SEP 27 1991

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

008728

SEP 27 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review Meeting on **ACETOCHLOR** 3rd

FROM: Esther Rinde, Ph.D. E.R.
Manager, Carcinogenicity Peer Review
Health Effects Division (H7509c)

TO: Addressees

Attached for your review is a package on **ACETOCHLOR** prepared by **Dr. Tim McMahon.**

A meeting to consider the carcinogenicity classification of **ACETOCHLOR** is scheduled for **Wednesday Oct. 16, 1991, at 10:00 am in Room 821, CM2.**

Addressees

P. Fenner-Crisp
W. Burnam
R. Engler
R. Hill
R. Beliles
K. Baetcke
L. Brennecke
M. Van Gemert
M. Copley
K. Dearfield
J. Parker
H. Pettigrew
W. Sette
G. Ghali
B. Fisher
J. Du
Y. Woo
G. Burin
J. Quest
E. Saito (for microfiche-with one-liner)
T. McMahon
M. Ioannou

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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Issues Addressed to the Peer Review Committee in Connection with the Classification of Acetochlor as a Carcinogen.

TO: Esther Rinde, Ph.D.
Manager, Peer Review for Carcinogenicity

FROM: Timothy F. McMahon, Ph.D. 9/25/91
Toxicologist, Review Section I
Toxicology Branch III
Health Effects Division (H7509C)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head 9/25/1991
Review Section I, Toxicology Branch II
Health Effects Division (H7509C)

Attached is an overview of the carcinogenic potential of Acetochlor, including data on mutagenicity, metabolism, and developmental and reproductive toxicity. These data are based upon studies submitted to the Agency by ICI Central Toxicology Laboratory, Cheshire, UK. Evaluation of carcinogenicity and other relevant data from a previous carcinogenicity assessment of acetochlor are also attached as additonal supporting data.

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TABLE OF CONTENTS

I. Scientific Issues Considered by Toxicology Branch II, Health Effects Division, in Connection with the Classification of Acetochlor as a Carcinogen.

A. Background

Acetochlor, or 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide, is an herbicide intended for control of annual grasses and certain broadleaf weeds in crops such as corn, soybeans, sorghum, and peanuts grown in high organic matter soils. This chemical has been previously classified by the Toxicology Branch Peer Review Committee (PRC) as a Group B2- Probable Human Carcinogen, based upon the findings of increased incidence of malignant or combined malignant and benign tumors in multiple species, positive mutagenic effects, and the activity of structurally related known carcinogens (attachment A). These conclusions were based upon data submitted to the Agency by Monsanto Chemical Company.

The registrant (ICI Agricultural Products, Wilmington, Delaware) has submitted an application to the Agency for an Experimental Use Permit (new chemical food/feed use) and G petition (temporary tolerance) for use of acetochlor on corn and ornamental shrubs in commercial nurseries.

B. Evaluation of Carcinogenicity Data

1. Two Year Chronic Toxicity/Carcinogenicity Study in Rats (Attachment B)

Reference: Virgo, D.M. and Broadmeadow, A., 1988. SC-5676: Combined Oncogenicity and Toxicity Study in Dietary Administration to CD Rats for 104 Weeks.
Study # 88/SUC017/0348. Life Science Research, Ltd., Suffolk, England. MRID # 415920-04.

In this study, technical SC-5676 was administered to male and female rats in the diet for 104 weeks at doses of 0, 18, 175, and 1750 ppm (0, 0.8, 7.9, and 79.6 mg/kg/day active ingredient). Tumorigenic responses were observed in both sexes at the 1750 ppm dose level. These responses are detailed in the following table (Table 1):

TABLE 1

Incidence of Neoplastic Lesions in Male and Female Rats Given Dietary SC-5676 for 104 Weeks (Terminal Sacrifice Group; Decedent + Surviving)[a]

	Males				Females			
Dose (ppm)	0	18	175	1750	0	18	175	1750
No. animals examined:	50	49	50	50	50	50	49	49
Adenoma of Nasal Epithelium	0[b] (0)[c]	0(0)	0(0)	30(60)[e]	0(0)	0(0)	0(0)	28(57)[e]
Carcinoma of Nasal Epithelium	0 (0)	0(0)	0(0)	2(4)	0 (0)	0(0)	0(0)	1(2)
Thyroid- No. animals examined	50	50	48	50	50	50	50	49
follicular cell adenoma	2(4)	1(2)	2(4)	5(10)	1(2)	1(2)	3(6)	5(10)

[a]data from Table 13l, pages 220-224 of registrant report.

[b]number of rats with lesion; [c]percent of rats with lesion

[d] $p < 0.05$ vs control; [e] $p < 0.01$ vs control; [f] $p < 0.001$ vs control.

A significant increase in adenomas of the nasal epithelium was observed in both male and female rats at the 1750 ppm dose level ($p < 0.01$). This increase in nasal epithelial adenomas was also significant when decedent and surviving rats were considered separately, supporting the treatment related nature of the effect. The finding of follicular cell adenomas of the thyroid was apparently treatment related only when decedent and surviving rats were combined. The trend of increased thyroid follicular cell adenomas was significant for female rats as analyzed by the Cochran-Armitage test ($p < 0.05$, page 38 of registrant report), but was not statistically significant for male rats, even though the percentage of rats with this tumor was equivalent between sexes at the 1750ppm dose level (10%). The incidence of thyroid follicular cell adenoma at the 1750 ppm dose level in females was outside the historical control range for this tumor type (see historical control data, attachment B).

Two rare tumor types were also observed in this study. Benign chondroma of the femur was found in 1 male rat which died during the study and in 1 female rat surviving to week 104. Basal cell tumors of the stomach were also found in 1 male and 1 female rat which died during the study. The rarity of these tumor types supports the finding that these were related to administration of test material.

The highest dose of test article examined in this study was 1750 ppm in both male and female rats. This dose caused a body weight decrement of approximately 12-14% during the first 13 weeks of treatment in both sexes of rats. This weight gain decrement persisted throughout the study in both sexes. In addition, decreased food efficiency, ophthalmoscopic abnormalities, clinical effects on GGT and cholesterol, and increased organ:body weight ratios were also observed in both sexes at 1750 ppm test article. In light of these systemic effects, the high dose level of 1750 ppm is considered to be an adequate dose for assessing the carcinogenic potential of acetochlor in rats.

2. Seventy-Eight Week Carcinogenicity Study in Mice (Attachment C)

Reference: Amyes, S.J., 1989. SC-5676: 78 Week Feeding Study in CD-1 Mice. Study # 87/SUC0012/0702. Life Science Research, Ltd., Suffolk, England. MRID # 415651-19.

Technical SC-5676 was administered in the diet to male and female CD-1 mice for 78 weeks at dietary levels of 0 ppm, 10 ppm (1.1 mg/kg/day active ingredient - males; 1.4 mg/kg/day active ingredient - females), 100 ppm (11 mg/kg/day active ingredient - males; 13 mg/kg/day active ingredient - females), and 1000 ppm (116 mg/kg/day active ingredient - males; 135 mg/kg/day active ingredient - females). An increased incidence of pulmonary adenomas was observed in male and female mice, as shown in the following table (Table 2):

TABLE 2
Incidence of Neoplastic Lesions in Male and Female Mice Given Dietary SC-5676 for 78 Weeks[a]

	Males				Females			
Dose (ppm)	0	10	100	1000	0	10	100	1000
No. animals examined:	50	50	50	50	50	50	50	50
Lungs								
pulmonary adenoma	5(10)[c]	4(8)	11(22)	12(24)	1(2)[c]	3(6)	5(10)	7(14)[b]
pulmonary carcinoma	5(10)	4(8)	3(6)	4(8)	4(8)	0(0)	2(4)	4(8)
adenoma + carcinoma	10(20)[c]	7(14)	14(28)	16(32)	5(10)[c]	3(6)	7(14)	11(22)

[a]data taken from Table 24 of registrant report.

[b]$p < 0.05$ vs control by Fisher's exact test.

[c]$p < 0.05$ vs control by Cochran-Armitage test for significant trend.

As shown in table 2, a signficant trend of increase in pulmonary adenomas was observed in both male and female mice. In addition, the incidence of pulmonary adenomas in female mice from the 1000 ppm dose group was significantly different vs control. A significant increase in this tumor type was not found in male mice at the 1000 ppm dose level.

A dose adequate for the assessment of carcinogenicity of acetochlor in mice was not achieved in this study. However, review of a six-week range-finding study in mice with acetochlor showed decreases in body weight gain of 9% and 12% at 600 ppm and 1200 ppm Acetochlor, respectively for male mice. In female mice from this study, a significant decrease in body weight gain (21%) was not observed until the 2400 ppm dose level. Thus, based upon the results of the range-finding study, a dose adequate for the assessment of carcinogenicity of acetochlor can be considered to have been achieved for male mice, but not for female mice.

C. Additional Toxicology Data

1. Chronic Toxicity in Dogs (Attachment D)

Reference: Broadmeadow, A., 1988. SC-5676: Toxicity Study by Oral (Capsule) Administration to Beagle Dogs for 52 Weeks. Study # 88/SUC018/0136. Life Sciences Research, Ltd. MRID # 415651-18.

SC-5676 was administered to male and female beagle dogs by gelatin capsule for 52 weeks at dose levels of 0, 2.0, 10.0, and 50.0 mg/kg/day. Significant neurological effects were evident at the high dose level. These included abnormal head movements, stiffness and rigidity of the hindlimbs, ataxia, tremor, depressed righting, hopping, and flexor reflexes, and exaggerated tonic neck reflex. Two of five males and four of five females in the high dose group were killed between weeks 39 and 51 due to marked ataxia. Examination of the brains of these dogs for histopathologic changes showed degeneration of the granular layer in the deeper parts of the vermis cerebellum. In addition, the two males and two of the four females were also observed with depletion of Purkinje cells in areas adjacent to the granular cell degeneration. In dogs surviving to the end of treatment, granular layer degeneration and Purkinje cell depletion were observed in two male dogs. No significant inhibition of brain and plasma cholinesterase was observed after 52 weeks of treatment.

2. Mutagenicity (Attachments E, F, G, H)

a) Reference: Challander, R.D. and priestley, K.P., 1989. Acetochlor: An Evaluation in the Salmonella Mutation Assay. Study # YV2370/VV2423. ICI Central Toxicology Laboratory, Cheshire, UK. MRID # 415651-21.

Acetochlor induced a reproducible, positive, mutagenic response in strain TA1538 of *Salmonella typhimurium* with metabolic activation at 1000 µg/plate (less than 2x background mutation but significant at $p < 0.05$). Significant increases in the number of revertant colonies were not induced in strains TA1535, TA1537, TA98, and TA100.

b) Reference: Randall, V., 1989. Acetochlor: An evaluation in the Mouse Micronucleus Test. Study # SM0339. ICI Central Toxicology Laboratory, Cheshire, U.K. MRID # 415651-23.

Acetochlor was not clastogenic in the mouse micronucleus test at the doses tested (898 and 1436 mg/kg in males; 1075 and 1719 mg/kg in females). This study was classified as unacceptable as additional information was requested in order to upgrade this study.

c) Reference: Howard, C.A., 1989. An Evaluation of the In Vitro Cytogenetic Assay with Acetochlor in Human Lymphocytes. Study # SV0336. ICI Central Toxicology Laboratory, Cheshire, U.K. MRID # 415651-22.

Acetochlor was clastogenic in cultured human lymphocytes in both the presence and absence of S9 mix at 100 µg/ml, and in the absence of S9 mix at 50 µg/ml.

d) Reference: Trueman, R.W., 1989. Acetochlor; Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes In Vivo. Study # SR0357. ICI Central Toxicology Laboratory, Cheshire, U.K. MRID # 415651-24.

Acetochlor induced a weak DNA repair (as measured by UDS) in rat hepatocytes derived from animals exposed *in vivo* at 2000 mg/kg (20 hour time point).

2. Metabolism (Attachment I)

Reference: Hawkins, D.R., Kirkpatrick, D., and Dean, G. Five reports:

[1]: Laboratory Project No. HRC/STR 18/88502, "The Biokinetics of 14-C Acetochlor After Oral Administration to Rats at a Nominal Level of 10 mg/kg."

[2]: Laboratory Project No. HRC/STR 18/89184, "The Biokinetics of 14-C Acetochlor After Oral Administration to Rats at a Nominal Level of 200 mg/kg."

[3]: Laboratory Project No. HRC/STR 18/89487, "The Distribution and Excretion of Radioactivity after Oral Administration of 14-C Acetochlor at 10 mg/kg to Rats Pre-treated with Non-Radiolabelled Acetochlor."

[4]: Laboratory Project No. HRC/STR 18/89603, "The Metabolism of 14-C Acetochlor in the Rat after Oral Administration."

[5]: Laboratory Project No. CTL/P/2809, "Acetochlor: Biotransformation Study in the Rat."

Disposition of 14-C acetochlor was examined in CD Sprague-Dawley rats at single oral doses of 10 and 200 mg/kg, and at 10 mg/kg x 14 days. Metabolites of acetochlor were characterized and identified in urine, feces, and bile. Acetochlor was well absorbed after oral administration at both 10 and 200 mg/kg. A majority of a radioactive dose (50-60%) was eliminated in male and female rats in urine after 24 hours, with a significant percentage (13-22%) in feces. The percentage in urine was decreased at 200 mg/kg aftyer 24 hours (40-50%), with an increase in the percentage in feces (26-37%). Repeated oral dosing at 10 mg/kg had no significant effect on disposition of acetochlor. Tissue concentrations after 5 days were highest in those tissues well-perfused with blood, due apparently to the avid binding of 14-C acetochlor derived radioactivity to red blood cells (blood: plasma ratio = or > 100).The major biotransformation product in urine at 10 and 200 mg/kg was the mercapturic acid conjugate of acetochlor after removal of the ethoxymethyl side chain. Glucuronide and glutathione conjugates of acetochlor were identified in bile, with the glucuronide conjugate as the major metabolite in bile. Fecal metabolites were complex and difficult to identify. Enterohepatic recirculation of acetochlor was suggested from these studies.

3. Reproductive and Developmental Studies

Reference: Brooker, A.J, Stubbs, A., and John, D.M., 1989. Acetochlor: Teratogenicity Study in the Rat. Study # RR 0431. Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire. MRID # 415920-05.

The developmental toxicity of acetochlor was assessed by oral administration of acetochlor to pregnant female rats on gestation days 6 through 15, inclusive, at doses of 0, 40, 150, and 600 mg/kg/day. Maternal toxicity was evident at the high dose (600 mg/kg/day) in the form of clinical signs, and reduced body weight gain and food consumption. Additional data are needed in order to assign a Maternal and Developmental NOEL and LEL.

Reference: A.J, Stubbs, A., and John, D.M., 1989. Acetochlor: Teratogenicity Study in the Rabbit. Study # RB 0432. Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire. MRID # 415920-06.

The developmental toxicity of acetochlor was assessed by oral administration of acetochlor to pregnant New Zealand White Rabbitson gestation days 6 through 18, inclusive, at doses of 0, 30, 100, and 300 mg/kg/day. Additional data are required in order to determine the Maternal and Developmental NOEL and LEL.

Reference: Willoughby, C.R. SC-5676: Effects Upon Reproductive Performance of Rats Treated Continuously Throughout Two Successive Generations. Study # 89/0414. Life Science Research, Ltd., Suffolk, England. MRID # 415651-20.

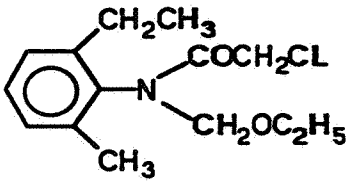
SC-5676 was administered to groups of male and female CD rats in the diet at dose levels of 18 ppm (1.6 mg/kg/day), 175 ppm (21 mg/kg/day) and 1750 ppm (160 mg/kg/day). Systemic toxicity was observed in high dose parental males and females, and consisted of reductions in body weight, food consumption, and increases in relative organ weights.

Reproductive performance and the rate of physical development of offspring were not affected by administration of SC-5676 in the diet. However, compound-related reductions in lactational day 21 body weight and total body weight gain during lactation were observed in high-dose pups from both generations.

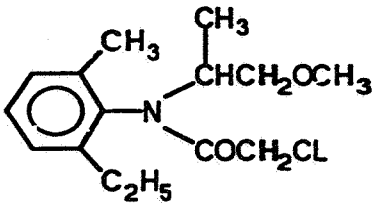
Parental toxicity NOEL = 175 ppm; Parental toxicity LEL = 1750 ppm.
Reproductive toxicity NOEL = 175 ppm; Reproductive toxicity LEL = 1750 ppm.

D. Structure-Activity Considerations

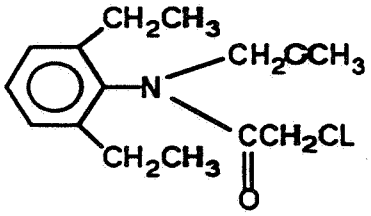
Acetochlor is structurally related to Metolachlor, Alachlor, Allidochlor, Butachlor, Propachlor, and SAN 582H, as illustrated below:



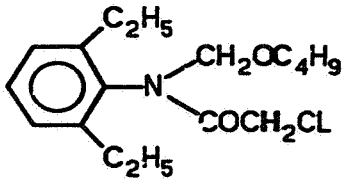
Acetochlor



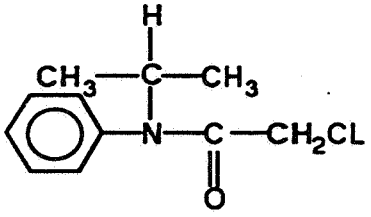
Metolachlor



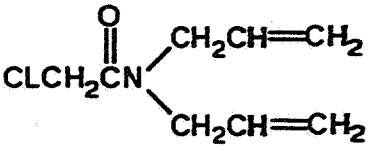
Alachlor



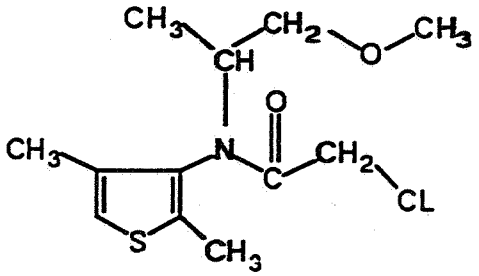
Butachlor



Propachlor



Allidochlor



SAN 582H

Alachlor is carcinogenic 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, an increased incidence of liver tumors was observed at 260 mg/kg in females. Alachlor gave a positive mutagenic response in one Ames assay (negative in 4 others), and in a DNA damage/repair (UDS) assay. Negative findings were reported from other bacterial assays, *in vitro* cytogenetics, HGPRT assay, and microsome plate incorporation. The Peer Review Committee has classified Alachlor as a B2 carcinogen and Alachlor has undergone Special Review (PD4 has been completed).

