors	35	26	43	40[b]	23[c]	31	36[d]	31[e]
- Harderian gland	(60)[f]	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Adenoma	8	7	7	9	3	1	5	4
- Kidneys	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Adenocarcinoma	0	0	2	1	0	0	0	0
Adenoma	2	1	1	2	0	0	0	3[b]
Sarcoma	0	0	0	0	0	0	0	2
- Total malignant kidney tumors	0	0	2	1	0	0	0	2
- Liver	(60)[f]	(59)	(60)	(59)	(60)	(60)	(60)	(58)
Adenoma	8	4	9	7	2	0	0	4
Carcinoma	6	7	10	22[b,d]	1	0	0	4[b]
- Lungs	(60)[f]	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Adenoma	6	10	12	5	2	6	8[g]	4
Carcinoma	7	3	4	3	0	5[g]	3	7[b,g]
Histiocytic sarcoma	0	0	0	0	0	0	1	0
- Total lung tumors	13	13	16	8	2	11[d]	12[d]	11[b,d]
- Lymphatic System	(60)[f]	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Lymphoma	4	2	2	4	6	7	12	1
- Ovaries	-	-	-	-	(59)[f]	(60)	(60)	(58)
Adenoma	-	-	-	-	0	0	1	0
Granulosa cell tumor	-	-	-	-	0	0	3	2
Luteoma	-	-	-	-	0	0	1	1
- Total benign ovarian tumors	-	-	-	-	0	0	5[g]	3[b]

TABLE 11. Frequently Occurring Neoplastic Lesions in Mice Fed MON-097 for 23 Months[a] (continued)

Organ/Lesion	Males/Dose (ppm) 0	500	1500	5000	Females/Dose (ppm) 0	500	1500	5000
- Pituitary gland	(58)[f]	(49)	(58)	(54)	(58)	(57)	(55)	(51)
Adenoma	0	0	0	1	2	2	0	0
- Uterus	-	-	-	-	(59)[f]	(60)	(60)	(59)
Endometrial stromal polyp	-	-	-	-	1	2	2	2
Histiocytic sarcoma	-	-	-	-	0	6[d]	6[d]	5[g]
Leiomyosarcoma	-	-	-	-	3	0	2	0

[a] Neoplastic lesions that occurred at a frequency of no more than one per dose group were excluded from this table unless significance was noted for total tumors of a given organ

[b] Statistically significant linear trend ($p \leq 0.01$) using the Peto analysis. It should be noted that the animals scheduled for interim sacrifice at 12 months (10/sex/dose) were included in the above compilation even though they would be at low risk of developing neoplasms by month 12. The sponsors indicated, however, that statistical analysis by the Peto method has the advantage of utilizing survival and time to tumor information.

[c] Corrected value found by our reviewers; this value was reported as 22 by the sponsor.

[d] Statistically significant increase compared to control ($p \leq 0.01$) using the Chi-square test (uncorrected for continuity).

[e] Reanalysis by our reviewers indicated a statistically significant linear trend ($p < 0.05$) using the Cochran-Armitage test; the analysis reported by the sponsor had used the value of 22 females with tumors at 0 ppm and had reported a significance of $p \leq 0.01$ using the Peto analysis.

[f] Number in parentheses is the number of animals from which tissue was examined histologically.

[g] Significantly different from control by Fisher's exact test $p < 0.05$.

The sponsor provided statistical analysis of incidence of neoplasms using chi-square (without continuity correction), and analysis of linear trend using the Peto analysis. With the chi-square analysis there was a statistically increased incidence ($p \leq 0.01$) in the following:

- liver carcinomas in high-dose males (22/59) as compared to controls (6/60);
- total lung tumors in the low- (11/60), mid- (12/60) and high-dose females (11/59) as compared to controls (2/60)
- histiocytic sarcomas of the uterus in the low- (6/60) and mid-dose (6/60) female groups as compared to controls (0/59).

By the Peto trend analysis there were significant linear trends for the following:

- females with kidney adenomas (0/60, 0/60, 0/60, and 3/59 in control, low-, mid-, and high-groups groups;
- males with liver carcinomas (6/60, 7/58, 10/60, and 22/59 in the controls, low-, mid-, and high-dose groups;
- females with liver carcinomas (1/60, 0/60, 0/60, and 4/58) in control, low-, mid-, and high-dose groups;
- females with total lung tumors (2/60, 11/60, 12/60, and 11/59 in controls, low-, mid-, and high-dose groups);
- females with lung carcinomas (0/60, 5/60, 3/60, and 7/59 in controls, low-, mid-, and high-dose groups);
- females with benign ovarian tumors (0/59, 0/60, 5/60, and 3/58) in control, low-, mid-, and high-dose groups);
- females with histiocytic sarcomas of the uterus (0/59, 6/60, 6/60, and 5/59 in control, low-, mid-, and high-dose groups).

The incidence of frequent non-neoplastic lesions is summarized in Table 12. The report authors stated that there was a dose-related increased incidence of interstitial nephritis in all dosed groups of males and females.

The sponsor provided statistical analysis of nonneoplastic lesions which indicated significant increases ($p \leq 0.01$) in the incidence of interstitial nephritis compared to controls in both males and females receiving the highest dose Analysis of trend (Peto test) indicated

TABLE 12. Frequently Occurring Nonneoplastic Lesions in Mice Fed MON-097 for 23 Months

Organ/Lesion	Males/Dose (ppm) 0	500	1500	5000	Females/Dose (ppm) 0	500	1500	5000
- Adrenal Gland	(58)[a]	(59)	(58)	(59)	(59)	(60)	(60)	(59)
Amyloidosis	0	2	1	1	1	1	4	1
- Bone Marrow	(60)	(60)	(60)	(60)	(60)	(60)	(59)	(59)
Fibrous Osteodystrophy	0	0	0	0	8	11	6	6
- Cecum	(59)	(56)	(60)	(57)	(56)	(59)	(59)	(58)
Typhlitis	0	3	0	0	0	1	0	1
- Colon	(59)	(56)	(59)	(59)	(57)	(60)	(59)	(59)
Nematodiasis	10	9	1	5	3	1	0	1
- Duodenum	(60)	(56)	(60)	(57)	(57)	(59)	(59)	(58)
Amyloidosis	0	0	0	0	0	1	2	1
Duodenitis	0	2	2	0	0	1	1	3
- Eyes	(60)	(60)	(60)	(59)	(60)	(60)	(60)	(59)
Cataract	3	0	1	0	1	0	1	1
Keratitis	0	1	1	1	2	0	0	0
Panophthalmitis	2	0	0	0	1	0	0	0
Retinal Degeneration	4	3	6	3	2	3	1	8[c]
- Harderian Gland	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Dacryoadenitis	3	3	0	3	4	1	1	0
- Heart	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Cardiomyopathy	2	3	1	5	2	1	0	0
Endocarditis	0	2	0	0	0	0	0	0
Myocarditis	0	2	2	2	1	0	2	1
Thrombosis	1	1	3	2	0	2	1	0
- Ileum	(59)	(56)	(59)	(57)	(56)	(59)	(59)	(58)
Amyloidosis	2	0	0	1	2	2	0	2
Ileitis	0	2	0	0	0	0	0	0
- Jejunum	(59)	(56)	(58)	(57)	(57)	(59)	(59)	(58)
Amyloidosis	4	5	3	2	0	2	3	3

[a] Number in parentheses is the number of animals from which tissue was examined histologically.

[c] Statistically significant linear trend ($p \leq 0.01$) using the Cochran-Armitage test; analyses by our reviewers.

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TABLE 12. Frequently Occurring Nonneoplastic Lesions in Mice Fed MON-097 for 23 Months (continued)

Organ/Lesion	Males/Dose (ppm) 0	500	1500	5000	Females/Dose (ppm) 0	500	1500	5000
Kidneys	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)[d]
Amyloidosis	1	3	2	1	2	2	4	2
Cysts	5	10	6	2	3	1	0	1
Hydronephrosis	3	1	5	3	2	5	1	6
Infarction	0	6	0	0	1	0	0	0
Interstitial Nephritis	30	35	42	50[b,c]	31	33	31	45[b,c]
Nephrocalcinosis	0	0	0	0	0	1	0	2
Liver	(60)	(59)	(60)	(59)	(60)	(60)	(60)	(58)
Cell Focus	2	0	0	0	1	1	0	0
Cysts	5	1	3	2	1	1	2	0
Fatty Infiltration	2	0	1	0	0	2	0	0
Hepatitis	3	3	0	2	2	3	5	1
Necrosis	2	5	0	3	5	3	1	4
Lungs	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Bronchopneumonia	0	0	0	2	0	0	0	0
Interstitial Pneumonia	5	6	10	3	3	3	4	5
Lymphocytosis	2	1	1	0	4	1	1	2
Precipitate, Alveolar	2	0	0	0	0	0	1	0
Lymph Nodes	(57)	(55)	(57)	(52)	(55)	(55)	(55)	(51)
Angiectasis	0	1	2	0	4	0	0	0
Congestion	1	2	1	0	2	2	0	1
Lymphadenitis	2	2	1	1	3	0	6	4
Lymphoid Hyperplasia	0	5	2	2	6	0	9	1
Middle Ear	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Otitis Media	3	1	1	1	1	1	1	3
Nose	(60)	(59)	(59)	(60)	(60)	(60)	(60)	(59)
Rhinitis	0	4	3	0	3	2	5	0

Statistically significant increase compared to control ($p \leq 0.01$) using the Chi-square test (uncorrected for continuity).

Correct value determined by our reviewers.

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TABLE 12. Frequently Occurring Nonneoplastic Lesions in Mice Fed MON-097 for 23 Months (continued)

Organ/Lesion	Males/Dose (ppm) 0	500	1500	5000	Females/Dose (ppm) 0	500	1500	5000
Ovaries	--	--	--	--	(59)	(60)	(60)	(58)
Amyloidosis	--	--	--	--	2	1	2	0
Cyst, follicular	--	--	--	--	2	2	1	2
Cyst, hemorrhagic	--	--	--	--	11	10	9	7
Cyst, simple	--	--	--	--	29	26	21	19
Peripheral Nerve	(55)	(46)	(48)	(51)	(54)	(52)	(57)	(57)
Neuropathy	0	0	0	0	2	0	0	0
Pituitary Gland	(58)	(49)	(58)	(54)	(58)	(57)	(55)	(51)
Hyperplasia	0	0	0	0	1	0	2	0
Prostate Gland	(60)	(59)	(60)	(60)	--	--	--	--
Prostatitis	3	2	6	3	--	--	--	--
Salivary Gland	(60)	(60)	(60)	(59)	(60)	(59)	(60)	(57)
Sialoadenitis	3	2	0	2	0	1	3	1
Seminal Vesicles	(60)	(60)	(60)	(60)	--	--	--	--
Seminal Vesiculitis	3	1	6	1	--	--	--	--
Skin	(60)	(59)	(60)	(59)	(57)	(60)	(60)	(59)
Dermatitis	4	1	8	3	6	5	2	5
Spleen	(59)	(56)	(55)	(59)	(57)	(60)	(59)	(57)
Hematopoiesis, Extramedullary	2	0	1	2	4	5	5	4
Hemosiderosis	0	1	0	2	5	4	2	8
Stomach	(60)	(59)	(60)	(60)	(59)	(60)	(59)	(59)
Adenomatous Hyperplasia	2	8	6	1	2	3	0	4
Gastritis	1	2	3	6	7	4	6	2
Testes	(60)	(60)	(60)	(60)	--	--	--	--
Atrophy	12	14	14	3	--	--	--	--
Degeneration	1	5	0	3	--	--	--	--
Mineralization	0	2	0	1	--	--	--	--
Thymus	(50)	(45)	(48)	(45)	(48)	(51)	(49)	(43)
Lymphoid Hyperplasia	0	0	0	0	4	0	1	0

TABLE 12. Frequently Occurring Nonneoplastic Lesions in Mice Fed MON-097 for 23 Months (continued)

Organ/Lesion	Males/Dose (ppm)				Females/Dose (ppm)			
	0	500	1500	5000	0	500	1500	5000
- Thyroid Gland	(59)	(58)	(58)	(57)	(58)	(58)	(59)	(59)
Cyst, follicular	0	1	0	0	1	2	0	2
Thyroiditis	0	0	0	0	2	0	1	1
- Uterus	--	--	--	--	(59)	(60)	(60)	(59)
Cystic Endometrial Hyperplasia	--	--	--	--	42	30	34	22
Endometritis	--	--	--	--	2	1	4	3
- Vagina	--	--	--	--	(58)	(60)	(59)	(58)
Epidermoid Dysplasia	--	--	--	--	5	6	1	1
Vaginitis	--	--	--	--	2	4	4	4
- Zymbal's Gland	(49)	(56)	(55)	(56)	(58)	(53)	(56)	(55)
Adenitis	3	1	3	0	1	0	1	0

a significant positive trend ($p \leq 0.01$) for interstitial nephritis in males (30/60, 35/60, 42/60, and 50/60 in control, low-, mid- and high-dose groups) and in females (31/60, 33/60, 31/60, and 45/60 in control, low-, mid-, and high-dose groups) and a positive trend ($p \leq 0.01$) for retinal degeneration in females (2/60, 3/60, 1/60, and 8/59 in control, low-, mid-, and high-dose groups). However, analysis by pairwise comparison did not indicate a significant increase ($p < 0.01$) in the incidence of retinal degeneration in high-dose females.

10. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The authors concluded that when MON-097 was fed in the diet to random bred Swiss albino CD-1 mice at 0, 500, 1500, and 5000 ppm in the diet it was oncogenic under the conditions of the study. It caused a dose-related increase in pulmonary adenomas in males receiving 500 and 1500 ppm and in all female test groups, a dose-related increase in pulmonary carcinomas in all female test groups, an increase in hepatic carcinomas in all female test groups and in high-dose males, and a dose-related increase in uterine histiocytic sarcomas in all female test groups. A dose-related increase in interstitial nephritis was also seen in all test groups of males and females. It caused a persistent decrease in body weight and body weight gain in male and female groups receiving 5000 ppm but not in low- or mid-dose groups. The only clinical laboratory data considered to be compound related was a decrease in RBC, Hgb, and Hmct values in high-dose females at 23 months; the authors considered that "this anemia may be indirectly compound related as it was associated with the presence of tumors, particularly of the liver, and of renal disease." The only changes in organ weight values and organ-to-body weight ratios considered related to treatment were absolute and relative liver and kidney weight ratios in dosed males and absolute and relative kidney weights in dosed females; this was stated to be based on histopathological correlations. Signs of toxicity, food consumption fluctuations, clinical chemistry values in test groups differing from controls and weight changes in adrenals and thyroids were not considered compound related.

A quality assurance statement, signed and dated May 4, 1983, was present

11. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We conclude that the experimental design and conduct of this bioassay for the oncogenicity of MON-097 was generally in accord with Pesticide Assessment Guidelines. We classify the study as Core Minimum. Deficiencies are as follows:

1. The mice used in the study were received in three different shipments and were acclimated for different periods of time; however, they were all approximately the same age at study initiation.

2. Abnormal clinical findings with the date of observation were entered on individual animal disposition records, but summary incidences were not tabulated nor were weekly observation forms available.

3. There was no evidence that blood smears were obtained from 10 animals/sex/dose group at 18 months as suggested by the guidelines.

4. Organ weights were obtained after fixation. It is common practice, however, to weigh organs before fixation.

5. Necropsy findings were summarized by organ systems rather than by individual tissues or organs.

Food efficiency in high-dose males was approximately half of the control value; however, because the variability was so great the difference was not significant (ANOVA $\alpha = 0.05$, analysis by our reviewers).

Our statistician indicated that it was inappropriate for the study authors to analyze the clinical chemistry, hematology, organ weight, and organ-to-body weight data by the independent, two-sided Student's t-test However, our statistician's reanalysis of these data by a more appropriate method (ANOVA followed by Duncan's multiple range test) did not change any of the conclusions as to which values in dosed animals were significantly different from control values.

Due to the small size of the blood samples, pooling of 3 to 5 individual samples was necessary for the clinical chemistry analyses at interim and terminal sacrifices; as a result, only 2 or 3 pools were analyzed for each dose group at each sacrifice. We conclude that the availability of only 2-3 values for each clinical chemistry parameter makes the statistical tests (ANOVA, tests to determine differences between means, and trend analyses) of these measurements unstable.

The sponsor concluded that blood chemistry effects indicative of liver damage were observed in dosed animals. This conclusion was based on observations of increased serum alkaline phosphatase in high-dose females at months 12 and 23, increased serum glutamic oxaloacetic transaminase in high-dose males at month 12, and slightly increased total bilirubin in mid- and high-dose mice of both sexes at month 23. The study authors discounted these observations, concluding that these abnormal findings were for the most part randomly distributed and on many occasions lacked any definitive histopathological correlations.

The study authors did not consider effects on organ weights and organ-to-body weight ratios to be test compound-related unless there was correlative histopathology. With this criterion, the study authors considered only liver and kidney weight and organ-to-body weight ratio increases to be test compound-related.

Differences in the accuracy of weighing the adrenals at 12 and 23 months were apparent; the weights of the adrenals were reported to an accuracy of only two digits after the decimal at month 12 (weights were 0.01-0.04 g with most weights being 0.01 or 0.02 g) but were reported to an accuracy of three digits after the decimal at month 23 (most weights were <0.01 g for males and <0.02 g for females). The mean weight of liver in control females at termination that was reported was unusually high and had a large standard deviation (Table 7). Examination of the individual liver weights revealed that one control female (#1508) had a liver weight of 8.57 g which was correlated with hepatocellular carcinoma (4 x 2.2 x 1.8 cm). If this value were omitted the mean was 1.54±0.325 and the high-dose females had a significantly higher ($p \leq 0.05$) mean liver weight than controls.

The study authors compiled the gross pathology results into four tables according to whether the animals were sacrificed or found dead or moribund. Two tables were for tumors and the other two for non-tumor lesions. They also reported the microscopic diagnoses by neoplasm and nonneoplastic lesions in different tables. As the gross lesions were tabulated as the number of lesions/sex/dose rather than animals with lesions/sex/dose, the gross pathology results were not amenable to statistical treatment. Our reviewers' examination of the individual animal data records revealed that where tissue masses (suspected tumors) were diagnosed grossly, a microscopic diagnosis was also made and usually confirmed the gross diagnosis with the appropriate cell type. The care with which lesions found grossly were processed through the histology laboratory and presented to the pathologist for microscopic examination appeared to be quite effective. Tables 13 and 14 were prepared by our reviewers to correlate the gross diagnoses with microscopic diagnoses. For practical purposes, the analysis for tumor incidence can appropriately be based solely upon the microscopic diagnoses.

The study authors concluded that dose-related increases occurred only for liver carcinomas (males at all doses and high-dose females), uterine histiocytic sarcomas (females at all doses), pulmonary adenomas (females at all doses and low- and mid-dose males), and pulmonary carcinomas (females at all doses). However, the study authors did not analyze histopathology data statistically. This analysis was provided by the sponsor.

The sponsor concluded that significant ($p \leq 0.01$) dose-related positive trends occurred for liver carcinomas (males and females), liver adenomas (males and females combined), uterine histiocytic sarcomas (females), ovary benign tumors (females), total lung tumors (females), lung carcinomas (females), kidney adenomas (females), malignant kidney tumors (males and females combined), and animals with tumors (males and females). Significantly increased incidences ($p \leq 0.01$) of neoplastic lesions in treated animals as compared to controls were found for liver carcinomas (high-dose males), uterine histiocytic sarcomas (low- and mid-dose females), lung tumors (females at all doses), and animals with tumors (mid-dose females). Although

TABLE 13. Correlation of Reported Gross Pathology (Tissue Masses) and Histopathological Diagnoses of Neoplastic Lesions[a]

LIVER (FEMALES)
- -gross masses of digestive system in control and all dose groups
- -carcinomas
  - --significant dose-related positive trend in incidence

LIVER (MALES)
- -gross masses of digestive system in control and all dose groups
- -carcinomas
  - --significant dose-related positive trend in incidence
  - --significantly increased incidence at high dose

LIVER (MALES PLUS FEMALES)
- -gross masses of digestive system in control and all dose groups
- -adenomas
  - --significant dose-related positive trend in incidence

LUNG (FEMALES)
- -gross pulmonary masses in control and all dose groups
- -carcinomas
  - --significant dose-related positive trend in incidence
- -total lung tumors (including carcinomas)
  - --significant dose-related positive trend in incidence
  - --significantly increased incidence at all doses

KIDNEY (FEMALES)
- -gross urinary tract masses in mid- and high-dose groups
- -adenomas
  - --significant dose-related positive trend in incidence

KIDNEY (MALES PLUS FEMALES)
- -gross urinary tract masses in control and in mid- and high-dose groups
- -malignant tumors
  - --significant dose-related positive trend in incidence

OVARIES (FEMALES)
- -gross reproductive tract masses in control and all dose groups
- -benign tumors
  - --significant dose-related positive trend in incidence

UTERUS (FEMALES)
- -gross reproductive tract masses in control and all dose groups
- -histiocytic sarcomas
  - --significant dose-related positive trend in incidence
  - --significantly increased incidence at low and mid doses

TABLE 13. Correlation of Reported Gross Pathology (Tissue Masses) and Histopathological Diagnoses of Neoplastic Lesions[a] (Continued)

TOTAL TUMORS (FEMALES)
-gross tissue masses in control and dose groups
-animals with tumors
--significant dose-related positive trend in incidence
--significantly increased incidence at mid dose.
TOTAL TUMORS (MALES)
-gross tissue masses in control and all dose groups
-animals with tumors
--significant dose-related positive trend in incidence

TABLE 14. Correlation of Reported Gross Pathology (Tissue Lesions) and Histopathological Diagnoses of Nonneoplastic Lesions[a]

EYES (FEMALES)
-gross ocular lesions in control and in mid and high dose groups
-retinal degeneration
--significant dose-related positive trend in incidence
KIDNEYS (MALES AND FEMALES)
-gross urinary tract lesions in control and in all dose groups for both sexes
-interstitial nephritis
--significant dose-related positive trend in incidence for both sexes
--significantly increased incidence at high dose for both sexes

[a] Table prepared by our reviewers.

there are some differences between the study authors' conclusions and those of the sponsor (see Table 15 for tabular presentation), they both unequivocally agree that MON was carcinogenic, causing definite increases in liver carcinomas, lung tumors, and histiocytic sarcomas of the uterus. The sponsors analysis of tumors used a p value of 0.01 for significance. Analysis by our reviewers indicated that in addition to the findings of the sponsor the following neoplasms were significant at a p level of 0.05: carcinoma of lungs in low- and high-dose females, histiocytic sarcoma of the uterus in high-dose females and total ovarian benign tumors of the uterus in mid-dose females.

In addition to total number of tumors occuring in an organ system as related to exposure, an examination as to possible acceleration of tumor development was attempted by our reviewers. This issue had not been addressed by either the sponsor or the study authors. The latency of tumors could only be estimated based upon tumors observed in animals dying during particular time periods. Tables 16 and 17 present a detailed breakdown of the main tumors of concern as related to their observation with time of death and dose level. Tables 18 and 19 present the data in a somewhat different manner allowing a quicker assessment of the early-appearing tumors. Especially notable were the frequencies of high-dose males that died at 20-23 months with liver carcinomas (10/17) (Table 16) and dosed females that died with uterine histiocytic sarcomas at 13-16 months (5/16) and at 17-19 months (4/15) (Table 17).

When we considered the animals that died or were killed before the terminal sacrifice, combining the tumors for all treated groups to assess differences from controls, a slightly different pattern emerged in that tumors of the lung also become prominent. Thirty-six (3%) animals (combined males and females) with lung tumors (adenomas plus carcinomas) were observed in 243 early deaths for a 15% incidence versus only 2 out of 59 controls (3%). The number of early uterine sarcomas still remains substantial, 14 of 125 (11%) versus 0 of 29 controls (0%). However, comparing total early liver tumors in the dosed groups with the controls diminishes the evidence for a substantial early development. Based upon these data, it is concluded that earlier-appearing tumors were observed with greater frequency as related to treatment in three organ systems - liver, lungs, and uterus.

A NOEL for chronic toxicity could not be established due to increased liver and kidney weights at the low-dose level. The LOEL for chronic toxicity of MON-097 in mice was 500 ppm in the diet (lowest dose tested).

12. CBI APPENDIX:

Appendix A, Materials and Methods, CBI Vol. I, pp. 9-21.
Appendix B, Protocol, CBI Vol. III, pp. 126-187.

TABLE 15. Analysis of Neoplastic Response

	MALES							
	Study Author				Sponsor			
	Trend	L	M	H[a]	Trend	L	M	H
Liver adenoma					X[b]			
Liver carcinoma		X	X	X	X			X
Lung adenoma		X	X					
Lung carcinoma								
Total lung tumors								
Kidney adenoma								
Malignant kidney tumors					X[b]			
Total animals with tumors					X			

	FEMALES							
	Study Author				Sponsor			
	Trend	L	M	H	Trend	L	M	H
Liver adenoma					X[b]			
Liver carcinoma				X	X			
Lung adenoma		X	X	X				
Lung carcinoma		X	X	X	X			
Total lung tumors					X	X	X	X
Kidney adenomas					X			
Malignant kidney tumors					X[b]			
Uterine histiocytic sarcomas		X	X	X	X	X	X	
Benign ovary tumors					X			
Total animals with tumors					X		X	

[a] L, low dose; M, mid dose; H, high dose.

[b] Indicates statistics performed using combined male and female groups.

TABLE 16. Frequencies of Tumors in Males as Related to Time of Death[a]

	0-12 Months				12 Month Sacrifice				13-16 Months				17-19 Months				20-23 Months				23 Month Sacrifice			
Dose Level (ppm)	0	500	1500	5000	0	500	1500	5000	0	500	1500	5000	0	500	1500	5000	0	500	1500	5000	0	500	1500	5000
No. of Animals Examined	0	7	2	6	10	10	10	10	3	5	3	6	2	5	5	8	15	9	15	17	30	24	25	13
No. of Tumors Observed	0	0	0	0	2	0	2	2	0	4	2	7[b]	1	3	10	7	13	5	8	19	25	22	25	20
Harderian Gland																								
Adenoma	0	0	0	0	0	0	1[c]	0	0	1	0	1	1	0	1	2	1	1	2	2	6	5	3	5
Kidneys																								
Adenocarcinoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0
Adenoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0	1	1	0
Liver																								
Adenoma	0	0	0	0	1	0	0	1	0	0	0	1	0	0	2	0	0	0	1	1	7	4	6	4
Carcinoma	0	0	0	0	0	0	0	0	0	1	0	1	0	1	2	3	5	1	2	10	1	4	6	8
Lungs																								
Adenoma	0	0	0	0	0	0	1	0	0	1	0	1	0	1	1	1	0	0	2	1	6	8	8	2
Carcinoma	0	0	0	0	0	0	0	0	0	0	1	1	0	1	3	0	2	2	0	1	5	0	0	1
Histiocytic Sarcoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymphatic System																								
Lymphoma	0	0	0	0	1	0	0	0	0	1	1	2	0	0	1	1	3	1	0	1	0	0	0	0
Pituitary Gland																								
Adenoma	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Table prepared by our reviewers.
[b] Total number of tumors may exceed the number of animals examined due to multiplicity of tumors in some animals.
[c] Number of animals in which a given tumor type was found upon histopathological examination.

TABLE 17. Frequencies of Tumors in Females as Related to Time of Death[a]

	0-12 Months				12 Month Sacrifice				13-16 Months				17-19 Months				20-23 Months				23 Month Sacrifice			
Dose Level (ppm)	0	500	1500	5000	0	500	1500	5000	0	500	1500	5000	0	500	1500	5000	0	500	1500	5000	0	500	1500	5000
No. of Animals Examined	3	3	2	6	10	10	10	10	2	3	5	8	5	6	4	5	9	13	22	18	31	25	17	12
No. of Tumors Observed	0	0	2	1	1	1	7	0	0	2	2	4	2	5	9[b]	5	4	10	16	12	10	12	11	15
Harderian Gland																								
Adenoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2[c]	0	0	0	1	2	2	1	2	2
Kidneys																								
Adenocarcinoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Adenoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Liver																								
Adenoma	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	3
Carcinoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	1
Lungs																								
Adenoma	0	0	0	0	0	0	1	0	0	0	0	0	0	1	3	1	0	2	3	1	2	3	1	2
Carcinoma	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2	0	2	0	3	3	3
Histiocytic Sarcoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymphatic System																								
Lymphoma	0	0	2	1	0	0	1	0	0	0	1	0	2	2	2	0	2	3	6	0	2	2	0	0
Pituitary Gland																								
Adenoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0
Ovaries																								
Adenoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Granlosa Cell Tumor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	1	1
Luteoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
Uterus																								
Endometrial Stromal Polyp	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	2
Histiocytic Sarcoma	0	0	0	0	0	0	0	0	0	1	1	3	0	?	1	1	0	0	4	1	0	1	0	0
Leiomyosarcoma	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	2	1	0	1	0	1	0

[a] Table prepared by our reviewers.

[b] Total number of tumors may exceed the number of animals examined due to multiplicity of tumors in some animals.

[c] Number of animals in which a given tumor type was found upon histopathological examination.

TABLE 18. Summary of Males With Tumors as Related to Time of Death

	Months 0-12	12	13-16	17-19	20-23	23	Total
Liver Adenoma							
Control	-	1	-	-	-	7	8
L	-	-	-	-	-	4	4
M	-	-	-	2	1	6	9
H	-	1	1	-	1	4	7
	0	2	1	2	2	21	28
Liver Carcinoma							
Control	-	-	-	-	5	1	6
L	-	-	1	1	1	4	7
M	-	-	-	2	2	6	10
H	-	-	1	3	10	8	22
	0	0	2	6	18	19	45
Lung Adenoma							
Control	-	-	-	-	-	6	6
L	-	-	1	1	-	8	10
M	-	1	-	1	2	8	12
H	-	-	1	1	1	2	5
	0	1	2	3	3	24	33
Lung Carcinoma							
Control	-	-	-	-	2	5	7
L	-	-	-	1	2	-	3
M	-	-	1	3	-	-	4
H	-	-	1	-	1	1	3
	0	0	2	4	5	6	17
Kidney Adenoma							
Control	-	-	-	-	2	-	2
L	-	-	-	-	-	1	1
M	-	-	-	-	-	1	1
H	-	-	-	-	2	-	2
	0	0	0	0	4	2	6

L = low dose
M = mid dose
H = high dose

TABLE 19. Summary of Females with Tumors as Related to Time of Death

	Months 0-12	12	13-16	17-19	20-23	23	Total
Liver Carcinomas							
Control	-	-	-	-	-	1	1
L	-	-	-	-	-	-	0
M	-	-	-	-	-	-	0
H	-	-	-	-	3	1	4
	0	0	0	0	3	2	5
Lung Adenomas							
Control	-	-	-	-	-	2	2
L	-	-	-	1	2	3	6
M	-	1	-	3	3	1	8
H	-	-	-	1	1	2	4
	0	1	0	5	6	8	20
Lung Carcinoma							
Control	-	-	-	-	-	-	-
L	-	-	-	-	2	3	5
M	-	-	-	-	-	3	3
H	-	-	1	1	2	3	7
	0	0	1	1	4	9	15
Lymphomas							
Control	-	-	-	2	2	2	6
L	-	-	-	2	3	2	7
M	2	1	1	2	6	-	12
H	1	-	-	-	-	-	1
	3	1	1	6	11	4	26
Uterus Hist. Sarcomas							
Control	-	-	-	-	-	-	0
L	-	-	1	2	-	1	4
M	-	-	1	1	4	-	6
H	-	-	3	1	1	-	5
	0	0	5	4	5	1	15

L = low dose
M = mid dose
H = high dose

ATTACHMENT G
007697

Data Evaluation Record

Study Type: Gene mutation in CHO/HGPRT cells.

Study Identification: "CHO/HGPRT Gene Mutation Assay with MON 097."
Lab. performing study: Monsanto Environmental Health Lab.
St. Loius, MO 63110
Sponsor: Monsanto Agricultural Products Co.
St. Louis, MO. 63167
Study no.: ML-82-281
Project no.: EHL-830013
Accession no.: 071970
Report date: 6/9/83
Submitted to EPA: 9/22/83
Study director: A.P. Li, Ph.D.

Reviewed By: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

DSS 8/2/85

Approved By: Irving Mauer, Ph.D.
Geneticist, Toxicology Branch
Hazard Evaluation Division (TS-769)

8/2/85

Conclusions: The submitted study demonstrated that acetochlor was weakly mutagenic in this test system, with or without metabolic activation. Although the trend for a dose-response relationship was statistically significant ($p < 0.05$), the evidence for a dose-dependent effect was minimal.

Classification: Acceptable

Materials

1) Test chemicals: Acetochlor (MON 097), a "yellowish-brown liquid"; sample #T830020; 96.3% a.i.

Positive controls: Ethyl methanesulfonate (EMS)- no metabolic activation
Benzo(a)pyrene (BP)- with metabolic activation

Metabolic activation: S-9 fraction (9,000 x g supernatant) from Arochlor 1254-induced rat livers, purchased from Litton Bionetics.

2) Doses tested: Acetochlor- 25, 75, 100, 125, and 150 ug/ml without S-9.
25, 50, 75, 100, and 125 ug/ml with S-9.

vehicle control- ethanol

positive control- 100 ug/ml EMS without S-9
1 ug/ml 3-MC with S-9

3) Test system: CHO cells, cloned K1BH4, originally obtained from Dr. A. W. Hsie of Oak Ridge National Laboratories.

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Methods

A photocopy of the submitted methods is appended. The methods were reviewed, and the following point(s) were noted:

(1) The choice of ethanol as a solvent control in this assay is questionable, since incubation of cells with ethanol in the presence of S-9 fraction produced about a two-fold increase in mutation frequency in two separate experiments.

Results/Discussion

The selection of doses and concentration of S-9 fraction was based on preliminary studies which demonstrated an optimum of 10% S-9. The cytotoxicity of acetochlor increased as the concentration of S-9 increased.

Incubation of CHO cells with acetochlor in the presence or absence of S-9 caused an increase in mutation frequency when compared to untreated controls (Table 2, photocopied from submitted study report). Doses of 125 or 150 ug/ml without S-9 produced a 3.8 and 2.7x increase in mutation frequency, respectively, and were judged to be statistically significant ($p < 0.05$). A substantial decrease in relative survival was noted at the highest dose of 150 ug/ml, 39% of control as compared to 71-95% of control at lower concentrations test article.

In the presence of S-9 fraction, a statistically significant increase in mutation frequency of 3x control ($p < 0.05$) was observed only at the high dose of 125 ug/ml. Acetochlor was more cytotoxic in the presence of S-9, as doses of 75, 100, and 125 ug/ml produced decreases in cell survival of 34%, 30%, and 7% of control.

The investigators stated that "[t]he dose-response relationship was found to be linear ($p < 0.05$) for both treatment with and without S-9."

The positive controls induced the appropriate responses, demonstrating that the test system could respond to direct and indirect mutagens.

The submitted data are considered to be evidence of a weak mutagenic potential of acetochlor, as clear evidence of mutagenicity is seen only at cytotoxic concentrations.

Classification: Acceptable

ACETOCHLOR Tox review 007697

Page_____ is not included in this copy.

Pages 179 through 183 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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

ATTACHMENT H

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Data Evaluation Record

Study Type: Gene mutation in mouse lymphoma (L5178Y) cells. 004586

Study Identification: "An Evaluation of Mutagenic Potential of MON 097 Employing the L5178Y TK+/- Mouse Lymphoma Assay."
Lab. performing study: SRI International
Menlo Park, CA 94025
Sponsor: Monsanto Agricultural Products Co.
St. Louis, MO. 63167
Study no.: SR 81-150
Project no.: LSC-2575 (SRI)
Accession no.: 071970
Report date: August, 1982
Submitted to EPA: 9/22/83
Study director: Ann D. Mitchell, Ph.D.

Reviewed By: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

DS 8/2/85

Approved By: Irving Mauer, Ph.D.
Geneticist, Toxicology Branch
Hazard Evaluation Division (TS-769)

8/2/85

Conclusions: Positive for gene mutations in mouse lymphoma cells (L5178Y) only in the presence of metabolic activation. Negative for gene mutations in the absence of metabolic activation. The purity of the test substance was not stated, therefore it was not clear whether the technical grade of active ingredient was tested in this assay.

Classification: Acceptable

Materials

1) Test chemicals: Acetochlor (MON 097), a "plum-colored liquid"; lot NBP 1924845; % a.i. not stated.

Positive controls: Ethyl methanesulfonate (EMS)- no metabolic activation
3-methylcholanthrene (3-MC)- with metabolic activation

Metabolic activation: S-9 fraction (9,000 x g supernatant) from Arochlor 1254-induced rat liver (male Fischer-344 rats).

2) Doses tested: Acetochlor- 20, 30, 45, 60, 76, 100 and 400 ul/l without S-9.
5, 15, 20, 30, 40, 50, 100 and 250 ul/l with S-9.

vehicle control- 1% DMSO.

positive control- 500 ug/ml EMS without S-9
6 ug/ml 3-MC with S-9

3) Test system: Mouse lymphoma L5178Y cells, heterozygous for thymidine kinase.

Methods

A photocopy of the submitted methods is appended. The methods were reviewed and the following point(s) are noted:

None.

Results/Discussion

The selection of doses tested in the presence or absence of metabolic activation (S-9) were based on the results of a range-finding assay. Doses of acetochlor of ≥100 ul/l were cytotoxic as evidenced by relative suspension growth of less than 5% of the solvent control group in the range finding assay.

In the primary study, incubation of mouse lymphoma cells with acetochlor in the absence of S-9 did not produce any effect on mutation frequency at any of the tested doses (data not shown). Doses of 100 ul/l resulted in average relative suspension growth of about 10% of control, and were therefore cytotoxic. The positive control without S-9, EMS, induced an increase in mutation frequency of about 5.6x control values, demonstrating that the test system could respond appropriately to a direct-acting mutagen.

Incubation of lymphoma cells with acetochlor in the presence of S-9 produced an apparent dose-related increase in mutation frequency (Table 1). Doses of 40 ul/l and above were apparently cytotoxic as evidenced by dose-dependent decreases in relative suspension growth and relative cloning efficiency. The positive control with S-9, 3-MC, caused an average increase in mutation frequency of 5.4x control values, demonstrating that the test system could respond appropriately to a mutagen requiring metabolic activation.

Table 1. Effect of Acetochlor and S-9 on Mutation Frequency[a]

Test Material	Dose	Relative Suspension Growth (%)[b]	Relative Cloning Efficiency (%)[c]	Mutation Frequency (% control)
DMSO	1%	100.0	100.1	-
3-MC	6 ug/ml	47.7	35.6	538.8
MON 097	5 ul/l	82.9	97.8	102.0
MON 097	15 ul/l	89.1	85.4	150.3
MON 097	20 ul/l	82.2	77.8	155.9
MON 097	30 ul/l	45.3	70.6	220.3
MON 097	40 ul/l	12.6	36.1	427.1
MON 097	50 ul/l	7.9	17.2	523.2
MON 097	100 ul/l	3.2	NC	-
MON 097	250 ul/l	3.3	NC	-

[a]data excerpted from submitted study.

Classification: Acceptable

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Pages 186 through 192 are not included.

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____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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ATTACHMENT 1
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 10 1987

005977

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of additional data for and the registrant's response to a review of an In Vivo bone marrow chromosome study in rats with Acetochlor and a review of an In Vivo micronucleus assay in mice with Acetochlor. EPA ID #'s 3F2966 and 524-GUI; EPA Record #'s 185604 and 185606; EPA Accession #'s 266002 and 263233; Caswell #3B; Tox Branch Project 7-0375.

TO: Robert Taylor/Vickie Walters (PM #25)
Herbicide - Fungicide Branch
Registration Division (TS-767C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/8/87
Pharmacologist, Review Section V
Toxicology Branch/HED (TS-769C)

THRU: Irving Mauer, Ph.D. 7-8-87
Mutagenicity Secondary Reviewer (Review Section VI)
and
Quang Q. Bui, Ph.D., D.A.B.T. 7/9/87
Acting Section Head, Review Section V
and
Theodore M. Farber, Ph.D., D.A.B.T. 7/10/87
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: Monsanto Company
1101 17th Street, N.W.
Washingotn, D.C. 20036

Action Requested: Review additional data and the registrant's response to an In Vivo bone marrow chromosome study in rats with Acetochlor and review an In Vitro micronucleus assay in mice with Acetochlor.

Recommendations:

For the In Vivo bone marrow chromosome study in rats: under conditions of this study and the additional information provided by the registrant (Ammended Final Report MSL-5724 and the IN Vivo Micronucleus Assay in Mice with Acetochlor, HL-84-405/241-207), this study (Report MSL-5724, P.D. 686) is upgraded to an Acceptable study. Toxicity was demonstrated at the high dose (500 mg/kg) by evidence of a statistically significant body weight loss in both males and females at 48 hours.

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For the In Vivo micronucleus assay in mice: under conditions of this test the high dose level of MON 097 (2000 mg/kg) exhibited mortality and signs of clinical toxicity. No evidence of an increase in micronucleated polychromatic erythrocytes was noted at the dose levels tested in this study. This study is classified as Acceptable.

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Discussion:

Each of the deficiencies stated in the Agency DER in reference to the "IN VIVO Bone Marrow Chromosome Study in Rats with Acetochlor (MON 097) [Study No. HL 83-006, Project No. 241-143]" for classifying the study as Unacceptable are addressed by first stating the Agency concern, then giving the registrant's response, followed by the Agency comment on the response.

1. Agency Concern:

From the "Methods" section, the first point:

"1) The tested doses were based on a range-finding study (submitted as an appendix) which demonstrated that 2/2 males and 1/2 females injected i.p. with a high dose of 1000 mg/kg died within 24 hours of treatment, whereas equal numbers of males and females treated with 100 or 300 mg/kg survived without any apparent toxic signs. It was reported that "the test compound produced no apparent effects on the mitotic indices of the animals which survived", however the "normal" range to which the investigators compared these results was not stated."

Registrant's Response:

Monsanto responded by stating that:

"This conclusion, which pertains to the results of the range finding study only, was undoubtedly based on the experience of the Study Director and not a comparison with a normal range of values. A normal range of values for mitotic index was not available for the period during which this study was conducted.

However, the following normal range of values for mitotic index was compiled from studies conducted at Hazleton Laboratories during 1985.

	X ± S.E.	Range	N(# of test groups)
Males	4.7 ± 0.3	1.1 - 8.6	42
Females	5.2 ± 0.4	1.8 - 9.7	26

(supplied by Dr. J.L. Ivett, Hazleton Biotechnologies)

The mean mitotic index calculated for each of the 3 groups in the range finding study fall well within the ranges cited above."

Agency Comment:

The explanation provided by the registrant is acceptable.

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2. Agency Concern:

The second point from the "Methods" section:

"2) It was stated in the submitted protocol that rats were to be sacrificed at 48 hours after treatment for analysis of bone marrow cells. Although slides were prepared for these animals, they were not examined."

Registrant's Response:

Monsanto responded by stating that:

"As stated on p. 13 of the original report, under 'Cytogenetic Analysis' the slides from the 48 hour sacrifice were not analyzed because there was no evidence of compound induced mitotic delay at the earlier time points. Table 5 displays the group mean mitotic indices and the results of the statistical analyses for the 6, 12 and 24 hour sacrifices. No statistically or biologically significant differences from control values were noted."

Agency Comment:

The explanation provided by the registrant is acceptable. However, it should be noted that the protocol for a study should make reference to changes in procedure when there is evidence that further work is uneccessary.

3. Agency Concern:

The third point from the "Methods" section:

"3) Although pre-test body weights were provided, body weights at study termination were not reported."

Registrant's Response:

Monsanto responded by stating that:

"Terminal body weights were recorded for the animals sacrificed at 24 and 48 hours as per Hazleton standard procedures. However, for reasons unknown, terminal body weight measurements were not required by the protocol for this study and were consequently not included in the final report. These weights are included in the amended report (Section II)."

Agency Comment:

The appended "Summary Table of Body Weight Difference..." from the investigators Amended Final Report shows that at 48 hours both the males and females of the 500 mg/kg dose group exhibited statistically significant lower body weights than that of the control groups.

4. Agency Concern:

The fourth point from the "Method" section:

"4) Although 6 rats/sex/dose were treated and sacrificed, and bone marrow slides were prepared for all treated rats, generally slides were examined from only 5 rats (or less) of each dose group."

Registrants's Response:

Monsanto responded by stating that:

"The original final report states why only 5 rats/sex group were examined in some cases (p. 9 under 'Chromosome Evaluation'): 'At least sixty cells in metaphase were examined from five of the six rats chosen randomly for each sex and group. In some instances, it was not possible to locate 60 spreads, so as many spreads as could be found were analyzed. If 60 spreads were not found from any of the animals initially analyzed for each sex and group, the sixth animal of that group was then analyzed."

The design for this study basically incorporated an extra animal of each sex per group as insurance against having an inadequate number of metaphase spreads upon which to make an evaluation.

In a few cases it was not possible to find the desired 5 slides per group which each containing a sufficient number of spreads for analysis."

Agency Comment:

The explanation provided by the registrant is acceptable.

5. Agency Concern:

The first deficiency stated in the "Results" section is as follows:

"A. General Observations: No effects of treatment on physical appearance were apparent. Data for clinical signs were submitted as individual animal data. Data for body weights were submitted as a summary table of pre-treatment weights; no differences between test groups were apparent. The effect of treatment of body weights could not be assessed since post-treatment values were not submitted."

Registrant's Response:

Monsanto responded by stating that:

"Terminal body weights have been included in the amended report (Section II) for this study. A statistical comparison of treated and control group mean body weight change has been conducted by Monsanto (Section III). The results of this analysis indicate a statistically significant reduction in body weight gain in both male and female high dose animals when compared to controls at the 48 hour interval."

Agency Comment:

The Agency comment is discussed in point 3 above.

6. Agency Concerns:

The second deficiency stated in the "Results" section is as follows (under B. Cytogenetic Analyses):

"Numerous discrepancies between the number of animals examined, as reported in the summary tables, and the number of animals for which data were reported in the individual animal data were noted. The number of animals reported as examined in the summary tables in many cases was less than the number of animals with individual data reported. In some cases, data were reported for animals from which "0" cells were examined, e.g. #D77707 (Group 2, 24 hours) and #D77771 (Group 5, 24 hours)."

Registrant's Response:

Monsanto responded to the first part of the Agency concern concern by stating that:

"These apparent deficiencies stem from a possible misinterpretation of the values in column 3 (i.e. Number of animals analyzed per group) of Table 2 (i.e. Summary of Aberration Data). The values in this column represent the number of animals for which slides were prepared and scanned for 60 metaphase spreads. In those cases where 60 metaphase spreads were found in the first 5 animals/sex/group examined, it was not necessary to examine the slide prepared from the sixth animal in that group.

The values in column 3 also include slides from animals that were scanned but in which 60 metaphase spreads were not found. In these cases further chromosomal analysis for aberrations was not conducted."

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They further responded to the second part of the Agency concern by stating that:

"These errors have been corrected in the amended report (Section II)."

Agency Comment:

The explanation provided by the registrant is acceptable, however, the "total number of cells analyzed" on Table 5 is incorrect, it should read 540 rather than 480.

7. Agency Concern:

It was stated in the discussion that:

"No effect of treatment on the incidence of chromosomal abnormalities was apparent. No effect of treatment on the mitotic index was apparent, therefore there is no evidence that the test substance reached the target tissue, the bone marrow, in sufficient concentration to produce a toxic effect. Although slides were reportedly prepared for the 48 hour sacrifice, they apparently were not examined."

Registrant's Response:

Monsanto responded by stating that:

"Although an effect on mitotic index was not apparent in this study, other appropriate endpoints were available for determining the adequacy of the dose levels tested. The TSCA Health Effects Testing Guidelines 40 CFR Part 798 (FR Vol. 50, No. 188 p. 39445) state under 'Dose Levels' (§798.5385 (d)(5)(ii)) '...the dose being the maximum tolerated dose or that producing some indication of cytotoxicity...' As evidence of having approximated the MTD, a statistically significant decrease in body weight gain was observed at the high dose level. According to a report issued by the U.S. EPA's GeneTox Program on Mammalian in vivo and in vitro cytogenetic assays: (Mutat. Res. 87:143, 1981) '...the doses selected should extend over at least a single-log range, with the maximum dose no less than a factor of 2 less than a dose producing a significant level of toxicity.' The high dose selected for the definitive study (i.e. 500 mg/kg) was within a factor of 2 of the dose that produced 75% mortality in the range finding study (i.e. 1000 mg/kg). It should also be noted that the mitotic index was not reduced in the lone survivor at 1000 mg/kg on the range finding study."

Agency Comment:

The explanation provided by the registrant is acceptable.

The registrant (Monsanto) went into further discussion by 145 stating:

> "It is Monsanto's opinion that the dose levels employed on this study were appropriate and adequate for assessing the potential of acetochlor to induce structural chromosomal aberrations."

and further:

> "Additional information concerning the clastogenic potential of acetochlor and which supports the adequacy of this study can be found in a report entitled In Vivo Micronucleus Assay in Mice with Acetochlor (HL-84-405/241-207). This micronucleus assay is being submitted concurrently under separate letter and is identified as R.D. No. 685 and Special Report MSL-5723."

Recommendations:

Under conditions of this study and the additional information provided by the registrant (Ammended Final Report MSL-5724 and the IN VIVO Micronucleus Assay in Mice with Acetochlor, HL-84-405/241-207), this study (Report MSL-5724, R.D. 686) is upgraded to an Acceptable study. Toxicity was demonstrated at the high dose (500 mg/kg) by evidence of a statistically significant body weight loss in both males and females at 48 hours.

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Page 201 is not included in this copy.

Pages _____ through _____ are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

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Data Evaluation Record

Study Type: In vivo cytogenetics in rats.

Study Identification: "In Vivo Bone Marrow Chromosome Study in Rats with Acetochlor (MON 097)".
Lab. performing study: Hazelton Laboratories America, Inc. Vienna, VA 22180
Sponsor: Monsanto Agricultural Products Co. St. Louis, MO. 63167
Study no.: HL 83-006
Project no.: 241-143 (Hazelton)
Accession no.: 071970
Report date: May 24, 1983
Submitted to EPA: 9/22/83
Study director: Michael G. Farrow, Ph.D.

Reviewed By: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

DSS 8/2/85

Approved By: Irving Mauer, Ph.D.
Geneticist, Toxicology Branch
Hazard Evaluation Division (TS-769)

8/2/85

Conclusions: No effect on the incidence of chromosomal abnormalities was apparent. No effect on mitotic index was apparent, therefore there was no evidence that the test material reached the bone marrow in sufficient concentration to produce a toxic effect. Numerous discrepancies in the results reported in the summary tables vs. individual animal data were noted.

Classification: Unacceptable Deficiencies as noted.

Materials and Methods

A. Materials- (1) Test chemicals: Acetochlor (MON 097), a "brown liquid", 96.3% a.i.

Positive control: Mitomycin C (Sigma Chem. Co.), assumed 100% a.i.

Mitotic arrest- colchicine; supplier, purity not stated.

(2) Doses tested: Acetochlor- 40, 150, and 500 mg/kg by i.p. injection.

vehicle control- corn oil, 5 ml/kg.

positive control- Mitomycin C, 5 mg/kg

mitotic arrest- colchicine 2 mg/kg.

(3) Test animal: Male and female Sprague-Dawley CD albino rats, obtained from Charles River Breeding Laboratories, Kingston, N.Y.

Methods

A photocopy of the submitted methods is appendad. The methods were reviewed and the following points were noted:

1) The tested doses were based on a range-finding study (submitted as an appendix) which demonstrated that 2/2 males and 1/2 females injected i.p. with a high dose of 1000 mg/kg died within 24 hours of treatment, whereas equal numbers of males and females treated with 100 or 300 mg/kg survived without any apparent toxic signs. It was reported that "the test compound produced no apparent effects on the mitotic indices of the animals which survived", however the "normal" range to which the investigators compared these results was not stated.

2) It was stated in the submitted protocol that rats were to be sacrificed at 48 hours after treatment for analysis of bone marrow cells. Although slides were prepared for these animals, they were not examined.

3) Although pre-test body weights were provided, body weights at study termination were not reported.

4) Although 6 rats/sex/dose were treated and sacrificed, and bone marrow slides were prepared for all treated rats, generally slides were examined from only 5 rats (or less) of each dose group.

Results

A. General observations: No effects of treatment on physical appearance were apparent. Data for clinical signs were submitted as individual animal data. Data for body weights were submitted as a summary table of pre-treatment weights; no differences between test groups were apparent. The effect of treatment on body weights could not be assessed since post-treatment values were not submitted.

B. Cytogenetic Analyses: Cells were examined only for 6, 12, and 24 hours after treatment with acetochlor; cells from animals sacrificed 48 hours after treatment were not examined for cytogenetic abnormalities. Data were submitted as summary tables and as individual animal findings.

No effect of treatment on the frequency of chromosomal aberrations, the modal number (i.e. the average number of chromosomes/metaphase), or the mitotic index was apparent at 6, 12 or 24 hours after treatment.

Numerous discrepancies between the number of animals examined, as reported in the summary tables, and the number of animals for which data were reported in the individual animal data were noted. The number of animals reported as examined in the summary tables in many cases was less than the number of animals with individual data reported. In some cases, data were reported for animals from which "0" cells were examined, e.g. #D77707 (Group 2, 24 hours) and #D77777 (Group 5, 24 hours).

These data are tabulated in Table 1 of this review.

Table 1. Number of Animals Examined for Chromosomal Abnormalities[a]

Group	Test material	Dose	Time of Sacrifice 6 hours	12 hours	24 hours
1	Corn Oil	5 ml/kg	9/12[b]	10/10	10/11
2	Mitomycin C	5 mg/kg	-	-	8/11
3	Acetochlor	40 mg/kg	9/11	10/11	10/11
4	Acetochlor	150 mg/kg	6/12	10/10	10/10
5	Acetochlor	500 mg/kg	11/11	10/10	8/12

[a]data excerpted from submitted study.