Butachlor is carcinogenic in rats. In a dietary administration study (interim report only, dated 1982), stomach tumors were induced at 3000 ppm (150 mg/kg) in females. Butachlor was found to be weakly mutagenic in one Ames assay, and negative in a rec assay and reversion. 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 in females (neoplastic nodules and carcinomas, combined) at 150 mg/kg.

Allidochlor has no acceptable chronic or mutagenicity studies to support the chemical (all IBT). The Peer Review Committee has not reviewed this chemical.

Propachlor, in a two year chronic toxicity/carcinogenicity study in rats showed evidence of an increased incidence of thyroid and ovarian neoplasia; however, the study did not use high enough dose levels to adequately assess the carcinogenic potential of Propachlor. A carcinogenicity study in mice also used doses below those necessary to adequately assess the carcinogenicity of Propachlor in mice. Propachlor was not mutagenic in a chromosome aberration assay, cytogenetic assay, gene mutation test, and two UDS assays.

SAN 582H, in a chronic toxicity/carcinogenicity study in rats, was found to cause increased incidence of benign tumors of the liver in male rats at 700 and 1500 ppm. In female rats, benign tubular adenomas of the ovary were observed in increased incidence at 1500 ppm. In a 94 week dietary administration study in mice, no increase in the incidence of treated mice with benign or malignant tumors was observed.

SAN 582H was not mutagenic in the Ames Salmonella assay, but caused positive UDS activity at dose levels well below the cytotoxic level in one study. A second UDS study showed that SAN 582H did not induce any significant increase in net nuclear grain counts. Other studies on the mutagenicity of SAN 582H (a third UDS assay, in vitro transformation of BALB/3T3 cells with S9 activation, and in vitro micronucleus test in mouse bone marrow) did not show any mutagenic effects, but were all classified as unacceptable by the Agency.

E. Weight of Evidence Considerations:

The Committee is asked to consider the following regarding toxicology data on Acetochlor in a weight-of-evidence determination of carcinogenic potential:

1. In the rat chronic toxicity/carcinogenicity study, acetochlor was associated with a significant increase in adenomas of the nasal epithelium at a dose of 1750 ppm (79.6 mg/kg/day a.i.) in both male and female rats. Carcinoma of the nasal epithelium was also observed in 2 male and 1 female rat at the 1750 ppm dose level, but not at lower doses. A significant positive trend for the incidence of thyroid follicular cell adenomas was also observed in female rats at the 1750 ppm dose level. In male rats, a similar incidence of thyroid follicular cell adenomas was found at the 1750 ppm dose, but was not statistically significant from control, but nonetheless related to treatment with acetochlor.

2. Significant increases in the incidence of nasal epithelial hyperplasia, kidney pelvic hyperplasia, and degeneration of the outer retinal nuclear layer were observed in both male and female rats at the 1750 ppm dose level. The presence of nasal epithelial hyperplasia and adenomas occurred together in 13 male rats at the 1750 ppm dose level, and in 9 female rats at the 1750 ppm dose level.

2. In the 78 week carcinogenicity study in mice, a significant increase in the incidence of pulmonary adenomas was observed in female mice at the 1000 ppm dose level, as well as a significant positive trend for the increase in this tumor type. An increased incidence of pulmonary adenomas was observed in male mice from the 100 and 1000 ppm dose groups, although statistical significance was not achieved for this sex.

3. In the Ames Salmonella assay, acetochlor induced a reproducible, positive, mutagenic response in strain TA1538 of *Salmonella typhimurium* at 1000 µg/plate. In the unscheduled DNA synthesis assay, acetochlor induced a weak DNA repair response in rat hepatocytes from animals exposed at 2000 mg/kg. In vitro cytogenetic experiments with human lymphocytes showed that acetochlor was clastogenic in both the presence and absence of S9 mix at 100 µg/ml, and in the absence of S9 mix at 50 µg/ml. Acetochlor was not clastogenic in the mouse micronucleus test in male or female mice.

4. Acetochlor has been previously classified by the Peer Review Committee as a Group B2 Carcinogen (probable human carcinogen). In addition, the structurally related compound Alachlor has also been given a B2 classification for carcinogenicity. The classification of acetochlor as a Group B2 carcinogen was based upon data submitted by Monsanto Chemical Company (see Attachment A), whose results from carcinogenicity testing of acetochlor are similar to those results obtained by ICI.

5/31/89

(13)

Attachment A 008728

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

FILE COPY

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 evalmate 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 masal turbinate papillary adencmas im male and female Albino rats given 1000 ppm im 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 _____

14

(14)

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Robert Beliles

Lynnard Slaughter

Marcia Van Gemert

William Sette

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

Stephen C. Dapson

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

Diane Beal

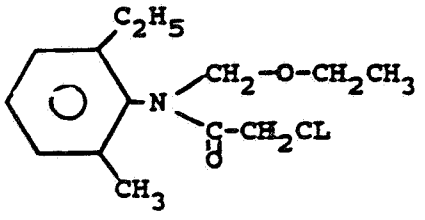
Richard Hill —

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, amd 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= 0.1124	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

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
Observations:					
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.

(23) Attachment B 008728

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Reviewed by: Timothy F. McMahon, Ph.D. 7/3/91
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 7/9/91 008478
Section I, Toxicology Branch II (H7509C)

Data Evaluation Report

Study type: Combined Carcinogenicity/Chronic Toxicity - rats
Guideline: 83-5

EPA ID Numbers: MRID number: 415920-04
Caswell No: 003B-ICI
HED Project Nos: 0-1999 –

Test material: SC-5676

Synonyms: 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide; Acetochlor

Study number(s): 88/SUC017/0348

Sponsor: ICI Central Toxicology Laboratory
Macclesfield
Cheshire, UK

Testing Facility: Life Science Research, Ltd.
Suffolk, England

Title of report: SC-5676: Combined Oncogenicity and Toxicity Study in Dietary Administration to CD Rats for 104 Weeks

Author(s): D.M. Virgo (senior author); Alan Broadmeadow (study director)

Study Completed: March 18, 1988

Conclusions:

Technical SC-5676 was administered to male and female rats in the diet for 104 weeks at doses of 0, 18, 175, and 1750 ppm (0, 0.8, 7.9, and 79.6 mg/kg/day active ingredient). In males and females,systemic toxicity in the form of reduced body weight gain, decreased food efficiency, ophthalmologic abnormalities, elevated GGT and cholesterol, and increased organ:body weight ratios were evident at the 1750ppm dose level, establishing this as a maximum tolerated dose for the study. Tumorigenic responses were observed in both sexes from administration of

1750ppm SC-5676. These included a significant increase in the incidence of nasal epithelial adenomas and thyroid follicular cell adenomas. Nasal carcinomas were observed in a total of 3 rats (2 males and 1 female). Rare tumors in the form of chondroma of the femur and basal cell tumors of the stomach were also observed. Non-neoplastic histopathology in the kidney, retina, pancreas, and nasal epithelium was also increased at the 1750ppm dose level.

The data in this study support the conclusion of limited evidence of carcinogenicity for technical SC-5676, based upon the occurrence of increased incidence of benign thyroid follicular cell tumors and benign and malignant nasal tumors only in high dose male and female rats, and the occurrence of benign chondroma and basal cell tumors in male and female rats.

The No Observed Effect Level (NOEL) = 175 ppm

The Lowest Observed Effect Level (LEL) = 1750 ppm (males and females; decreased body weight gain, decreased food efficiency, increased organ:body weight ratios, increased plasma GGT and cholesterol).

The Maximum Tolerated Dose (MTD) = 1750 ppm (males and females; decreased body weight gain).

Classification: Core Minimum

This study satisfies the guideline requirements (83-5) for a combined carcinogenicity/ chronic toxicity study in rats.

I. MATERIALS AND METHODS

A. Test Material

SC-5676; description:dark brown viscous liquid oil (batch #s 1 and 3); Purity (by pre-study analysis provided by sponsor: 91.0% [page 285 of report]).

Test article was stated as stable under the storage conditions employed. This was verified from analysis of samples of test material obtained prior to and following the study by ICI Western Research Laboratories, Richmond, California. Results of this analysis (pages 285-286) demonstrated the stability of test material under the storage conditions employed by the performing laboratory.Test material was found to be stable in rodent diet when kept at -20 °C, but was shown to have limited shelf life (between 8-11 days; page 292 of report). Thus, test diets were prepared weekly.

B. Test Animals

Two hundred ninety four male and 295 female CD rats (remote Sprague-Dawley origin) Source: Charles River U.K. Limited, Kent, England. Age: approximately 4-5 weeks old upon receipt. Weight range (at time of dosing): males, 108-170g; females, 98-153g.

C. Animal Husbandry

A total of 589 rats (294 males and 295 females) were employed in this study. On arrival, rats were assigned to treatment groups at random and individually identified. Health status of the rats was assessed daily during the 9 day acclimation period prior to commencement of dosing. During the first 54 weeks of treatment, rats were housed in polypropylene cages measuring 38 x 25 x 18cm. From week 55 onward, rats were housed in similar cages with dimensions of 45 x 28 x 20cm. into suspended cages with wire mesh floors so that each cage contained 5 rats of the same sex.

All rats had free access to food (Labsure Laboratory Animal Diet No. 2, Cambridgeshire) which was ground by the manufacturer. Water in polyethylene bottles was also supplied *ad libitum*. Food and water were withheld only overnight preceeding blood or urine collection. Animals were housed in temperature (18 - 25 °C) and humidity (40 - 70%) controlled rooms, and permanent daily recordings were made of these parameters. A 12 hour light/dark cycle was employed. No significant deviations in these parameters was observed during the study.

Cages were dispersed so that possible environmental influences arising from their distribution were equilibrated.

D. Dietary Mixtures

SC-5676 was administered by admixture with the diet in powdered form. Dietary pre-mixes were prepared weekly by incorporation of a weighed quantity of SC-5676 into untreated test diet. This premix was stirred for

20 minutes in a Hobart A200 mixer, and was then further diluted with test diet to give a final concentration of technical material of 1750ppm. Mixing at this stage was continued for 15 minutes in a Gardner 50L mixer. This diet was used for the high dose group, while serial dilution of this prepared diet was performed to attain the dosing concentrations of test article for other treatment groups.

D. Stability and Homogeneity

Stability of dietary test mixtures was performed on trial diets prior to study initiation. A trial dietary mixture of 18ppm was prepared and sampled for analysis prior to the study. Stability was checked after storing the diet at room temperature for 7, 10, and 14 days, as well as after storage for four days at -20 °C followed by 3 and 11 days storage at room temperature. Results of this analysis (page292 of report) showed a usable shelf life of between 10-11 days when stored at room temperature (i.e. found concentration within 10% of nominal). No alteration in test diet concentration resulted from storage at -20 °C. Therefore, diets were prepared weekly, and each prepared batch was divided into two batches, of which the first was used for days 1-4 of the week, and the second batch stored at -20 °C until used on the last 3 treatment days of the week.

Representative samples (100g) of test diets were taken at weeks 1, 2, 3, and 4, and at four week intervals thereafter to assess achieved concentration of test diet over the course of the study. Results of analysis for achieved concentration of test article (page 301 of report) showed the following results:

	Range (% nominal)	Mean (% nominal)
Group 2 (low dose):	92-114	101± 5.7
Group 3 (mid dose):	87-109	97 ± 5.3
Group 4 (high dose):	84-107	98 ± 5.4

Homogeneity of dietary test mixtures at the low and high dose level was conducted in representative samples taken from six positions in the mixer. Results of this analysis (page 291 of report) showed that the concentration of test material at the six positions sampled for each dose level was within 10% of the nominal concentration for each dose level.

E. Experimental Design and Dosing

Rats were assigned to one of eight dose groups. Tumorigenic potential of SC-5676 was assessed in groups of fifty males and 50 females who received test chemical for 104 weeks in the diet at the following four dose levels:

Group #	Dose Level (ppm)	No. of Rats male	female
1	0	50	50
2	18	50	50
3	175	50	50
4	1750	50	50

In addition to the above, a further ten males and females were treated at the 18 and 175ppm dose levels for 52 weeks, as were a group of 20 males and females at the 0 and 1750ppm dose levels.

Dose levels for this study were selected based upon results obtained from a subchronic oral toxicity study in rats (MRID# 415920-04) in which dietary levels of 0, 20, 200, and 2000ppm test article were used.

It was not stated whether rats were housed within the same animal room for the entire course of the study. SC-5676 was administered continuously in the diet to treatment groups. Control rats received untreated diet of the same batch and at the same frequency as test article treated rats.

An additional 10 male and female rats were selected from the total rats ordered and used as veterinary controls to monitor the potential outbreak of disease during the study. According to the registrant (page 18), no outbreak of disease occurred during the study.

E. Statistical Analysis

A copy of the statistical procedures employed in this study is attached to this review.

F. Compliance

A signed statement of no data confidentiality claims was provided.

A signed statement of GLP compliance was provided.

A signed statement of quality assurance was provided.

A signed statement of flagging studies for potential adverse effects was provided.

II. OBSERVATIONS AND RESULTS

A. **Mortality**

Rats were observed early each morning and again in the afternoon for signs of mortality and/or moribundity.

On weekends, the second observation time was made at midday. Any animal showing signs of debility or intoxication was killed by CO_2 asphyxiation and subjected to detailed macroscopic examination. Tissues were preserved in 10% buffered formalin where possible.

Cumulative mortality in male and female rats is summarized in the following Table (Table 1):

TABLE 1

Cumulative Mortality in Rats Given SC-5676 in the Diet for 104 Weeks[a]

Week of Study	Males 0	Males 18	Males 175	Males 1750	Females 0	Females 18	Females 175	Females 1750
1	0(0)[b]	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
13	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(2)
26	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	3(6)
52	1(2)	0(0)	1(2)	0(0)	0(0)	2(4)	1(0)	5(10)
78	13(26)	10(20)	10(20)	8(16)	10(20)	11(22)	9(18)	10(20)
104	39(78)	38(76)	41(82)	28(56)	30(60)	30(60)	30(60)	32(64)

[a]data calculated from pages 55-57 and 318-336 of registrant report.

[b]cumulative mortality (percent mortality)

The registrant stated (page 31 of report) that a total of 147 males and 133 females died or were killed during the study. In males, mortality was from the main dose group only; in females, 128 rats from the main dose group died or were killed during the study, and 5 females from the interim sacrifice group died or were killed.

No significant differences in mortality were observed between control and treated rats of either sex over the duration of treatment. However, mortality among control male rats and those in the 175ppm dose group was slightly higher than expected. The registrant stated that the slightly higher mortality could not be associated with treatment. The higher than expected incidence of mortality in control male rats deserves additional explanation from the registrant.

B. Body Weights

Rats were weighed on the date of study initiation, weekly thereafter for the next 14 weeks, bi-weekly thereafter, and again before necropsy. Group mean body weights at selected times are presented in Table 2.

TABLE 2

Group Mean Body Weights in Male and Female Rats Given SC-5676 in the Diet for 104 Weeks[a]

Week of Study	Males (g)				Females (g)			
	0	18	175	1750	0	18	175	1750
0	143	145	149	146	129	126	126	126
1	202	202	206	196	158	154	155	151
13	540	524	538	496	278	274	277	255
26	656	635	654	588	315	313	316	281
52	811	774	810	702	410	410	406	340
104	870	857	820	773	610	625	606	448

[a]data taken from Table 4a, pages 66-69 of registrant report.

Body weight in male and female rats at the 1750ppm dose level was decreased relative to control rats throughout the study. The percentage decrease in absolute body weight was progressive in both sexes up to week 52. At week 104, body weight in male rats was decreased 12% vs controls, while in female rats this percentage was higher (decreased 27% vs control). Body weight in male rats at the 18ppm dose level was also decreased relative to control from week 13 of the study. This was apparently not related to treatment, as body weights in the 175ppm dose group of males were not affected.

Effects of test article treatment on body weight gain in male and female rats are summarized in the following Table (Table 3):

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TABLE 3

Group Mean Body Weight Gains in Male and Female Rats Given SC-5676 in the Diet for 104 Weeks[a]

	Males				Females			
	0	18	175	1750	0	18	175	1750
Body weight (week 0)	143	145	149	146	129	126	126	126
Weight gain (grams):								
0-13	396	379[b]	390	349[c]	150	147	151	129[c]
%control	-	96	98	88	-	98	101	86
0-52	668	629[b]	662	555[c]	281	283	279	213[c]
%control	-	94	99	83	-	101	99	76
0-104	727[d]	712[d]	671[d]	627[a,d]	481	499	479	322[c]
%control	-	98[d]	92[d]	86[d]	-	104	100	67

[a]data taken from Table 4b, pages 70-71 of registrant report.