[b]number of animals with results reported in individual animal data/ number of animals with results reported in summary table.

Discussion

No effect of treatment on the incidence of chromosomal abnormalities was apparent. No effect of treatment on the mitotic index was apparent, therefore there is no evidence that the test substance reached the target tissue, the bone marrow, in sufficient concentration to produce a toxic effect. Although slides were reportedly prepared for the 48 hour sacrifice, they apparently were not examined.

Significant discrepancies were noted by the reviewer between the results of chromosomal examinations as reported in the summary table and the data contained in the individual animal data appendices. For 8/13 reported results, the number of animals with actual data was less than the number of animals reported as examined in the summary table. For two animals, data were reported although the individual data indicated that "0" cells were examined for these animals. These discrepancies are sufficient cause for an audit of the supporting raw data.

Classification: Unacceptable Deficiencies as noted.

ACETOCHLOR Tox review 007697

Page_____ is not included in this copy.

Pages 205 through 214 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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

____ The document is not responsive to the request.

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

A. MATERIALS:

A copy of the "material and methods" section from the investigators' report is appended.

1. Test compound: Acetochlor(MON 097), Description: Amber-Purple Liquid, Lot#: Dayton RDNT 08001, Purity: 96.1%, contaminants: not provided.

EHL Substance Identification Code: T830072
Stated Stability: Stable for > 2 years @ 80°F
Received: Aug. 24, 1983 from Monsanto Agricultural Products Co.

2. Test animals: Species: Albino Rat, Strain: Sprague-Dawley, Age: approximately 26 weeks, Weight: Males:198.0 to 248.0 gms, Females:141.0 to 180.0 gms (at start of study).

Received: September 6, 1983.
The animals were kept under standard animal care conditions (see attached materials and methods.)
Source: Charles River Breeding Laboratory, Portage, MI.

B. STUDY DESIGN:

1. Animal assignment

A total of 70 animals per sex per dose group were used. The animals were assigned by computer randomization (EHL KRONIX) to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 24 months		Interim Sac. 12 months	
		male	female	male	female
1 Cont.	0	60	60	10	10
2 Low (LDT)	40	60	60	10	10
3 Mid (MDT)	200	60	60	10	10
4 High(HDT)	1000	60	60	10	10

2. Diet Preparation

Diet was prepared "approximately weekly" and stored at room temperature (apparently). Samples of treated food were analyzed for stability at room temperature and when refrigerated. "Dietary Level Verification" was checked on all dietary levels at during first 6 weeks and week 89 and on "one level/week otherwise".

Results - A signed cover sheet for the Appendix (III) containing the chemistry data was provided. Methods for determination were provided.

Test material stability was found to range from 94.6 to 99.8% purity over a 2 year period.

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Primary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/8/87 160
Review Section V, Toxicology Branch/HED (TS-769C)

Mutagenicity Secondary Reviewer: Irving Mauer, Ph.D.
Review Section VI, Toxicology Branch/HED (TS-769C)

Section Head Sign Off: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, Review Section V, Toxicology Branch/HED (TS-769C)

I. Study Type: Mutagenicity - IN VIVO Mouse Micronucleus Test
Guideline §84-2

Study Title: IN VIVO Micronucleus Assay in Mice with Acetochlor

EPA Identification Numbers: EPA ID No. 3F2966 and 534-GUI
EPA Accession No. 266002
EPA Record No. 185604
Shaughnessy No.
Caswell No. 3B
Tox. Branch Project No. 7-0375
Document No.

Sponsor: Monsanto Company
1101 17th Street, N.W.
Washington, D.C. 20036

Testing Laboratory: Hazelton Biotechnologies Corporation
9200 Leesburg Turnpike
Vienna, Virginia 22180

Study Number(s): Report No. HL-84-405
Project No. 241-207
R.D. No. 685
Special Report No. MSL-5723

Study Date(s): June 2, 1986 (March 13, 1985)

Study Author(s): Compiled by F.L. Groya
Joy Cavagnaro, Ph.D.
Thomas Cortina

Test Compound: MON 097 (also known as Acetochlor)
Purity = 96.7%
Description: a dark red liquid
Lot Number: Dayton Batch 18 (BA-18)
Date: June 1, 1983

Vehicle(s): Corn Oil
from C.F. Sauer Co.
Lot No. 52500

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Positive Control(s): Cyclophosphamide
from Sigma
Lot No. 123F-0283

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Dose(s): Vehicle Control - Corn Oil at 10 ml/kg
Positive Control (Cyclophosphamide) 40 mg/kg
MON 097 - single doses of 200, 660 or 2000 mg/kg administered by oral gavage at a dosing volume of 10 ml/kg.

Test Animal(s): Male and female CD®-1 mice
received from Charles River Breeding Laboratories, Inc., Kingston, New York, October 17, 1984.
141 animals per sex
approximately 32-40 days old

This study was designed to evaluate the mutagenicity potential of MON 097, administered orally to male and female mice as determined by micronuclei production in polychromatic erythrocytes (PCE).

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

Animals were kept under standard animal care conditions. They were acclimated 41 days prior to study initiation. They received Waynes F-6 Rodent Blox and "water" ad libitum.

The animals were "randomized via computer-generated random numbers" to the following groups:

Group	n Male	n Females	Dose
1 - Vehicle Control	27	27	Corn Oil
2 - Positive Control	9	9	Cyclophosphamide 40 mg/kg
3 - Low Dose - MON 097	27	27	200 mg/kg
4 - Mid Dose - MON 097	27	27	660 mg/kg
5 - High Dose - MON 097	27	27	2000 mg/kg

All animals received 10 ml/kg once by oral gavage. Dosing solutions were prepared fresh on day of administration. Dose levels were based on range-finding study (provided in report, see following "Results" section).

Clinical observations for "general appearance, behavior, toxic and pharmacological effects" were noted "twice daily or prior to sacrifice." Body weights were taken prior to treatment and at sacrifice.

Nine animals per sex were sacrified at 24, 48 and 72 hours (except for positive control where all animals were sacrificed at 24 hours).

The cytogenetic techniques used are described in the attached "materials and methods." Six-hundred and twenty-five polychromatic erythrocytes (PCE) were scored for the presence of micronuclei from 8 of the 9 mice, chosen randomly, for each group and sex. The numbers of normochromatic erythrocy' s (NCE) were also recorded.

Statistical procedures were described, see attached "materials and methods". Methods appear to be adequate to interpret results.

A Quality Assurance Statement was included.

III. Results

A. IN VIVO Micronucleus Assay Dose Range Finding Study

Six animals per sex were used (CD® -1 mice approximately 32 to 40 days old). They received either 1000, 2000 or 3000 mg/kg of MON 097 as a single oral dose in corn oil.

1. Mortality

One male in the 2000 mg/kg group and both females of the 3000 mg/kg group died.

2. Clinical Observations

The following clinical signs were noted (Table I).

Table I: Clinical Observation Data[a]

Dose (mg/kg):	1000	2000	3000
n =	4	3	3
Observations:			
Urine Stains	4(2)†	20(3)	12(3)
Soft Feces	2(2)	4(2)	2(2)
Slightly Depressed	2(1)	14(3)	4(3)
Depressed	--	--	2(1)
Rough Coat	--	5(3)	3(2)
Distended Abdomen	--	10(1)	--
Eyes Squinted	--	--	2(1)
Prostrate	--	--	2(1)
Tremors	--	--	2(1)
Labored Respiration	--	--	1(1)

† = # observation (# animals)
[a] = Data extracted from HLA Project No. 241-207 Table 1.

There was an apparent dose-related increase in clinical observations.

3. Body Weight

There was a dose related decrease in body weight gain (Table II).

Table II: Body Weight Gain[a]

Dose (mg/kg)	Body Weight Gain	
	Male	Female
1000	1.0 gm	0.5 gm
2000	0.5 gm	-3.5 gm
3000	-1.5 gm	--*

* = animals died
[a] = Data extracted from HLA Project No. 241-207 Table 2.

4. Conclusions:

The investigators determined that 2000 mg/kg was the MTD, and thus was the highest level to be tested in the primary study.

B. Primary Study

1. Mortality

Eleven males and 12 females of the 2000 mg/kg group (HDT) died.

2. Clinical Observations

No abnormal observations were noted in the control and low dose group at 24, 48 or 72 hours or in the positive control at 24 hours. The mid dose group had a few animals with urine stains or rough coat. The high dose, however, had significantly increased observations involving, urine stains, soft stool, rough coat, depression, labored respiration, red stains on nose and/or eyes, distended abdomen, tremors, and ataxia.

3. Body Weight

The following Table III presents the body weight gain data. The investigators provided both group mean and individual animal data.

Table III: Body Weight Gain (gms)[a]

Dose(mg/kg)	Hours		
	24	48	72
Control	-1.3/-1.3[†]	0.3/-0.7	-0.1/-0.4
Pos. Cont.	-1.6/-1.2	--	--
200	-0.6/-1.3	-0.7/-0.9	-0.1/-0.7
660	-1.3/-0.5	0/1.5	0.9/0.9
2000	-2.9/-0.6	0.9/-3.1	-0.2/-2.4

[†] = Male/Female
[a] = Data extracted from HLA Project No. 241-207 Table 5.

The provided data were variable, and therefore no definite conclusion could be drawn. The investigators stated that: "In general, animals weighed at 24 hours, including control, exhibited slight weight loss following treatment, while 48 and 72 hour animals varied between slight body weight loss and slight gain. The group 5 (2,000 mg/kg) 24 hour males, 48 hour females, and 72 hour females showed a slightly larger loss of body weight".

4. Cytogenetic Analysis

The investigators provided summary and individual animal data. The attached Table 2 from the investigators report presents the summary data. No effect on incidence of micronucleated polychromatic erythrocytes was noted between the 3 MON 097 test groups compared to the solvent control. The positive control, however, showed a statistically significant increase in PCE micronuclei. The ratio of polychromatic (PCE) to normochromatic (NCE) erythrocytes is presented on attached Table 4 (from the investigators report). The investigators noted a decrease in PCE:NCE ratio in the high dose at 72 hours. They attributed this to the onset of stem cell toxicity. They further stated that "According to Schmid (1976), the ratio of PCE:NCE in animals of similar age as used for this study should be about 1.1".

IV. Conclusions

Under conditions of this test the high dose level of MON 097 (2000 mg/kg) exhibited mortality and signs of clinical toxicity. No evidence of an increase in micronucleated polychromatic erythrocytes was noted at the dose levels tested in this study.

V. Core-Classification: Acceptable.

ACETOCHLOR Tox review 007697

Page_____ is not included in this copy.

Pages 221 through 230 are not included.

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____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

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____ Identity of the source of product ingredients.

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____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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ATTACHMENT J

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

FEB 3 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 524-EUP-56: Renewal of temporary tolerances and an Experimental Use Permit (EUP) for Harness® Herbicide (Acetochlor). Caswell #38, Accession # - . Tox. Proj. #7-0206

TO: Robert Taylor (12)
Registration Division (TS-767C)

FROM: Winnie Teeters, Ph.D. Winnie Teeters 1-29-87
Pharmacologist, Section V
Tox./HED (TS-769C)

THRU: Quang Bui, Ph.D. Quang Bui 2-3-87
Acting Section Head, Section V
Tox./HED (TS-769C)

2/3/87

and

Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

CHEMICAL: Acetochlor (2-chloro-N-[ethoxymethyl]-N-[2-ethyl-6-methyl phenyl] acetamide), MON 097, CP 55097. Harness® is an EC formulation containing 86.4 - 86.5% A.I.

ACTION REQUESTED: Review requests for renewal of temporary tolerances and an EUP for Harness® Herbicide. No additional data were submitted.

The EUP Program: An amended experimental program is being proposed, with only 2200 pounds being requested for use under the renewed program. This amount will be applied over a two-year program in 13 states to 1100 acres.

Requested Renewal of the following Temporary Tolerances:

Corn (grain)	0.1 ppm
Corn (forage and fodder)	0.5 ppm
Eggs	0.02 ppm
Milk	0.02 ppm
Beef tissue	0.02 ppm
Hog tissue	0.02 ppm
Chicken tissue	0.02 ppm

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History: On March 20, 1980, the Agency approved a crop destruct EUP for aceto-chlor (now Harness® Herbicide) for use on corn, soybeans, peanuts and

grain sorghum. On Feb. 10, 1982, temporary tolerances for acetochlor on corn(all grain 0.1 ppm) and soybean grain (0.4 ppm) were granted. The EUP and temporary tolerances were allowed to lapse on March 20, 1985. A petition (3F2966 & 524-GUI) for permanent tolerances and data submitted with an EUP and Petition (524-EUP-65/2G2797 and 3G2791) for temporary tolerances were reviewed earlier (memo of Teeters to Taylor, Aug., 5, 1985) and it was recommended that the permanent tolerances were not supported by the available data; furthermore, acetochlor was found to be an oncogen in both the rat and mouse.

Recommendations: Studies for acetochlor in our files are adequate to support the requested renewal of the EUP and temporary tolerances, except for an acute inhalation study with the formulation, a subchronic, or longer term, feeding study in a non-rodent (the 119-day dog feeding study [Pharmocopathics Res. Labs. #7920, 10-10-80] did not establish a NOEL and the 1-year dog feeding study [Pharmocopathics Res. Labs. #PR-80-008, 10-14-81] was classified as Supplementary Data) and a mutagenicity study for chromosome aberration (Hazleton Labs. America #83-006, 5-14-83 was Unacceptable).

However, the following information should be considered when decisions are made regarding these requests:

Acetochlor had been found in chronic studies to be oncongenic in both mice and rats, and chronic systemic toxicity has not been adequately defined. A new study is necessary to establish a NOEL for chronic toxicity. See memo of Teeters to Taylor, 8-5-85.

A risk assessment (memo of Lacayo to Teeters, 5-4-86) based on the findings in these oncogenic studies estimates a worst case potency of Q_1^* (mg/kg/day) to be 10^{-2} for humans; for mice and rats the corresponding values are 10^{-3} and 10^{-4}, respectively.

In an informal note from Lacayo using tolerances the same as the ones requested in this action, except that 0.02 ppm each in goat, horse and sheep tissues were included, the dietary risk to humans was estimated to be 3.3×10^{-6} (see memo of Teeters to Taylor, 5-15-86), indicating there appears to be only a minor risk from dietary exposure at requested tolerance levels (including the extra tissues). But the risks to applicators have not been assessed.

Furthermore, acetochlor is one of a series of closely related analogs having oncogenic activities (memo of Teeters to Engler, 8-23-85). In a draft "Peer review of Acetochlor" (Jan. 21, 1987) it was concluded that there is evidence that acetochlor meets the criteria for Group B2- Probable Human Carcinogen.

Summary of data in our files to support these requests:

Memo of Teeters to Taylor, 8-5-85. (Petition 3F2966 & 524-GUI and 524-EUP-56/2G2797 and 3G2791.) Studies with acetochlor (MON 097, CP 55097).

1. Subchronic 21-day dermal, IRDC Study #IR 80-356, 12-11-81.

   LOEL for systemic effects (mortality and decreased weight gain): 1200 mg/kg (HDT).
   NOEL for systemic effects: 400 mg/kg

LOEL for dermal irritaton· 100 mg/kg (LDT)
NOEl for dermal irritation· not established

Core minimum.

2. Dermal sensitization. (MON 097 technical). Bio/Dynamics, Inc. Study #BD-82-204. 4-13-83.
   Positive dermal sensitizer
   Core minimum

3. Dermal sensitization. (MON 097, 8 lbs/gal EC formulation, Harness®). Bio/Dynamics, Inc. Study #BD-82-205, 4-13-83.
   Positive dermal sensitizer
   Core minimum

4. In Vivo bone marrow chromosome study, Hazleton Labs. America, Inc. Study #HL83-006, 5-24-83.
   No evidence of chromosome abnormalities induced, but the study is Unacceptable.

5. Rat hepatocyte primary culture/DNA repair test, Pharmacon Res. Internat'l, Inc. Study #PK-52-151, 2-17-83.
   No evidence of inducement of unscheduled DNA synthesis.
   Study is Acceptable.

6. Mouse lymphoma assay, SRI Internat'l Study #SR-81-150, Aug.-82.
   Positive mutagen only in the presence of metabolic activation.
   Study is Acceptable.

7. CHO/HGPRT gene mutation assay, Monsanto Environmental Health Lab. Study #ML-82-281, 6-9-83.
   Weakly positive at near-toxic doses, but the vehicle used (alcohol) did not appear to be inert in the assay.
   Study is Acceptable.

8. Rabbit teratology studies, IRDC, Pilot Studies #IR-79-292, Primary Study #IR-79-293, 11-24-81.
   Two pilot studies are Invalid Data; the third pilot study is Supplementary Data as a range-finding study.

   The primary study is also Supplementary Data and a new study is requested; insufficient numbers of litters were available to fully assess the teratogenic potential.

9. Two generation reproduction study in rats, IRDC Study #IR-80-053, 12 16-82.
   Reproductive NOEL: 500 ppm
   Reproductive LOEL: 1500 ppm (based on decreased body weight gain of F_{2b} pups)
   Systemic NOEL: <500 ppm based on absolute and relative organ weight: decreases for ovary weights in F_1 females, decreases for pituitary weights for F_1 and F_{2b} males, increases for thyroid weights in F_{1b} and F_{2b} pups.

   Minimum Data

10. Metabolism study with rats, Hazleton Raltech, Inc. Study #MSL-2824, June-83.

Little (0.5%) eliminated via lungs: >70% excreted within 48 hrs., preferentially in urine. Elimination is biphasic with a fast half-life of < 10 hrs. and a slow half-life of 128-286 hrs. Early metabolites are mainly mercapturates: later ones were sulfoxides, sulfones, and sulfates; over 20 metabolites were identified. Less than 1% of parent compound is excreted unchanged in feces. There was retention of 2-2.5% of dose in RBC due to covalent binding to hemoglobin.

Core Guideline

11. One year feeding study in dogs, Pharmacopathics Res. Lab. Study #PR-80-008, 10-14-81.

Dogs at 40 mg/kg (HDT) showed testicular atrophy accompanyied by decreased absolute and relative (to body weight) testicular weight, decreased body weight gain of males and decreased terminal body weight of females. There is also suggestive evidence at this level for anemia and hepatotoxicity but a NOEL and LOEL cannot conclusively be determined for these effects at lower levels because of control data variability and the wide range of normal values for these parameters established at the testing facility.

Supplementary Data

12. Chronic toxicity and oncogenicity study in rats, Pharmacopathics Res. Labs. Study #PR-80-006, 5-20-83.

Oncogenic NOEL: 1500 ppm
Oncogenic LOEL: 5000 ppm -increased incidence of liver carcinomas and thyroid follicular cell adenomas in males.
There were positive trends for hepatic carcinomas in females and thyroid follicular cell adenomas in males.
Systemic LOEL: 500 ppm (LDT) based on organ weight effects and decreased body weight in males.
Systemic NOEL was not established. One must be established in a new study.
The high level (5000 ppm) also increased incidences of polyarteritis of the testes and arteries of males and liver necrosis and alveolar histiocytosis in females. Mortality was increased in females and food consumption was decreased in both sexes.

Minimum Data

13. Oncogenicity study in mice, Pharmacopathics Res. Labs. Study #PR-80-007, 5-4-83.

Oncogenic NOEL: < 500 ppm (LDT). There were increased incidences of: liver carcinomas in high level males, total tumors in females of all levels, carcinomas of the lungs in low and high level females, uterine histiocytic sarcomas in females of all levels and total benign ovarian tumors in mid level females. There were positive linear trends for: liver carcinomas in both sexes, and pulmonary carcinomas, total lung tumors, ovarian

benign tumors and kidney adenomas in females.
Non-neoplastic lesions included an increase in interstitial nephritis in both sexes of the high level(5000 ppm).
Systemic LOEL: 500 ppm(LDT) based on increased liver and kidney weights in males.

Minimum Data

Memo of Dykstra to Taylor, dated 3-21-84 for PP# 1G 2454. These data are summarized as follows:

1. Acute oral LD_{50}. Rat, Mon 097, 2953 mg/kg (both sexes), Category II, Minimum Data. Environmental Health Laboratory Report #80-49, 10-15-80.

2. Acute dermal LD_{50}, Rabbit, Mon 097, 3667 mg/kg (both sexes), Category III, Minimum Data. Environmental Health Laboratory Report #80-48, 10-15-80.

3. Primary dermal irritation, Mon 097, P.I.=0.6/8.0, Category IV, Minimum Data. Environmental Health Laboratory Report #80-50, 10-15-80.

4. Primary eye irritation, Mon 097, scores for unwashed = 18.8/110, for washed = 1.2/110, Category II, Minimum Data. Environmental Health Laboratory Report #80-51, 10-15-80.

5. 91-Day feeding, Rat, CP-55097, NOEL = 800 ppm
   LOEL = 2000 ppm based on body weight loss and food consumption decrease, Minimum Data. Pharmacopathics Report #7914, 10-10-80.

6. 119-Day feeding, Dog, CP-55097, NOEL <25 mg/kg/day (LDT), dose-related elevated SGPT - Minimum Data. Pharmacopathics Report #7920, 10-10-80.

7. Teratology, Rat, CP-55097, Negative at 400mg/kg/day
   Fetotoxic NOEL = 200 mg/kg/day
   Maternal NOEL = 200 mg/kg/day
   Minimum Data. IRDC Report #401-066, 10-15-80.

8. Mutagenicity, Ames Salmonella Assay, CP-55097, Negative for strains TA-98, 100, 1535 and 1537, with and without mouse and rat microsomal preparations. Minimum Data. Monsanto Report # MRC-DA-838, 12-5-78.

ATTACHMENT K

007697

Data Evaluation Record

C04586

Study Type: In vivo cytogenetics in rats.

Study Identification:	"In Vivo Bone Marrow Chromosome Study in Rats with Acetochlor (MON 097)".
Lab. performing study:	Hazelton Laboratories America, Inc. Vienna, VA 22180
Sponsor:	Monsanto Agricultural Products Co. St. Louis, MO. 63167
Study no.:	HL 83-006
Project no.:	241-143 (Hazelton)
Accession no.:	071970
Report date:	May 24, 1983
Submitted to EPA:	9/22/83
Study director:	Michael G. Farrow, Ph.D.
Reviewed By:	D. Stephen Saunders Jr., Ph.D. Toxicologist, Section V TOX/HED (TS-769)
Approved By:	Irving Mauer, Ph.D. Geneticist, Toxicology Branch Hazard Evaluation Division (TS-769)

DSS 8/2/85

8/2/85

Conclusions: No effect on the incidence of chromosomal abnormalities was apparent. No effect on mitotic index was apparent, therefore there was no evidence that the test material reached the bone marrow in sufficient concentration to produce a toxic effect. Numerous discrepancies in the results reported in the summary tables vs. individual animal data were noted.

Classification: Unacceptable Deficiencies as noted.

Materials and Methods

A. Materials- (1) Test chemicals: Acetochlor (MON 097), a "brown liquid", 96.3% a.i.

Positive control: Mitomycin C (Sigma Chem. Co.), assumed 100% a.i.

Mitotic arrest- colchicine; supplier, purity not stated.

(2) Doses tested: Acetochlor- 40, 150, and 500 mg/kg by i.p. injection.

vehicle control- corn oil, 5 ml/kg.

positive control- Mitomycin C, 5 mg/kg

mitotic arrest- colchicine 2 mg/kg.

(3) Test animal: Male and female Sprague-Dawley CD albino rats, obtained from Charles River Breeding Laboratories, Kingston, N.Y.

Table 1. Number of Animals Examined for Chromosomal Abnormalities[a]

Group	Test material	Dose	Time of Sacrifice 6 hours	12 hours	24 hours
1	Corn Oil	5 ml/kg	9/12[b]	10/10	10/11
2	Mitomycin C	5 mg/kg	-	-	8/11
3	Acetochlor	40 mg/kg	9/11	10/11	10/11
4	Acetochlor	150 mg/kg	6/12	10/10	10/10
5	Acetochlor	500 mg/kg	11/11	10/10	8/12

[a]data excerpted from submitted study.

[b]number of animals with results reported in individual animal data/ number of animals with results reported in summary table.

Discussion

No effect of treatment on the incidence of chromosomal abnormalities was apparent. No effect of treatment on the mitotic index was apparent, therefore there is no evidence that the test substance reached the target tissue, the bone marrow, in sufficient concentration to produce a toxic effect. Although slides were reportedly prepared for the 48 hour sacrifice, they apparently were not examined.

Significant discrepancies were noted by the reviewer between the results of chromosomal examinations as reported in the summary table and the data contained in the individual animal data appendices. For 8/13 reported results, the number of animals with actual data was less than the number of animals reported as examined in the summary table. For two animals, data were reported although the individual data indicated that "0" cells were examined for these animals. These discrepancies are sufficient cause for an audit of the supporting raw data.

Classificat n: Unacceptable Deficiencies as noted.

ACETOCHLOR Tox review 007697

Page_____ is not included in this copy.

Pages 238 through 242 are not included.

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____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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ATTACHMENT L 187

007697 004586

Data Evaluation Record

Study Type: In vitro rat hepatocyte DNA repair assay.

Study Identification: "Rat Hepatocyte Primary Culture/DNA Repair Test."

Lab. performing study:	Pharmakon Research International, Inc. Waverly, PA 18471
Sponsor:	Monsanto Agricultural Products Co. St. Louis, MO. 63167
Study no.:	PK 82-151 (Monsanto)
Project no.:	PH 311-MO-001-82 (Pharmakon)
Accession no.:	071970
Report date:	2/17/83
Submitted to EPA:	9/22/83
Study director:	Robert W. Naismith, Ph.D.
Reviewed By:	D. Stephen Saunders Jr., Ph.D. Toxicologist, Section V TOX/HED (TS-769)
Approved By:	Irving Mauer, Ph.D. Geneticist, Toxicology Branch Hazard Evaluation Division (TS-769)

DSS 8/2/85

Conclusions: No effect of treatment on the rate of thymidine incorporation was apparent at doses of 0.032 to 3.2 ug/well. Doses of 10.6 to 320 ug/well were reported as "cytotoxic", however the criteria for this assessment, or any data obtained from these cells, were not submitted. The purity and method of dose calculation for the test article were not supplied, and it is not clear whether the technical grade of active ingredient was tested in this assay.

Classification: Unacceptable Deficiencies as noted.

Materials

(1) Test chemicals: Acetochlor (MON 097), a "colorless, pale yellow liquid", Lot #NBP1737813, % a.i. not stated.

Positive control: 2-AAF (Aldrich Chem. Co.), % a.i. not stated.

Vehicle control- DMSO (Mallinkrodt, Inc.), purity not stated.

(2) Doses tested: Acetochlor- 0.032 ug/well to 320 ug/well.

vehicle control- DMSO

positive control- 2-AAF, 1×10^{-4}M.

(3) Test system: Rat hepatocytes isolated from the liver of male Fischer-344 rats, obtained from Charles River Breeding Laboratories, Wilmington, Mass., "or any USDA acceptable source".