[b]significantly different vs control ($p < 0.05$)

[c]significantly different vs control ($p < 0.001$)

[d]data recalculated from Table 4a, pages 66-69 of registrant report

As shown above, body weight gain was significantly decreased in both male and female rats in the 1750ppm dose groups, and was consistently affected throughout the study. Weight gain in male rats in the 1750ppm dose group was decreased by 12% over the 0-13 week period of the study, and in female rats was affected similarly (14% decrease). Weight gain in male rats from weeks 0-52 was decreased 17% vs control, and in female rats was affected to a slightly greater degree (decrease of 24% vs control). For the entire study period (weeks 0-104), body weight gain was decreased 14% in male rats vs control, and was decreased 33% in females vs control. Thus, effects of test article treatment at 1750ppm on body weight gain appeared slightly greater in female than male rats.

There were significant effects on body weight gain in male rats in the 18ppm dose group from weeks 0-13 (4%

decrease vs control) and 0-52 (6% decrease vs control). These changes were statistically significant at the 0.05 level of probability, but were not likely related to treatment with test article, as no effect was observed at the next highest dose level (175ppm). However, it should be noted that overall weight gain in male rats at the 175ppm dose level was decreased 8% vs control when data were recalculated. Thus, there appears to be a dose-related trend in body weight gain decrease for both male and female rats.

C. **Food Consumption and Efficiency**

Food consumption was calculated for each rat on a weekly basis by measurement of the amount of food given and that remaining in the hoppers. Scattered food was estimated twice each week and included in the food residue for calculation of the food consumption. Food efficiency was calculated as the weight of food consumed per unit gain in body weight. Food efficiency was calculated for the first 14 weeks of treatment and for 13 week periods until study termination.

Group mean food consumption data are presented in Table 4 below:

TABLE 4

Group Mean Food Consumption in Male and Female Rats Given SC-5676 in the Diet for 104 Weeks[a]

	Food consumption (g/rat/week)							
Weeks	Males				Females			
of Study	0	18	175	1750	0	18	175	1750
1-13	190	189	190	181[d]	133	133	135	128[b]
%control	-	99	100	95	-	100	101	96
1-52	186	181	183	173[d]	134	133	133	126[d]
%control	-	97	98	93	-	99	99	94
1-78	198	189[c]	190[b]	178[d]	143	140	141	132[d]
% control	-	95	96	90	-	98	99	92
1-104	204	187[c]	188[c]	177[d]	145	145	146	133[c]
%control	-	92	92	87	-	100	101	92

[a]data from Table 3, pages 58-65 of registrant report.

[b]significantly different vs control ($p < 0.05$)

[c]significantly different vs control ($p < 0.01$)

[d]significantly different vs control ($p < 0.001$)

As shown in Table 4, group mean food consumption in male and female rats was affected primarily at the 1750pp dose level. Significant effects were noted in male rats continuously from weeks 0-97, and in female rats from weeks 104. From study week 0-13, food consumption was decreased by 5% in male rats vs control, and by 4% in female over this time period. From weeks 0-78, food consumption was decreased 10% overall in male rats, and 8% overall female rats. For the entire study period, food consumption was decreased 13% in male rats at the 1750ppm dose lev and 8% in female rats at this dose level.

Food efficiency (total food consumed / total weight gain) among male and female rats during the first 25 weeks of the study is shown below in the following Table (Table 5):

TABLE 5

Group Mean Food Efficiency in Male and Female Rats Given SC-5676 in the Diet for 104 Weeks[a]

Weeks	Food efficiency (g food/g body weight gain)							
	Males				Females			
of Study	0	18	175	1750	0	18	175	1750
1-7	4.9	5.1	5.0	5.3	9.3	9.1	9.1	10.2
7-14	13.3	13.8	13.9	15.5	34.2	29.5	27.8	44.9
1-14	9.1	9.5	9.5	10.4	21.7	19.3	18.4	27.5

[a]data recalculated from Table 5, page 72 of registrant report.

From the above recalculated data, food efficiency was apparently affected slightly in males from the 1750ppm dose group from weeks 1-14 of the study (10.4 vs 9.1). A similar effect was observed in female rats from the 1750ppm dose group, except that the magnitude of the effect appeared somewhat greater at this dose (27.5 vs 21.7). This pattern of change appeared to extend throughout the study period in both sexes, although it is to be

noted that overall food efficiency for the study duration was unaffected in male rats at the 1750 ppm dose level vs control (29.2 vs 29.5), while in female rats food efficiency at the 1750ppm dose level was different from control (43.0 vs 31.4, page 73 of report).

The combined observations of decreased body weight gain, food consumption, and food efficiency supports the conclusion of test article toxicity. While food consumption and body weight gain decreases in male rats are closely parallel for the study duration (12% decrease in body weight, 13% decrease in food consumption), food consumption was decreased 8% in female rats for weeks 0-104 of the study, but body weight gain was decreased 33%, as reflected in the overall decreased food efficiency in female rats, both for weeks 1-14 and for the study duration. Thus, treatment with test article at 1750ppm may be more toxic to female rats as shown by these data.

D. Intake of SC-5676

The group mean intake of SC-5676 for male and female rats over the course of the study is summarized in the following table (Table 6):

TABLE 6

Group Mean Achieved Dosage of SC-5676 in Male and Female Rats Over 104 Weeks[a]

Dose Group (ppm)	Nominal mg/kg/day (ppm/20)	Average Intake (weeks 1-104) (mg/kg/day) males	females
0	0	0	0
18	0.9	0.67	0.88
175	8.75	6.37	8.53
1750	87.5	66.9	92.1

[a]data taken from Table 6, page 77 of registrant report.

Achieved dosage of SC-5676 was based upon concentration of the active ingredient in dietary formulations (91%). Thus, the actual nominal dose levels received by rats in this study were 0, 16, 159, and 1589 ppm (0, 0.8, 7.96, and 79.6 mg/kg/day). Group mean achieved intake of SC-5676 was between 80-84% of nominal for male rats in all dose groups, while an achieved intake of between 107-115% was calculated in female rats in all dose groups.

Note: The approximately 20% difference in achieved intake of SC-5676 between male and female rats could explain in part the apparently greater effects of test article on female rats at the 1750ppm dose level.

E. **Ophthalmoscopic Examination**

Both eyes of all rats were examined prior to initiation of the study by means of a Fisons Binocular Indirect Ophthalmoscope after instillation of 0.5% tropicaimide. Nine males and 12 females with relatively severe ocular abnormalities were discarded prior to the study and replaced with spare animals bearing no abnormalities. During the course of treatment, surviving rats were examined at week 23, 49, 76, and 101. An additional examination was performed on all surviving males after 97 weeks of treatment.

As stated by the registrant (page 29 of report), observations of hyaloid remnants and persistent hyaloid arteries were omitted from the results as they were considered normal developmental structures and there was no indication of a treatment related disturbance.

Ophthalmic examination after 76 weeks of treatment revealed a high proportion (24 of 43 rats, 55%) of female rats in the 1750ppm dose group with hyperreflection ($p < 0.001$ vs controls). This ocular lesion was also present in female rats at 101 weeks (15 of 25 rats, 60%; $p < 0.05$ vs control). In the majority of affected female rats this lesion was bilateral. The incidence of this lesion in female rats at the 18 and 175ppm dose levels was not significantly different from control values.

In male rats at the 1750ppm dose level, foci or plaques in the vitreous or on the posterior capsule of the lens (page 32, section 5.7 of report) were observed in increased incidence beginning at 76 weeks, and were observed in increased incidence in this dose group at subsequent examination times. At 76 weeks, 12 of 42 male rats in the 1750ppm dose group (28%; $p < 0.05$ vs control) were observed with this lesion, and 10 of 26 remaining rats (38%) were observed with this lesion at 97 weeks. At 101 weeks, 9 of 25 remaining rats (36%) were observed with this lesion. In most cases, this lesion was bilateral. Male rats at the other dose levels were not observed with an increased incidence of this lesion when compared to control values.

With the exception of those lesions described above, no other ocular lesions were attributed to administration of SC-5676 at any dose level in either male or female rats.

F. **Clinical Signs and Pathology**

Examination of rats for any sign of ill health or systemic toxicity was recorded twice daily. Examination for palpable masses was made once a week. The location, size, consistency, time of first observation and subsequent course were noted for each palpable mass.

Blood samples were obtained from the orbital sinus of 20 male and 20 female rats with the highest identity numbers from each dose group. Blood was withdrawn at weeks 13, 24, 50, 78, and 102 under light ether anesthesia following an overnight fast.

Collected blood was mixed with EDTA anticoagulant for hematological examination, and with heparin anticoagulant for biochemical measurements. An Ortho ELT-8ds automated hematology analyzer was utilized for hematology measurements.

a) **Hematology**

The following CHECKED hematological parameters were examined:

_x_ total leucocyte count*	_-_ total plasma protein*
_x_ erythrocyte count*	_x_ leukocyte differential*
_x_ hemoglobin*	_x_ mean corpuscular HGB
_-_ hematocrit*	_x_ mean corpusc. HGB conc.
_x_ platelet count	_-_ mean corpusc. volume
_x_ packed cell volume	_-_ methemoglobin
_-_ reticulocyte count	_-_ sulfa-hemoglobin

*EPA guideline requirement "-" not analyzed

13 weeks

Male rats in the 1750 ppm dose group showed an increased total white blood cell count vs control (17.8 vs 15.4 1000 cells/cm^2, $p < 0.05$). However, no such effect was observed at the 1750ppm dose level. Female rats in the 1750ppm dose group showed decreases in platelet counts (579 vs 627 1000/cm^2 in control, $p < 0.05$) packed cell volume (45 vs 47% in control, $p < 0.05$), and an increase in mean corpuscular hemoglobin concentration 35 vs 34%, $p < 0.01$ vs control).

24 weeks

Hemoglobin concentration in male rats was significantly ($p < 0.05$) increased at the 18ppm dose level vs control, as was red blood cell number (8.93 vs 8.65 mil/cmm). Platelet number was observed to be low at the 175 and 1750ppm dose level (565 and 591 vs 647 1000/cmm); however, the control value had a high standard deviation (103) which was due to 2 abnormally high values. Thus, the intergroup differences observed at 175 and 1750ppm were not considered treatment related.

No significant hematological effects were observed in female rats at 24 weeks.

50 weeks

No significant hematologic effects were observed.

78 weeks

In male rats, no significant effects were observed.

In female rats, an increase in platelet count was observed at the 18ppm dose level vs control (638 vs 573 1000/cmm).

102 weeks

In male rats, mean corpuscular volume in the 1750ppm dose group was significantly ($p < 0.05$) decreased vs control (53 vs 57%). No other changes were reported as significant.

In female rats, mean corpuscular hemoglobin concentration was significantly increased at all doses vs control, and mean corpuscular volume was significantly ($p < 0.001$) decreased in the 1750ppm dose group vs control (56 vs 62cμ).

The registrant stated (page 33) that abnormally low erythrocyte characteristics (packed cell volumes, hemoglobin concentration, and erythrocyte counts) were reported after 50, 78, and 102 weeks of treatment, especially in males. While low but non-statistically significant values for these parameters were observed in male rats at 50 weeks in the 175 and 1750ppm dose groups, such a trend was not apparent at 78 or 102 weeks upon inspection of the data (Table 8c-8 pages 97-102 of report).

Note: In the subchronic toxicity study with acetochlor in rats (MRID# 415920-04), an apparent dose-related trend towards increased hemoglobin levels and decreased platelet levels in male rats was observed at 12 weeks, as was an increase in red blood cells in female rats. These changes were significant at the highest dose in this study (2000ppm). However, such changes are not apparent at 13 weeks in the present study.

b) **Clinical Chemistry:**

Blood samples were obtained for blood chemistry measurements at week 24, 50, 78 and 102 under the same conditions governing the sampling of blood for hematological analysis, except that lithium heparin was used as the anticoagulant. The following CHECKED parameters were measured:

_x_ glucose*
_-_ albumin*
_-_ globulin (calculated)
_-_ creatinine
_x_ total bilirubin*
_-_ direct bilirubin
_-_ indirect bilirubin
_x_ urea nitrogen*
_x_ total protein*
_x_ cholesterol*
_-_ triglycerides
_x_ electrophoretic protein fractions
_x_ AST(SGPT)*
_x_ ALT(SGOT)*
_x_ alkaline phosphatase
_x_ creatine phosphokinase*
_-_ lactate dehydrogenase
_-_ sorbitol dehydrogenase
_x_ gamma glutamyl trans-peptidase
_x_ ornithine carbamyl transferase

_x_ calcium*
_x_ inorganic phosphate*
_x_ sodium*
_x_ potassium*
_x_ chloride*

*EPA guideline requirement "-" not examined

Note: Measurement of albumin as recommended by the guidelines (83-5) was replaced by measurment of electrophoretic protein fractions.

Significant findings in blood chemistry measurements are summarized below for male and female rats (Table 7):

Table 7
Blood Chemistry in Male and Female Rats Administered SC-5676 in the Diet for 104 Weeks[a]

	Males				Females			
	0	18	175	1750	0	18	175	1750
24 Weeks								
GGT (iu/l)	2±1	2±1	2±1	4±1[b]	3±1	3±1	3±1	4±1
cholesterol (mg%)	77±27	83±18	88±17	91±10	99±10	99±21	92±26	105±23
50 Weeks								
GGT (iu/l)	2±1	1±2	1±1	3±2[b]	1±1	1±1	0±0	1±1
cholesterol (mg%)	85±31	80±22	84±23	90±27	90±33	98±21	86±26	106±36
AST(iu/l)	58±9	70±13[b]	67±16	72±13[b]	71±17	62±8	69±15	71±27
78 Weeks								
GGT (iu/l)	4±3	2±1	5±6	8±6[b]	0±0	0±1	0±0	0±1
cholesterol (mg%)	113±53	133±44	137±45	132±42	120±22	119±31	131±34	144±28
102 Weeks								
GGT (iu/l)	5±2	6±3	5±2	8±3[b]	2±1	3±2	2±1	3±2[b]
cholesterol (mg%)	115±20	149±82	136±57	180±67[b]	147±52	167±96	150±97	141±34

[a] data taken from Tables 9a-9d, pages 103-119 of report.

[b] significantly different vs controls, $p < 0.05$.

As summarized above, consistent and significant increases in GGT were observed in male rats in the 1750ppm dose group throughout the study. Blood cholesterol showed a similar trend, although the increase at 1750ppm was significant only at week 102.

In female rats, GGT was significantly increased in the 1750ppm dose group only at week 102, while blood cholesterol showed an apparent dose related increase up to week 78. However, this increase was not statistically significant over the course of the study for female rats.

c) **Urinalysis:**

Overnight urine samples were collected from the 10 males and females used for hematological analysis and blood chemistry when possible after 11, 23, 49, 77, and 101 weeks of treatment. Urine was collected from rats placed in individual metabolism cages under conditions of food and water deprivation (16 hours).

The following CHECKED parameters were examined:

_x_ appearance*	_x_ glucose*
_x_ volume*	_x_ pH
_x_ specific gravity*	_x_ bilirubin*
_x_ protein*	_x_ urobilinogen
_x_ ketone*	_x_ nitrate
_x_ blood*	___
_x_ sediment analysis*	___

*EPA guideline requirement "-" not examined

Note: Group mean values for urine volume, pH, and specific gravity are given in Tables 10a-10e, pages 119-123 of the registrant's report; values for the remaining urine parameters are found in Appendix 10a-10e, pages 964-983 of the report.

No apparent test article related effects on measured urinary parameters were reported during the course of this study.

G. **Macroscopic Observations** (Table 12a-12d, pages 132-163)

All rats were killed by carbon dioxide asphyxiation and subjected to gross necropsy. This procedure was performed at 52 weeks on all surviving rats in the satellite treatment groups, and at study termination (104 weeks) in all surviving rats in the main treatment groups.

Detailed examination of external features and orifices was made at necropsy, as well as examination of the neck and associated tissues, the cranial, thoracic, abdominal, and pelvic cavities and associated viscera, and the carcass. Abnormalities, interactions, and changes were recorded and tissues were preserved in fixative.

No treatment related macroscopic lesions were apparent either among those rats killed or dying during the

treatment period, or among those killed at 52 and 104 weeks of treatment.

H. **Organ Weights** (Tables 11a-11d, pages 124-131 of report).

Organs to be weighed were obtained from all animals dying or killed during the study and dissected free of fat and contiguous tissue before weighing. The following organs were weighed: adrenals, brain, heart, kidneys, liver, testes, and ovaries, prostate. Group mean and individual organ weights were provided. Organ/body weight ratios were also provided.

Absolute organ weights were unaffected at 52 weeks of treatment, as shown by the registrant in Tables 11a, pages 124-125 of the report. Organ:body weight ratios, however (Table 11b, pages 126-127 of report), were significantly affected for the brain, heart, kidneys, and liver of female rats at the 1750ppm dose level, and for the kidneys and liver of male rats at this dose level. Results are summarized below (Table 8):

Table 8

Organ:Body Weight Ratios at 52 Weeks in Male and Female Rats Administered SC-5676 in the Diet[a]

	Males				Females			
	0	18	175	1750	0	18	175	1750
brain	0.29±0.03	0.29±0.03	0.27±0.03	0.3±0.02	0.48±0.07	0.47±0.09	0.52±0.09	0.58±0.11[c]
heart	0.23±0.02	0.23±0.03	0.22±0.01	0.25±0.04	0.28±0.03	0.28±0.03	0.3±0.03	0.33±0.07[c]
kidneys	0.62±0.05	0.61±0.07	0.62±0.04	0.69±0.06[c]	0.67±0.1	0.65±0.11	0.71±0.03	0.82±0.09[d]
liver	3.19±0.7	3.16±0.28	3.30±0.24	3.56±0.4[b]	3.51±0.4	3.45±0.54	3.52±0.3	3.92±0.46[c]

[a]data taken from Table 11b, pages 126-127 of report.