Methods

A photocopy of the submitted methods is appended. The methods were reviewed and the following points were noted:

1) The criteria for assessing cytotoxicity were not stated.

2) The method for calculating doses was not stated. Since the doses were reported as "ug/well", and the test substance was supplied as a liquid, the density and purity of the test material were required to calculate doses. If the investigators assumed a density of 1.0 and purity of 100%, it should be so stated. Further, since the test material in the in vivo cytogenetics study (Monsanto #HL 83-006) was a brown liquid, with reported purity of 96.3% a.i., it is not clear whether the technical material was tested in the present study since it was described as a "pale yellow liquid".

Results/Discussion

No effect of treatment on the rate of incorporation of ^{3}H-thymidine by hepatocytes in vitro was apparent at doses of 0.032 ug/well to 3.2 ug/well. Doses of 10.6 ug/well and above were reported as cytotoxic, however the criteria for this assessment were not provided, nor were any effects of treatment on thymidine incorporation by these cells reported. The positive control, 2-AAF, induced about a 100-fold increase in incorporation of thymidine, demonstrating that the test system could respond appropiately to a known mutagen. These data are presented in the table below (photocopied from the submitted study report):

Treatment	Concentration	Net Nuclear Grains Triplicate Cultures x ± s.d.
Untreated		0.0 ± 0.1
DMSO		0.2 ± 0.2
2AAF	1×10^{-4} M	52.7 ± 9.5**
MON 097	0.032 ug/well	0.5 ± 0.4
MON 097	0.106 ug/well	0.3 ± 0.4
MON 097	0.32 ug/well	0.7 ± 0.6
MON 097	1.06 ug/well	0.6 ± 1.0
MON 097	3.2 ug/well	0.4 ± 0.5
MON 097	10.6 ug/well	Cytotoxic
MON 097	32.0 ug/well	Cytotoxic
MON 097	106.6 ug/well	Cytotoxic
MON 097	320.0 ug/well	Cytotoxic

**Positive finding. Mean net nuclear grain count of five or greater than the vehicle control.

Classification: Unacceptable Inadequate identification of test article; method of dose calculation not adequately described; criteria for cytotoxicity or data from these cells not submitted.

ACETOCHLOR Tox review 007697

Page_____ is not included in this copy.

Pages 245 through 248 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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

____ The document is not responsive to the request.

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

007697 192

CASWELL FILE

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

AUG 20 1986

005374

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Response to Toxicology Branch Evaluation of Acetochlor DNA Repair Assay in Hepatocytes. EPA ID No. 524-348; TOX PN #1411/1412; Caswell #003B

TO: Robert Taylor (25)
Registration Division (TS-767)

FROM: D. Stephen Saunders, Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

DSS 8/15/86

THRU: Irving Mauer, Ph.D.
Senior Geneticist, Toxicology Branch
and
Laurence D. Chitlik, DABT
Head, Section V, Toxicology Branch
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division

08-21-86

8-14-86

Action Requested

Review and comment on the response submitted by the Registrant regarding the Toxicology Branch evaluation of the rat hepatocyte DNA repair assay conducted with acetochlor, which was originally classified as Unacceptable data.

Recommendation

It is recommended that the acetochlor rat hepatocyte DNA repair assay (study #PK 82-151 [Monsanto]/PH 311-MO-001-82 [Pharmakon]) be upgraded to Acceptable status. No evidence of mutagenicity was presented in this study, and acetochlor should be considered as negative for DNA damage in this assay.

(con't)

Discussion

Cited Deficiency #1: The purity of the test material was not stated in the study report.

Company response: "The test article, acetochlor (MON 097) was supplied as a pale yellow liquid of 99.7% purity. ...This information was inadvertently omitted from ~~from~~ the final report and has since been added as an addendum (dated Sept. 11, 1985, see attached)."

EPA Comment: This deficiency is corrected by the submitted additional information.

Cited Deficiency #2: The method for calculating doses was not stated. Since the doses were reported as "ug/well", and the test substance was supplied as a liquid, the density and purity of the test material were required to calculate doses. If the investigators assumed a density of 1.0 and purity of 100%, it should be so stated.

Company response: "A weighed aliquot of the test article was dissolved in DMSO and serially diluted. 20 ul of each dilution were added to wells containing cells and media in a final volume of 2 ml. Final concentrations of the test article were expressed as ug/well or ug/ml. The Pharmakon dose preparation sheets have been included as part of the report addendum (attached)."

EPA Comment: This deficiency is corrected by the submitted additional information.

Cited Deficiency #3: The criteria for assessing cytotoxicity were not stated.

Company response: "As indicated in the attached letter from the study director: 'Cytotoxicity is noted by cell detachment, abnormal cell morphology, unusual cell staining and overall decrease in grains relative to the solvent and untreated controls.' Data on these cells in not routinely included in final reports but can be found in the raw data at the testing laboratory."

EPA Comment: This deficiency is corrected by the submitted additional information. The criteria used by the investigators to establish cytotoxicity (detachment, altered morphology, etc.) are fairly standard, and Toxicology Branch is satisfied that potentially positive data have not been discarded due to inappropriate definitions of cytotoxicity.

DSS:TOX/HED:8-15-86:FILE 2RESP3B

250

007697 ATTACHMENT M

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

195

006769

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of rat dominant lethal assay; Record no. 233009/223010/223017/223015; EPA ID no. 3F2966/6G3345/524-GUI/524-EUP-AT; MRID No. 403893-01; Proj. No. 8-0777; Caswell No. 3B

TO: Robert Taylor/V.K. Walters (PM 25)
Registration Division (TS-769C)

FROM: James N. Rowe, Ph.D. James N. Rowe 6/27/88
Section V, Toxicology Branch
Hazard Evaluation Branch (TS-769C)

THRU: Quang Q. Bui, Ph.D. Quang Q. Bui 6/27/88
Section Head
Section V, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Theodore M. Farber, Ph.D. 6/30/88
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ACTION: Expedited review of a rat dominant lethal study; Record no.233009/223010/223017/223015; EPA ID no.3F2966/6G3345/524-GUI/524-EUP-AT; Accession No. 40389301; Proj. No. 8-0777; Caswell No. 3B

RECOMMENDATIONS:

Unacceptably low pregnancy rates among control and low dose animals associated with low mating activities in control males limit the sensitivity of this study to adequately evaluate the potential dominant lethality/reproductive toxicity of MON 097.

This study is designated unacceptable data and cannot be upgraded. A new study is requested.

251

007697

006769

Reviewed By: James N. Rowe, Ph.D. James N. Rowe 6/27/88
Section V, Toxicology Branch (TS-769C)
Secondary Reviewer: Kerry Dearfield, Ph.D. Kerry J. Dearfield 6-27-88
Section V, Toxicology Branch (TS-769C)

196

DATA EVALUATION REPORT

Study type: Dominant Lethal
Test system: Rats, Sprague-Dawley
Guideline: 84-2

Study Title: DOMINANT LETHAL/FERTILITY STUDY OF MON 097 IN SPRAGUE-DAWLEY RATS

EPA ID NOS.: EPA ID NO. 3F2966/6G3345/524-GUI/524-EUP-AT
EPA MRID No. 403893-01
Caswell No. 3B
Project No. 8-0777

Sponsor: Monsanto Company
St. Louis, MO 63110

Testing Laboratory: Monsanto Environmental Health Laboratory
645 S. Newstead
St. Louis, MO 63110

Laboratory Project No.: EHL-86008

Final Report Date: 10/11/87

Date of Study Completion: 8/11/87

Study Author: M.W. Naylor, B.S., Study director

Quality Assurance: A statement of Quality Assurance is signed by Arthur F. Uelner, Manager, Quality Assurance at EHL

Compound: MON-097, ID code T860008, Lot No. XLF-396, purity 94.3% from Monsanto Agricultural Co., Date received 2/12/86, purple liquid; chemical name is acetochlor.

CONCLUSIONS - EXECUTIVE SUMMARY:

Unacceptably low pregnancy rates among control and low dose animals associated with low mating activities in control males limit the sensitivity of this study to adequately evaluate the potential dominant lethality/reproductive toxicity of MON 097.

Recommendation: This study is unacceptable.

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Methods:

A photocopy of the experimental methods is attached (see Attachment 1).

The test compound was administered in the diet (0, 100, 1000, 2000 ppm) for approximately 9 weeks to 4 groups of 30 male CD (SD) BR rats each. At the end of the exposure period, each male was co-housed with a single untreated virgin female for up to 5 days. After a recovery period of 2-6 days, the males were again co-housed with a second untreated virgin female. A concurrent positive control group of males (30) received a single intraperitoneal injection (0.3 mg/kg) of triethylenemelamine (TEM) 3 days before mating.

All animals were checked for mortality and morbidity twice daily. Detailed clinical observations for males were performed weekly in males until mating and in females immediately prior to mating. Body weights were measured weekly in males until mating, once again at the end of mating and prior to terminal sacrifice while females were weighed prior to mating. Food consumption was determined weekly in males only prior to mating. Evidence of copulation was determined by the presence of a copulatory plug or by vaginal smear.

At the scheduled sacrifice time, males were examined externally and internally by gross necropsy; female reproductive organs were examined for pregnancy status, number and state of nidations, early resorption sites and corpora lutea counts.

Vehicle: Dietary feed: Ralston Purina RODENT CHOW No. 5002

Statistical analysis:
1) Dunnett's Multiple Comparison Test for body weights and food consumption, 2) Mann-Whitney U test for preimplantation losses, viable and non-viable implants expressed as both per pregnant female and per corporea lutea/pregnant female, dead implants/ total implants and corporea lutea/pregnant female, 3) Chi-square test for fertile males, number pregnant/number co-housed and the number of females with >1 and >2 dead implants.

Results:

Analysis of test compound

Test compound purity and stablity and dietary test mixture homogeneity and stability were within acceptable limits. The stability of the test substance compared with an analytical standard was acceptable during the exposure period (97% of standard concentration and 93% of standard concentration during a three-month period). Analysis for homogeneity of acetochlor in dietary mix indicated an acceptable level of homogeneity for the

low and high dose groups (0-15% variation from nominal with average variation of 4.3%). The dietary test mixture was stable up to 14 days at room temperature (within 10% of day 0 concentration) and when stored in the refrigerator for 36 days (90-110% of day 0 value). Analysis of dietary concentration of MON 097 weekly during the test period indicated that the concentration in all dose groups was within acceptable limits of the target value (85-100% of target).

Mortality/morbidity

There were no mortalities reported in this study and no apparent treatment-related clinical signs of toxicity.

Body weights

At the 2000 ppm dose level (HDT) there was a statistically significant depression in mean male body weights (gm) by the end of one week of treatment as compared with the control group and which was evident throughout the exposure period, i.e.,:

	Week 1	Week 5	Week 9
Controls	443.0	528.5	588.9
HDT	419.1**	493.0**	544.0**

** Dunnett's Test (two-tailed), $p<.01$)

Food consumption

There was a statistically significant depression ($p<.01$) in the mean food consumption (gm/kg/day) during week one of test substance administration (control, 61.4 gm; 1000 ppm, 54.8 gm; 2000 ppm, 48.8 gm). No statistically significant difference in any dose-group was observed thereafter for mean food consumption. Mean food efficiency (% food consumed converted to body weight) was sporadic but generally lower in the high dose group as compared to controls.

Fertility Data

A copy of the summary fertility data from the study report (Table 4) is attached (Attachment 2).

Meaningful analysis of the effects of acetochlor upon fertility/dominant lethality is limited by the low pregnancy rate observed in the control females (30/60, 50%) as well as in the low dose group (28/60, 47%). A low fertility index in these males (21 males/30 pregnancies, 70%) was also noted in both dose groups. Further, mating activity was quite low in the mated control group (34/60 females co-housed, 57%). This suggests some difficulty in animal husbandry. As indicated by the study author (p. 11 of report) the pregnancy rate (# pregnant/# co-housed) in the negative controls was below the historical mean for this strain of rat in their laboratory. The 50% pregnancy rate in the

control group is not significantly different from the positive control. It is questionable whether meaningful dose-related data can be determined from the study. Also, it is uncertain that other factors may have compromised this study, e.g., to alter the control pregnancy rate. Therefore, the reviewer will not attempt to discuss the additional parameters presented. It is noted that the positive control did produce statistically significant effects for pregnancy rate, depressed number of corpora lutea, increased resorptions, etc. This indicates that the rat strain utilized is reponsive to the positive control, TEM.

Gross necropsy

No compound-related gross pathological effects were noted.

ACETOCHLOR Tox review 007697

Page_____ is not included in this copy.

Pages 256 through 259 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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

____ The document is not responsive to the request.

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

ATTACHMENT N

007697 (x)

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANC

MEMORANDUM

SUBJECT: Structural Similarity of ACETOCHLOR To Other Positive Oncogens

FROM: W. Teeters, Pharmacologist 8-23-85
Acting Head, Section V
Toxicology Branch (TS-769C)

TO: Reto Engler, Ph.D.
Chief, Scientific Mission Support Staff
Toxicology Branch (TS-769C)

On the following page are the chemical structures of ACETOCHLOR and several close analogues (taken from a memorandum of July 29, 1983 to R. Taylor from A. Mahfouz). All are substituted chloroacetanilides excepts Allidochlor, which is a chloroacetamide. Metolachlor is produced by Ciba-Geigy; the others are all Monsanto products.

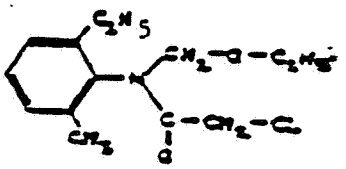
ACETOCHLOR is a positive carcinogen which induces an increased incidence of liver carcinomas and thyroid follicular cell adenomas in male rats (5000 ppm) and there was a positive trend for hepatic carcinomas in females. In mice at doses of 500, 1500 and 5000 ppm, there were increased incidences of liver carcinomas in high level males, total lung tumors in females of all levels, carcinomas of the lungs in low and high level females, uterine histiocytic sarcomas in females of all levels and total benign ovarian tumors in mid-level females. There were positive linear trends for: liver carcinomas in both sexes, and pulmonary carcinomas, total lung tumors, ovarian benign tumors and kidney adenomas in females (memorandum of August 5, 1985 to R. Taylor from W. Teeters).

Additionally, ACETOCHLOR was weekly positive in the CHO/HGPRT gene mutation assay at near toxic doses but the vehicle used (alcohol) had some activity. It was also positive (with activation only) in the mouse lymphoma test. Negative results were obtained in the Ames salmonella test and in two tests which were unacceptable: the bone marrow chromosome aberration and hepatocyte DNA repair tests.

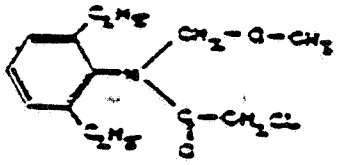
007697

125

#3B

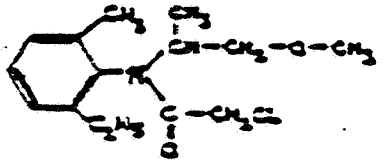


Acetochlor



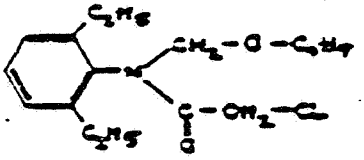
#11

Alachlor



Metolachlor

188DD



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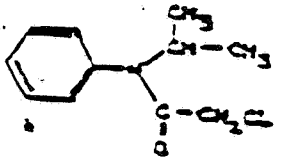
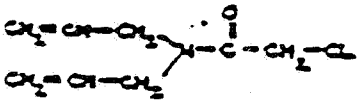
Butachlor



Propachlor

194D



Allidochlor

#984

007697

121

The rat teratology study with ACETOCHLOR was negative at a dose of 400 mg/kg, and the rabbit study must be repeated.

Decreased numbers of pups/litter were seen at a dose of 5000 ppm in a rat reproductive study with ACETOCHLOR.

ALACHLOR is also oncogenic in both rats and mice. In rats, it caused nasal turbinate (42 mg/kg) and stomach tumors (126 mg/kg) in both sexes and thyroid follicular adenomas in males (146 mg/kg). In mice, there was an increased incidence of lung tumors in females (260 mg/kg). (See memorandum of 6-16-82 to R. Taylor from A. Manfouz).

BUTACHLOR (Machete") causes stomach tumors (only defined as masses at necropsy) in female rats (3000 ppm). (See memorandum of 1-4-83 to R. Taylor from W. Dykstra.)

METOLACHLOR caused a significantly elevated incidence of proliferative liver lesions (neoplastic nodules plus carcinomas combined) at the highest dose level tested (3000 ppm) in female rats. The mouse oncogenic study was negative for proliferative lesions. (See "Peer Review of Metolachlor".)

PROPACHLOR (Ramrod") and ALLIDOCHLOR (Randox") were both tested by Industrial Bio-Test Laboratories and these long term studies must be repeated.

cc: Acetochlor File, Caswell #38

Reviewer's Peer Review Package for 1st Meeting

8/29/85

FILE COPY

007697

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

AUG 29 1985

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review on Acetochlor.

FROM: Reto Engler, Chief
Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Addressees

Attached for your review and consideration is a package on Acetochlor consisting of

1. SAR discussion

2. Summary on Tox data (instead of 1-liners)

3. DER's 4-mutagenicity studies
Metabolism study
Long-term rat study
Long-term mouse study

A meeting to discuss and evaluate the scientific issues and determine the weight of the evidence has been scheduled for Thursday, September 12, 1985 at 10:00 AM in Dr. Farber's office (Room 816, CM-2).

Attachments:

ADDRESSEES

Theodore Farber
William Burnam
John Quest
Donald Barnes
Winnie Teeters
Steve Saunders
Laurence Chitlik
Louis Kasza
Bertram Litt

#3

007697

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Structural Similarity of ACETOCHLOR To Other Positive Oncogens

FROM: W. Teeters, Pharmacologist
Acting Head, Section V
Toxicology Branch (TS-769C)

W Teeters 9-23-85

TO: Reto Engler, Ph.D.
Chief, Scientific Mission Support Staff
Toxicology Branch (TS-769C)

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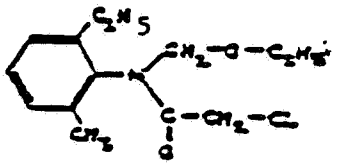
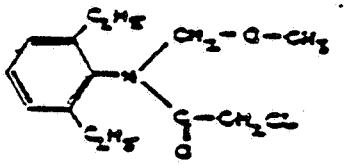
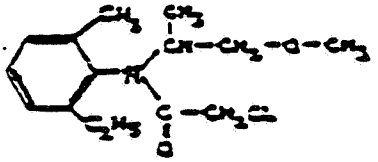


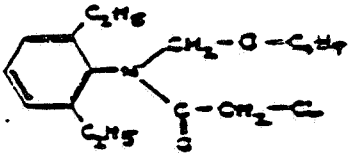
Acetochlor



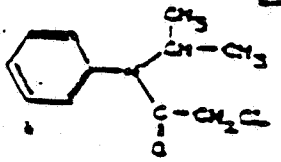
Alachlor



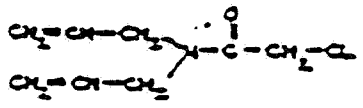
Metolachlor



Butachlor



Propachlor



Allidochlor

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PROPACHLOR (Ramrod™) and ALLIDOCHLOR (Randox™) were both tested by Industrial Bio-Test Laboratories and these long term studies must be repeated.

cc: Acetochlor File, Caswell #3

007697

AUG 5 1985

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

004586

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

AUG 5 1985

MEMORANDUM

SUBJECT: Petition (3F2966 & 524-GUI) for Permanent Tolerances (Acc#071962-72) and Review of Data Previously Submitted with an EUP and Petition (524-EUP-56/2G2797 and 3G2791) for Temporary Tolerances (Acc#248618-20) for Harness® (Acetochlor)

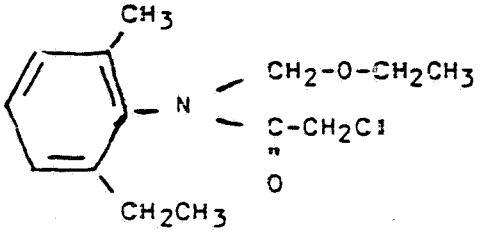
Caswell #38

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

FROM: Winnie Teeters, Ph.D. W. Teeters 8-5-85
Pharmacologist, Section V
Tox/HED (TS-769C)

THRU: Laurence D. Chitlik, D.A.B.T. LDC 8/5/85
Head, Section V
Tox/HED (TS-769C)
and
Theodore M. Farber, Ph.D. 8/5/85
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

CHEMICAL: 2 Chloro-N-Ethoxymethyl-N-(2 Ethyl-6-Methylphenyl) Acetamide



Synonyms: Acetochlor, MON 097, CP-55097
Harness® (an emulsifiable concentrate containing 86.5% a.i. [CP-550971])

ACTION REQUESTED: Review studies submitted previously with a den [illegible] EUP and petition for temporary tolerances and recently submitted studies to support Monsanto Chemical Company's request for registration

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and establishment of permanent tolerances for Harness® in/on the following raw agricultural commodities:

Corn (grain)	0.1 ppm
(forage & fodder)	0.8 ppm
Soybeans (grain)	0.4 ppm
(forage & hay)	5.0 ppm
Grain Sorghum (grain)	0.2 ppm
(forage & fodder)	3.0 ppm
Peanuts (nuts)	0.4 ppm
(hulls)	2.5 ppm
Eggs and chicken tissue	0.02ppm
Milk and beef tissue	0.02ppm
Hog tissue	0.02ppm

Recommendations:

At this time Toxicology Branch does not find the requested permanent tolerances supported by the available data. Further, Acetochlor has been found to be a carcinogen in both the rat and mouse. A risk assessment must be performed based on these findings; this has been tentatively scheduled for the week of August 29, 1985 by the Mission Support Staff.

Additionally, it is recommended that the recently submitted Pharmacopathics Research Laboratories' studies in the mouse, rat and dog (Report Nos. PR-80-007, PR-80-006, PR-80-008, respectively) be audited. The chromosome aberration assay performed by Hazleton Laboratories America is also a candidate study for an audit (Study #HL-83-006)

Summary of Reviewed Data

The following is a summary of data reviewed in this action submitted with the EUP and petition for temporary tolerances (Acc#248618-20; 524-EUP-56/2G2797 and 3G2791) and the petition for permanent tolerances (Acc#071962-72; 3F2966 and 524- GUI)

1. Subchronic 21-day dermal toxicity study in rabbits. Acc. #248620, International Research and Development Corp., Study #IR-80-356, 12-11-1981.

   The LOEL for systemic effects (mortality and decreased body weight) was 1200 mg/kg (HDT); the NOEL for systemic effects was 400 mg/kg. The LOEL for dermal irritation was 100 mg/kg (LDT) and a NOEL for dermal irritation was not established. The study is classified as Core-Minimum.

2. Dermal sensitization study in guinea pigs with MON 097 Technical. Acc. #071970, Bio-dynamics Incorp., Study # BD-82-204, 4-13-83.

Technical MON 097 was a positive dermal sensitizer.

3. Dermal sensitization study in guinea pigs with MON 097 8 lbs/gal. E.C. Acc. #071970, Bio-dynamics Incorp., Study # BD-82-205, 4-13-83.

   The E.C. formulation of MON 097 was a positive dermal sensitizer.

4. In Vivo bone marrow chromosome study in rats with Acetochlor (MON 097). Acc. #071970, Hazleton Laboratories America, Inc., Study #HL83-006, 5-24-83.

   Under conditions of the study, MON 097 gave no evidence that it induced chromosomal abnormalities, but the study is Unacceptable because of several deficiencies. This study is a candidate for audit. For details see pages 1 and 2 of review.

5. Rat hepatocyte primary culture/DNA repair test. Acc. #071970, Pharmacon Research International, Inc., Study #PK-52-151, 2-17-1983.

   Under the conditions of the study, MON 097 did not appear to induce unscheduled DNA synthesis, but the study is Unacceptable because of multiple deficiencies, See "Conclusion" in the review, page 1.

6. Evaluation of mutagenic potential of MON 097 employing the L5178Y $TK^{+}/-$ mouse lymphoma assay. Acc. #071970, SRI International, Study #SR-81-150, Aug.-1982

   MON 097 was a positive mutagen in this assay only in the presence of metabolic activation. The study is Acceptable.

7. CHO/HGPRT gene mutation assay with MON 097. Acc. #071970, Monsanto Environmental Health Lab., Study #ML-82-281, 6-9-1983.

   MON 097 was weakly positive at near-toxic doses. However, the vehicle used (alcohol) did not appear to be inert in the assay. The study is Acceptable.

8. Rabbit teratology studies. Acc. #248620; International Research and Development Corp.; Pilot Studies #IR-79-292, Final Report #s 401-103, 401-103a, 401-103b; Primary Study #IR-79-293, 11-24-81.

   There were 3 pilot teratology studies and a primary study. Two of the pilot studies (401-103,401-103a) are classified as Invalid Data. The third pilot study is classified Supplementary Data as a dose range-finding study. See

"Recommendation" on page 1 of review for an explanation of these classifications for these pilot studies.

The primary study is also classified as Supplementary Data and a new study is requested. Insufficient numbers of litters were available to fully assess the teratogenic potential so no conclusions were reached. See "Recommendations" on page 1 of the review for requested additional data for this primary study.

9. Two Generation Reproduction Study in Rats. Acc. #071969, International Research and Development Corp., Study # IR-80-053, 12-16-82.

The doses were 500, 1500 and 5000 ppm. A slight decrease (about 20%) in litter size was noted at the high dose in all matings. The high dose also caused decreases in pup body weight gain during lactation for both generations; this effect was also seen in male F_{2b} pups of the mid level.

Chronic nephritis was increased in females of the F_1 generation fed the high level and a slight increase in prostatis in this level may have been related to treatment.

Apparent treatment-related increases in thyroid weights were noted in low and mid dose F_{1b} (male) and F_{2b} (male and female) pups and in mid and high F_1 dams. Liver weights (nonsignificant in males) and ratios were increased in mid(not statistically significant) and high F_1 parents. Pituitary weights were decreased in all doses of F_1 adult males(mean absolute only at low and high doses), and in low and high dose F_{2b} male pups but were increased in low F_{1b} female pups. Decreases were also seen for ovary weights for adult F_1 females fed all levels.

The reproductive NOEL is 500 ppm and the LOEL is 1500 ppm based on decreased body weight gain of F_{2b} pups.

The systemic LOEL is 500 ppm based on absolute and relative organ weight: decreases for ovary weights in F_1 females, decreases for pituitary weights for F_1 and F_{2b} males and increases for thyroid weights in F_{1b} and F_{2b} pups. The systemic Noel was not established.

The study is classified as Core- Supplementary because of inadequate gross and histopathological examinations. See discussion under "Protocol" on page 1 of the review.

10. The Metabolism of Acetochlor in the Laboratory Rat. Acc. # 071971 and 071972, Hazleton Raltech Inc., Report # MSL-2824, June, 1983.

Acetochlor was rapidly excreted (>70% within 48 hours) with the urinary route accounting for about twice the percentage of the fecal route; pulmonary excretion was

insignificant. Elimination was biphasic with a rapid (t 1/2 < 10 hrs) and slow (t 1/2=128-286 hrs) phase.