[b]$p < 0.05$ vs control; [c]$p < 0.01$ vs control; [d]$p < 0.001$ vs control.

Absolute organ weights at terminal sacrifice (Table 11c, pages 128-129 of report) were not significantly altered in male rats except adrenal weight at the 1750ppm dose level, which decreased from 0.125±0.03g to 0.097±0.044g. In female rats, significant decreases in brain weight (1.92±0.1 vs 1.99±0.09g), heart weight (1.35±0.19 vs 1.61±0.27g), and liver weight (17.0±3.5 vs 20.1±4.1g) were observed at the 1750ppm dose

level.

Organ:body weight ratios were unaffected at 104 weeks except in the brain of female rats at the 1750ppm dose level, where the brain:body weight ratio increased from 0.34±0.06 in controls to 0.44±0.08 in the 1750ppm dose group.

I. Microscopic Observations

Samples of the following tissues were preserved in either 4% buffered formalin (all tissues except eyes) or Davidson's fixative (eyes and optic nerve). All macroscopically abnormal tissues were also preserved along with samples of normal tissue where appropriate.

Digestive	Respiratory	Urogenital
_x_ tongue	_x_ trachea	_x_ kidneys*
_x_ salivary glands*	_x_ lungs*	_x_ urinary bladder*
_x_ esophagus*	_x_ nasal cavity	_x_ testes*
_x_ stomach*		_x_ epididymides*
_x_ duodenum*	Cardiovascular	_x_ seminal vesicle*
_x_ jejunum*	_x_ aorta*	_x_ prostate
_x_ ileum*	_x_ heart*	_x_ ovaries
_x_ cecum*	_x_ bone marrow	_x_ uterus*
_x_ colon*	_x_ lymph nodes*	_x_ vagina
_x_ rectum*	_x_ spleen*	
_x_ liver*	_x_ thymus*	
_x_ pancreas*		
_x_ gall bladder*		

Neurologic	Glandular	Other
_x_ brain*	_x_ adrenals *	_x_ bone (femur)
_x_ peripheral nerve*	_x_ lacrimal gland	_x_ skeletal
_x_ spinal cord (3 levels)*	_x_ mammary gland	_x_ muscle
_x_ pituitary*	_x_ parathyroids*	_x_ skin*
_x_ eyes	_x_ thyroids*	_x_ all gross lesions*

*EPA guideline requirement

"-" not examined

Tissues were prepared for microscopic examination by embedding in paraffin wax, cutting thin sections (5µm), and staining with hematoxylin and eosin. Microscopic examination was performed on the above tissues from all rats in control and high dose groups sacrificed at 52 weeks, on all rats killed at 104 weeks, and on all decedent rats.

1a) Neoplastic and Non-Neoplastic Observations-52 Weeks (Interim Sacrifice Group)

Two female rats in the control group and 3 female rats in the 1750ppm dose group assigned to the 52 week sacrifice died before sacrifice. In these rats, there was no apparent effect of test article on tumor formation (Table 13A, page 164 of report). In those rats surviving the 52 week treatment period (Tables 13E and 13F, pages 193-202 of the report), increased incidences of nasal epithelial adenomas and nasal epithelial hyperplasia in both male and female rats at the 1750ppm dose level were observed, as summarized below (Table 9):

TABLE 9

Incidence of Neoplastic and Non-Neoplastic Lesions in Surviving Male and Female Rats Given Dietary SC-5676 for 52 Weeks (Interim Sacrifice Group)[a]

	Males				Females			
Dose (ppm)	0	18	175	1750	0	18	175	1750
Head								
No. of Animals Examined	20	10	10	20	18	9	10	16
Adenoma of Nasal Epithelium	0[b] (0)[c]	0 (0)	0 (0)	5 (25)[d]	0 (0)	0 (0)	0 (0)	8 (50)[f]
Hyperplasia of Nasal Epithelium	0 (0)	0 (0)	0 (0)	11 (55)[f]	0(0)	0(0)	0(0)	13(76)[f]

[a]data taken from Table13E and 13F, pages 193-202 of registrant report.

[b]number of rats with specified lesion.

[c]percentage of rats with specified lesion.

[d]$p < 0.05$ vs control; [f]$p < 0.001$ vs control

As shown in **Table 9, male and female rats at the 1750ppm dose level showed a significant increase in the incidence of nasal epithelial adenomas and nasal epithelial hyperplasia after 52 weeks of treatment. No other treatment related lesions at 52 weeks were reported.**

1b) Neoplastic and Non-Neoplastic Observations-104 Weeks (Terminal Sacrifice Group)

i. Rats Assigned to 104 Weeks of Treatment Dying or Killed During the 104 Week Treatment Period (Tables 13C and 13D, pages 168-192 of the report)

Adenoma of the nasal epithelium was observed in those rats dying or killed which had been assigned to the 104 week treatment period. This occurred solely in the 1750ppm dose group. In males, 17 of the 28 rats (60%)which died or were killed were found with this tumor type, while 18 of 31 female rats which died or were killed (58%)) were found with this tumor type. Carcinoma of the nasal epithelium was observed in 1 female rat at the 1750ppm dose level.

Benign chondroma of the femur was observed in 1 male rat from the 1750ppm dose group, as was benign basal cell tumor of the stomach in 1 male and 1 female from the 1750ppm dose group (Table 13C, pages 168-171 of report).

Non-neoplastic observations in those rats dying or killed during the 104 week treatment period included hyperplasia of the nasal epithelium (solely at the 1750ppm dose level in both sexes), purulent rhinitis (males only at the 1750ppm dose level), squamous metaplasia of the olfactory epithelium (males only at the 1750ppm dose level), pelvic epithelial hyperplasia in the kidney (dose related trend in both males and females), degeneration of the retinal outer nuclear layer in the eye (females in the 1750ppm dose group only), fatty infiltration of the pancreatic stroma (females in the 1750ppm dose group), and parafollicular hyperplasia of the cervical lymph nodes (dose-related trend in male rats). These findings are summarized below (Table 10):

TABLE 10

Incidence of Non-Neoplastic Lesions in Decedent Male and Female Rats Given Dietary SC-5676 for 104 Weeks[a]

	Males				Females			
Dose (ppm)	0	18	175	1750	0	18	175	1750
No. Animals Examined	40	37	41	28	30	31	34	31
Hyperplasia of Nasal Epithelium	0[b](0)[c]	0(0)	0(0)	17(60)[e]	0(0)	0(0)	0(0)	17(54)[e]
Purulent Rhinitis	0(0)	1(3)	3(7)	4(14)[d]	1(3)	1(3)	2(6)	1(3)
Metaplasia-Olfactory Epithelium	0(0)	0(0)	0(0)	4(14)[d]	0(0)	0(0)	1(3)	0(0)
Kidney								
No. animals examined	40	38	41	28	30	31	35	31
pelvic epithelial hyperplasia	5(12)	6(16)	9(22)	15(53)[e]	2(7)	4(13)	7(20)	8(26)
Eye								
No. animals examined	38	31	37	27	29	29	33	27
retinal outer nuclear layer degeneration	0(0)	0(0)	1(3)	3(11)	2(7)	2(7)	7(21)	11(40)[d]

Table 10, cont.

	Males				Females			
Dose (ppm)	0	18	175	1750	0	18	175	1750
Pancreas								
No. animals examined	40	37	41	27	30	31	35	31
stromal fatty infiltration	9(22)	6(16)	5(12)	2(7)	4(13)	7(22)	12(34)	13(42)[d]
Cervical lymph node								
No. animals examined	39	37	40	27	30	31	35	31
parafollicular hyperplasia	4(10)	5(13)	6(15)	9(33)[d]	1(3)	4(13)	6(17)	4(13)

[a]data taken from Table 13D, pages 172-192 of report.

[b]number of animals with specified lesion; [c]percent of animals with specified lesion

[d] $p < 0.05$ vs control; [e] $p < 0.001$ vs control.

As shown above, hyperplasia of the nasal epithelium was observed solely at the 1750ppm dose level in both sexes, while purulent rhinitis and metaplasia of the olfactory epithelium affected male rats only at the 1750ppm dose level. In contrast to these effects, pelvic hyperplasia of the kidney showed an apparent dose-related increase in both male and female rats, which was statistically significant only in male rats, although the incidence of this lesion increased from 7% in control females to 26% in 1750ppm dose group females.

Non-neoplastic lesions related to treatment with SC-5676 confined primarily to female rats included retinal outer nuclear layer degeneration (increased from 7% in controls to 40% at the 1750ppm dose level) and stromal fatty infiltration of the pancreas (increased from 13% in controls to 42% at the 1750ppm dose level). Parafollicular hyperplasia of cervical lymph nodes showed an apparent dose related increase in male rats, increasing from 10% in controls to 33% at the 1750ppm dose level.

Other changes stated by the registrant (page 36) included 2 cases of glandular hyperplasia with dystrophy and giant cell formation in the stomach of male rats from the 1750ppm dose group (reported as a rare finding), and a

higher incidence of females in the 1750ppm dose group which were in proestrous as evidenced by vaginal changes (page 186 of report).

ii. Rats Surviving the 104 Week Treatment Period (Terminal Sacrifice Group)

Neoplastic observations reported in rats which survivied the 104 week treatment period were confined to a significant increase in adenoma of the nasal epithelium of male and female rats at the 1750ppm dose level, the presence of a benign chondroma of the femur in 1 female rat at the 1750ppm dose level, and carcinoma of the nasal epithelium in 2 male rats from the 1750ppm dose group. Data are summarized by the registrant in Table 13G, pages 203-205 of the report, and are summarized in Table 11 below:

TABLE 11

Incidence of Neoplastic Lesions in Surviving Male and Female Rats Given Dietary SC-5676 for 104 Weeks (Terminal Sacrifice Group)[a]

	Males				Females			
Dose (ppm)	0	18	175	1750	0	18	175	1750
No. animals examined:	10	12	9	22	20	19	15	18
Adenoma of Nasal Epithelium	0[b] (0)[c]	0(0)	0(0)	13(59)[d]	0(0)	0(0)	0(0)	10(55)[e]
Carcinoma of Nasal Epithelium	0(0)	0(0)	0(0)	2(15)	0(0)	0(0)	0(0)	0(0)

[a] data from Table 13G, pages 203-205 of registrant report.

[b] number of rats with lesion; [c] percent of rats with lesion

[d] $p < 0.01$ vs control; [e] $p < 0.001$ vs control

Non-neoplastic findings in those rats surviving the 104 week treatment period are summarized by the reigistrant in Table 13H, pages 208-219 of the report, and also below (Table 12):

TABLE 12

Incidence of Non-Neoplastic Lesions in Surviving Male and Female Rats Given Dietary SC-5676 for 104 Weeks (Terminal Sacrifice Group)[a]

	Males				Females			
Dose (ppm)	0	18	175	1750	0	18	175	1750
No. animals examined:	10	12	9	22	20	19	15	18
Hyperplasia of Nasal Epithelium	0[b] (0)[c]	0(0)	0(0)	8(36)	0(0)	0(0)	0(0)	11(61)[f]
Adrenal Cortex-focal hyperplasia	4(40)	6(50)	4(44)	7(32)	6(30)	3(15)	6(40)	12(66)[d]
Adrenal Medulla-hyperplasia	2(20)	6(50)	1(11)	16(72)	1(5)	0(0)	3(20)	4(22)
Pancreas-stromal fatty infiltration	0(0)	5(41)[d]	3(33)	13(59)[e]	11(55)	12(63)	8(53)	10(55)

[a]data from Table 13H, pages 206-219 of registrant report.

[b]number of rats with lesion; [c]percent of rats with lesion

[d] $p < 0.05$ vs control; [e] $p < 0.01$ vs control; [f] $p < 0.001$ vs control.

The only lesions considered treatment related by the registrant included the significantly increased incidence of hyperplasia of the nasal epithelium in females from the 1750ppm dose group, and the increased incidence of stromal fatty infiltration of the pancreas in male rats from the 18 and 1750ppm dose groups. Increased incidence of focal cortical hyperplasia of the adrenal cortex in females from the 1750ppm dose group and increased incidence of hyperplasia of the adrenal medulla in male rats from the 1750ppm dose group were also observed as non-neoplastic lesions in surviving rats. While hyperplasia of the adrenal cortex was felt to be unrelated to treatment as stated on page 37 of the registrant's report, it was unclear whether hyperplasia of the adrenal medulla was also meant to be included, as this followed a similar pattern of incidence with dose.

A lower incidence of female rats in diestrus at the 1750ppm dose level was observed in relation to controls. This observation is supportive of evidence in females from the 52 week time point of the study in which a higher incidence of females in proestrus was observed at this same dose level, and suggests some type of test article

interference with the reproductive physiology of female rats.

iii. Neoplastic Observations Combined-104 Weeks

Combined neoplastic findings in decedent and surviving rats from 104 weeks of treatment is presented below (Table 13):

TABLE 13

Incidence of Neoplastic Lesions in Male and Female Rats Given Dietary SC-5676 for 104 Weeks (Terminal Sacrifice Group: Decedent + Surviving)[a]

	Males				Females			
Dose (ppm)	0	18	175	1750	0	18	175	1750
No. animals examined:	50	49	50	50	50	50	49	49
Adenoma of Nasal Epithelium	0[b] (0)[c]	0(0)	0(0)	30(60)[e]	0(0)	0(0)	0(0)	28(57)[e]
Carcinoma of Nasal Epithelium	0 (0)	0(0)	0(0)	2(4)	0 (0)	0(0)	0(0)	1(2)
Thyroid- No. animals examined	50	50	48	50	50	50	50	49
follicular cell adenoma	2(4)	1(2)	2(4)	5(10)	1(2)	1(2)	3(6)	5(10)

[a]data from Table 13I, pages 220-224 of registrant report.

[b]number of rats with lesion; [c]percent of rats with lesion

[d] $p < 0.05$ vs control; [e] $p < 0.01$ vs control; [f] $p < 0.001$ vs control.

As shown, a significant increase in adenomas of the nasal epithelium was observed in both male and female rats at the 1750ppm dose level. This increased in nasal epithelial adenomas was also significant when decedent (see section [1b], page 19 of DER) and surviving rats were considered separately, supporting the treatment related

nature of the effect. The finding of follicular cell adenomas of the thyroid was apparently treatment related only when decedent and surviving rats were combined. The trend of this increase was significant for female rats as analyzed by the Cochran-Armitage test ($p < 0.05$, page 38 of registrant report), but was not statistically significant for male rats, even though the percentage of rats with this tumor was equivalent between sexes at the 1750ppm dose level (10%). The incidence of thyroid follicular cell adenoma at the 1750ppm dose level in females was outside the historical control range for this tumor type (see historical control data attached to this DER).
A summary of non-neoplastic findings in decedent and surviving rats from 104 weeks of treatment follows (Table 14):

TABLE 14

Incidence of Non-Neoplastic Lesions in Male and Female Rats Given Dietary SC-5676 for 104 Weeks (Terminal Sacrifice Group: Decedent + Surviving)[a]

	Males				Females			
Dose (ppm)	0	18	175	1750	0	18	175	1750
No. animals examined:	50	50	50	50	50	50	50	49
Adrenal Cortex-								
focal hyperplasia	9[b](18)[c]	19(38)[d]	12(24)	11(22)	11(22)	9(18)	14(28)	21(42)[d]
Retina-								
No. animals examined	48	43	46	49	49	48	48	45
degeneration of outer nuclear layer	2(4)	1(2)	2(4)	7(14)	13(26)	7(14)	14(28)	24(48)[d]
Nasal Epithelium								
No. animals examined	50	49	50	50	50	50	49	49
hyperplasia	0 (0)	0(0)	0(0)	25(50)[f]	0(0)	0(0)	0(0)	28(57)[f]
Kidney-								
No. animals examined	50	50	50	50	50	50	50	49
pelvic epithelial hyperplasia	6(12)	7(14)	10(20)	22(44)[f]	4(8)	7(14)	9(18)	14(28)[e]

Table 14, cont.

	Males				Females			
Dose (ppm)	0	18	175	1750	0	18	175	1750
Pancreas-								
No. animals examined	50	49	50	49	50	50	50	49
stromal fatty infiltration	9(18)	11(22)	8(16)	15(30)	15(30)	19(38)	20(40)	23(47)

[a]data from Table 13J, pages 225-247 of registrant report.

[b]number of rats with lesion; [c]percent of rats with lesion

[d] $p < 0.05$ vs control; [e] $p < 0.01$ vs control; [f] $p < 0.001$ vs control.

As shown previously for rats considered separately at 104 weeks as either surviving (Table 12) or decedent (Table 10), significant increases in the incidence of nasal epithelial hyperplasia, kidney pelvic epithelial hyperplasia, and degeneration of the outer retinal nuclear layer were seen in either male or female rats at the 1750ppm dose level. Stromal fatty infiltration of the pancreas was not significantly increased at the 1750ppm dose level, but showed an apparent trend for an increase in both male and female rats with increasing dose of test article.

The most significant neoplastic observation in response to administration of test article was adenoma and carcinoma of the nasal epithelium, while hyperplasia of the nasal epithelium was most significantly affected in reponse to administration of test article.

It should be noted that upon inspection of individual animal data (Appendix 12B, pages 1468-1568 and 1855-1954; Appendix 12D, pages 2215-2284 and 2434-2490), the presence of nasal epithelial hyperplasia and adenomas occurred together in 13 male rats at the 1750ppm dose, and in 14 female rats at the same dose level. Multiple nasal adenomas were observed in 11 male rats at the 1750ppm dose level, and in 9 female rats at this dose level. The presence of nasal hemorrhage was evident in 6 male rats at the 1750ppm dose level, and in 2 female rats at this dose.