Acetochlor was extensively metabolized, with less than 1% unchanged compound found in the feces and none detectable in the urine. The early (<24 hour) metabolites were mostly mercapturates and later ones mostly sulfoxides, sulfones and sulfates; 20 metabolites were identified. Early conjugation with glutathione is assumed.

The only tissue retaining significant amounts of labeled Acetochlor (about 2.5%) was the erythrocytes; their turnover rate in the rat correlates well with the slow phase of Acetochlor elimination. The radioactivity was covalently bound to hemoglobin and retention data suggested a possible cumulative effect on erythrocyte function.

Repeated doses of Acetochlor had little effect on excretion kinetics; the single large dose increased the half-lives for the slow and rapid phases about 50%.

There did not appear to be any significant sex differences in the metabolism of Acetochlor.

The study is classified as Core Guideline.

11. A one-year feeding study in dogs with MON 097. Acc. #248618-19, Pharmacopathics Research Laboratories, Study # PR-80-008, 10-14-81.

The dogs at the high dose (40 mg/kg) showed testicular atrophy (6/6) accompanied by decreased absolute and relative (to body weight) testicular weight, decreased body weight gain of males and decreased terminal body weight of females. There is also suggestive evidence at the high level for anemia and hepatotoxicity but a NOEL and LOEL cannot conclusively be determined for these effects at lower dose levels because of the variability of control data during the study and the wide range of normal values for these parameters established at the testing facility. There is also suggestive evidence for effects on adrenal weights.

Additional data are requested and the study has been recommended for audit. See page 1 of the review for the requested information and the conclusions. The study is classified as Supplementary Data.

12. MON 097: Chronic toxicity and oncogenicity study in the rat. Acc. #071962-65, Pharmacopathics Research Laboratories, Inc., Study #PR-80-006, 5-20-1983.

The dose levels were 500, 1500 and 5000 ppm (25, 75 and 250 mg/kg). MON 097 was carcinogenic to the rat; the high level caused an increased incidence of liver carcinomas and thyroid follicular cell adenomas in males.

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There were positive trends for hepatic carcinomas in females and thyroid follicular cell adenomas in males. The high level also caused increased incidences of polyarteritis of the testes and arteries of males and liver necrosis and alveolar histiocytosis in females. It increased mortality in females and decreased food consumption in both sexes. There was a dose-related decrease in body weights of both sexes at the mid and high levels and a decrease in males only at the low level.

Based on organ weight effects and decreased body weight in males, the systemic LOEL is 500 ppm (25 mg/kg, LDT) and a NOEL was not established; one must be established in the rat in a new chronic study. The study is classified as Minimum Data, but it is recommended for audit.

13. MON 097: 24 month oncogenicity study in the mouse. Acc. #071966-68, Pharmacopathics Research Laboratories, Inc., Report # PR-80-007, 5-4-83.

Doses were 500, 1500 and 5000 ppm (75, 225 and 750 mg/kg/day). MON 097 was a carcinogen in mice. There were increased incidences of: liver carcinomas in high level males, total lung tumors in females of all levels, carcinomas of the lungs in low and high level females, uterine histiocytic sarcomas in females of all levels and total benign ovarian tumors in mid level females. There were positive linear trends for: liver carcinomas in both sexes, and pulmonary carcinomas, total lung tumors, ovarian benign tumors and kidney adenomas in females. There was an increase in interstitial nephritis in both sexes of the high level. Treatment decreased body weight and increased mortality of both sexes at the high level. Absolute and relative liver weights were increased in all levels of males and in high level females. Absolute and relative kidney weights were also increased in all levels of males.

Based on increased liver and kidney weights of males, the LOEL for systemic effects is 500 ppm(LDT) and a NOEL was not established. The study is classified as Minimum Data. It is recommended for audit.

Data Gaps

The following studies are presently data gaps:

1. Chronic rat
2. Chronic dog
3. Reproduction
4. Teratology in the rabbit
5. Mutagenicity- structural chromosome aberration
   - other genotoxicity (DNA repair)

Previously Reviewed Data

Data submitted previously were reviewed by William Dykstra in a memo of 3-24-81 for PP# 1G2454. These data are summarized as follows:

1. Acute oral LD_{50}, Rat, Mon 097, 2953 mg/kg (both sexes), Category II, Minimum Data. Environmental Health Laboratory Report #80-49, 10-15-80.

2. Acute dermal LD_{50}, Rabbit, Mon 097, 3667 mg/kg (both sexes), Category III, Minimum Data. Environmental Health Laboratory Report #80-48, 10-15-80.

3. Primary dermal irritation, Mon 097, P.I.=0.6/8.0, Category IV, Minimum Data. Environmental Health Laboratory Report #80-50, 10-15-80.

4. Primary eye irritation, Mon 097, scores for unwashed= 18.8/110, for washed=1.2/110, Category II, Minimum Data. Environmental Health Laboratory Report #80-51, 10-15-80.

5. 91-Day feeding, Rat, CP-55097, NOEL = 800 ppm
   LOEL = 2000 ppm based on body weight loss and food consumption decrease, Minimum Data. Pharmacopathics Report #7914, 10-10-80.

6. 119-Day feeding, Dog, CP-55097, NOEL <25 mg/kg/day (LDT), dose-related elevated SGPT - Minimum Data. Pharmacopathics Report #7920, 10-10-80.

7. Teratology, Rat, CP-55097, Negative at 400mg/kg/day Fetotoxic NOEL = 200 mg/kg/day, Maternal NOEL = 200 mg/kg/day, Minimum Data. IRDC Report #401-066, 10-15-80.

8. Mutagenicity, Ames Salmonella Assay, CP-55097, Negative for strains TA-98, 100, 1535 and 1537, with and without mouse and rat microsomal preparations, Minimum Data. Monsanto Report # MRC-DA-838, 12-5-78.

007697

004586

Data Evaluation Record

Study Type: In vivo cytogenetics in rats.

Study Identification:	"In Vivo Bone Marrow Chromosome Study in Rats with Acetochlor (MON 097)".
Lab. performing study:	Hazelton Laboratories America, Inc. Vienna, VA 22180
Sponsor:	Monsanto Agricultural Products Co. St. Louis, MO. 63167
Study no.:	HL 83-006
Project no.:	241-143 (Hazelton)
Accession no.:	071970
Report date:	May 24, 1983
Submitted to EPA:	9/22/83
Study director:	Michael G. Farrow, Ph.D.
Reviewed By:	D. Stephen Saunders Jr., Ph.D. Toxicologist, Section V TOX/HED (TS-769) DSS 8/2/85
Approved By:	Irving Mauer, Ph.D. Geneticist, Toxicology Branch Hazard Evaluation Division (TS-769) 8/2/85

Conclusions: No effect on the incidence of chromosomal abnormalities was apparent. No affect on mitotic index was apparent, therefore there was no evidence that the test material reached the bone marrow in sufficient concentration to produce a toxic effect. Numerous discrepancies in the results reported in the summary tables vs. individual animal data were noted.

Classification: Unacceptable Deficiencies as noted.

Materials and Methods

A. Materials- (1) Test chemicals: Acetochlor (MON 097), a "brown liquid", 96.3% a.i.

Positive control: Mitomycin C (Sigma Chem. Co.), assumed 100% a.i.

Mitotic arrest- colchicine; supplier, purity not stated.

(2) Doses tested: Acetochlor- 40, 150, and 500 mg/kg by i.p. injection.

vehicle control- corn oil, 5 ml/kg.

positive control- Mitomycin C, 5 mg/kg

mitotic arrest- colchicine 2 mg/kg.

(3) Test animal: Male and female Sprague-Dawley CD albino rats, obtained from Charles River Breeding Laboratories, Kingston, N.Y.

Methods

A photocopy of the submitted methods is appended. The methods were reviewed and the following points were noted:

1) The tested doses were based on a range-finding study (submitted as an appendix) which demonstrated that 2/2 males and 1/2 females injected i.p. with a high dose of 1000 mg/kg died within 24 hours of treatment, whereas equal numbers of males and females treated with 100 or 300 mg/kg survived without any apparent toxic signs. It was reported that "the test compound produced no apparent effects on the mitotic indices of the animals which survived", however the "normal" range to which the investigators compared these results was not stated.

2) It was stated in the submitted protocol that rats were to be sacrificed at 48 hours after treatment for analysis of bone marrow cells. Although slides were prepared for these animals, they were not examined.

3) Although pre-test body weights were provided, body weights at study termination were not reported.

4) Although 6 rats/sex/dose were treated and sacrificed, and bone marrow slides were prepared for all treated rats, generally slides were examined from only 5 rats (or less) of each dose group.

Results

A. General observations: No effects of treatment on physical appearance were apparent. Data for clinical signs were submitted as individual animal data. Data for body weights were submitted as a summary table of pre-treatment weights; no differences between test groups were apparent. The effect of treatment on body weights could not be assessed since post-treatment values were not submitted.

B. Cytogenetic Analyses: Cells were examined only for 6, 12, and 24 hours after treatment with acetochlor; cells from animals sacrificed 48 hours after treatment were not examined for cytogenetic abnormalities. Data were submitted as summary tables and as individual animal findings.

No effect of treatment on the frequency of chromosomal aberrations, the modal number (i.e. the average number of chromosomes/metaphase), or the mitotic index was apparent at 6, 12 or 24 hours after treatment.

Numerous discrepancies between the number of animals examined, as reported in the summary tables, and the number of animals for which data were reported in the individual animal data were noted. The number of animals reported as examined in the summary tables in many cases was less than the number of animals with individual data reported. In some cases, data were reported for animals from which "0" cells were examined, e.g. #D77707 (Group 2, 24 hours) and #D77[illegible] Group 5, 24 hours).

These data are tabulated in Table [illegible] of this review.

ACETOCHLOR Tox review 007697

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Pages _____ through _____ are not included.

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____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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Table 1. Number of Animals Examined for Chromosomal Abnormalities[a]

Group	Test material	Dose	Time of Sacrifice 6 hours	12 hours	24 hours
1	Corn Oil	5 ml/kg	9/12[b]	10/10	10/11
2	Mitomycin C	5 mg/kg	-	-	8/11
3	Acetochlor	40 mg/kg	9/11	10/11	10/11
4	Acetochlor	150 mg/kg	6/12	10/10	10/10
5	Acetochlor	500 mg/kg	11/11	10/10	8/12

[a]data excerpted from submitted study.

[b]number of animals with results reported in individual animal data/ number of animals with results reported in summary table.

Discussion

No effect of treatment on the incidence of chromosomal abnormalities was apparent. No effect of treatment on the mitotic index was apparent, therefore there is no evidence that the test substance reached the target tissue, the bone marrow, in sufficient concentration to produce a toxic effect. Although slides were reportedly prepared for the 48 hour sacrifice, they apparently were not examined.

Significant discrepancies were noted by the reviewer between the results of chromosomal examinations as reported in the summary table and the data contained in the individual animal data appendices. For 8/13 reported results, the number of animals with actual data was less than the number of animals reported as examined in the summary table. For two animals, data were reported although the individual data indicated that "0" cells were examined for these animals. These discrepancies are sufficient cause for an audit of the supporting raw data.

Classification: Unacceptable Deficiencies as noted.

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007697-007

004586

Data Evaluation Record

Study Type: In vitro rat hepatocyte DNA repair assay.

Study Identification: "Rat Hepatocyte Primary Culture/DNA Repair Test."

Lab. performing study:	Pharmakon Research International, Inc. Waverly, PA 18471
Sponsor:	Monsanto Agricultural Products Co. St. Louis, MO. 63167
Study no.:	PK 82-151 (Monsanto)
Project no.:	PH 311-MO-001-82 (Pharmakon)
Accession no.:	071970
Report date:	2/17/83
Submitted to EPA:	9/22/83
Study director:	Robert W. Naismith, Ph.D.
Reviewed By:	D. Stephen Saunders Jr., Ph.D. Toxicologist, Section V TOX/HED (TS-769)
Approved By:	Irving Mauer, Ph.D. Geneticist, Toxicology Branch Hazard Evaluation Division (TS-769)

DSS 8/2/85

8/2/85

Conclusions: No effect of treatment on the rate of thymidine incorporation was apparent at doses of 0.032 to 3.2 ug/well. Doses of 10.6 to 320 ug/well were reported as "cytotoxic", however the criteria for this assessment, or any data obtained from these cells, were not submitted. The purity and method of dose calculation for the test article were not supplied, and it is not clear whether the technical grade of active ingredient was tested in this assay.

Classification: Unacceptable Deficiencies as noted.

Materials

(1) Test chemicals: Acetochlor (MON 097), a "colorless, pale yellow liquid", Lot #NBP1737813, % a.i. not stated.

Positive control: 2-AAF (Aldrich Chem. Co.), % a.i. not stated.

Vehicle control- DMSO (Mallinkrodt, Inc.), purity not stated.

(2) Doses tested: Acetochlor- 0.032 ug/well to 320 ug/well.

vehicle control- DMSO

positive control- 2-AAF, 1×10^{-4}M.

(3) Test system: Rat hepatocytes isolated from the liver of male Fischer-344 rats, obtained from Charles River Breeding Laboratories, Wilmington, Mass., "or any USDA acceptable source".

Methods

A photocopy of the submitted methods is appended. The methods were reviewed and the following points were noted:

1) The criteria for assessing cytotoxicity were not stated.

2) The method for calculating doses was not stated. Since the doses were reported as "ug/well", and the test substance was supplied as a liquid, the density and purity of the test material were required to calculate doses. If the investigators assumed a density of 1.0 and purity of 100%, it should be so stated. Further, since the test material in the in vivo cytogenetics study (Monsanto #HL 83-006) was a brown liquid, with reported purity of 96.3% a.i., it is not clear whether the technical material was tested in the present study since it was described as a "pale yellow liquid".

Results/Discussion

No effect of treatment on the rate of incorporation of ^{3}H-thymidine by hepatocytes in vitro was apparent at doses of 0.032 ug/well to 3.2 ug/well. Doses of 10.6 ug/well and above were reported as cytotoxic, however the criteria for this assessment were not provided, nor were any effects of treatment on thymidine incorporation by these cells reported. The positive control, 2-AAF, induced about a 100-fold increase in incorporation of thymidine, demonstrating that the test system could respond appropiately to a known mutagen. These data are presented in the table below (photocopied from the submitted study report):

Treatment	Concentration	Net Nuclear Grains Triplicate Cultures x ± s.d.
Untreated		0.0 ± 0.1
DMSO		0.2 ± 0.2
2AAF	1×10^{-4} M	52.7 ± 9.5**
MON 097	0.032 ug/well	0.5 ± 0.4
MON 097	0.106 ug/well	0.3 ± 0.4
MON 097	0.32 ug/well	0.7 ± 0.6
MON 097	1.06 ug/well	0.6 ± 1.0
MON 097	3.2 ug/well	0.4 ± 0.5
MON 097	10.6 ug/well	Cytotoxic
MON 097	32.0 ug/well	Cytotoxic
MON 097	106.6 ug/well	Cytotoxic
MON 097	320.0 ug/well	Cytotoxic

**Positive finding. Mean net nuclear grain count of five or greater than the vehicle control.

Classification: Unacceptable Inadequate identification of test article; method of dose calculation not adequately described; criteria for cytotoxicity or data from these cells not submitted.

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007697

Data Evaluation Record

Study Type: Gene mutation in mouse lymphoma (L5.78Y) cells. 004566

Study Identification: "An Evaluation of Mutagenic Potential of MON 097 Employing the L5178Y TK+/- Mouse Lymphoma Assay."
Lab. performing study: SRI International
Menlo Park, CA 94025
Sponsor: Monsanto Agricultural Products Co.
St. Louis, MO. 63167
Study no.: SR 81-150
Project no.: LSC-2575 (SRI)
Accession no.: 071970
Report date: August, 1982
Submitted to EPA: 9/22/83
Study director: Ann D. Mitchell, Ph.D.

Reviewed By: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

DS 8/2/85

Approved By: Irving Mauer, Ph.D.
Geneticist, Toxicology Branch
Hazard Evaluation Division (TS-769)

8/2/85

Conclusions: Positive for gene mutations in mouse lymphoma cells (L5178Y) only in the presence of metabolic activation. Negative for gene mutations in the absence of metabolic activation. The purity of the test substance was not stated, therefore it was not clear whether the technical grade of active ingredient was tested in this assay.

Classification: Acceptable

Materials

1) **Test chemicals:** Acetochlor (MON 097), a "plum-colored liquid"; lot 3P 1924845; % a.i. not stated.

Positive controls: Ethyl methanesulfonate (EMS)- no metabolic activation
3-methylcholanthrene (3-MC)- with metabolic activation

Metabolic activation: S-9 fraction (9,000 x g supernatant) from Arochlor 1254-induced rat liver (male Fischer-344 rats).

2) **Doses tested:** Acetochlor- 20, 30, 45, 60, 76, 100 and 400 ul/l without S-9.
5, 15, 20, 30, 40, 50, 100 and 250 ul/l with S-9.

vehicle control- 1% DMSO.

positive control- 500 ug/ml EMS without S-9
6 ug/ml 3-MC with S-9

3) **Test system:** Mouse lymphoma L5178Y cells, heterozygous for thymidine kinase.

Methods

A photocopy of the submitted methods is appended. The methods were reviewed and the following point(s) are noted:

None.

Results/Discussion

The selection of doses tested in the presence or absence of metabolic activation (S-9) were based on the results of a range-finding assay. Doses of acetochlor of ≥100 ul/l were cytotoxic as evidenced by relative suspension growth of less than 5% of the solvent control group in the range finding assay.

In the primary study, incubation of mouse lymphoma cells with acetochlor in the absence of S-9 did not produce any effect on mutation frequency at any of the tested doses (data not shown). Doses of 100 ul/l resulted in average relative suspension growth of about 10% of control, and were therefore cytotoxic. The positive control without S-9, EMS, induced an increase in mutation frequency of about 5.6x control values, demonstrating that the test system could respond appropriately to a direct-acting mutagen.

Incubation of lymphoma cells with acetochlor in the presence of S-9 produced an apparent dose-related increase in mutation frequency (Table 1). Doses of 40 ul/l and above were apparently cytotoxic as evidenced by dose-dependent decreases in relative suspension growth and relative cloning efficiency. The positive control with S-9, 3-MC, caused an average increase in mutation frequency of 5.4x control values, demonstrating that the test system could respond appropriately to a mutagen requiring metabolic activation.

Table 1. Effect of Acetochlor and S-9 on Mutation Frequency[a]

Test Material	Dose	Relative Suspension Growth (%)[b]	Relative Cloning Efficiency (%)[c]	Mutation Frequency (% control)
DMSO	1%	100.0	100.1	-
3-MC	6 ug/ml	47.7	35.6	538.8
MON 097	5 ul/l	82.9	97.8	102.0
MON 097	15 ul/l	89.1	85.4	150.3
MON 097	20 ul/l	82.2	77.8	155.9
MON 097	30 ul/l	45.3	70.6	220.3
MON 097	40 ul/l	12.6	36.1	427.1
MON 097	50 ul/l	7.9	17.2	523.2
MON 097	100 ul/l	3.2	NC	-
MON 097	250 ul/l	3.3	NC	-

[a]data excerpted from submitted study.

Classification: Acceptable

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007697

Data Evaluation Record

Study Type: Gene mutation in CHO/HGPRT cells.

Study Identification: "CHO/HGPRT Gene Mutation Assay with MON 097."
Lab. performing study: Monsanto Environmental Health Lab.
St. Loius, MO 63110
Sponsor: Monsanto Agricultural Products Co.
St. Louis, MO. 63167
Study no.: ML-82-281
Project no.: EHL-830013
Accession no.: 071970
Report date: 6/9/83
Submitted to EPA: 9/22/83
Study director: A.P. Li, Ph.D.

Reviewed By: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

DSS 8/2/85

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8/2/85

Conclusions: The submitted study demonstrated that acetochlor was weakly mutagenic in this test system, with or without metabolic activation. Although the trend for a dose-response relationship was statistically significant ($p < 0.05$), the evidence for a dose-dependent effect was minimal.

Classification: Acceptable

Materials

1) Test chemicals: Acetochlor (MON 097), a "yellowish-brown liquid"; sample #T830020; 96.3% a.i.

Positive controls: Ethyl methanesulfonate (EMS)- no metabolic activation
Benzo(a)pyrene (BP)- with metabolic activation

Metabolic activation: S-9 fraction (9,000 x g supernatant) from Arochlor 1254-induced rat livers, purchased from Litton Bionetics.

(2) Doses tested: Acetochlor- 25, 75, 100, 125, and 150 ug/ml without S-9.
25, 50, 75, 100, and 125 ug/ml with S-9.

vehicle control- ethanol

positive control- 100 ug/ml EMS without S-9
1 ug/ml 3-MC with S-9

(3) Test system: CHO cells, cloned K1BH4, originally obtained from Dr. A. W. Hsie of Oak Ridge National Laboratories.

Methods

A photocopy of the submitted methods is appended. The methods were reviewed, and the following point(s) were noted:

(1) The choice of ethanol as a solvent control in this assay is questionable, since incubation of cells with ethanol in the presence of S-9 fraction produced about a two-fold increase in mutation frequency in two separate experiments.

Results/Discussion

The selection of doses and concentration of S-9 fraction was based on preliminary studies which demonstrated an optimum of 10% S-9. The cytotoxicity of acetochlor increased as the concentration of S-9 increased.

Incubation of CHO cells with acetochlor in the presence or absence of S-9 caused an increase in mutation frequency when compared to untreated controls (Table 2, photocopied from submitted study report). Doses of 125 or 150 ug/ml without S-9 produced a 3.8 and 2.7x increase in mutation frequency, respectively, and were judged to be statistically significant ($p < 0.05$). A substantial decrease in relative survival was noted at the highest dose of 150 ug/ml, 39% of control as compared to 71-95% of control at lower concentrations test article.

In the presence of S-9 fraction, a statistically significant increase in mutation frequency of 3x control ($p < 0.05$) was observed only at the high dose of 125 ug/ml. Acetochlor was more cytotoxic in the presence of S-9, as doses of 75, 100, and 125 ug/ml produced decreases in cell survival of 34%, 30%, and 7% of control.

The investigators stated that "[t]he dose-response relationship was found to be linear ($p < 0.05$) for both treatment with and without S-9."

The positive controls induced the appropriate responses, demonstrating that the test system could respond to direct and indirect mutagens.

The submitted data are considered to be evidence of a weak mutagenic potential of acetochlor, as clear evidence of mutagenicity is seen only at cytotoxic concentrations.

Classification: Acceptable

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007697

004586

Study: The Metabolism of Acetochlor in the Laboratory Rat

Accession No.: 071971/071972

Sponsor/Contracting Lab.: Monsanto/Hazelton Raltech Inc.

Report No./Date/Submitted: MSL-2824/6-83/9-22-83

Reviewer: D. Stephen Saunders Jr., Ph.D. DSS 7/31/85 8/3/85

Test Compound

Homogeneous mixture of 12-C, 13-C, and 14-C-Acetochlor, lot no. 2179029, >98% a.i. Specific activity of 14-C label = 9.8 mCi/mmole.

Methods

The methods employed in this study were determined to be adequate by this reviewer. A photocopy of the submitted methods is attached to this review.

The study was conducted with Charles River CD SD rats, divided into four experimental groups: Group A received a single oral dose by gavage of 400 mg/kg radiolabeled acetochlor. Elimination of radiolabel by the pulmonary route was monitored in this group. Group B rats were given a single dose of 10 mg/kg, and Group C rats were given a single dose of 400 mg/kg. Group D received daily doses of 10 mg/kg for 14 days, followed by a single dose of 10 mg/kg of radiolabeled test substance. For all test groups, elimination of label was monitored for 7 days after the last dose.

Also, the structural formulas for several of the principal metabolites were identified by conventional analytical techniques.

Results

A. Excretion- Expired air was collected from animals of group A (400 mg/kg by gavage). These animals excreted an average of 0.04% of the administered dose over 7 days by exhalation. Because of the insignificant release by this route, expired air was not monitored in subsequent analyses.

In all of the treatment groups, acetochlor was rapidly excreted, as more than 70% of the administered dose was excreted within 48 hours (Table 1 of this review). The distribution of metabolites between urine and feces favored the fecal route in group B males (10 mg/kg single dose), however females excreted approximately equal amounts of label by either route. In contrast, animals from group C (400 mg/kg single dose) and group D (10 mg/kg repeated dose) excreted a larger proportion of the administered dose through the urine.

Table 1. Distribution of Excreted Dose (%)[a,b]

Group		Urine		Feces		Total	
		0-2 days	0-7 days	0-2 days	0-7 days	0-2 days	0-7 days
B	Male	29.4	31.5	47.2	50.1	76.6	81.6
	Female	41.8	43.4	39.2	40.4	81.0	83.8
C	Male	42.5	46.7	26.0	28.9	68.5	75.6
	Female	46.5	49.7	24.7	26.6	71.2	76.3
D	Male	56.6	59.4	24.3	25.6	80.9	85.0
	Female	66.1	67.8	18.9	19.3	85.0	87.1

[a]data excerpted from submitted study.

[b]percent excreted days 0-2 calculated by reviewer.

Whole-body elimination of acetochlor was biphasic with a rapid and a slow phase. This type of excretion pattern is consistent with a two compartment model of distribution. Rapid excretion would be predicted from well-perfused organs such as heart, liver and kidney, and a longer half-life would be expected for excretion from tissues that do not receive as much blood flow such as fat. However, studies on tissue residues (see section C of this review) indicate that approximately 2.5% of the administered dose was associated with red blood cells, apparently due to binding to hemoglobin. The long half-life (approx. 180 hours) determined for the slow phase of excretion correlates with the half-life of red blood cell turnover in the rat. This fact led the investigators to speculate that the erythrocyte was the slow phase compartment. Repeated doses of acetochlor had little effect on the excretion kinetics as can be seen by comparisons of groups B and D. The half-lives for both the rapid and slow phases were about 50% longer for animals given the single high dose (group C) than for either of the low dose groups. This effect is consistent with saturation of metabolic enzymes of excretory mechanims. Kinetic data are presented in table 2.

Table 2. Kinetic Constants for Excretion of (14-C)-Acetochlor[a]

Group		t-1/2 (rapid)	t-1/2 (slow)
B	Male	7.1 hours	161.9 hours
	Female	5.8	182.4
C	Male	10.4	249.3
	Female	9.3	286.4
D	Male	7.1	128.6
	Female	5.4	186.3

[a]data excerpted from submitted study.

B. Metabolic Pathway- Acetochlor was extensively metabolized, with less than 1% of the administered dose excreted unchanged into the feces and no unmetabolized acetochlor detectable in the urine. Approximately 20 different metabolites were characterized from urine and feces. The most common metabolites excreted at early time points (<24 hours) were mercapturates. At later time points the relative proportion of mercapturates decreased as the proportion of other sulfur-containing metabolites (sulfoxides, sulfones, sulfates) increased. Based on these data it is apparent that an early step in the metabolism of acetochlor is conjugation with glutathione. The proposed metabolic pathway for acetochlor and identified structures of metabolites (photocopied from the submitted study) are depicted in figures 68-70, appended.