III. DISCUSSION

In the present study, male and female Sprague-Dawley rats were administered SC-5676 technical in the diet for 104 weeks at levels of 0, 18, 175, and 1750 ppm in order to determine potential carcinogenicity and chronic toxicity of this compound. Rats were monitored for treatment related effects on mortality, body weight gain, food consumption, food efficiency, palpable masses, and clinical signs of toxicity. Interim sampling of blood and urine was performed to monitor toxicity during the study. At study termination, rats were killed and blood samples were again obtained for hematological analysis. Appropriate organ weights were recorded, and tissues were examined for both neoplastic and non-neoplastic changes related to treatment with test article. Effects of test article treatment on neoplastic and non-neoplastic responses in decedent rats and those sacrificed at study termination was delineated, as was the effect of test article treatment on these responses in the combined groups.

Mortality was not significantly altered in test article treated rats of either sex in comparison to control rats. Mortality in control male rats, was, however, slightly higher over the course of the study (78%) than usual. No explanation was given by the registrant as to why this occurred.

While no adverse effects were seen on mortality in this study, there were effects on body weight and body weight gain in both male and female rats. Absolute body weight at 13 weeks was decreased in both male and female rats in the 1750ppm dose group by 9%. At 52 weeks of the study, this decrease was 14% and 18% for males and females, respectively. Overall absolute body weight was decreased 12% in males and 27% in females for weeks 0-104 of the study.

Body weight gain was also affected throughout the study, primarily in male and female rats from the 1750ppm dose group. Weight gain for weeks 0-13 was decreased 12% in male rats at the 1750ppm dose level, and was decreased 14% in female rats at this dose level. At week 52 of the study, body weight gain in male and female rats from the 1750ppm dose level was decreased 17% and 24%, respectively. Overall body weight gain for male and female rats at the 1750ppm dose level was decreased 14% and 33%, respectively, in comparison to controls. Thus, the effects of test article treatment on body weight and body weight gain were most apparent at the 1750ppm dose level.

The decrease in body weight gain for male and female rats at the 1750ppm dose levels during the first 13 weeks of the study was paralleled by a decrease in food consumption in these dose groups, which suggests adverse palatability of the diet at these dose levels. However, food efficiency was also affected during this time period, primarily at the 1750ppm dose level. The combined observations of decreased body weight gain, food consumption, and food efficiency supports the conclusion of test article toxicity. While food consumption and body weight gain decreases in male rats were closely parallel for the study duration (12% decrease in body weight, 13% decrease in food consumption), food consumption was decreased 8% in female rats for weeks 0-104 of the study, but body weight gain was decreased 33%, as reflected in the overall decreased food efficiency in female rats, both for weeks 1-14 and for the study duration. Efficiency of food conversion was less affected by test article treatment in male rats.

Ophthalmologic effects from administration of test article were evident in both male and female rats in the 1750ppm dose level beginning at week 76. In male rats, this lesion consisted of foci or plaques in the vitreous or on the posterior capsule of the lens, and was often observed bilaterally. No apparent histopathological lesion was

asssociated with this ocular alteration. In females, the primary ocular change observed was hyperreflection of the ocular fundus. According to the registrant (page 39), this observation is often associated with a reduced thickness of the retinal layer and is supported by the histopathological observation of degeneration of the retinal outer layer. Thus, significant ocular effects from test article administration were observed in both male and female rats at the 1750ppm dose level.

Some hematological parameters were altered at each time point of examination during the study, such as platelet counts, mean corpuscular hemoglobin concentration, and packed cell volume. However, these effects were not consistent over time and did not display a dose related trend in most cases. The relative lack of hematologic effects in this study is supported by previous results from subchronic administration of SC-5676 to male and female rats, where a trend towards increased hemoglobin levels and decreased platelets was observed in male rats, and increases in red blood cells were observed in female rats after 13 weeks. These results were achieved with an oral dose of 2000ppm test article. Thus, the highest dose used in this study, 1750ppm, was apparently not high enough to produce significant hematologic effects as those produced in the subchronic study with SC-5676. It is apparent that SC-5676 would cause hematologic effects at high enough doses.

Most clinical blood chemistry parameters were unaffected over the course of treatment with SC-5676. However, a consistent increase in gamma-glutamyl transpeptidase (GGT) was observed in male rats at the 1750ppm dose level, as was a consistent increase in blood cholesterol at this dose. Similar changes were observed in female rats, but did not reach statistical significance at the 1750ppm dose level, except for GGT at 102 weeks. A dose-related trend for increased blood cholesterol was also observed in the subchronic rat study with SC-5676, but no changes in GGT were observed.

Analysis of urine during the study did not reveal any significant changes in any parameter measured in either male or female rats.

Treatment related effects on observed macroscopic lesions were also not apparent over the course of the study.

Significant effects of test article administration on absolute organ weights were not apparent at 52 weeks of treatment. However, organ:body weight ratios for the brain, heart, liver, and kidneys were significantly increased in male rats at the 1750ppm dose level, and were significantly increased in female rats at this dose level for the kidneys and liver. As with many other effects noted in this study, the significant effects occurred primarily at the 1750ppm dose level.

Nasal epithelial hyperplasia, adenoma, and carcinoma were the most significant non-neoplastic and neoplastic observations resulting from administration of SC-5676. Hyperplasia and nasal adenoma were present in both male and female rats from the 1750ppm dose level at 52 weeks. This effect was seen only at the 1750ppm dose level. These same effects were seen again in decedent and surviving rats assigned to the 104 week treatment groups, with the same finding that nasal effects were limited to the 1750ppm dose level. When decedent and surviving rats treated for 104 weeks were combined, a total of 60% of males and 57% of females in the 1750ppm dose group were found with nasal adenoma. Four percent of males and 2% of females were found with nasal carcinoma at the 1750ppm dose level. Follicular cell adenoma of the thyroid was also a significant neoplastic response in both male and female rats. Ten percent of both male and female rats from the 1750ppm dose level were found with this tumor type at 104 weeks when decedent and surviving rats were combined. A significant dose-related trend was found in female rats for this tumor, but not in males. That thyroid follicular cell adenoma

was related to treatment was supported by historical control data provided by the registrant (page 40 of report and attached Table), where the highest incidence of this tumor was found to be 6%, below the 10% incidence found in this study.

Two rare tumor types were also observed in this study. Benign chondroma of the femur was found in 1 male rat which died during the study and in 1 female rat surviving to week 104. Basal cell tumors of the stomach were also found in 1 male and 1 female rat which died during the study. The rarity of these tumor types supports the finding that these were related to administration of test material.

Treatment of male and female rats with SC-5676, especially at the 1750ppm dose level, was also apparently responsible for increases in the incidence of a number of non-neoplastic lesions in both sexes. These included epithelial hyperplasia of the kidney pelvis (increased from 6% in control males to 22% in 1750ppm males), nasal epithelial hyperplasia (increased from 0% in control males and females to 8% and 11% in 1750ppm males and females, respectively), and stromal fatty infiltration of the pancreas (increased from 9% to 15% in 1750ppm males, and from 15% to 23% in 1750ppm females). The non-neoplastic lesions of focal hyperplasia of the adrenal cortex and degeneration of the outer nuclear layer of the retina appeared to affect females in the 1750ppm dose group primarily.

The highest dose of test article examined in this study was 1750 ppm in both male and female rats. This dose caused a body weight decrement of approximately 12-14% during the first 13 weeks of treatment in both sexes of rats. This weight gain decrement persisted throughout the study in both sexes. In addition, decreased food efficiency, ophthalmoscopic abnormalities, clinical effects on GGT and cholesterol, and increased organ:body weight ratios were also observed in both sexes at 1750ppm test article. In light of these systemic effects, the 1750ppm dose level is considered a maximum tolerated dose (MTD) for the test article in this study.

IV. CONCLUSIONS

Technical SC-5676 was administered to male and female rats in the diet for 104 weeks at doses of 0, 18, 175, and 1750 ppm (0, 0.8, 7.9, and 79.6 mg/kg/day active ingredient). In males and females, systemic toxicity in the form of reduced body weight gain, decreased food efficiency, opthalmologic abnormalities, elevated GGT and cholesterol, and increased organ:body weight ratios were evident at the 1750ppm dose level, establishing this as a maximum tolerated dose for the study. Tumorigenic responses were observed in both sexes from administration of 1750ppm SC-5676. These included a significant increase in the incidence of nasal epithelial adenomas and thyroid follicular cell adenomas. Nasal carcinomas were observed in a total of 3 rats (2 males and 1 female). Rare tumors in the form of chondroma of the femur and basal cell tumors of the stomach were also observed. Non-neoplastic histopathology in the kidney, retina, pancreas, and nasal epithelium was also increased at the 1750ppm dose level.

The data in this study support the conclusion of limited evidence of carcinogenicity for technical SC-5676, based upon the occurrence of increased incidence of benign thyroid follicular cell tumors and benign and malignant nasal tumors only in high dose male and female rats , and the occurrence of benign chondroma and basal cell tumors in male and female rats.

The No Observed Effect Level (NOEL) = 175 ppm

The Lowest Observed Effect Level (LEL) = 1750 ppm (males and females; decreased body weight gain, decreased food efficiency, increased organ:body weight ratios, increased plasma GGT and cholesterol)

The Maximum Tolerated Dose (MTD) = 1750 ppm (males and females; decreased body weight gain).

V. CLASSIFICATION

Core Minimum

This study satisfies the guideline requirements (83-5) for a combined carcinogenicity/ chronic toxicity study in rats.

4.5.13 Statistical evaluation

Tests for the significance of difference between each treatment group and the corresponding controls were conducted as follows:

For bodyweight gain, food consumption, haematology, blood chemistry, urinalysis and organ weight data, a series of Student's t-tests was performed using a pooled within-group error variance. The least significant difference was calculated at the 0.1%, 1% and 5% levels of significance. Statistical significances for eosinophil, basophil and monocyte count are not reported as these data are not normally distributed.

For macroscopic and microscopic changes, inter-group differences in incidences were evaluated by Fisher's Exact Test (two-tailed for macroscopic and non-neoplastic findings, one-tailed for neoplastic microscopic findings). Tarone's extension of Cox's test (Biometrika, 62, 679-684, 1975) was used to examine linear trend on dose and to assess deviation for linearity.

Time-to-event analysis of mortality was by Cox's test, applied as an overall test for homogeneity of survival curves and for pair-wise comparison against control.

Cochran-Armitage trend test was applied to the incidences of neoplastic and non-neoplastic findings for animals allocated for terminal study.

4.6 Raw data

All raw data and specimens pertaining to this study, except those used or generated in the course of any supplier's or sponsor's analysis, are stored in the archives of Life Science Research.

Text Table 1

Historical data for control animals

Study		CDR39(1)		CDR39(2)		CDR40		CDR41(1)		CDR41(2)	
		M	F	M	F	M	F	M	F	M	F
No. examined :		50	50	50	50	50	50	50	50	50	50
Thyroid											
Follicular adenoma	No.	2	3	2	1	3	0	1	3	3	0
	%	4	2	4	2	6	0	2	6	6	0
Follicular carcinoma	No.	0	1	0	0	1	0	0	0	0	0
	%	0	2	0	0	2	0	0	0	0	0

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Attachment C

Primary Review by: Patricia A. Turck, M.S. 7/16/91
Review Section I, Toxicology Branch II (H7509C)
Secondary Review by: Yiannakis M. Ioannou, Ph.D. 7/16/91
Review Section I, Toxicology Branch II (H7509C)

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DATA EVALUATION REPORT

STUDY TYPE: Oncogenicity §83.2

MRID NO.: 415651-19 TOX. CHEM. NO.: 003B HED PROJECT NO.: 0-1999

TEST MATERIAL: SC-5676

SYNONYMS: N-Ethoxymethyl-N-(2-methyl 6 ethylphenyl)chloracetamide, acetochlor.

STUDY NUMBER: 87/SUC0012/0702.

SPONSOR: ICI Americas Inc., Wilmington, DE.

TESTING FACILITY: Life Science Research Ltd., Suffolk, England.

TITLE OF REPORT: SC-5676: 78 Week Feeding Study in CD-1 Mice.

AUTHOR(S): S.J. Amyes.

REPORT ISSUED: June 9, 1989.

CONCLUSION: In a 78-week feeding study designed to evaluate the carcinogenic potential of SC-5676, groups of 50 CD-1 mice/sex/dose were administered the test material at concentrations of 0, 10, 100, or 1000 ppm. In males, a dose-related increase in absolute and relative (to body weight) kidney weight was observed and was accompanied by significant increases in renal tubular basophilia at all dietary levels in males. In females, the only compound-related finding was a significant increase in anterior polar vacuoles in the lens of the eye at the high-dose level. Under the conditions of this study, the dietary exposure of SC-5676 resulted in a significant increase in pulmonary adenomas in female mice, and significant positive trends toward the development of pulmonary adenomas in both males and females. However, a definitive assessment of the carcinogenic potential was not possible because laboratory historical control data and a definitive characterization of pulmonary tumors, i.e., site and type, were not provided. Therefore, until these data are provided, the reviewers cannot make a definitive assessment of the carcinogenic potential of SC-5676.

Based on these results, the LOEL for systemic toxicity in males is 10 ppm (1.1 mg/kg/day); a NOEL was not established. In females, the NOEL and LOEL for systemic toxicity were 100 (13 mg/kg/day) and 1000 ppm (135 mg/kg/day), respectively.

The Maximum Tolerated Dose was not achieved in this study. Although increased kidney weight associated with tubular basophilia was observed in male mice at the 10ppm dose level, this effect was not considered sufficient for an MTD in this study. Review of a six-week range finding study in mice with acetochlor (Life Science Res. report # 85/SUC008/496) showed decreases in body weight gain of 9% and 12% at 600ppm and 1200ppm acetochlor, respectively, for male mice. In female mice from this study, a significant decrease in body weight gain (21%) was not observed until the 2400ppm dose level. Thus, based upon the results of the range-finding study, the MTD can be considered to have been achieved for male mice, but not for female mice.

CORE CLASSIFICATION: Supplementary; this study does not meet the minimum requirements set forth under guideline 83.2 for a carcinogenicity study in mice. This study may be upgraded upon submission and review of the additional data.

A. MATERIALS:

1. Test compound: SC-5676; Description - Dark brown viscous liquid; Batch Nos - 1 and 3; Purity - 90.5%.

2. Test animals: Species - Mouse; Strain - CD-1; Age - 35-42 days at study initiation; Weight - Males, 23-31 and Females, 20-27 g at study initiation; Source - Charles River (UK) Ltd., Kent, England.

3. Mice were individually housed in suspended polypropylene cages with stainless steel mesh bottoms. Wood shavings were used as bedding. Temperature and relative humidity were maintained at approximately 21°C and 55% throughout the study. A minimum of 20 air changes/hour and a 12-hour light/12-hour dark cycle was achieved.

4. Animals received food (Laboratory Animal Diet No. 2; Labsure, Cambridgeshire, England) and water ad libitum.

5. Statistics: The following procedures were utilized in analyzing the numerical data:

   -Body weight gain, hematology, and organ weight data--Student's t-test using a pooled within-treatment error variance;

   -Mortality--Cox's test and Tarone's extension of Cox's test (analysis of trend). Adjusted mortality was estimated using the Kaplan-Meier method; and

   -Ophthalmic and gross and microscopic pathological findings (nonneoplastic and neoplastic lesions)--Fisher's Exact Probability test.

6. Signed Quality Assurance and GLP Compliance statements, dated June 9, 1989, and a No Data Confidentiality Claim statement, dated June 25, 1990, were presented.

B. STUDY DESIGN:

1. Selection of dietary levels: Dietary concentrations were chosen based on the results of a preliminary rangefinding study in which mice (strain and number/sex/group not reported) were fed diets containing 300, 600, 1200, 2400, 4800, or 9600 ppm for 6 weeks. At 1200 ppm or higher, reductions in body weight gain (≥12%) and red cell characteristics were observed in males. Females receiving 2400 ppm had severe reductions (20%) in body weight, but females administered 1200 ppm were not affected. Males receiving 600 ppm exhibited reduced body weight gain and increased relative kidney weight. At 300 ppm, slight reductions (11%) in body weight were also observed.

2. Animal assignment: Animals were assigned using a computer-generated randomization procedure to the following test groups:

Test Group	Dietary Concentration (ppm)	Main Study 78 Weeks		Interim Sac. 52 Weeks	
		Males	Females	Males	Females
Control	0	50	50	10	10
Low (LDT)	10	50	50	10	10
Mid (MDT)	100	50	50	10	10
High (HDT)	1000	50	50	10	10

The test material was administered continuously in the diet for 78 weeks.

3. Diet preparation: Test diets were prepared weekly and stored at -20°C until use. Homogeneity of the test material in diets containing the lowest and highest concentrations was determined prior to study initiation. Stability of the test material in the low-dose diet after 7 and 14 days of storage at ambient temperature was determined prior to initiation of the study and again assayed after storage for 0 and 7 days (4 days at -20°C and 3 days at room temperature) during week 18 of the main study. Samples of test diets were analyzed for concentration during weeks 1, 13, 26, 39, 52, 65, and 78.

Results: Preliminary stability analysis indicated that the low dose (10 ppm) was unstable; 21-23% of the test material was lost after 14 days of storage at room temperature. Therefore, the test diets for the main study were stored at -20°C until use, and diet was replaced in the feeders every 4 days. This appeared to reduce the stability problem; no significant loss in active ingredient was detected after storage and feeding procedures were changed.

Homogeneity of the test diets was acceptable; aliquots were within 3 to 7% of each other, and the coefficient of variation ranged from 3.9 to 4.9%. Concentrations of the test diets were within acceptable ranges; actual concentrations ranged from -15 to +12% of target concentrations.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for clinical signs of toxicity and mortality. Furthermore, animals were subjected to detailed physical examinations weekly to detect palpable masses.