C. Tissue Residues- The only tissue which retained significant amounts of radiolabeled acetochlor was the red blood cell. Acetochlor apparently bound covalently to hemoglobin, as determined by gel electrophoresis. The amount of label retained by other tissues was proportional to tissue mass and/or degree of perfusion. Relatively little label was retained in body fat, suggesting that bioaccumulation due to fat storage is not a factor with this compound. These data are depicted in table 3.

Table 3. Tissue Residues of 14-C Acetochlor[a]

	Group B		Group C		Group D	
Tissue	Male	Female	Male	Female	Male	Female
Brain	2743[b] (0.005)[c]	3268 (0.007)	15316 (0.006)	15429 (0.007)	2471 (0.003)	2809 (0.006)
Heart	20885 (0.024)	24000 (0.026)	107407 (0.026)	89873 (0.022)	29677 (0.027)	27209 (0.026)
Kidney	12466 (0.028)	13067 (0.025)	62500 (0.033)	65658 (0.032)	12491 (0.025)	12083 (0.022)
Liver	12342 (0.157)	13900 (0.134)	46094 (0.148)	50414 (0.135)	10881 (0.125)	10919 (0.104)
Lung	25035 (0.036)	24112 (0.033)	112409 (0.035)	113514 (0.040)	20839 (0.023)	18692 (0.027)
Spleen	27246 (0.019)	23256 (0.013)	131612 (0.018)	146087 (0.021)	25000 (0.012)	26800 (0.015)
GI tract	3209 (0.032)	2558 (0.026)	12440 (0.029)	13356 (0.032)	3467 (0.027)	2208 (0.020)
GI contents	1252 (0.061)	623 (0.025)	10420 (0.093)	5286 (0.052)	4710 (0.132)	812 (0.020)

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Table 3. Tissue Residues of 14-C Acetochlor (con't.)

	Group B		Group C		Group D	
Tissue	Male	Female	Male	Female	Male	Female
Eyes	ND -	ND -	8845 (0.0005)	8395 (0.0007)	ND -	ND -
Gonads	1602 (0.004)	ND -	6603 (0.005)	50480 (0.002)	1154 (0.002)	ND -
Fat	1314 (0.051)	1788 (0.069)	9744 (0.092)	11580 (0.110)	1125 (0.044)	1218 (0.047)
Muscle	2517 (0.273)	2110 (0.229)	9277 (0.245)	10640 (0.284)	1948 (0.212)	2072 (0.225)
Femur	2700 **	3314 **	14770 **	20670 **	3338 **	3566 **
Sternum	4872 **	5393 **	28960 **	29600 **	4298 **	4480 **
Whole blood	173000 (2.54)	166000 (2.45)	766000 (2.77)	811000 (2.95)	131000 (1.95)	142000 (2.10)
Plasma	684 **	660 **	3671 **	4684 **	1016* **	1012* **
Total % Retained	3.23	3.04	3.50	3.69	2.58	2.79

[a]data excerpted from submitted study.

[b]dpm/g tissue, calculated by reviewer from average organ weights.

[c]percent of administered dose.

ND = Not detectable

*data not included in Group D summary table, obtained by reviewer from raw data.

**total body mass of this tissue has not been estimated for the rat.

Discussion and Conclusion

Acetochlor was rapidly eliminated from the rat, with >70% of the administered dose excreted within 48 hours for most of the groups. Animals given a single high dose (Group C, 400 mg/kg) or repeated low doses (Group D, 10 mg/kg for 15 consecutive days) appeared to preferentially eliminate radiolabel via the urine, with little difference between sexes. Group B males (single dose of 10 mg/kg) appeared to excrete more label in the feces than in urine, whereas females excreted approximately equal amounts in urine or feces. The kinetics of excretion were biphasic, with a rapid phase ($t_{1/2}$ = 5.4-10.4 hours) and a slow phase ($t_{1/2}$ = 128.6-286.4 hours). Animals given the high dose (400 mg/kg) had somewhat larger half-lives for both phases, consistent with saturation of metabolic enzymes and/or excretion mechanisms.

Acetochlor was extensively metabolized, with <1% of the administered compound excreted unchanged in the feces. An early step in the proposed metabolic pathway is conjugation with glutathione, and the majority of the excreted metabolites were mercapturic acid derivatives. The remainder of excreted metabolites were other sulfur-containing derivatives of acetochlor such as sulfates, sulfoxides and sulfones.

The only tissue which accumulated significant amounts of acetochlor was the red blood cell, which retained about 2.5% of an administered dose. This percentage was not dose-dependent (although the absolute amount retained obviously was) since similar percentages were retained by all three dosage groups. A slightly smaller percentage was retained by group D animals as compared to group B animals. This effect is consistent with competition for target receptor sites between labeled and unlabeled chemical, and induction of metabolic and/or excretory mechanisms. This conclusion is supported by the findings that group D rats, compared to group B animals, had slightly higher levels of radioactivity retained in the plasma in conjunction with a higher percentage of administered dose excreted in the urine at 2 days and a slightly higher percentage excreted overall at 2 or 7 days (Tables 1 and 3). The radioactivity was determined to be covalently bound to the hemoglobin fraction of the erythrocyte. Since a significant amount of label was bound even after 14 consecutive doses of unlabeled chemical (group D), these data suggest that a cumulative effect of acetochlor on red blood cell function is possible.

Classification: Core-Guideline

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EPA: 68-01-6561
TASK: 109
July 29, 1985

DATA EVALUATION RECORD

ACETOCHLOR

Chronic Feeding Toxicity and Oncogenicity Study in the Rat

STUDY IDENTIFICATION: Ahmed, F. E., Seely, J. C. MON 097: Chronic toxicity and oncogenicity study in the rat. (Unpublished study No. PR-80-006, prepared by Pharmacopathics Research Laboratories, Inc., Laurel, MD, for Monsanto Company, St. Louis, MO; dated May 20, 1983.) Accession Nos. 071962 - 071965.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: Irvin Edwards/fa

Date: 7/26/85

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1. CHEMICAL: MON 097, 2-chloro-N(ethoxymethyl)-6'ethyl-ortho-aceto-toluidine, a herbicide (acetochlor).

2. TEST MATERIAL: The test material was from Lot # NBP 1737874 with 94.5% purity. The compound was described as a maroon liquid with a characteristic odor.

3. STUDY/ACTION TYPE: Chronic feeding toxicity and oncogenicity study in the rat.

4. STUDY IDENTIFICATION: Ahmed, F. E., Seely, J. C. MON 097: Chronic toxicity and oncogenicity study in the rat. (Unpublished study No. PR-80-006, prepared by Pharmacopathics Research Laboratories, Inc., Laurel, MD, for Monsanto Company, St. Louis, MO; dated May 20, 1983.) Accession Nos. 071962 - 071965.

5. REVIEWED BY:

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Date: 7-30-85

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EPA Section Head

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Date: 8/5/85

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7. CONCLUSIONS:

A. Under the conditions of this chronic/oncogenicity feeding study with Sprague-Dawley rats, there was increased mortality in females receiving the high dose (5000 ppm). There was a significant ($p < 0.05$) dose-related decrease in the mean body weights of males and females receiving the mid (1500 ppm) and high doses, and a significant ($p < 0.05$) decrease in food consumption by males and females receiving the high dose. A decrease in the mean body weight of males receiving the low dose (500 ppm) also reached a significant ($p < 0.05$) level at the end of the study (weeks 103 to 115). Histopathologic examination of the tissues indicated increased incidences of polyarteritis of the testis and arteries of males and liver necrosis and alveolar histiocytosis in females receiving the high dose ($p < 0.05$). There was also a statistically significant increase in the incidences of liver carcinomas and thyroid adenomas in males receiving the high dose ($p < 0.05$). In addition, a compound-related positive trend ($p < 0.05$) was noted for the incidences of liver carcinomas in males and females and thyroid follicular cell adenomas in males.

Based on body and organ weight data, the LOEL for chronic effects is 500 ppm (LDT).

B. The study is classified as core minimum, although a NOEL for non-neoplastic effects was not established; one must be established in a new chronic study.

8. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

A photocopy of the detailed materials and methods used in this study are presented in Appendix A; the following is a brief description:

1. The test material, MON-097, was mixed with the basic diet at specified amounts and provided to the rats ad libitum. Diets were prepared fresh weekly to give dietary levels of 0, 500, 1500, and 5000 ppm.

2. The rats were random-bred Sprague Dawley, Cesarean-derived weanlings purchased from Charles River Breeding Laboratories, Wilmington, MA. Of the 640 rats purchased, 20 were used for baseline studies, 560 were used for the lifetime phase of the study, and the remaining 60 rats were sacrificed on day zero. Animals were acclimatized to laboratory conditions and randomly assigned into four groups, based on body weight; each consisted of 70 males and 70 females.

3. Test diets were analyzed at various intervals throughout the study (weeks 1, 2, 3, 4, 6, 8, 12, 18, 24, 30, 36, 42, 48, 52, 60, 78, 90, and 104) for MON-097 concentrations, homogeneity and 7-day stability.

4. Clinical observations were performed twice daily, and body weight and food consumption were determined weekly during the first 13 weeks and biweekly thereafter. Clinical chemistry, hematology and urinalysis determinations were performed on 10 males and 10 females per group one week prior to study initiation and at 6, 12, 18, and 24/27 months.

5. All rats that died or that were sacrificed when moribund, sacrificed at month 12 (interim, 10/group/sex), or sacrificed at termination were necropsied and tissues were examined histologically. Organ weights were determined for animals sacrificed at month 12 and at termination.

6. Food consumption and body weight data were statistically analyzed by one-way analysis of variance (ANOVA) using F-test for variance comparison, and significant differences were further analyzed by Dunnett's test. Clinical chemistry, hematology and organ-weight data were analyzed by the independent, two-sided t-test. The histopathology data were analyzed for statistical significance by the sponsor using the Cochran-Armitage test for linear trend, the chi-square test and the Peto method which utilizes survival and time to tumor information; these analyses utilized a p value of < 0.01 for significance.

9. REPORTED RESULTS:

A. Clinical Observation and Mortality: The most frequently observed clinical signs in both sexes in all groups were opaque eyes and alopecia. Opaque eyes were observed much earlier, i.e., by week 9, and occurred at a higher incidence in males than females; whereas alopecia was observed much earlier and at a higher incidence in females than in males. However, these effects were apparently not compound-related. Skin lesions and tumors were also evident by month 7 for both males and females. The incidences were progressively higher in females than in males, but were similar among dosed and control animals.

Ophthalmology examinations during the study did not reveal any compound-related effects.

Increased mortality was noted in females receiving the high (5000 ppm) dose by month 12 when compared to control animals (Table 1). Due to increased mortality in the high-dose group, all females were sacrificed by week 103 of the study when survival was

TABLE 1. Percent Survival of Rats Fed Diets Containing MON-097 for 24/27 Months

Dietary Level (ppm)	Percent Survival (No. of Dead Animals) at Month			
	12[a]	18	24[b]	27
Males				
Control	98.6(1)	86.7(8)	53.3(28)	31.7(41)
500	100.0(0)	90.0(6)	55.0(27)	33.3(40)
1500	97.1(2)	86.7(8)	65.0(21)	45.0(33)
5000	94.3(4)	86.7(8)	58.3(25)	25.0(45)
Females				
Control	98.6(1)	91.7(5)	41.7(35)	—
500	98.6(1)	71.7(17)	31.7(41)	—
1500	97.1(2)	83.3(10)	43.3(34)	—
5000	91.4(6)	68.3(19)	18.3(49)	—

[a] Interim sacrifice animals (10/sex/group) included in percent survival calculations.

[b] Females were sacrificed during week 103 of the study.

18.3% in the high-dose group. There were no compound-related mortalities noted throughout the study in males when compared to controls. By month 24, there were still sufficient males in each group to allow the study to proceed to month 27.

B. Diet Analyses: The concentrations of MON-097 in freshly prepared diets throughout the study were within acceptable limits of the theoretical. Mixing efficiency values ranged between 85 - 113 percent with a few exceptions. Analyses conducted 7 days after diet preparation throughout the study to determine the compound's stability were variable (range 83-118) and no specific pattern, i.e., decrease or increase, compared to day 1 could be detected.

C. Body Weight Determinations: A significant compound-related decrease in mean body weights of males receiving the mid and high doses was noted throughout the study when compared to controls (Table 2). Similarly a significant compound-related decrease in mean body weights of females receiving the high dose was noted during the study when compared to controls. Females receiving the mid-dose (1500 ppm) showed decreased mean body weight between weeks 31 and 103 of the study, but not all intervals were statistically different from control values. There were no significant compound-related effects on body weight in animals receiving the low dose, except in males at study termination. Mean body weights of low-dose males decreased gradually after week 103 of the study and were approximately 14% lower than the control group by termination

D. Food Consumption: Compound-related decreases in mean food consumption were noted at a few time intervals during the study in males and females receiving the high dose. A few isolated incidences of reduced food consumption were also noted for animals receiving the mid dose. There were no other changes noted (Table 3). Feed efficiency data for the first 13 weeks of the study indicated that high-dose animals of both sexes did not utilize feed as efficiently as the other groups. This was in agreement with reduced body weight data. Compound intake data indicated that the amount of compound consumed at the early weeks of the study was higher, as expected, because of the fast rate of growth. The time-weighted average was 22, 69, and 250 mg/kg body weight for males and 30, 93, and 343 mg/kg/body weight for females receiving the low- (25 mg/kg), mid- (75 mg/kg), and high- (250 mg/kg) doses, respectively.

E. Hematology: Females receiving the high dose showed a slight but significant decrease in hemoglobin and hematocrit values for months 6, 12 and 18, but not for month 24 of the study (Table 4). There were no other compound-related changes noted in the parameters investigated for males and females at months 6, 12, 18, or 24/27 of the study.

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TABLE 2. Selected Mean Body Weights of Rats Fed Diets Containing MON-097 for 24/27 Months

Dietary Level (ppm)	Group Mean Body Weight (g) at Week[a]						
	0	13	27	53	79	103[b]	115
Males							
Control	174.5	511.5	584.0	693.1	752.4	751.8	745.4
	18.4	50.5	67.4	88.2	102.4	130.2	104.1
500	170.6	495.7	568.7	677.8	731.5	721.0	640.9*
	17.5	47.8	62.6	93.9	110.9	117.5	131.4
1500	170.4	472.1*	538.0*	631.0*	678.6*	664.7*	618.7*
	17.1	46.3	49.6	77.3	91.0	111.6	126.1
5000	172.1	418.9*	479.5*	534.5*	545.9*	529.2*	479.8*
	16.6	49.1	49.2	62.9	69.6	67.3	65.1
Females							
Control	147.0	315.1	358.0	450.6	483.6[c]	449.5	--
	14.1	32.6	43.0	77.4	93.5	92.3	
500	144.6	318.7	354.9	445.4	491.2	503.3	--
	12.0	27.7	41.7	75.3	85.1	75.4	
1500	147.9	307.8	342.3	416.6*	437.4*	431.4	--
	10.3	33.2	45.4	75.9	93.9	101.4	
5000	146.5	269.4*	284.0*	302.8*	308.2*	308.1*	--
	11.1	22.8	32.7	48.8	60.5	42.3	

[a] Mean value and standard deviation.

[b] Females were sacrificed during week 103 of the study.

[c] Value corrected by reviewers (original value being 486.5).

* Statistically different from control value ($p < 0.05$).

TABLE 3. Selected Mean Food Consumption of Rats Fed Diets containing MON-097 for 24/27 Months

Dietary Level (ppm)	Mean Food Consumption (g/rat/week) at Week						
	0	13	27	53	79	103[a]	115
Males							
Control	154.1	171.5	170.4	160.4	175.0	180.4	165.6
500	153.6	170.7	163.0	170.6*	181.4	176.0	151.8
1500	156.4	165.6	158.9	166.1	181.0	171.7	166.1
5000	124.2*	164.4	162.4	145.8*	159.4*	155.1*	161.1
Females							
Control	131.4	131.2	149.9	160.9	178.4	153.6	—
500	135.1	140.4	147.0	158.7	186.6	151.4	—
1500	133.8	135.4	146.4	155.1	170.2	161.7	—
5000	110.4*	130.1	141.6	119.6*	137.4*	138.9	—

[a] Females were sacrificed during week 103 of the study.

* Significantly different from control value ($p < 0.05$)

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TABLE 4. Mean Hemoglobin and Hematocrit Values of Female Rats Fed Diets Containing MON-097 for 24 Months

Dietary Level (ppm)	Mean Hemoglobin (g %) and Hematocrit (pc %) values on months 0		6		12		18		24	
	Hgb	Hct	Hgb	Hct	Hgb	Hct	Hgb	Hct	Hgb	Hct
Control	12.2	39.0	15.0	44.3	14.1	45.0	13.9	41.2	12.9	39.6
500	-	-	14.8	42.7*	14.3	43.5	14.1	41.0	13.3	40.2
1500	-	-	13.9*	43.9	14.3	42.1	13.3	40.5	13.1	40.2
5000	-	-	14.2*	41.6*	12.5*	40.1*	11.4*	34.8*	12.7	39.5

*Statistically different from control value ($p < 0.05$).

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F. Clinical Chemistry: There were some isolated significant differences noted in blood chemistry parameters among control and dosed groups. These differences were not consistent over time and were apparently not compound-related.

G. Urinalysis: There were no compound-related changes noted in urine chemistry values and microscopic examination of urine sediments of males and females throughout the study.

H. Gross Examination: Gross pathology findings were summarized for each organ system instead of specific tissues and lesions were not specified, except on individual animal pathology sheets. However, individual animal data indicate that all gross lesions were examined histologically. There was a slight increase in urinary lesions noted in animals receiving the high dose at the 12-month interim sacrifice. For high-dose animals that died or were sacrificed moribund during the second year of the study, an increase in the number of lesions in the following systems was noted: the cardiovascular system of males and females, the endocrine system of males, and urinary and reproductive systems of females. In addition, an increase in the number of lesions of the urinary system of all dosed male groups was observed. At terminal sacrifice, increased lesions were noted in the urinary system of the mid- and high-dose males and high-dose females.

I. Organ Weights: There were no significant differences in organ weights and organ-to-body weight ratios among control and dosed males at the one-year interim sacrifice. In females, lower mean adrenal weights in mid- (0.13 g) and high-dose (0.10 g) animals (and lower mean adrenal-to-body weight ratios in high-dose animals) were observed when compared to control (0.19 g). However, the authors stated that the mean adrenal weight of the corresponding female control at month 12 was almost twice as high as the value for historical control rats (0.08 - 0.11 g) of that age.

At final sacrifice, the mean brain and heart weights of mid- and high-dose males and the mean brain weight of mid- and high-dose females were lower than the control values. These decreases were accompanied by corresponding increases in organ-to-body weight ratios (Table 5) The mean pituitary, heart, and adrenal weights of high-dose females were lower than the control values, but the organ-to-body weight ratios were similar among control and dosed animals. The mean thyroid/parathyroid weights and organ-to-body weight ratios in all dosed females were significantly higher when compared to control values (Table 5). In addition, the mean relative weights of thyroid in mid- and high-dose males, and the relative weights of liver, adrenals, kidneys, and testis in high-dose males and liver and kidneys in high-dose females were significantly higher than control values.

TABLE 5. Organ Weight Data for Rats Fed Diets containing MON-097 for 24/27 Months

Dietary Level (ppm)	Organ									
	Brain		Thyroid		Heart		Adrenals		Gonads	
	W[a] (g)	RW (%)	W (g)	RW (%)	W (g)	RW (%)	W (g)	RW (%)	W (g)	RW (%)
Males										
Control	2.23	3.03	0.05	0.07	2.12	2.83	0.10	0.14	3.48	4.72
	0.097	0.472	0.020	0.026	0.368	0.371	0.037	0.049	0.522	0.969
500	2.19	3.45*	0.06	0.09	2.01	3.14	0.12	0.19	3.52	5.44
	0.125	0.774	0.034	0.05	0.453	0.898	0.069	0.125	0.761	1.272
1500	2.15*	3.65*	0.05	0.09*	1.87*	3.13	0.20	0.36	3.26	5.41
	0.109	0.835	0.010	0.022	0.324	0.766	0.408	0.788	0.751	1.441
5000	2.11*	4.56*	0.07	0.15*	1.61*	3.42*	0.09	0.20*	4.45	9.85*
	0.115	0.888	0.024	0.053	0.266	0.663	0.018	0.067	3.180	8.522
Females										
Control	2.19	5.01	0.03	0.06	1.66	3.75	0.21	0.48	0.25	0.55
	0.131	1.286	0.007	0.013	0.285	0.912	0.163	0.362	0.407	0.809
500	2.13	4.39	0.04*	0.08*	1.68	3.42	0.17	0.36	0.17	0.35
	0.119	0.690	0.008	0.018	0.324	0.583	0.097	0.220	0.177	0.415
1500	2.09*	5.11	0.04*	0.09*	1.60	3.84	0.17	0.42	0.33	0.68
	0.084	1.058	0.012	0.030	0.441	1.118	0.101	0.249	0.927	1.718
5000	1.99*	6.59*	0.04*	0.12*	1.20*	3.91	0.10*	0.34	0.13	0.45
	0.097	1.150	0.013	0.044	0.231	0.748	0.021	0.100	0.114	0.390

[a] W - weight.
RW - organ-to-body weight ratio.

* Statistically different from control value ($p < 0.05$).

J. Histopathology: At interim sacrifice, an increase in the incidence of prostatitis was noted in males and hemosiderosis of the spleen in females receiving the high dose. There were no other effects noted. A summary of the most frequently observed non-neoplastic lesions at 24/27 months is presented in Table 6. There was a significant increase in the incidence of liver necrosis ($p < 0.05$) and alveolar histiocytosis ($p < 0.05$) in females receiving the high dose. There was also a significant linear trend ($p < 0.05$) in the incidences of peripheral nerve neuropathy, heart thrombosis, and stomach fibrosis. In males, a significant increase in the incidences of polyarteritis of the testes and in polyarteritis of the arteries ($p < 0.05$) was noted in animals receiving the high dose. A significant linear trend ($p < 0.05$) was also noted for these lesions.

A summary of the most frequently observed neoplastic lesions is presented in Table 7. The incidence of hepatocellular carcinomas was significantly higher (Fisher Exact test) in males receiving 5000 ppm when compared to control and there was a significant dose-related trend. The incidence was also higher in females receiving 5000 ppm, and although there was a significant dose-related trend ($p < 0.05$), the incidence was not significantly different from control using the Fisher Exact test. The data also indicated an increase in the incidence of liver adenomas in the concurrent control (and dosed) males when compared to historical controls from the testing laboratory. The latter were reported to be 2 of 401 (0.5%) at final sacrifice, whereas in concurrent controls the incidence was 3 of 19 (15.8%) males. There was also an increase in the incidence of follicular cell adenoma of the thyroid in males receiving the high dose (Fisher Exact test, $p < 0.05$) and a dose-related trend (Cochran-Armitage test, $p < 0.05$). In addition, an increase in the incidence of interstitial cell tumors of the testes was noted in males receiving the high dose, but the increase was not statistically significant and did not show a linear trend.

10. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that MON-097 fed to this strain of rat caused a statistically significant decrease in food consumption in both high-dose groups, and a decrease in body weights of mid- and high-dose males and females. In addition, dose-related increases in thyroid follicular cell adenomas in mid- and high-dose males, hepatic carcinomas in low-, mid-, and high-dose males and high-dose females, and testicular interstitial cell tumors in all dosed males were observed. These neoplastic changes were considered by the authors to be compound related due to the increased incidences noted when compared to the concurrent and historical control data (see Histopathology Section). Statistical analysis of the histopathology data was not performed by the study authors but was conducted by the sponsor; these analyses are discussed below.

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TABLE 6. Summary of Most Frequently Observed Nonneoplastic Lesions in Rats Fed Diets Containing MON-097 for 24/27 Months (continued)

Organ/Lesion		Males				Females			
		0	500	1500	5000	0	500	1500	5000
Peripheral nerve	N[a]	69	67	70	67	66	70	67	63
Neuropathy		1	0	1	1	0	0	0	4[d]
Pituitary	N	68	70	70	70	70	70	70	67
Hyperplasia		3	5	3	3	4	6	6	4
Prostate	N	70	70	70	69	-	-	-	-
Prostatitis		19	18	18	15				
Skin	N	70	69	70	70	70	69	70	70
Granuloma foot-pad		15	17	22	11	19	10	10	0
Spleen	N	70	70	70	70	70	70	70	70
Hemosiderosis		18	13	10	10	20	15	20	27
Stomach	N	70	70	70	70	70	70	70	70
Fibrosis		13	10	13	14	4	5	7	12[d]
Testes	N	70	70	70	70	-	-	-	-
Polyarteritis		7	11	12	17*[b,d]				
Thyroid	N	69	69	70	70	69	69	69	69
C-cell hyperplasia		2	3	2	3	0	0	1	0
Uterus		-	-	-	-	70	70	70	70
Endometritis						10	13	11	5

[a]Number of tissues examined, including interim sacrifice animals.

[b]Statistical analysis conducted by our reviewers, using the Fisher Exact test.

[c]Statistical analysis conducted by the sponsor.

[d]Significant linear trend using the Cochran-Armitage test ($p < 0.05$).

*Statistically different than control values ($p < 0.05$).

TABLE 7. Summary of Most Frequently Observed Neoplastic Lesions in Rats Fed Diets Containing MON-097 for 24/27 Months

Organ/Lesion		Males				Females			
		0	500	1500	5000	0	500	1500	5000
Adrenals	N[a]	70	70	70	70	70	70	70	70
Pheochromocytoma (benign)		4	5	4	1	0	0	0	1
Liver	N	70	70	70	70	70	70	70	70
Hepatocellular adenoma		6	2	5	7	0	2	2	2
Hepatocellular carcinoma		0	2	3	6*[bd]	1	1	1	5[bd]
		6	4	8	13	1	3	3	7
Hemangiosarcoma		0	0	0	0	1	0	0	1
Mammary gland	N	12	18	10	11	67	69	67	55
Adenoma		0	0	0	0	7	12	7	2
Fibroadenoma		1	0	0	0	50	61	64	39
Adenocarcinoma		0	0	0	0	13	13	13	7
Pancreas	N	69	70	70	70	70	70	70	70
Islet cell adenoma		10	11	10	8	2	1	0	1
Pituitary	N	68	70	70	70	70	70	70	57
Adenoma		23	18	23	19	35	41	34	24
Carcinoma		13	9	5	4	17	6	13	4
Testes	N	70	70	70	70	-	-	-	-
Interstitial cell tumor		2	4	4	7				
Thyroid	N	69	69	70	70	69	69	69	69
C-cell adenoma		7	2	4	4	4	1	1	0
Follicular cell adenoma		0	0	3	5*[bd]	2	0	0	3
Uterus		-	-	-	-	70	70	70	70
Adenocarcinoma						1	0	1	4

[a]Number of tissues examined, including interim sacrifice animals.

[b]Statistical analysis conducted by our reviewers, using the Fisher Exact test.

[c]Statistical analysis conducted by the sponsor.

[d]Significant linear trend using the Cochran-Armitage test ($p < 0.05$).

*Statistically different than control values ($p < 0.05$) using the Fisher Exact test

For other parameters investigated in this study, the sponsor was in agreement with the conclusions of the authors except for some hematology results. The study authors considered that only the statistically significant decrease at month 18 in the mean hemoglobin count in females receiving the high-dose was compound-related, whereas, the sponsor indicated that the decrease in both hemoglobin and hematocrit values in this group at months 6, 12, and 18 was compound-related.

B. Quality assurance inspections were performed periodically throughout the study.

11. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Our evaluation of the results of this chronic toxicity/oncogenicity study with MON-097 in rats indicates that it was adequately conducted and reported yet there were some deficiencies noted e.g., summarizing gross examination data by systems, rather than by organ or tissues, weighing organs after fixation and use of a $p < 0.01$ rather than 0.05 for statistical significance of histopathologic data. The conclusions of the authors are supported by the data.

The results indicate increased mortality in females receiving 5000 ppm of test material in the diet (high dose). However, adequate numbers of animals were alive after month 18 to allow for evaluation of late-developing tumors. There was also a compound-related decrease in the mean body weights of males and females receiving 1500 and 5000 ppm of test material, although the decrease in mid-dose females was less pronounced. In addition a decrease in the mean body weights of males receiving the low dose by about week 103 was noted; this effect appears to be biologically significant and compound-related. Decreased food consumption and food efficiency was also observed in males and females receiving the high dose throughout the study. There were no compound-related effects in clinical signs and eye examinations, hematology, blood chemistry, and urinalysis noted in dosed animals, except for lower hemoglobin and hematocrit values in females receiving the high dose at months 6, 12, and 18, but not month 24. Changes in organ weights and organ-to-body weight ratios were usually associated with lower body weights of dosed animals. However, it should also be noted that the organs were weighed after fixation in 10% buffered formalin. Consequently, these changes could not be definitively related to compound administration, except for the liver, thyroid, and testis, where the animals exhibited histopathologic changes.

Individual animal data indicate that all gross lesions were further examined histologically. There were some compound-related effects

noted during histologic examination of the tissues. Based on statistical analysis conducted by these reviewers, nonneoplastic lesions included increased incidences of polyarteritis of the testes and arteries of males and liver necrosis and alveolar histiocytosis of females receiving the high dose ($p < 0.01$). In addition, the statistical analyses of the data by the sponsor indicated significant linear trends for these lesions ($p < 0.01$) as well as significant increases in the incidences of liver necrosis and alveolar histiocytosis and inflammation of the tongue in females receiving the high dose.

Neoplastic lesions were also noted and the data were analyzed statistically by our reviewers. A significant increase ($p < 0.05$) in liver carcinomas and thyroid follicular cell adenomas was noted in males receiving 5000 ppm using the Fisher Exact test. A statistically significant increase in the incidences of liver carcinomas in the females receiving the high dose was not observed by our reviewers using either the Fisher Exact test or the Peto method at a $p < 0.05$. However, a significant positive trend (Cochran-Armitage test) in the incidences of liver carcinomas in females ($p < 0.05$) as well as in the incidence of liver carcinomas and thyroid follicular cell adenomas in males ($p < 0.05$) was noted.

The statistical analyses of neoplastic lesions conducted by the sponsor indicated only a linear trend in the incidence of liver carcinomas for both sexes combined ($p < 0.01$) and a significant increase in the incidence of liver carcinomas for both sexes combined using the Peto method ($p < 0.01$). In addition, a positive trend was noted for the thyroid follicular cell adenomas. It should be noted, however, that the sponsor utilized a p value of 0.01 instead of 0.05 which is more commonly accepted, and is an EPA policy.

The following deficiencies were noted: organs were weighed following fixation in 10% buffered formalin and necropsy data were reported as the number of lesions per organ system.

Based on body and organ weight data the LOEL for chronic effects is 500 ppm (LDT) of test material in the diet, and a NOEL cannot be established.

12. CBI APPENDIX: Appendix A, Materials and Methods, CBI, pp. 9-22.

APPENDIX A

Materials and Methods

ACETOCHLOR Tox review 007697

Page_____ is not included in this copy.

Pages 358 through 417 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

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____ Sales or other commercial/financial information.

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

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-01-6561
TASK: 109
August 5, 1985

DATA EVALUATION RECORD

ACETOCHLOR (Harness)

Oncogenicity Study in Mice

STUDY IDENTIFICATION: Ahmed, F. E., Tegeris, A. S., Seely, J. C. MON-097: 24-month oncogenicity study in the mouse. (Unpublished report No. PR-80-007 prepared by Pharmacopathics Research Laboratories, Inc., Laurel, MD, for Monsanto Agricultural Products Company, St. Louis, MO; dated May 4, 1983.) Accession Nos. 071966-071968.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: August 2, 1985

007697

004586

1. CHEMICAL: Acetochlor: 2-chloro-N-(ethoxymethyl)-6'-ethyl-ortho-acetotoluidine.

2. TEST MATERIAL: MON-097, purity 94.5%; Lot No. NBP 1737874.

3. STUDY/ACTION TYPE: Oncogenicity study in mice.

4. STUDY IDENTIFICATION: Ahmed, F. E., Tegeris, A. S., Seely, J. C. MON-097: 24-month oncogenicity study in the mouse. (Unpublished report No. PR-80-007 prepared by Pharmacopathics Research Laboratories, Inc., Laurel, MD, for Monsanto Agricultural Products Company, St. Louis, MO; dated May 4, 1983.) Accession Nos. 071966-071968.

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7. CONCLUSIONS:

A. Under the conditions of this study, treatment of random-bred Swiss albino CD-1 mice with MON-097 resulted in a definite increase in tumors of the liver, lung, and uterus with suggestive increased tumors of the ovaries and kidneys:

1. Definite increases based on pairwise comparison using the chi square test or Fisher exact test.

a) liver carcinomas, high-dose males ($p \leq 0.01$)

b) total lung tumors, females at all doses ($p \leq 0.01$)

c) carcinomas of the lung in low and high dose females ($p \leq 0.05$)

d) uterine histiocytic sarcomas, low- and mid-dose females ($p \leq 0.01$) and high dose females ($p \leq 0.05$)

e) Total benign tumors of the ovaries in mid-dose females ($p \leq 0.05$)

2. Only suggestive increases based on linear trend analysis using the Peto method ($p < 0.01$)

a) liver carcinomas, females and males
b) lung carcinomas, females
c) total lung tumors in females
d) ovary benign tumors
e) kidney adenomas, females

Changes in other parameters that appeared to be related to dosing included: 1) an increased mortality in both high-dose males and females; 2) decreased mean body weights in high-dose males and females; 3) decreased red blood cell count, hematocrit, and hemoglobin in high-dose females at terminal sacrifice; 4) increased white blood cell count in high-dose males at terminal sacrifice; 5) increased platelet count in mid- and high-dose females at terminal sacrifice; 6) increased mean liver weights and liver-to body weight ratios at study termination in all dosed groups of males and in high-dose females as well as an increase in liver-to-body weight ratios in all dosed males and females at 12 months; an increase in absolute and relative kidney weights in all dosed groups of males at termination; and an increase in absolute and relative adrenal weights in all groups of males and in high-dose females at study termination; 7) an increase in interstitial nephritis in high-dose males and females.

A NOEL for chronic toxicity could not be established based on increased liver, and kidney, weights at the low-dose level. The LOEL for chronic toxicity of MON-097 in mice was 500 ppm (lowest dose tested).

Core Classification: Core Minimum.

8. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

For details of the author's Materials and Methods see Appendix A of this review.

The test material was MON-097 (CP 55097, NBP 1737874), a maroon liquid with a stated purity of 94.5%. The major component is 2-chloro-N-(ethoxymethyl)-6'-ethyl-ortho-aceto-toluidine. The basic experimental design consisted of the exposure of Swiss albino CD-1 mice to MON-097 in the diet for up to 23 months at dose levels of 0, 500, 1500, and 5000 ppm. Five hundred random-bred Swiss albino CD-1 weanling mice were inspected upon arrival, quarantined for 22-23 days, and randomized by weight into the experimental groups prior to dosing. Twenty mice were sacrificed before the start of dosing to determine baseline gross pathology and histopathology, with the remainder assigned to groups of 60 male and 60 female mice at each dose level. Ten of each group were sacrificed at 12 months so that the long-term study, in effect, consisted of 50 animals per group fed the indicated doses for up to 23 months. The diets were prepared weekly.

Animals were observed twice daily for mortality or other signs of toxicity. Body weights and food consumption were determined once pre-test, weekly during the first 13 weeks, and biweekly thereafter. Terminal body weights were those determined at necropsy or weights taken within 7 days before sacrifice. Organ weights were determined at the interim and the terminal sacrifices on fixed tissues.

Urinalysis, hematology, and blood chemistry values were determined in 10 mice/sex/dose at a 12-month interim sacrifice and at study termination. Blood was pooled from 3-4 mice for chemistry determinations.

Complete gross pathology examinations and histopathological evaluations were performed on each animal.

Body weight and food consumption data were analyzed statistically by one way analysis of variance using F test for comparison of variances and Dunnett's test was used to determine which means were significantly different from controls. Clinical laboratory data and organ weight data were analyzed by a two-sided Student's t-test. Neither the protocol nor materials and methods indicated

that the study author analyzed histopathology data. The study sponsor analyzed the incidence of tumor and nontumor lesions to detect statistically significant ($p \leq 0.01$) dose-related linear trends and differences between control and dosed animal values.

B. Protocol:

See Appendix B for Protocol details.

9. REPORTED RESULTS:

Analysis of Diets: The analytical procedure for MON-097 was validated prior to initiation of the study. The response was linear in the range to be used for the analysis, and diet analyses prior to the study were reasonably reproducible. For nominal values of 500, 1500, and 5000 ppm, the respective reproducibilities were 110.83%, 109.17%, and 88.89%; the respective standard deviations expressed as percent were 9.77, 12.69, and 12.80. Mixing was efficient and test compound was stable in the diet for 14 days. Diet analyses during the study indicated MON-097 was stable in the diets for at least one week (diets were prepared weekly) and was homogeneously mixed with the diets. MON-097 in the diet was analyzed at weeks 1, 2, 3, 4, 6, 8, 12, 18, 24, 30, 36, 42, 48, 52, 60, 78, and 90. The means and standard deviations for the study, as calculated by our reviewers, were:

Nominal (ppm)	Analytical (ppm)	Coefficient of Variation (%)	Range (ppm)
500	492.06± 37.14	7.5	454.92 - 529.2
1500	1468.71± 93.09	6.3	1375.62 - 1561.80
5000	4894.29±307.90	6.3	4586.39 - 5202.19

Clinical Observations: No unusual clinical signs were observed that were considered to be related to dosing. The most frequently observed signs were alopecia, skin lesions, and distended abdomens; these were random in occurrence.

Mortality: Mortality data at selected intervals are summarized in Table 1. A general increase in mortality began to appear after month 12 in dosed animals as compared with controls. Survival at 18 months ranged from 66-94% in males groups and 60-86% in female groups. The study was terminated at 23 months when survival was 26% in both males and females receiving 5000 ppm (high-dose).

TABLE 1. Mortality and Percent Survival at Selected Intervals in Mice Fed MON-097 for 23 Months[a]

Groups/Dose (ppm)	Mortality (and % Survival) at End of Month				
	1	6	12	18	23
Males					
0	0 (100%)	0 (100%)	0 (100%)	3 (94%)	20 (60%)
500	0 (100%)	3 (94%)	7 (86%)	16 (68%)	25 (50%)
1500	0 (100%)	0 (100%)	2 (96%)	6 (88%)	25 (50%)
5000	0 (100%)	3 (94%)	5 (90%)	17 (66%)	37 (26%)
Females					
0	1 (98%)	1 (98%)	3 (94%)	7 (86%)	19 (62%)
500	0 (100%)	0 (100%)	2 (96%)	9 (82%)	25 (50%)
1500	0 (100%)	0 (100%)	2 (96%)	11 (78%)	33 (34%)
5000	1 (98%)	2 (96%)	7 (86%)	20 (60%)	37 (26%)

[a]Fifty animals/sex/group; animals scheduled for sacrifice at 12 months were not included in mortality calculations.

Body Weights: Body weights at selected intervals are summarized in Table 2. Significantly ($p \leq 0.05$) lower body weights in dosed animals as compared with controls were observed in the following groups: mid-dose males at 53 and 79 weeks; high-dose males and females at all selected time intervals. The mean body weights of high-dose males and females was approximately 80% of control at study termination. In mid-dose males mean body weights were decreased 6.5% at 18 months but only 3% at 23 months as compared to controls.

Food and Water Consumption: Water consumption was not measured. Food consumption data at selected intervals are summarized in Table 3. Although significant ($p \leq 0.05$) sporadic changes in food consumption were found in both sexes, they were not consistent and there were no changes that were related to dose level.

Food Efficiency: Mean food efficiencies during the first 13 weeks of study are summarized in Table 4. There were no changes in food efficiency that were related to dose level during this early phase of the study. Food efficiency was not studied beyond week 13.

Hematology: Except for a decrease in red cell parameters (RBC, Hmct, and Hb) in high-dose females at month 23, which the authors correlated with anemia, and an increase in white cell count in high-dose males at month 23, which the authors indicated to correlate with hepatocellular carcinoma, other changes were not consistent with time or dose and not considered compound related (authors). The following significant ($p \leq 0.05$) decreases in hematology parameters were observed (Table 5): red blood cell count (RBC) in high-dose females at months 12 and 23; hemoglobin (Hgb) in high-dose females at month 23; hematocrit (Hmct) in high-dose females at month 23 and in mid- and high-dose males at month 12. Significant increases in the following hematology parameters were also observed (Table 5): white blood cell count (WBC) in high-dose males at month 23; RBC in mid-dose females at month 12; platelet count (Plt Ct) in low- and mid-dose males at month 12 and in mid- and high-dose females at month 23.

Clinical Chemistry: For serum alkaline phosphatase (SAP), serum glutamic oxaloacetic transaminase (SGOT), and total bilirubin (TB), some significant increases were observed (Table 6) as follows: SAP in high-dose females at month 12; SGOT in high-dose males at month 12; TB in mid-dose females at month 23. The authors attributed changes in total protein to hemolysis of blood samples. There were no good correlations between SAP, SGOT, SGPT, and TB and histologic findings. Since all values were from pooled blood samples of 3-4 animals, direct animal correlations of chemistry and histologic changes could not be made (CBI pp 52-57).

TABLE 2. Selected Mean Body Weights for Mice Fed MON-097 for 23 Months

Groups/Dose (ppm)	Body Weights (g) at Week:			
	27	53	79	99
Males				
0	35.683 ± 3.427[a]	37.017 ± 3.895	36.787 ± 3.526	35.500 ± 3.214
500	34.947 ± 2.649	36.547 ± 2.932	36.088 ± 2.843	35.880 ± 3.206
1500	34.950 ± 2.873	35.293[b] ± 3.195	34.386[b] ± 3.059	34.720 ± 3.156
5000	31.386[b] ± 2.527	31.018[b] ± 2.621	30.545[b] ±2.251	29.286[b] ± 2.054
Females				
0	29.441 ± 2.866[a]	30.368 ± 2.932	32.744 ± 2.945	31.545 ± 4.131
500	28.433 ± 2.936	31.069 ± 3.722	33.000 ± 3.413	32.040 ± 2.993
1500	29.267 ± 3.156	30.448 ± 3.039	31.974 ± 2.716	31.750 ± 2.826
5000	25.931[b] ± 2.183	26.830[b] ± 2.293	28.933[b] ± 2.753	28.267[b] ± 3.788

[a]Standard deviation.

[b]Significantly different from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

TABLE 3. Selected Mean Food Consumption in Mice Fed MON-097 for 23 Months

Group/Dose (ppm)	Grams of Food/Mouse/Week at Week 13	27	53	79	99
Males					
0	35.2 ±4.6[a]	38.0 ±3.8	37.4 ±4.5	39.0 ±4.4	38.1 ±4.6
500	34.6 ±4.9	38.0 ±3.0	36.7 ±3.6	36.9 ±3.4	35.5 ±3.9
1500	33.2* ±3.2	37.7 ±3.3	36.5 ±3.8	37.0 ±4.8	36.9 ±4.2
5000	35.7 ±4.7	39.2 ±5.4	36.5 ±4.8	36.1* ±4.4	37.0 ±4.6
Females					
0	35.1 ±4.5	40.3 ±5.0	39.4 ±6.0	39.2 ±4.0	36.5 ±5.9
500	32.9* ±3.8	39.7 ±4.4	40.6 ±6.4	40.4 ±3.1	38.7 ±3.2
1500	33.7 ±4.0	40.0 ±4.1	40.1 ±4.8	38.9 ±3.9	37.8 ±4.0
5000	37.9* ±5.1	45.4* ±7.4	41.9 ±8.5	37.4 ±4.6	35.5 ±8.1

[a] Standard deviation.

* Significantly different from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

TABLE 4. Mean Food Efficiency (Change in Body Weight/Food Consumed/Week) During the First 13 Weeks of a 23-Month Study of MON-097 Oncogenicity[a]

Dose (ppm)	Males	Females
0	0.015[b] ±0.025[c]	0.011 ±0.027
500	0.015 ±0.019	0.011 ±0.023
1500	0.013 ±0.025	0.014 ±0.023
5000	0.008 ±0.022	0.011 ±0.030

[a] Statistical analysis of these data by our reviewers indicated no significant differences in mean food efficiencies between control and dosed groups (ANOVA followed by Duncan's multiple range test) and no dose-related trends (regression analysis).

[b] g body weight/g food consumed/week, mean for first 13 weeks of study.

[c] Standard deviation.

TABLE 5. Determination of Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Hemoglobin (Hgb), Hematocrit (Hmct), and Platelet Count (Plt Ct) in Mice Fed MON-097

	Males Dose (ppm)				Females Dose (ppm)			
	0	500	1500	5000	0	500	1500	5000
RBC ($\times 10^6/mm^3$)								
12 months	7.4 ±1.1[a]	7.0 ±0.8	7.3 ±1.0	6.7 ±1.1	7.3 ±0.7	7.7 ±0.6	8.0[b] ±0.5	6.5[b] ±0.9
23 months	5.3 ±1.3	5.0 ±0.6	5.4 ±1.2	4.5 ±0.6	4.8 ±0.6	5.4 ±0.8	5.4 ±1.0	3.8[b] ±0.7
WBC ($\times 10^3/mm^3$)								
12 months	6.9 ±1.7	9.1 ±4.1	7.8 ±2.8	7.8 ±2.8	8.7 ±2.5	9.8 ±4.9	9.3 ±3.1	10.0 ±4.5
23 months	9.7 ±2.4	13.6 ±5.9	12.0 ±3.8	14.5[b] ±3.5	15.2 ±4.4	14.2 ±8.8	13.9 ±4.8	26.0 ±30.0
Hgb (g/dL)								
12 months	12.7 ±0.9	12.7 ±1.0	11.8 ±1.3	11.6 ±1.5	11.9 ±1.9	12.7 ±0.8	12.7 ±1.2	11.7 ±0.7
23 months	12.0 ±2.2	12.0 ±1.2	12.7 ±1.7	10.9 ±1.7	12.0 ±1.5	13.0 ±1.8	12.5 ±2.0	9.3[b] ±2.0
Hmct (pc/dL)								
12 months	40.0 ±3.2	37.9 ±3.0	36.6[b] ±3.4	37.4[b] ±2.2	39.2 ±4.1	39.5 ±2.7	39.5 ±2.2	38.0 ±2.9
23 months	35.8 ±9.6	36.7 ±3.5	37.1 ±4.4	32.6 ±5.5	37.8 ±4.5	38.9 ±5.4	39.5 ±4.0	29.1[b] ±6.4
Plt Ct ($\times 10^3/mm^3$)								
12 months	437 ±262	710 [b] ±180	847 [b] ±153	565 ±242	513 ±303	579 ±261	454 ±187	662 ±168
23 months	456 ±281	408 ±187	547 ±170	478 ±179	302 ±129	309 ±99	484 [b] ±185	482 [b] ±212

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[a] Standard deviation.

[b] Significantly different from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

TABLE 6. Serum Levels of Alkaline Phosphatase (SAP), Glutamic Oxaloacetic Transaminase (SGOT), and Total Bilirubin (TB) in Mice Fed MON-097

	Males Dose (ppm)				Females Dose (ppm)			
	0	500	1500	5000	0	500	1500	5000
SAP (IU/L)								
12 months	206 ±42 [a]	212 ±31	211 ±12	199 ±48	167 ±11	182 ±54	183 ±20	195 ±13 [b]
23 months	273 ±113 [c]	192 ±27	227 ±36	179 ±5	198 ±56	246 ±55	243 ±83	351 ±180 [c]
SGOT (IU/L)								
12 months	75 ±8	85 ±14	84 ±18	105 ±7 [b]	88 ±16	82 ±13	98 ±1	102 ±7
23 months	116 ±40 [c]	77 ±20	106 ±24	103 ±16	82 ±18	61 ±46	109 ±20	85 ±18 [c]
TB (mg/dL)								
12 months	0.4 ±0.0	0.4 ±0.1	0.4 0.0	0.4 0.0	0.4 ±0.0	0.5 ±0.2	0.4 ±0.1	0.4 ±0.1
23 months	0.4 ±0.1 [c]	0.4 ±0.0	0.6 ±0.1	0.6 ±0.2	0.4 ±0.1	0.5 ±0.3	0.6 ±0.1 [b]	0.6 ±0.1 [c]

[a] Standard deviation.

[b] Significantly different from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

[c] Only 2 pools were analyzed.

Urinalysis: There were no changes in urinary parameters related to dosing.

Organ Weights: At month 12, parallel increases in organ weights and organ-to-body weight ratios were reported for livers (Table 7), and adrenals in dosed females (Table 8). At month 23, parallel increases in organ weights and organ-to-body weight ratios were observed for male livers (Table 7), male and female adrenals (Table 8), male kidneys (Table 9), and female thyroids/parathyroids (Table 10). There were no treatment-related weight changes in the other organs that were examined (brain, pituitary, heart, and gonads).

Gross Pathology: Summary tabulation of gross pathology findings by the report authors (CBI pp II83-II86) did not include the number of animals per group with specific lesions in a particular tissue but tabulated the number of animals with neoplastic or nonneoplastic lesions in an organ system (e.g., digestive, endocrine, reproductive) by dose and sex. Individual pathology data records contained more specific data. Gross observations at necropsy included: 1) an increase in urinary tract lesions in males (scheduled sacrifices for all dose groups; those that died or were sacrificed in moribund condition in the high-dose group) and in the females (scheduled sacrifices for mid- and high-dose groups; those that died or were sacrificed in moribund condition in the high-dose group); 2) an increase in digestive tract (primarily liver) masses in males (scheduled sacrifices for mid- and high-dose groups); 3) an increase in pulmonary masses in females (scheduled sacrifices and animals that died or were sacrificed in moribund condition for all dose groups); and 4) reproductive tract masses in females (scheduled sacrifices for high-dose group). The author stated that a variety of other lesions and masses were observed but were not considered treatment-related.

Histopathology: Table 11 presents a summary of the incidence of neoplastic lesions. If only one animal in any dose group had a tumor, it was not included in the table. The report authors did not indicate any statistical analysis of the data. However, they concluded that there was a dose-related increase in the incidence of the following neoplasms:

- o histiocytic sarcomas of the uterus in low-, mid-, and high-dose females
- o lung adenomas and carcinomas combined in all groups of dosed females
- o lung adenomas in low- and mid-dose groups of males and low-, mid-, and high-dose groups of females
- o hepatic carcinomas in low-, mid- and high-dose groups of males and in high-dose females

TABLE 7. Mean Liver Weights and Liver-to-Body Weight Ratios in Mice Fed MON-097[c]

Dietary Level (ppm)	Males		Females	
	Liver Weight (g)	g Liver / 1000 g body weight	Liver Weight (g)	g Liver / 1000 g body weight
12-Month Sacrifice				
0	1.49 ±0.265[a]	41.964 ±7.865	1.30 ±0.240	48.861 ±7.212
500	1.58 ±0.123	49.636[b] ±6.313	1.45 ±0.235	55.653[b] ±7.245
1500	1.44 ±0.262	52.166[b] ±6.536	1.62[b] ±0.316	60.340[b] ±8.056
5000	1.68 ±0.279	56.324[b] ±12.418	1.53[b] ±0.098	70.994[b] ±5.370
23-Month Sacrifice				
0	1.62 ±0.327[a]	45.507 ±8.169	1.76 ±1.303	54.357 ±30.808
500	2.10[b] ±1.031	58.464[b] ±27.742	1.72 ±0.260	53.886 ±7.989
1500	1.96[b] ±0.451	56.271[b] ±12.167	1.62 ±0.258	50.873 ±7.723
5000	2.52[b] ±0.852	87.088[b] ±32.926	1.92 ±0.311	65.595 ±7.695

[a] Standard deviation.

[b] Significantly different from control ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test; analysis by report authors.

[c] Performance of Bartlett's test by our reviewers indicated inhomogeneous variances for these data; transformation of data to achieve homogeneity of variance was performed by our reviewers prior to reanalysis by ANOVA followed by Duncan's multiple range test. The means and standard deviations are presented as the values before transformation.

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TABLE 8. Mean Adrenal Weights and Adrenal-to-Body Weight Ratios in Mice Fed MON-097

Dietary Level (ppm)	Males		Females	
	Adrenal Weight (g)	g Adrenal / 1000 g body weight	Adrenal Weight (g)	g Adrenal / 1000 g body weight
12-Month Sacrifice				
0	0.01 ±0.004[a]	0.342 ±0.134	0.01 ±0.003	0.420 ±0.131
500	0.01 ±0.005	0.439 ±0.154	0.02[b] ±0.009	0.834[b] ±0.426
1500	0.01 ±0.003	0.411 ±0.167	0.02[b] ±0.005	0.606[b] ±0.219
5000	0.01 ±0.005	0.471 ±0.181	0.02[b] ±0.004	0.831[b] ±0.187
23-Month Sacrifice				
0	0.007 ±0.002[a]	0.191 ±0.068	0.013 ±0.003	0.431 ±0.106
500	0.009[b] ±0.003	0.246[b] ±0.077	0.014 ±0.004	0.443 ±0.106
1500	0.009[b] ±0.003	0.259[b] ±0.105	0.015 ±0.004	0.470 ±0.123
5000	0.010[b] ±0.003	0.360[b] ±0.122	0.016[b] ±0.003	0.556[b] ±0.127

[a] Standard deviation.