Results: Mortality is summarized in Table 1. A slight increase (32%) in mortality was observed in high-dose males when compared with controls (20%); however, the increase was not statistically significant. Furthermore, a similar increase was not observed in females. The clinical signs observed with similar frequency in all test groups, including controls, during the study were those commonly seen in mice of this strain and age and therefore were not considered to be compound related. Palpable swellings noted during weekly clinical examinations occurred with similar frequency in control and test groups and were unrelated to treatment.

2. Body weight: Animals were weighed at study initiation, once weekly for the first 14 weeks, and bimonthly for the remainder of the study.

Results: Body weight gain data are summarized in Table 2. No statistically significant reductions in body weight or body weight gain were observed during the study. Slight increases in total body weight gain were observed in low-dose males and mid-dose females.

3. Food consumption and compound intake: Food consumption was determined weekly during the study. Efficiency was calculated at four weekly intervals for the first 12 weeks of the study; compound intake was calculated using food consumption and body weight data.

Results: Food consumption data are summarized in Table 3. No changes in food consumption or food efficiency were observed in the test groups relative to controls. The mean daily dosages, based on percent active ingredient, were approximately 0, 1.1, 11, and 116 mg/kg/day for males and 0, 1.4, 13, and 135 mg/kg/day for females from the control, low-, mid-, and high-dose groups, respectively.

4. Ophthalmological examination: Ophthalmic examinations were performed prior to study initiation on all animals using a Fisons binocular indirect ophthalmoscope and 0.5% tropicamide. After weeks 13, 24, 50, and 76, the eyes from all animals in the control and high-dose groups were similarly examined. Prior to study termination at week 79, ophthalmic examination of all surviving animals was performed.

Results: Ophthalmic findings after 76 weeks of treatment are summarized in Table 4. At 13 and 24 weeks, ophthalmic findings occurred with similar frequency in control and high-dose groups. At the 50-week interval, a statistically significant increase ($p < 0.05$) in the incidence of hyaloid remnant was observed in high-dose males (40/55) when compared with controls (28/56). This was not considered to be compound related, however, because incidences of hyaloid remnant observed in control and high-dose females were similar to that observed in high-dose males, suggesting that the low incidence seen in

TABLE 1. Summary of Cumulative Mortality (Percent Survival) for Mice (Main Study) Fed SC-5676 for 78 Weeks[a]

Dietary Concentration (ppm)	Study Week: 18	26	52	76	80+[b]
			MALES		
0	0	0	3(94)	9(82)	10(80)
10	0	0	1(98)	11(78)	11(78)
100	0	0	3(94)	11(78)	14(72)
1000	0	2(96)	5(90)	16(68)	16(68)
			FEMALES		
0	0	0	2(96)	14(72)	17(66)
10	0	0	3(94)	7(86)	9(82)
100	0	0	3(94)	10(80)	12(76)
1000	0	0	1(98)	13(74)	13(74)

[a]Data were extracted from study No. 87/0702, Table 2.

[b]These animals were awaiting terminal sacrifice.

TABLE 2. Summary of Body Weight Gain Data (g) in Mice Fed SC-5675 for 78 Weeks[a]

Dietary Concentration (ppm)	Study Week: 0-13	13-52	52-78	0-78
	MALES			
0	12.7	7.9	1.4	20.8
10	13.4	8.4	0.8	24.1
100	12.9	6.2	0.6	19.4
1000	11.9	7.9	0.3	20.2
	FEMALES			
0	7.3	4.8	2.6	15.2
10	7.6	4.3	2.1	12.7
100	8.2	4.8	3.2	16.3
1000	8.2	3.6	2.2	14.2

[b]Body weight gains were calculated by the reviewers using individual animal data, Appendix 5.

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TABLE 3. Summary of Food Consumption Data (g/mouse/week) in Mice Fed SC-5676 for 78 Weeks

Dietary Concentration (ppm)	Study Week: 1	7	14	28	50	64	78
			MALES				
0	39	39	35	35	35	34	34
10	39	38	38	36	36	35	36
100	38	38	38	34	35	35	34
1000	39	38	37	35	36	34	35
			FEMALES				
0	35	38	35	35	35	32	32
10	35	37	35	33	33	34	34
100	34	37	36	32	32	31	31
1000	35	38	35	34	34	30	30

[a]Data were extracted from study No. 87/0702, Table 4.

TABLE 4. Summary of Ocular Effects Observed at 76 Weeks in Surviving Mice Fed SC-5676 Continuously for 78 Weeks[a]

	Dietary Concentration (ppm)							
	Males				Females			
Finding	0	10b	100[b]	1000	0	10[b]	100[b]	1000
Cornea:								
Superficial opacity	9(22)[c]	7(18)	11(30)	10(29)	13(36)	15(36)	5(13)*	10(27)
Lens:								
Hyaloid remnant	14(34)	7(18)	6(16)	15(44)	10(28)	6(14)	5(13)*	14(38)
Anterior polar vacuole(s)	10(24)	11(28)	12(32)	9(29)	7(19)	10(24)	12(32)	20(54)**
Anterior polar opacity	2(5)	4(10)	1(3)	1(3)	5(14)	4(10)	0(0)	1(3)
Posterior polar vacuole(s)	2(5)	2(5)	2(5)	0(0)	0(0)	3(7)	5(13)	1(3)
Posterior polar opacity	2(5)	3(8)	2(5)	3(9)	13(36)	4(10)**	13(34)	17(46)

[a]Data were extracted from study No. 87/0702, Table 7D.
[b]Examination of the 10- and 100-ppm groups was performed during week 78.
[c]Number in parentheses represents the % incidence.

* Significantly different from controls (0.05).
** Significantly different from controls (0.01).

control males at this interval was atypical. Furthermore, the increase did not persist to the end of the study. Also observed at this interval was a significant increase ($p < 0.01$) in the incidence of rosettes in high-dose females. Since a similar increase was not observed at study termination, this was not considered to be compound related. No other adverse effects were observed at 50 weeks.

At the 76-week interval, a significant increase (<0.01) in the incidence of vacuoles in the anterior polar region of the lens was observed in high-dose females. According to the study author, ophthalmic examination of females from the low- and mid-dose groups did not reveal a similar increase; however, although the incidences were not statistically significant, a dose-related pattern was evident. No increase in the incidence of vacuoles in the anterior polar region of the lens was observed in males. In addition, the incidence of anterior polar opacity was not increased.

5. Hematology analysis: Blood was collected from the retro-orbital sinus during week 51 from all animals selected for interim sacrifice and during weeks 78 or 79 from 20 animals/sex/group for hematology analysis. The animals selected were nonfasted and lightly anaesthetized with ether. The CHECKED (X) parameters were examined.

a. Hematology:

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Erythrocyte count*
X Leukocyte count*
X Platelet count*
X Leukocyte differential count*
X Packed cell volume (PCV)
Coagulation: Thromboplastin time (PT)*
X Reticulocyte count (RETIC)
Red cell morphology
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Mean corpuscular hemoglobin (MCH)

* Required for subchronic and chronic studies

Results: Hematology data from terminal sacrifice are summarized in Table 5. At the 51-week interval, packed cell volume, hemoglobin, and erythrocyte count were slightly reduced (7%) in males and significantly reduced ($p < 0.05$ or 0.01) in females from the high-dose group. After 77 weeks, packed cell volume and erythrocyte count were significantly reduced ($p < 0.05$) in mid- and high-dose males. In females, statistically significant reductions ($p < 0.05$) in neutrophil count at the low-dose and MCHC and MCV at the mid-dose were observed.

6. Sacrifice and Pathology: All animals that died or were sacrificed moribund or on schedule were subjected to a gross pathological examination. The CHECKED (X) tissues were collected for histological examination and fixed in 4% neutral buffered formaldehyde solution, except for the eyes with optic nerve attached and Harderian gland, which were saved in Davidson's fixative. In addition, the (XX) organs were weighed.

TABLE 5. Summary of Hematology Data Obtained During Terminal Sacrifice from Mice Fed SC-5676 in the Diet for 78 Weeks[a]

Dietary Concentration (ppm)	PCV (%)	Hb (g%)	RBC (mil/cmm)	MCHC (%)	MCV (cμ)	MCH (pg)	Platelets (1000/cmm)
			MALES[b]				
0	42 ± 6	14.7 ± 2.4	8.5 ± 1.0	35 ± 1	50 ± 3	17 ± 1	607 ± 106
10	40 ± 5	14.0 ± 1.7	8.1 ± 1.1	35 ± 0	50 ± 2	18 ± 1	700 ± 159
100	39 ± 4*	13.7 ± 1.4	7.8 ± 1.0*	35 ± 1	50 ± 3	18 ± 1	651 ± 163
1000	39 ± 4*	13.6 ± 1.5	7.7 ± 1.1*	35 ± 1	51 ± 3	18 ± 1	722 ± 163*
			FEMALES[b]				
0	40 ± 6	13.9 ± 2.3	7.9 ± 1.4	35 ± 1	51 ± 2	18 ± 1	524 ± 158
10	42 ± 5	14.6 ± 1.5	8.3 ± 1.0	35 ± 1	50 ± 2	17 ± 1	472 ± 88
100	40 ± 3	14.3 ± 1.2	8.3 ± 0.6	35 ± 1*	49 ± 3*	17 ± 1	465 ± 127
1000	41 ± 4	14.2 ± 1.4	8.1 ± 0.8	35 ± 1	50 ± 2	18 ± 1	509 ± 114

[a]Data were extracted from study No. 87/0702, Table 8B.
[b]Blood was collected from males during week 77 and from females during week 78.

*Significantly different from controls ($p < 0.05$).

Digestive System	Cardiovasc./Hemat.	Neurologic
X Tongue	X Aorta*	XX Brain*)
X Salivary glands*	X Heart*	X Periph. nerve*
X Esophagus*	X Bone marrow*	X Spinal cord*
X Stomach*	X Lymph nodes*	X Pituitary*
X Rectum*	X Spleen*	X Eyes* (optic nerve)
X Colon*	X Thymus	
X Cecum*		
X Ileum*		
X Jejunum*	Urogenital	Glandular
X Duodenum*	XX Kidneys*)	XX Adrenals*
XX Liver*)	X Urinary bladder*	Lacrimal gland
X Gallbladder*	XX Testes*)	X Mammary gland*
X Pancreas*	X Epididymis*	X Thyroid gland*
	X Prostate*	X Parathyroid*
Respiratory	X Seminal vesicle*	X Harderian gland
X Trachea*	X Ovaries*	
X Lung*	X Uterus*	Other
X Nasal cavity	Vagina	X Bone (sternum & femur)*
		X Skeletal muscle*
		X Skin*
		X All gross lesions & masses*

* Recommended by Subdivision F (October 1982) Guidelines.
Organ weight required in chronic studies.

The above tissues, except for the tongue, bone marrow, and mammary gland, from all control and high-dose animals dying or sacrificed moribund or on schedule and any animals from the low- or mid-dose groups dying or sacrificed moribund during the study were microscopically examined, as were gross lesions, kidney, liver, and lung from low- and mid-dose animals sacrificed on schedule.

a. Organ weight: Organ weight data collected following terminal sacrifice are summarized in Table 6.

At the interim sacrifice, a compound-related and statistically significant increase ($p < 0.01$) in relative (to body weight) kidney weight, associated with nephropathy in 4/9 animals examined, was observed in high-dose males. The observed significant increases ($p < 0.05$) in relative brain and adrenal weight in high-dose males may have been due to the reduction (12%) in body weight since no microscopic changes were observed; therefore, these reductions were not considered to be compound related. No other significant changes in organ weight were observed.

TABLE 6. Summary of Absolute and Relative Organ Weights in Mice Fed SC-5676 Continuously for 78 Weeks[a]

Organ	Dietary Concentration (ppm)							
	Males				Females			
	0	10	100	1000	0	10	100	1000
Terminal Body Weight (g)	47.0	48.3	46.1	45.3	37.4	36.4	38.1	36.7
Kidney:								
Absolute (g)	0.85	0.92*	0.96**	1.12***	0.53	0.56	0.55	0.49*
Relative (%)	1.83	1.93	2.12*	2.50***	1.48	1.56	1.46	1.36
Liver:								
Absolute (g)	2.4	2.9*	2.6	2.8	1.8	1.8	1.8	1.9
Relative (%)	5.07	5.96*	5.72	6.27*	4.90	5.01	4.77	5.18
Adrenals:								
Absolute (mg)	3.0	3.0	3.0	3.0	7.0	7.0	7.0	7.0
Relative (%)	5.7	6.0	5.9	6.5	18.2	18.7	18.6	18.8
Brain:								
Absolute (g)	0.52	0.51	0.51	0.51	0.52	0.53	0.52	0.50*
Relative (%)	1.12	1.08	1.12	1.14	1.45	1.50	1.41	1.39

[a]Data were extracted from study No. 87/0702, Table 12.

*Significantly different from controls ($p < 0.05$).
**Significantly different from controls ($p < 0.01$).
***Significantly different from controls ($p < 0.001$).

At terminal sacrifice, dose-related and statistically significant increases in absolute kidney weight were observed in low-, mid-, and high-dose males when compared with controls. Relative kidney weight was also significantly increased ($p < 0.001$) in males at the mid- and high-dose levels. In addition, dose-related increases in absolute and relative liver weight were observed in males. The increases in liver weight were accompanied by histopathological findings which probably affected the organ weight in low-dose animals. However, after exclusion of high-dose animals with hepatic carcinomas, adenomas, or hyperplastic nodules, liver weight was still significantly higher than controls. Other significant changes in organ weight were not considered to be compound related.

b. Gross pathology: Statistically significant increases in the incidences of enlarged kidneys (verified by increase in kidney weight) in high-dose males ($p < 0.05$) and distension of the coagulating gland of the seminal vesicles in low-dose males ($p < 0.01$) were observed. In addition, a slight increase in dark Harderian glands was observed in treated females when compared with controls (9, 28, 21, and 19 for control, low-, mid-, and high-dose groups, respectively). However, the reviewers considered only the enlarged kidneys to be compound related; the other findings were not corroborated by histopathological evidence and are commonly seen in mice of this age and strain.

c. Microscopic pathology: Nonneoplastic and neoplastic lesions found in the lungs, liver, and kidneys are summarized in Tables 7 and 8, respectively.

1. Nonneoplastic - At the 52-week interim sacrifice, nephropathy was observed in 44% (4/9) of high-dose males, compared with 0% of controls. Twenty percent of the high-dose females were affected, compared with 10% of control females.

   Statistically significant increases in the incidences of interstitial fibrosis, hyaline cysts, and cortical mineralization were observed in high-dose males when compared with controls. In addition, dose-related and statistically significant increases in the incidence of tubular basophilia were observed in all treatment groups compared to controls. Similar changes were not noted in females. Other significant changes in incidences of nonneoplastic findings in the adrenals, salivary glands, lungs, ovaries, and stomach were not considered by the study author to be compound related. However, a statistically significant increase in bronchiolar hyperplasia was observed in mid- and high-dose males, which may be associated with the increased incidence of pulmonary tumors.

However, although a statistically significant increase in pulmonary adenomas was noted in high-dose females, the incidence of bronchiolar hyperplasia was similar between control and high-dose females, while being slightly higher in low- and mid-dose females.

2. Neoplastic - At the interim sacrifice, a pulmonary adenoma was observed in one high-dose female and one mid-dose male. In addition, a hepatocytic adenoma was observed in 1/10 low-dose males. The number of animals (incidence) dying or killed during the study with pulmonary adenomas is presented below; all the animals developing adenomas survived to at least week 63.

Dose	0	10	100	1000
Males	0	0	4(29)	3(19)
Females	0	1(10)	0	1(7)

In animals assigned to terminal sacrifice (included animals found dead or sacrificed prior to study termination), a significant increase ($p < 0.05$) in the incidence of pulmonary adenomas was observed in high-dose females when compared with controls. Moreover, although not statistically significant, an increased incidence was also observed in mid- and high-dose males and mid-dose females. As a result, a significant positive trend was observed in both male and female mice. Combining pulmonary carcinomas and adenomas did not significantly change the pattern; although the incidence in high-dose females was no longer statistically significant, statistically significant positive trends ($p < 0.05$) were observed in both males and females. Hemangiosarcomas of the liver were observed in 2/50 high-dose males; although historical control incidences were not presented with the study report, the incidence of hemangiosarcomas is slightly above the range normally seen in mice of this age and strain (0-2.8%). Slight increases in the incidences of other neoplasms, including hepatocytic adenomas or carcinomas and malignant lymphoma in the hematopoietic tissue, were observed in treated males when compared with controls, but the incidences were within the range generally observed in animals of this strain and age and therefore, were not considered to be compound related.

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TABLE 7. Summary of Nonneoplastic Lesions in the Lungs, Kidney and Liver of Male Mice Fed SC-5676 Continuously for 78 Weeks[a]

	Dietary Concentration (ppm)			
Finding	0	10	100	1000
No. examined	50	50	50	50
Lung:				
Bronchiolar hyperplasia	5(13)[b]	4(10)	14(39)*	13(38)*
Kidney:				
Cortical mineralization	12(30)	12(31)	11(31)	23(68)**
Hyaline cast(s)	5(13)	4(10)	6(17)	12(35)*
Tubular basophilia	2(5)	13(33)*	10(28)**	15(44)**
Interstitial fibrosis	6(15)	7(18)	10(28)	17(50)*
Tubular epithelial hyper.	0	0	1(3)	4(12)*
Liver:				
Nodular hyperplasia	3(8)	6(15)	2(6)	2(6)
Focal hepatocytic hyper.	0	2(5)	0	1(3)
Periacinar hyperplasia	8(20)	11(28)	6(17)	9(26)

[a]Data were extracted from study No. 87/0702, Table 17.
[b]Numbers in parentheses represent % incidence.

* Significantly different from controls ($p < 0.05$).
** Significantly different from controls ($p < 0.01$).

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TABLE 8. Summary of Neoplastic Lesions in Mice Fed SC-5676 Continuously for 78 Weeks[a]

Finding	Dietary Concentration (ppm) 0	10	100	1000
MALES				
No. examined	50	50	50	50
Kidney:				
Adenoma	0	2(4)[b]	1(2)	1(2)
Liver:				
Hepatocytic adenoma	2(4)	4(8)	3(6)	5(10)
Hepatocytic carcinoma	1(2)	3(6)	2(4)	3(6)
Adenoma + carcinoma[c]	3(6)	7(14)	5(10)	8(16)
Hemangiosarcoma	0	0	0	2(4)
Lungs:				
Pulmonary carcinoma	5(10)	4(8)	3(6)	4(8)
Pulmonary adenoma	5(10)*	4(8)	11(22)	12(24)
Adenoma + carcinoma[c]	10(20)*	7(14)	14(28)	16(32)
FEMALES				
No. examined	50	50	50	50
Kidney:				
Adenoma	0	0	0	0
Liver:				
Hepatocytic adenoma	1(2)	2(4)	0	1(2)
Hepatocytic carcinoma	0	1(2)	0	0
Adenoma + carcinoma[c]	1(2)	3(6)	0	1(2)
Hemangiosarcoma	0	0	1(2)	0
Lungs:				
Pulmonary carcinoma	4(8)	0	2(4)	4(8)
Pulmonary adenoma	1(2)*	3(6)	5(10)	7(14)*
Adenoma + carcinoma[c]	5(10)*	3(6)	7(14)	11(22)

[a]Data were extracted from study No. 87/0702, Table 24. Includes only mice scheduled for sacrifice at 78 weeks.
[b]Numbers in parentheses represent % incidence.
[c]Calculated by the reviewers and analyzed using Fisher's Exact test and Cochran-Armitage trend test.

* Significantly different from controls ($p < 0.05$). Significant trends are denoted at the controls.

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D. REVIEWERS' DISCUSSION/CONCLUSIONS:

The data reveal a clear sex-related difference in systemic toxicity. In males, ingestion of SC-5676 resulted in dose-related increases in kidney effects which were manifested as increases in absolute and relative kidney weights and were associated with increases in the incidence of renal tubular basophilia at all dietary levels. In females, the only compound-related systemic effect was an increase in the incidence of anterior polar vacuoles in the lens of the eye at the high-dose level. Based on the dose-related increases in kidney weight and incidence of tubular basophilia, the LOEL for systemic effects in male mice was 10 ppm (1.1 mg/kg/day); the NOEL was not established. For female mice, the NOEL and LOEL for systemic toxicity were 100 (13 mg/kg/day) and 1000 ppm (135 mg/kg/day), respectively, based on an increase in ocular effects at 1000 ppm.

The Maximum Tolerated Dose was not achieved in this study. Although increased kidney weight associated with tubular basophilia was observed in male mice at the 10ppm dose level, this effect was not considered sufficient for an MTD in this study. Review of a six-week range finding study in mice with acetochlor (Life Science Res. report # 85/SUC008/496) showed decreases in body weight gain of 9% and 12% at 600ppm and 1200ppm acetochlor, respectively, for male mice. In female mice from this study, a significant decrease in body weight gain (21%) was not observed until the 2400ppm dose level. Thus, based upon the results of the range-finding study, the MTD can be considered to have been achieved for male mice, but not for female mice.

A significant increase in the incidence of pulmonary adenomas was observed in females. This was associated with significant positive trends in both males and females toward the development of pulmonary adenomas. However, a significant increase in the incidence of pulmonary carcinomas was not observed, and the incidence of combined pulmonary adenomas and carcinomas was not statistically significant in high-dose females, although statistically significant positive trends were still evident for both males and females. Since the study was terminated at 78 weeks (19.5 months), even though survival was greater than 68% in all groups, the observation period may have been insufficient for development of carcinomas. Conversely, the tumor incidences observed in the lungs may have been within historical control ranges. However, historical control data for the laboratory were not presented. Furthermore, the tumors were described as pulmonary adenomas or carcinomas without further characterizing them as to location in the lung; consequently, published historical control data for this strain of mouse were of little use in evaluating spontaneous tumor incidences. Therefore, until historical control data and definitive characterization of the pulmonary tumors, i.e., type and site, are provided, the reviewers are unable to make an assessment of the carcinogenic potential of SC-5676; the study is classified as supplementary and may be upgraded upon submission and review of the required data.

E. **STUDY DEFICIENCIES:**

The following deficiencies in the conduct or reporting of the study were noted:

1. **Historical control data on histopathological findings were not presented.**

2. **Definitive characterization, i.e., type and site, of pulmonary tumors was not performed.**

F. **CLASSIFICATION:** CORE Supplementary data.
Systemic NOEL (Males) = Not established.
Systemic LOEL (Males) = 10 ppm (approx. 1.1 mg/kg/day).
Systemic NOEL (Females) = 100 ppm (approx. 13 mg/kg/day).
Systemic LOEL (Females) = 1000 ppm (approx. 135 mg/kg/day).

Maximum Tolerated Dose- achieved in male mice, not achieved in female mice.

TABLE 28

Historical control data for selected tumours of the kidneys, liver, lungs and haemopoietic tissue in CD-1 mice generated at LSR

Code	:	017A	017B	022
Commenced (year)	:	85	85	86
Source	:	--- Charles River UK ---		
Housing (per cage)	:	1	1	4
Study duration (weeks)	:	78	78	78
No. of mice examined	:	50	50	60
MALES				
Tissue and neoplasm				
Kidney				
Adenoma		0	0	0
Liver				
Haemangioma		2	1	0
Haemangiosarcoma		0	0	0
Hepatocytic adenoma		9	6	1
Hepatocytic carcinoma		2	2	5
Lungs				
Pulmonary adenoma		5	2	7
Pulmonary carcinoma		5	6	2
Haemopoietic tissue				
Malignant lymphoma		4	2	3
FEMALES				
Tissue and neoplasm				
Lungs				
Pulmonary adenoma		2	2	2
Pulmonary carcinoma		2	4	1

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Attachment D

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Reviewed By: Ray Landolt 7/15/91
Section I, Toxicology Branch II - HFAS (H7509C)
Secondary Reviewer: Mike Ioannou 7/15/91
Section I, Toxicology Branch II - HFAS (H7509C)

DATA EVALUATION REPORT

Study Type: One-Year Oral Toxicity Dog (83-1)

TOX Chem. No. 003B
MRID No.:415651-18
Project No. 0-1999

Test Material: N-ethoxymethyl-N-(2-methyl-6-ethylphenyl) chloroacetamide

Classification: Preemergence Herbicide -

Common Name: Acetochlor, SC-5676

Study No.: LSR Report No. 88/SUC018/0136

Date of Study: December 2, 1988

Sponsor: Imperial Chemical Industries, Inc.

Testing Facility: Life Science Research, Ltd.

Title of Report: SC-5676: Toxicity Study by Oral (Capsule) Administration to Beagle Dogs for 52 Weeks.

Author: Alan Broadmeadow

Quality Assurance: D.L.M. Weller

Conclusion: Classification of Data: Guideline

This study satisfies the guideline data requirement (83-1) for a chronic nonrodent oral toxicity study.

This study meets the criteria of 40 CFR 158.34 for neurotoxicity (6) at the 50 mg/kg/day level (HDT).

NOEL = 2 mg/kg/day

LEL = 10 mg/kg/day with salivation, significant increase in alanine aminotransferase and ornithine carbamyl transferase activity accompanied by a significant increase in triglyceride and decreased blood glucose values.

Histopathological changes at this level were in the kidneys (interstitial nephritis and chronic vasculitis), testes (tubular degeneration), epididymides (hypospermia) and liver (reduced glycogen).

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A. Materials:

1. Test Compound - Technical SC-5676 of batch one and three with a purity of 91 percent was used in the study. The test material, a dark brown viscous liquid, was stored in an amber glass container for preparation of the gelatin capsules. Analysis of SC-5676 at the conclusion of the study indicated a concentration of 90.5 percent.

2. Test Animals - Twenty male (8.6 to 11.2 kg) and 20 female (8.0 to 10.9 kg) 20 week old pure-bred beagle dogs were used in this study.

B. Study Design:

1. Allocation of Animals:

Dose Level (mg/kg/day)	Male	Female
Control	5	5
2.0	5	5
10.0	5	5
50.0	5	5

The selection of dose levels for this study was based on a LEL of 60 mg/kg/day demonstrated in a 90-day oral toxicity study in dogs (MRID No. 415651-16).

All animals were housed individually with temperature and humidity controlled to provide a uniform environment. A 12-hour light/dark cycle was provided. Each dog received 400 g of a dry pelleted diet each morning before treatment. The uneaten food was withdrawn and weighed the following morning. Water was available ad libitum. All dogs were vaccinated against hepatitis, leptospirosis, distemper and canine parvovirus at 6, 9 and 12 weeks of age and against Bordatella bronchiseptica at 12 weeks of age.

2. Treatment - The liquid test material was weighed into size 00 gelatin capsules based on the most recent recorded bodyweight. The capsules were administered orally once each day after feeding, seven days a week for 52 weeks. Control animals received empty size 00 capsules.

3. Statistical - The significance of intergroup differences in body weight change, hematology, clinical chemistry, and urinalysis was assessed by Student's t-test using a pooled error variance. The significance of intergroup differences in organ weights was assessed by Dunnett's test.

C. Methods and Results:

1. Observations - Individual daily observations were recorded before and after each dose. In addition, the animals were observed periodically during the day for signs of toxicity.

a. Gross observations include excessive salivation observed within 30 minutes to five hours after dosing and abnormal shaking of the head associated with salivation in the high-dose males and females. The incidence of excessive salivation among males of the mid- and high-dose levels was less frequent and less severe than observed in the high-dose female dogs during the study. Males and females of the high dose level appeared emaciated during the latter half of the study.

b. Neurological examination, as a consequence of the signs of toxicity observed, was performed after 47 weeks with the following parameters evaluated:

Cranial nerve reflex
- Pupillary light and consensual light
- Palpebral-blink and corneal
- Gag
- General examination of the head

Segmental reflex
- Flexor (withdrawal) and crossed extensor
- Patellar
- Extensor tone

Postural reactions
- Placing-visual and tactile
- Extensor postural thrust
- Righting
- Tonic neck reactions
- Hopping reflex

General observations
- Behavior changes
- Abnormalites of gait and stance
- Tremor or other dyskinesis

Neurological changes were observed at the 50 mg/kg level during the last six weeks of the study. These changes were severe in 2/5 male and 3/5 female dogs. Neurological changes were less severe in 2/5 males and 1/5 females and not apparent in 1/5 males and 1/5 females at the 50 mg/kg dose level. These neurological changes comprised of "swaying or shaking of the body and head, head oscillation, stiffness and rigidity of the hindlimbs resulting in incoordination, ataxia, tremor and high-stepping gait, depressed righting, hopping and flexor reflexes and exaggerated tonic neck reflex". The two males exhibiting these neurological changes were killed during week 46 and the four females during week 39*, 46, 48, and 51 of the study. These neurological changes were not appearent at the mid and low levels.

c. Mortality - Six animals exhibiting neurological changes at the 50 mg/kg level were killed during the last six weeks of the study.

Dose Level (mg/kg)	Males	Females
Control	0/5	0/5
2.0	0/5	0/5
10.0	0/5	0/5
50.0	2/5	4/5

*A neurological examination was not performed for this female dog, however, this animal was ataxic prior to death during week 39.

d. Food Consumption - Each dog received 400 g of a dry pelleted diet each morning before treatment and the uneaten food was withdrawn and weighed the following morning.

A 23 to 28 percent decrease in food consumption was observed for females dosed the 50 mg/kg level during the last 39 weeks of the study. At the 10 mg/kg level a 13 percent decrease in food intake was observed for females during the last 13 weeks of the study. Food consumption of the low dosed females and the three test levels for males were comparable to their respective control values for the 52-week period.

e. Water intake was monitored following an increase in urinary volume after 24 weeks of treatment. "Water consumption was recorded over a three day period using polyethylene bottles fitted with Lixit valves" during weeks 30, 39, and 52.

An increase in water intake was observed during weeks 30-52 at the 50 mg/kg level in males and females by 57 and 23 percent, respectively, as compared to the control values.

f. Body Weight - All animals were weighed weekly before feeding and then prior to necropsy regardless of feeding cycle.

A significant ($p < 0.001$) decrement in body weight change was reported for the high dose females during weeks 26 and 39 by 90 and 81 percent, respectively. A decrease in food intake of 23 to 28 percent was observed during this period.

A significant ($p < 0.05$) decrement in body weight change was reported by the 39th week for the high dose males by 65 percent.

Mean body weight (Kg) values and percent change (%) as compared to the control values are presented in the following table.

	Male (mg/kg)				Female (mg/kg)			
Week	0	2.0	10.0	50.0	0	2.0	10.0	50.0
13	2.6	2.1(19)	2.1(19)	1.7(35)	2.3	2.3(0)	2.0(13)	1.5(35)
26	3.0	2.1(30)	2.4(20)	1.5(50)	3.1	3.0(3)	2.5(19)	0.3(90)**
39	3.1	2.1(32)	2.3(26)	1.1(65)*	3.7	3.3(11)	3.2(14)	0.7(81)**
52	3.2	2.4(25)	2.7(16)	2.2(31)	4.1	3.6(12)	3.4(17)	0.1(98)

* $p < 0.05$
** $p < 0.001$

g. Ophthalmoscopy examinations were performed initially, at 24 and 50 weeks of the study.

No treatment-related findings were observed between the treatment and control groups.

2. Clinical Findings - Blood was collected for hematology and clinical chemistry prior to administration of the oral dose from animals fasted overnight. Blood samples were collected one week before the initial dose, then after the 12th, 24th, and 50th week of the study. In the study report, terminal hematology and clinical chemistry values from the decedent animals were reported separately from the animals surviving 50 weeks of treatment. The clinical findings of the decedent animals were not included in this Data Evaluation Report.

a. Hematology parameters examined: The checked (*) parameters are recommended by Subdivision F guidelines of November 1989.

* Erythrocyte count
* Hemoglobin
Erythrocyte sedimentation rate
* Hematocrit
Reticulocyte count
Prothrombin time
Activated partial thromboplastin time
* Leucocyte count
* Leucocyte differential count
* Platelet count
Mean corpuscular hemoglobin
Mean corpuscular volume
Mean corpuscular hemoglobin concentration

These animals were not Factor VII deficient as reported in the subchronic dog study (MRID 415651-16).

No dose related hematological findings were reported for those animals that survived 50 weeks of compound administration.

b. Clinical chemistry parameters examined: The checked (*) parameters are recommended by Subdivision F guidelines of November 1989.

* Alkaline phosphatase (AP)
* Alanine aminotransferase (ALT)
* Aspartate aminotransferase (AST)
Cholinesterase-plasma and RBC
Sorbitol dehydrogenase (SDH)
Ornithine carbamyl transferase (OCT)
Gamma-glutamyl transpeptidase (GGT)
* Urea
* Creatinine
* Glucose
* Albumin
* Creatine phosphokinase (CPK)
* Total bilirubin
Total triglyceride
* Total cholesterol
* Total protein
Electrophoretic protein
* Sodium
* Chloride
* Potassium
* Inorganic phosphorus
* Calcium

Parameter recommended but not reported: lactic dehydrogenase

Alanine aminotransferase (ALT) activity was significantly elevated in excess of 100 percent at the 50 mg/kg level, as compared to the control values, in males ($p < 0.05$) during weeks 12, 24, and 50 and in females ($p < 0.01$) during weeks 12 and 24 of the study. Increased ALT activity was observed at the 10 mg/kg level in males by 32, 19, and 70 percent during weeks 12, 24 and 50, respectively. Female ALT activity was significantly ($p < 0.01$) elevated at the 10 mg/kg level in excess of 100 percent at the termination of the study.

Gamma-glutamyl transpeptidase (GGT) activity was significantly ($p < 0.05$) elevated in males at the 50 mg/kg level by 100, 25, and 75 percent during weeks 12, 24, and 50, respectively.

Ornithine carbamyl transferase (OCT) activity was significantly elevated in excess of 100 percent at the 50 mg/kg level in males ($p < 0.01$) during weeks 12, 24, and 50 of the study and in females ($p < 0.05$) at the 10 mg/kg level by week 50.

Blood urea values were elevated at the 50 mg/kg level significantly in males ($p < 0.01$) by 58 and 80 percent during weeks 24 and 50, respectively, and in females ($p < 0.05$) by 93 percent at the termination of the study.

Blood creatinine values were elevated at the 50 mg/kg level significantly in males ($p < 0.001$) by 50 percent at the termination of the study and in females ($p < 0.05$) by 33 and 83 percent during weeks 24 and 50, respectively.

Blood glucose values were decreased at the 50 mg/kg level significantly in males ($p < 0.05$) by 10 and 14 percent during weeks 12 and 24, respectively, and in females ($p < 0.05$) by 11 to 14 percent during weeks 12, 24, and 50 of the study. Female blood glucose values were significantly ($p < 0.05$) depressed at the 10 mg/kg level by 7 and 9 percent during weeks 24 and 50, respectively. In addition, female blood glucose values were significantly ($p < 0.01$) decreased at the low level during week 50 by 8 percent.

Triglyceride values at the 50 mg/kg level were significantly ($p < 0.01$) elevated in males by 46, 59, and 67 percent, during weeks 12, 24, and 50, respectively. In females the triglyceride values were significantly ($p < 0.05$) elevated by the 12 week interval (only) at the 10 and 50 mg/kg levels by 34 and 38 percent, respectively.