[b] Statistically significant from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

TABLE 9. Mean Kidney Weights and Kidney-to-Body Weight Ratios in Mice Fed MON-097

Dietary Level (ppm)	Males		Females	
	Kidney Wt. (g)	g kidney / 1000 g body wt.	Kidney Wt. (g)	g kidney / 1000 g body wt.
12-Month Sacrifice				
0	0.73 ±0.108[a]	20.705 ±2.869	0.46 ±0.064	17.403 ±2.485
500	0.89[b] ±0.143	27.762[b] ±4.999	0.54 ±0.105	20.695[b] ±3.483
1500	0.78 ±0.179	28.001[b] ±4.625	0.55[b] ±0.090	20.504[b] ±2.494
5000	0.81 ±0.180	26.983[b] ±5.667	0.41 ±0.047	19.172 ±2.401
23-Month Sacrifice				
0	0.76 ±0.127[a]	21.352 ±2.833	0.55 ±0.099	17.330 ±2.716
500	1.06[b] ±0.183	29.696[b] ±5.254	0.64[b] ±0.084	19.978 ±2.829
1500	1.08[b] ±0.270	31.028[b] ±6.423	0.52 ±0.050	16.283 ±1.397
5000	0.87[b] ±0.178	29.657[b] ±5.696	0.60 ±0.100	20.748[b] ±3.345

[a] Standard deviation.

[b] Significantly different from control ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test.

TABLE 10. Mean Thyroid/Parathyroid Weights and Thyroid/Parathyroid-to-Body Weight Ratios in Mice Fed MON-097

Dietary Level (ppm)	Males		Females	
	Thyroid/ Parathyroids Wt. (g)	g thyroid/parathyroids / 1000 g body wt.	Thyroid/ Parathyroids Wt. (g)	g thyroid/parathyroids / 1000 g body wt.
12-Month Sacrifice				
0	0.005 ±0.002[a]	0.133 ±0.047	0.005 ±0.003	0.196 ±0.100
500	0.005 ±0.002	0.170 ±0.071	0.005 ±0.002	0.205 ±0.079
1500	0.005 ±0.002	0.191[b] ±0.064	0.007 ±0.002	0.277[b] ±0.069
5000	0.005 ±0.002	0.156 ±0.074	0.006 ±0.002	0.384[b] ±0.083
23-Month Sacrifice				
0	0.007 ±0.002[a]	0.212 ±0.070	0.007 ±0.002	0.212 ±0.071
500	0.009[b] ±0.003	0.253 ±0.092	0.008[b] ±0.002	0.247 ±0.068
1500	0.007 ±0.002	0.206 ±0.065	0.009[b] ±0.002	0.296[b] ±0.089
5000	0.008 ±0.002	0.277[b] ±0.073	0.010[b] ±0.003	0.356[b] ±0.106

[a] Standard deviation.

[b] Significantly different from control value ($p \leq 0.05$) using ANOVA followed by Dunnett's t-test; analysis by report authors.

TABLE 11. Frequently Occurring Neoplastic Lesions in Mice Fed MON-097 for 23 Months[a]

Organ/Lesion	Males/Dose (ppm) 0	500	1500	5000	Females/Dose (ppm) 0	500	1500	5000
No. of animals examined microscopically	60	60	60	60	60	60	60	59
No. of animals with tumors	35	26	43	40[b]	23[c]	31	36[d]	31[e]
- Harderian gland	(60)[f]	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Adenoma	8	7	7	9	3	1	5	4
- Kidneys	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Adenocarcinoma	0	0	2	1	0	0	0	0
Adenoma	2	1	1	2	0	0	0	3[b]
Sarcoma	0	0	0	0	0	0	0	2
- Total malignant kidney tumors	0	0	2	1	0	0	0	2
- Liver	(60)[f]	(59)	(60)	(59)	(60)	(60)	(60)	(58)
Adenoma	8	4	9	7	2	0	0	4
Carcinoma	6	7	10	22[b,d]	1	0	0	4[b]
- Lungs	(60)[f]	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Adenoma	6	10	12	5	2	6	8[g]	4
Carcinoma	7	3	4	3	0	5[g]	3	7[b,g]
Histiocytic sarcoma	0	0	0	0	0	0	1	0
- Total lung tumors	13	13	16	8	2	11[d]	12[d]	11[b,d]
- Lymphatic System	(60)[f]	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Lymphoma	4	2	2	4	6	7	12	1
- Ovaries	-	-	-	-	(59)[f]	(60)	(60)	(58)
Adenoma	-	-	-	-	0	0	1	0
Granulosa cell tumor	-	-	-	-	0	0	3	2
Luteoma	-	-	-	-	0	0	1	1
- Total benign ovarian tumors	-	-	-	-	0	0	5[g]	3[b]

TABLE 11. Frequently Occurring Neoplastic Lesions in Mice Fed MON-097 for 23 Months[a] (continued)

Organ/Lesion	Males/Dose (ppm) 0	500	1500	5000	Females/Dose (ppm) 0	500	1500	5000
- Pituitary gland	(58)[f]	(49)	(58)	(54)	(58)	(57)	(55)	(51)
Adenoma	0	0	0	1	2	2	0	0
- Uterus	-	-	-	-	(59)[f]	(60)	(60)	(59)
Endometrial stromal polyp	-	-	-	-	1	2	2	2
Histiocytic sarcoma	-	-	-	-	0	6[d]	6[d]	5[g]
Leiomyosarcoma	-	-	-	-	3	0	2	0

[a] Neoplastic lesions that occurred at a frequency of no more than one per dose group were excluded from this table unless significance was noted for total tumors of a given organ.

[b] Statistically significant linear trend ($p \leq 0.01$) using the Peto analysis. It should be noted that the animals scheduled for interim sacrifice at 12 months (10/sex/dose) were included in the above compilation even though they would be at low risk of developing neoplasms by month 12. The sponsors indicated, however, that statistical analysis by the Peto method has the advantage of utilizing survival and time to tumor information.

[c] Corrected value found by our reviewers; this value was reported as 22 by the sponsor.

[d] Statistically significant increase compared to control ($p \leq 0.01$) using the Chi-square test (uncorrected for continuity).

[e] Reanalysis by our reviewers indicated a statistically significant linear trend ($p < 0.05$) using the Cochran-Armitage test; the analysis reported by the sponsor had used the value of 22 females with tumors at 0 ppm and had reported a significance of $p \leq 0.01$ using the Peto analysis.

[f] Number in parentheses is the number of animals from which tissue was examined histologically.

[g] Significantly different from control by Fisher's exact test $p < 0.05$.

The sponsor provided statistical analysis of incidence of neoplasms using chi-square (without continuity correction), and analysis of linear trend using the Peto analysis. With the chi-square analysis there was a statistically increased incidence ($p \leq 0.01$) in the following:

- liver carcinomas in high-dose males (22/59) as compared to controls (6/60);
- total lung tumors in the low- (11/60), mid- (12/60) and high-dose females (11/59) as compared to controls (2/60)
- histiocytic sarcomas of the uterus in the low- (6/60) and mid-dose (6/60) female groups as compared to controls (0/59).

By the Peto trend analysis there were significant linear trends for the following:

- females with kidney adenomas (0/60, 0/60, 0/60, and 3/59 in control, low-, mid-, and high-groups groups;
- males with liver carcinomas (6/60, 7/58, 10/60, and 22/59 in the controls, low-, mid-, and high-dose groups;
- females with liver carcinomas (1/60, 0/60, 0/60, and 4/58) in control, low-, mid-, and high-dose groups;
- females with total lung tumors (2/60, 11/60, 12/60, and 11/59 in controls, low-, mid-, and high-dose groups);
- females with lung carcinomas (0/60, 5/60, 3/60, and 7/59 in controls, low-, mid-, and high-dose groups);
- females with benign ovarian tumors (0/59, 0/60, 5/60, and 3/58) in control, low-, mid-, and high-dose groups);
- females with histiocytic sarcomas of the uterus (0/59, 6/60, 6/60, and 5/59 in control, low-, mid-, and high-dose groups).

The incidence of frequent non-neoplastic lesions is summarized in Table 12. The report authors stated that there was a dose-related increased incidence of interstitial nephritis in all dosed groups of males and females.

The sponsor provided statistical analysis of nonneoplastic lesions which indicated significant increases ($p \leq 0.01$) in the incidence of interstitial nephritis compared to controls in both males and females receiving the highest dose Analysis of trend (Peto test) indicated

TABLE 12. Frequently Occurring Nonneoplastic Lesions in Mice Fed MON-097 for 23 Months

Organ/Lesion	Males/Dose (ppm) 0	500	1500	5000	Females/Dose (ppm) 0	500	1500	5000
- Adrenal Gland	(58)[a]	(59)	(58)	(59)	(59)	(60)	(60)	(59)
Amyloidosis	0	2	1	1	1	1	4	1
- Bone Marrow	(60)	(60)	(60)	(60)	(60)	(60)	(59)	(59)
Fibrous Osteodystrophy	0	0	0	0	8	11	6	6
- Cecum	(59)	(56)	(60)	(57)	(56)	(59)	(59)	(58)
Typhlitis	0	3	0	0	0	1	0	1
- Colon	(59)	(56)	(59)	(59)	(57)	(60)	(59)	(59)
Nematodiasis	10	9	1	5	3	1	0	1
- Duodenum	(60)	(56)	(60)	(57)	(57)	(59)	(59)	(58)
Amyloidosis	0	0	0	0	0	1	2	1
Duodenitis	0	2	2	0	0	1	1	3
- Eyes	(60)	(60)	(60)	(59)	(60)	(60)	(60)	(59)
Cataract	3	0	1	0	1	0	1	1
Keratitis	0	1	1	1	2	0	0	0
Panophthalmitis	2	0	0	0	1	0	0	0
Retinal Degeneration	4	3	6	3	2	3	1	8[c]
- Harderian Gland	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Dacryoadenitis	3	3	0	3	4	1	1	0
- Heart	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Cardiomyopathy	2	3	1	5	2	1	0	0
Endocarditis	0	2	0	0	0	0	0	0
Myocarditis	0	2	2	2	1	0	2	1
Thrombosis	1	1	3	2	0	2	1	0
- Ileum	(59)	(56)	(59)	(57)	(56)	(59)	(59)	(58)
Amyloidosis	2	0	0	1	2	2	0	2
Ileitis	0	2	0	0	0	0	0	0
- Jejunum	(59)	(56)	(58)	(57)	(57)	(59)	(59)	(58)
Amyloidosis	4	5	3	2	0	2	3	3

[a] Number in parentheses is the number of animals from which tissue was examined histologically.

[c] Statistically significant linear trend ($p \leq 0.01$) using the Cochran-Armitage test; analyses by our reviewers.

TABLE 12. Frequently Occurring Nonneoplastic Lesions in Mice Fed MON-097 for 23 Months (continued)

Organ/Lesion	Males/Dose (ppm) 0	500	1500	5000	Females/Dose (ppm) 0	500	1500	5000
- Kidneys	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)[d]
Amyloidosis	1	3	2	1	2	2	4	2
Cysts	5	10	6	2	3	1	0	1
Hydronephrosis	3	1	5	3	2	5	1	6
Infarction	0	6	0	0	1	0	0	0
Interstitial Nephritis	30	35	42	50[b,c]	31	33	31	45[b,c]
Nephrocalcinosis	0	0	0	0	0	1	0	2
- Liver	(60)	(59)	(60)	(59)	(60)	(60)	(60)	(58)
Cell Focus	2	0	0	0	1	1	0	0
Cysts	5	1	3	2	1	1	2	0
Fatty Infiltration	2	0	1	0	0	2	0	0
Hepatitis	3	3	0	2	2	3	5	1
Necrosis	2	5	0	3	5	3	1	4
- Lungs	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Bronchopneumonia	0	0	0	2	0	0	0	0
Interstitial Pneumonia	5	6	10	3	3	3	4	5
Lymphocytosis	2	1	1	0	4	1	1	2
Precipitate, Alveolar	2	0	0	0	0	0	1	0
- Lymph Nodes	(57)	(55)	(57)	(52)	(55)	(55)	(55)	(51)
Angiectasis	0	1	2	0	4	0	0	0
Congestion	1	2	1	0	2	2	0	1
Lymphadenitis	2	2	1	1	3	0	6	4
Lymphoid Hyperplasia	0	5	2	2	6	0	9	1
- Middle Ear	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(59)
Otitis Media	3	1	1	1	1	1	1	3
- Nose	(60)	(59)	(59)	(60)	(60)	(60)	(60)	(59)
Rhinitis	0	4	3	0	3	2	5	0

[b] Statistically significant increase compared to control ($p \leq 0.01$) using the Chi-square test (uncorrected for continuity).

[d] Correct value determined by our reviewers.

TABLE 12. Frequently Occurring Nonneoplastic Lesions in Mice Fed MON-097 for 23 Months (continued)

Organ/Lesion	Males/Dose (ppm) 0	500	1500	5000	Females/Dose (ppm) 0	500	1500	5000
- Ovaries	—	—	—	—	(59)	(60)	(60)	(58)
Amyloidosis	—	—	—	—	2	1	2	0
Cyst, follicular	—	—	—	—	2	2	1	2
Cyst, hemorrhagic	—	—	—	—	11	10	9	7
Cyst, simple	—	—	—	—	29	26	21	19
- Peripheral Nerve	(55)	(46)	(48)	(51)	(54)	(52)	(57)	(57)
Neuropathy	0	0	0	0	2	0	0	0
- Pituitary Gland	(58)	(49)	(58)	(54)	(58)	(57)	(55)	(51)
Hyperplasia	0	0	0	0	1	0	2	0
- Prostate Gland	(60)	(59)	(60)	(60)	—	—	—	—
Prostatitis	3	2	6	3	—	—	—	—
- Salivary Gland	(60)	(60)	(60)	(59)	(60)	(59)	(60)	(57)
Sialoadenitis	3	2	0	2	0	1	3	1
- Seminal Vesicles	(60)	(60)	(60)	(60)	—	—	—	—
Seminal Vesiculitis	3	1	6	1	—	—	—	—
- Skin	(60)	(59)	(60)	(59)	(57)	(60)	(60)	(59)
Dermatitis	4	1	8	3	6	5	2	5
- Spleen	(59)	(56)	(55)	(59)	(57)	(60)	(59)	(57)
Hematopoiesis, Extramedullary	2	0	1	2	4	5	5	4
Hemosiderosis	0	1	0	2	5	4	2	8
- Stomach	(60)	(59)	(60)	(60)	(59)	(60)	(59)	(59)
Adenomatous Hyperplasia	2	8	6	1	2	3	0	4
Gastritis	1	2	3	6	7	4	6	2
- Testes	(60)	(60)	(60)	(60)	—	—	—	—
Atrophy	12	14	14	3	—	—	—	—
Degeneration	1	5	0	3	—	—	—	—
Mineralization	0	2	0	1	—	—	—	—
- Thymus	(50)	(45)	(48)	(45)	(48)	(51)	(49)	(43)
Lymphoid Hyperplasia	0	0	0	0	4	0	1	0

TABLE 12. Frequently Occurring Nonneoplastic Lesions in Mice Fed MON-097 for 23 Months (continued)

Organ/Lesion	Males/Dose (ppm)				Females/Dose (ppm)			
	0	500	1500	5000	0	500	1500	5000
- Thyroid Gland	(59)	(58)	(58)	(57)	(58)	(58)	(59)	(59)
Cyst, follicular	0	1	0	0	1	2	0	2
Thyroiditis	0	0	0	0	2	0	1	1
- Uterus	--	--	--	--	(59)	(60)	(60)	(59)
Cystic Endometrial Hyperplasia	--	--	--	--	42	30	34	22
Endometritis	--	--	--	--	2	1	4	3
- Vagina	--	--	--	--	(58)	(60)	(59)	(58)
Epidermoid Dysplasia	--	--	--	--	5	6	1	1
Vaginitis	--	--	--	--	2	4	4	4
- Zymbal's Gland	(49)	(56)	(55)	(56)	(58)	(53)	(56)	(55)
Adenitis	3	1	3	0	1	0	1	0

a significant positive trend ($p \leq 0.01$) for interstitial nephritis in males (30/60, 35/60, 42/60, and 50/60 in control, low-, mid- and high-dose groups) and in females (31/60, 33/60, 31/60, and 45/60 in control, low-, mid-, and high-dose groups) and a positive trend ($p \leq 0.01$) for retinal degeneration in females (2/60, 3/60, 1/60, and 8/59 in control, low-, mid-, and high-dose groups). However, analysis by pairwise comparison did not indicate a significant increase ($p < 0.01$) in the incidence of retinal degeneration in high-dose females.

10. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The authors concluded that when MON-097 was fed in the diet to random bred Swiss albino CD-1 mice at 0, 500, 1500, and 5000 ppm in the diet it was oncogenic under the conditions of the study. It caused a dose-related increase in pulmonary adenomas in males receiving 500 and 1500 ppm and in all female test groups, a dose-related increase in pulmonary carcinomas in all female test groups, an increase in hepatic carcinomas in all female test groups and in high-dose males, and a dose-related increase in uterine histiocytic sarcomas in all female test groups. A dose-related increase in interstitial nephritis was also seen in all test groups of males and females. It caused a persistent decrease in body weight and body weight gain in male and female groups receiving 5000 ppm but not in low- or mid-dose groups. The only clinical laboratory data considered to be compound related was a decrease in RBC, Hgb, and Hmct values in high-dose females at 23 months; the authors considered that "this anemia may be indirectly compound related as it was associated with the presence of tumors, particularly of the liver, and of renal disease." The only changes in organ weight values and organ-to-body weight ratios considered related to treatment were absolute and relative liver and kidney weight ratios in dosed males and absolute and relative kidney weights in dosed females; this was stated to be based on histopathological correlations. Signs of toxicity, food consumption fluctuations, clinical chemistry values in test groups differing from controls and weight changes in adrenals and thyroids were not considered compound related.

A quality assurance statement, signed and dated May 4, 1983, was present

11. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We conclude that the experimental design and conduct of this bioassay for the oncogenicity of MON-097 was generally in accord with Pesticide Assessment Guidelines. We classify the study as Core Minimum. Deficiencies are as follows:

1. The mice used in the study were received in three different shipments and were acclimated for different periods of time; however, they were all approximately the same age at study initiation.

2 Abnormal clinical findings with the date of observation were entered on individual animal disposition records, but summary incidences were not tabulated nor were weekly observation forms available.

3. There was no evidence that blood smears were obtained from 10 animals/sex/dose group at 18 months as suggested by the guidelines.

4. Organ weights were obtained after fixation. It is common practice, however, to weigh organs before fixation.

5. Necropsy findings were summarized by organ systems rather than by individual tissues or organs.

Food efficiency in high-dose males was approximately half of the control value; however, because the variability was so great the difference was not significant (ANOVA $\alpha = 0.05$, analysis by our reviewers).

Our statistician indicated that it was inappropriate for the study authors to analyze the clinical chemistry, hematology, organ weight, and organ-to-body weight data by the independent, two-sided Student's t-test. However, our statistician's reanalysis of these data by a more appropriate method (ANOVA followed by Duncan's multiple range test) did not change any of the conclusions as to which values in dosed animals were significantly different from control values.

Due to the small size of the blood samples, pooling of 3 to 5 individual samples was necessary for the clinical chemistry analyses at interim and terminal sacrifices; as a result, only 2 or 3 pools were analyzed for each dose group at each sacrifice. We conclude that the availability of only 2-3 values for each clinical chemistry parameter makes the statistical tests (ANOVA, tests to determine differences between means, and trend analyses) of these measurements unstable.

The sponsor concluded that blood chemistry effects indicative of liver damage were observed in dosed animals. This conclusion was based on observations of increased serum alkaline phosphatase in high-dose females at months 12 and 23, increased serum glutamic oxaloacetic transaminase in high-dose males at month 12, and slightly increased total bilirubin in mid- and high-dose mice of both sexes at month 23. The study authors discounted these observations, concluding that these abnormal findings were for the most part randomly distributed and on many occasions lacked any definitive histopathological correlations.

The study authors did not consider effects on organ weights and organ-to-body weight ratios to be test compound-related unless there was correlative histopathology. With this criterion, the study authors considered only liver and kidney weight and organ-to-body weight ratio increases to be test compound-related.

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Differences in the accuracy of weighing the adrenals at 12 and 23 months were apparent; the weights of the adrenals were reported to an accuracy of only two digits after the decimal at month 12 (weights were 0.01-0.04 g with most weights being 0.01 or 0.02 g) but were reported to an accuracy of three digits after the decimal at month 23 (most weights were <0.01 g for males and <0.02 g for females). The mean weight of liver in control females at termination that was reported was unusually high and had a large standard deviation (Table 7). Examination of the individual liver weights revealed that one control female (#1508) had a liver weight of 8.57 g which was correlated with hepatocellular carcinoma (4 x 2.2 x 1.8 cm). If this value were omitted the mean was 1.54±0.325 and the high-dose females had a significantly higher ($p \leq 0.05$) mean liver weight than controls.

The study authors compiled the gross pathology results into four tables according to whether the animals were sacrificed or found dead or moribund. Two tables were for tumors and the other two for non-tumor lesions. They also reported the microscopic diagnoses by neoplasm and nonneoplastic lesions in different tables. As the gross lesions were tabulated as the number of lesions/sex/dose rather than animals with lesions/sex/dose, the gross pathology results were not amenable to statistical treatment. Our reviewers' examination of the individual animal data records revealed that where tissue masses (suspected tumors) were diagnosed grossly, a microscopic diagnosis was also made and usually confirmed the gross diagnosis with the appropriate cell type. The care with which lesions found grossly were processed through the histology laboratory and presented to the pathologist for microscopic examination appeared to be quite effective. Tables 13 and 14 were prepared by our reviewers to correlate the gross diagnoses with microscopic diagnoses. For practical purposes, the analysis for tumor incidence can appropriately be based solely upon the microscopic diagnoses.

The study authors concluded that dose-related increases occurred only for liver carcinomas (males at all doses and high-dose females), uterine histiocytic sarcomas (females at all doses), pulmonary adenomas (females at all doses and low- and mid-dose males), and pulmonary carcinomas (females at all doses). However, the study authors did not analyze histopathology data statistically. This analysis was provided by the sponsor.

The sponsor concluded that significant ($p \leq 0.01$) dose-related positive trends occurred for liver carcinomas (males and females), liver adenomas (males and females combined), uterine histiocytic sarcomas (females), ovary benign tumors (females), total lung tumors (females), lung carcinomas (females), kidney adenomas (females), malignant kidney tumors (males and females combined), and animals with tumors (males and females). Significantly increased incidences ($p \leq 0.01$) of neoplastic lesions in treated animals as compared to controls were found for liver carcinomas (high-dose males), uterine histiocytic sarcomas (low- and mid-dose females), lung tumors (females at all doses), and animals with tumors (mid-dose females). Although

TABLE 13. Correlation of Reported Gross Pathology (Tissue Masses) and Histopathological Diagnoses of Neoplastic Lesions[a]

LIVER (FEMALES)
- -gross masses of digestive system in control and all dose groups
- -carcinomas
  - --significant dose-related positive trend in incidence

LIVER (MALES)
- -gross masses of digestive system in control and all dose groups
- -carcinomas
  - --significant dose-related positive trend in incidence
  - --significantly increased incidence at high dose

LIVER (MALES PLUS FEMALES)
- -gross masses of digestive system in control and all dose groups
- -adenomas
  - --significant dose-related positive trend in incidence

LUNG (FEMALES)
- -gross pulmonary masses in control and all dose groups
- -carcinomas
  - --significant dose-related positive trend in incidence
- -total lung tumors (including carcinomas)
  - --significant dose-related positive trend in incidence
  - --significantly increased incidence at all doses

KIDNEY (FEMALES)
- -gross urinary tract masses in mid- and high-dose groups
- -adenomas
  - --significant dose-related positive trend in incidence

KIDNEY (MALES PLUS FEMALES)
- -gross urinary tract masses in control and in mid- and high-dose groups
- -malignant tumors
  - --significant dose-related positive trend in incidence

OVARIES (FEMALES)
- -gross reproductive tract masses in control and all dose groups
- -benign tumors
  - --significant dose-related positive trend in incidence

UTERUS (FEMALES)
- -gross reproductive tract masses in control and all dose groups
- -histiocytic sarcomas
  - --significant dose-related positive trend in incidence
  - --significantly increased incidence at low and mid doses

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TABLE 13. Correlation of Reported Gross Pathology (Tissue Masses) and Histopathological Diagnoses of Neoplastic Lesions[a] (Continued)

TOTAL TUMORS (FEMALES)
-gross tissue masses in control and dose groups
-animals with tumors
--significant dose-related positive trend in incidence
--significantly increased incidence at mid dose.
TOTAL TUMORS (MALES)
-gross tissue masses in control and all dose groups
-animals with tumors
--significant dose-related positive trend in incidence

TABLE 14. Correlation of Reported Gross Pathology (Tissue Lesions) and Histopathological Diagnoses of Nonneoplastic Lesions[a]

EYES (FEMALES)
-gross ocular lesions in control and in mid and high dose groups
-retinal degeneration
--significant dose-related positive trend in incidence
KIDNEYS (MALES AND FEMALES)
-gross urinary tract lesions in control and in all dose groups for both sexes
-interstitial nephritis
--significant dose-related positive trend in incidence for both sexes
--significantly increased incidence at high dose for both sexes

[a] Table prepared by our reviewers.

there are some differences between the study authors' conclusions and those of the sponsor (see Table 15 for tabular presentation), they both unequivocally agree that MON was carcinogenic, causing definite increases in liver carcinomas, lung tumors, and histiocytic sarcomas of the uterus. The sponsors analysis of tumors used a p value of 0.01 for significance. Analysis by our reviewers indicated that in addition to the findings of the sponsor the following neoplasms were significant at a p level of 0.05: carcinoma of lungs in low- and high-dose females, histiocytic sarcoma of the uterus in high-dose females and total ovarian benign tumors of the uterus in mid-dose females.

In addition to total number of tumors occuring in an organ system as related to exposure, an examination as to possible acceleration of tumor development was attempted by our reviewers. This issue had not been addressed by either the sponsor or the study authors. The latency of tumors could only be estimated based upon tumors observed in animals dying during particular time periods. Tables 16 and 17 present a detailed breakdown of the main tumors of concern as related to their observation with time of death and dose level. Tables 18 and 19 present the data in a somewhat different manner allowing a quicker assessment of the early-appearing tumors. Especially notable were the frequencies of high-dose males that died at 20-23 months with liver carcinomas (10/17) (Table 16) and dosed females that died with uterine histiocytic sarcomas at 13-16 months (5/16) and at 17-19 months (4/15) (Table 17).

When we considered the animals that died or were killed before the terminal sacrifice, combining the tumors for all treated groups to assess differences from controls, a slightly different pattern emerged in that tumors of the lung also become prominent. Thirty-six (36) animals (combined males and females) with lung tumors (adenomas plus carcinomas) were observed in 243 early deaths for a 15% incidence versus only 2 out of 59 controls (3%). The number of early uterine sarcomas still remains substantial, 14 of 125 (11%) versus 0 of 29 controls (0%). However, comparing total early liver tumors in the dosed groups with the controls diminishes the evidence for a substantial early development. Based upon these data, it is concluded that earlier-appearing tumors were observed with greater frequency as related to treatment in three organ systems - liver, lungs, and uterus.

A NOEL for chronic toxicity could not be established due to increased liver and kidney weights at the low-dose level. The LOEL for chronic toxicity of MON-097 in mice was 500 ppm in the diet (lowest dose tested).

12. CBI APPENDIX:

Appendix A, Materials and Methods, CBI Vol. I, pp. 9-21.
Appendix B, Protocol, CBI Vol. III, pp. 126-187.

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