Cholesterol values at the 50 mg/kg level were significantly ($p < 0.05$) elevated in males by 26, 40, and 36 percent during weeks 12, 24, and 50, respectively.

Plasma cholinesterase values were significantly ($p < 0.05$) elevated in females at the 50 mg/kg level for acetyl activity during week 24 by 34 % and for butytyl activity during weeks 12 and 24 by 17 and 32%, respectively.

A dose related trend was observed for an increased acetyl and butyryl activity in females at the 10 mg/kg level. Male plasma cholinesterase values at the 50 mg/kg level were elevated during weeks 24 and 50 for for acetyl activity by 19 to 23% and for butyryl activity by 22%.

A summary of the statistically significant blood chemistry changes reported for the mid- and high-dose levels are presented in the following table.

Sex	Male						Female					
Dose (mg/kg/day)	10			50			10			50		
Interval (weeks)	12	24	50	12	24	50	12	24	50	12	24	50
Parameter:												
Alanine aminotransferase				↑	↑	↑			↑	↑	↑	
Gamma-glutamyl transferase				↑	↑	↑						
Ornithine carbamyl transferase				↑	↑	↑			↑			
Urea					↑	↑						↑
Creatinine						↑					↑	↑
Glucose				↓	↓			↓	↓	↓	↓	↓
Triglyceride				↑	↑	↑	↑			↑		
Cholesterol				↑	↑	↑						
Plasma cholinesterase												
acetyl											↑	
butyryl										↑	↑	

c. Urinalysis - Urine was collected prior to the initial treatment then during weeks 11, 23, and 49 from animals housed individually in metabolism cages overnight without food or water. The following were examined: The checked (*) parameters are recommended by Subdivision F guidelines of November 1989.

* Appearance	* Protein	* Bilirubin
* Volume	Reducing substances	Nitrites
pH	* Glucose	* Blood
* Specific gravity	* Ketones	* Microscopic sediment

An increase in urinary volume was reported at the 50 mg/kg level for females and significantly ($p < 0.05$) for males during weeks 23 and 49. This increased urinary volume was accompanied by a decrease in specific gravity and appearence "considered to be paler than normal".

d. Fecal examination for occult blood - Feces were collected over a three day period initially then during weeks 12, 24, and 50.

Feces from male and female dogs dosed at 2, 10, and 50 mg/kg were negative for blood.

3. Terminal Observation - On completion of the experimental period, all animals were anesthetized with sodium pentobarbital and killed by exsanguination. The following tissues were collected for histopathological examination and the (X) checked organs were weighed. Organ weights for decedent animals were included for the calculation of group means and standard deviations. The checked (*) parameters were recommended by Subdivision F guidelines of November 1989.

*	X	Adrenals	*	X	Liver	*		Skeletal muscle
		Aorta (thoracic)	*	X	Lung	*		Skin
*	X	Brain	*		Lymph-cervical	*		Spinal Cord -
		Bronchi			mesenteric			Cervical, thoracic,
*		Caecum			peribronchial			and Lumbar
		Colon	*		Mammary Glands	*	X	Spleen
*		Duodenum			Nasopharynx	*		Sternal bone and marrow
*		Epididymides	*	X	Ovaries	*		Stomach - Pylorus
*		Esophagus	*		Pancreas			and fundus
*		Eyes and optic nerve			Para-nasal sinus	*	X	Testes
		Femur	*	X	Pituitary	*		Thymus
		Gallbladder		X	Prostate	*	X	Thyroid and
*	X	Heart	*		Rectum			parathyroid
*		Ileum	*		Salivary Gland			Tongue
		Jejunum			Sciatic Nerve	*		Trachea
*	X	Kidneys						Turbinates
						*		Urinary Bladder
						*	X	Uterus
								Vagina

a. Brain Cholinesterase - Brain tissues taken at necropsy from the mid-line region of the cortex and both hemispheres were examined by the Ellman method. No dose-relared changes were reported in brain acetyl cholinesterase activity at the dose levels tested.

b. Organ Weights - Terminal body weights were reduced at the 50mg/kg level as compared to the controls for males by 16 % and significantly ($p < 0.01$) for females by 37%. Statistically significant changes in relative organ weights were limited to animals at the 50 mg/kg level. Male and female relative kidney weights were comparable to their respective control values. However, a significant ($p < 0.01$) decrease in absolute kidney weight of 37% was reported for females at the 50 mg/kg level.

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Female relative organ weights at the 50 mg/kg level were significantly ($p < 0.01$) increased for brain and heart by 55 and 27%, respectively. Male absolute heart weights were significantly ($p < 0.05$) reduced by 14 % at the high dose level.

At the 50 mg/kg level relative adrenal weights were increased for males by 28 % and significantly ($p < 0.01$) for females by 78 %.

Relative liver weights at the 50 mg/kg level were increased for females by 17 percent and significantly ($p < 0.01$) for males by 32 %. Female absolute liver weights were significantly ($p < 0.05$) reduced by 27% at the high dose level.

A significant increase in relative lung weights was reported at the 50 mg/kg level for males ($p < 0.05$) and females ($p < 0.01$) by 16 and 32 %, respectively. Female absolute lung weights were significantly reduced at the 10 ($p < 0.05$) and 50 ($p < 0.01$) mg/kg levels by 13 and 19%, respectively.

At the 50 mg/kg level a significant decrease in relative ($p < 0.05$) and absolute ($p < 0.01$) testes weight was reported by 34% and 48%, respectively. Relative thyroid weights of males at the 50 mg/kg level were significantly ($p < 0.05$) increased by 28%.

The percent increase and/or decrease in relative organ and absolute ( ) weights, for those respective organs that demonstrated a statistically significant change at the 50 mg/kg level, are presented in the following table.

	% Increase						% Decrease				
	Adrenal	Brain	Heart	Liver	Lung	Thyroid	Heart	Kidney	Liver	Lung	Testes
Male				32	16	28	(14)				34(48)
Female	78	55	27		32			(37)	(27)	(19)	

c. Macroscopic pathology findings were limited to the 50 mg/kg level. The animals killed prematurely were emaciated in appearence. The kidneys of males and females were abnormal in shape with multiple pale areas on the surface. Cervical lymph nodes of males (3/5) and females (3/5) were dark in appearance. The gallbladder was distended in males (1/5) and females (2/5). Adrenals were enlarged in 2/5 females. The liver of 2/5 males was pale in appearence.

d. Histopathological Examination - Treatment-related histopathological changes in the brain and kidneys of both sexes and in the testes of males were reported for the 50 mg/kg level. In the cerebellum, there were degeneration of the granular layer and depletion of the Purkinje cells. Kidney changes consisted of collecting duct hyperplasia, transitional cell hyperplasia, cortical atrophy with fibrosis and scarring accompanied by chronic vasculitis, interstitial nephritis, dilatation of Bowman's space and lipofusin pigment in the cortical tubules.

Testicular findings consisted of the degeneration of seminiferous tubules, maturation arrest and the formation of spermatid giant cells within the tubules. Hypospermia in the epididymides was also reported at the high-dose level.

Histopathological changes at the 10 mg/kg level were limited to the kidneys, epididymides, and testes of males. Kidney changes consist of interstitial nephritis, and chronic vasculitis. Hypospermia of the epididymides and seminiferous tubule degeneration were reported at the mid-dose level.

A dose related decrease in glycogen content in the liver was reported for males of all three dose levels and females of the mid and high dose levels.

The following table (from this study, table 11, pages 102-114) summarizes the the incidence of histopathological findings in the test groups of a greater frequency than observed in the control animals. Two males and four females at the 50 mg/kg level were killed during the experimental period.

Sex	Male				Female			
Dose (mg/kg/day)	0	2	10	50	0	2	10	50
Brain, Vermis cerebellum								
Degeneration of the granular layer				4/5				3/5
Depletion of Purkinje cells				4/5				2/5
Demyelination and degeneration of granule cell axons				1/5				
Kidney								
Interstitial nephritis			2/5	5/5	1/5			4/5
Collecting duct hyperplasia				5/5				5/5
Chronic vasculitis			3/5	4/5				5/5
Cortical fibrosis and/or scarred areas				4/5				5/5
Dilatation of Bowman's space				4/5				4/5
Cortical atrophy				4/5				5/5
Lipofuchsin pigment in cortical tubules		1/5		4/5				3/5
Dilatation of capilary blood vessels				1/5				2/5
Collecting duct cortical dilatation				1/5				2/5
Transitional cell hyperplasia				5/5				5/5
Papillary necrosis								1/5
Focal necrosis								1/5
Liver								
Reduced glycogen		1/5	2/5	4/5	1/5		1/5	4/5
Pigment in hepatocytes				1/5				1/5
Epididymides								
Hypospermia		1/5	2/5	5/5				
Testes								
Tubular degeneration			4/5	5/5				
Maturation arrest				5/5				
Spermatial giant cells within tubules		1/5		4/5				

Conclusion: The toxicity of SC-5676, when administered orally in capsules to dogs at 2, 10 or 50 mg/kg for 7 days per week for 52 weeks, was evident by treatment related changes in the brain, kidney and testes of animals dosed at the high level. Renal toxicity was manifest after 24 weeks of treatment at the 50 mg/kg level by increased water intake, urinary volume, blood urea and creatinine values. Alterations in kidney macroscopic and microscopic pathological findings were reported at the termination of the study. Neurological effects were appearent after 39 weeks of treatment at the 50 mg/kg level by changes in posture, gait, reflexes and ataxia. Two males and four females were killed between weeks 39 and 51 due to the severity of the neurological effects. These neurological changes were associated with the histopathological findings in the vermis cerebellum. Testicular effects consisted of decreased relative testes weight, atrophy and alterations in histopathological findings at the 50 mg/kg level. Increased clinical chemistry values, increased relative liver weight and reduced glycogen content characterized the liver effects. In addition, animals at the high dose level exhibited excessive salivation and weight loss (emaciation).

NOEL = 2 mg/kg/day

LEL = 10 mg/kg/day with salivation, significant increase in alanine aminotransferase and ornithine carbamyl transferase activity accompanied by a significant increase in triglyceride and decreased blood glucose values.

Histopathological changes at this level were in the kidney (interstitial nephritis and chronic vasculitis), testes (tubular degeneration), epididymides (hypospermia) and liver (reduced glycogen).

Classification of Data: Guideline

This study satisfies the guideline data requirement (83-1) for a chronic nonrodent oral toxicity study.

This study meets the criteria of 40 CFR 158.34 for neurotoxicity (6) at the 50 mg/kg level (HDT) with neurological behavioural changes, death of 2/5 males and 4/5 females between weeks 39 and 51 due to the severity of the neurological effects and neuropathology reported at this level.

(88)

Attachment E

Guideline Series 84: MUTAGENICITY

Reviewed by: John H.S. Chen, D.V.M. John H S Chen 6/11/91
Section I, Toxicology Branch II (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. Y.M.I 6/12/91
Section I, Toxicology Branch II (H7509C)

~~003478~~

DATA EVALUATION REPORT

CHEMICAL: Acetochlor

Tox. Chem. No.: 0038

MRID No.: 415651-21

EPA File Symbol:

STUDY TYPE: Salmonella/mammalian activation gene mutation assay

ACCESSION NUMBER:

SYNONYMS/CAS No.:

SPONSOR: ICI Americas, Inc., Wilmington, Delaware

TESTING FACILITY: ICI Central Toxicology Laboratory, Cheshire, UK

TITLE OF REPORT: Acetochlor: An Evaluation in the Salmonella Mutation Assay

AUTHOR(S): R.D. Challander & K.P. Priestley

STUDY NUMBER(S): YW2370/VW2423

REPORT ISSUED: July 19, 1989

CONCLUSION(S) - Executive Summary:

Acetochlor (89.9% purity) was found to induce reproducible, positive, mutagenic response to TA1538 strains of Salmonella typhimurium with metabolic activation system at 1000 ug/plate. Although this effect was less than 2X background mutation rates in each experiment, the increases in the observed number of revertant colonies in strain TA1538 at 1000 ug/plate in the presence of metabolic activation were statistically significant ($P<0.05$). Therefore, acetochlor gave a weak positive response in this study.

Study: Acceptable

This study satisfies the guideline requirements, 84-2, for a mutagenicity study (gene mutation).

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SALMONELLA

A. MATERIALS Acetochlor

1. Test Material: Name:
Description (e.g. technical, nature, color, stability):
Brown black liquid
Batch #: B2993/15 Purity: 89.9%
Contaminants: if reported, list in CBI appendix
Solvent used: DMSO
Other comments:

2. Control Materials:
Negative: DMSO
Solvent/final concentration:
Positive: Non-activation:

MNNG	[redacted]	1-5	ug/plate TA100, TA1535
Dauno Rubicin	[redacted]	0.2-1	ug/plate TA98, [redacted]
ICR-191	9-Aminoacridine	0.5-2	ug/plate [redacted] TA1537
4-NPD	[redacted]	1-5	ug/plate TA1538

MNNG = N-Methyl-N'-Nitro-N-Nitrosoquanidine; 4-NPD = 4-Nitro-O-Phenylenediamine

Activation:
2-Aminoanthracene (2-anthramine) 0.2 - 1 ug/plate
usually all strains
Other (list):

3. Activation: S9 derived from
__X__ Aroclor 1254 __X__ induced Male__ rat __X__ liver
___ phenobarbital ___ non-induced ___ mouse ___ lung
___ none ___ hamster ___ other
___ other ___ other
If other, describe below
Describe S9 mix composition (if purchased, give details):

S9 fraction	3 ml	Co-factor solution:		
Sucrose-Tris-EDTA Buffer	7 ml	Na_2HPO_4	100 mM	$MgCl_2$ 8 r
Co-factor solution	20 ml	KCl	33 mM	
		Glucose-6-phosphate	5 mM	
		NADP	4 mM	

4. Test organisms: S. typhimurium strains
___ TA97 __X__ TA98 __X__ TA100 ___ TA102 ___ TA104
__X__ TA1535 __X__ TA1537 __X__ TA1538 ; list any others:
Properly maintained? (Y) / N (circle one)
Checked for appropriate genetic markers (rfa mutation, R factor)? (Y) / N (circle one)

5. Test compound concentrations used:
Non-activated conditions: 1.6, 8, 40, 200, 1000, & 5000 ug/plate

Activated conditions : Same as non-activated conditions

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SALMONELLA

B. TEST PERFORMANCE

1. Type of Salmonella assay:
   - x standard plate test
   - ___ pre-incubation (___ minutes)
   - ___ "Prival" modification (i.e. azo reduction method)
   - ___ spot test
   - ___ other (describe in a.)

a. Protocol (brief description, or attach copy to appendix, if appropriate; e.g. include mediums used, incubation times, assay evaluation):

The tests were carried out in accordance with the method described by Maron and Ames (the revised methods for the Salmonella mutagenicity test; Mutation Res. 113, 173-215, 1983). The experimental procedures used for this study is attached (Lab report pages 28-33).

2. Preliminary cytotoxicity assay (include concentration ranges, activation and nonactivation; strain(s) used; reported results, e.g. cytotoxicity indices (effect on background lawn; reduction in revertants) and solubility):

Although the preliminary cytotoxicity test was not performed in this study, an acceptable maximum dosage level of test material (5 mg/plate) has been used.

3. Mutagenicity assay (reported results, e.g. induction of revertants - individual plate counts and/or summary given; appropriateness of positive and background (concurrent and/or historical) revertant levels; number of concentration levels used; number of cultures per concentration; include representative table, if appropriate):

The mutagenicity of Acetochlor technical in the Ames test was evaluated using five tester strains of S. typhimurium (TA98, TA100, TA1535, TA1537 & TA1538) at 6 concentrations (1.6 to 5000 ug/plate) either in the presence or absence of metabolic activation system from three separate experiments. Results obtained from these three experiments, the compound did not induce any significant, reproducible increases in the numbers of revertant colonies in strains TA1535, TA1537, TA98 and TA100 either in the presence or absence of metabolic activation system (S9 activation). However, significant positive responses ($P<0.05$) were repeatedly observed in strain TA1538 at 1000 ug/plate in the presence of S9 mix (See results of the 2nd and 3rd Experiments in Tables 2 and 3). Although less than 2X background mutation rates were noted in each experiment, the general effect was consistently observed between experiments. In the first experiment (See results in Table 1), the compound induced a significant increase ($P<0.01$) in the observed number of revertant colonies in strain TA1538 at 1000 ug/plate in the absence of S9 mix. This effect was not reproduced in the 2nd and 3rd experiments and was not considered to be compound-induced mutation.

The study Author concluded that "under the conditions of this assay, acetochlor gave a weak but positive, mutagenic response in the presence of auxiliary metabolising system (S9) with Salmonella typhimurium tester strain TA1538."

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SALMONELLA

4. **Reviewer's discussion/conclusions** (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

(A) The spontaneous revertant colonies for each of the five tester strains of Salmonella typhimurium are found within the normal ranges of revertant colonies recommended by the Ames Salmonella mutagenicity test (Ames et al., Mutation Res. 31: 347-364, 1975).

(B) The strain specific control compounds (MNNG, ICR191, 4-Nitro--o-phnylenediamine and Dauno Rabicin) and the positive control compound (2-AA) to ensure the efficacy of the activation system have given significantly positive responses as expected. These positive control values demonstrated the sensitivity of the assay system with or without metabolic activation.

(C) Statistically significant increases ($P<0.05$; less than 2X background mutation rates) in the number of revertant colonias for the strain TA1538 were observed in the presence of metabolic activation at 1000 ug/plate in Experiments 2 and 3 (See results given in Tables 2 and 3). We agree with the study Author's conclusion that acetochlor gave a weak but positive response with strain TA1538 in the presence of S9 mix only.

(D) The study was conducted properly to generate valid reults and satisfies the guideline requirements, 84-2, for a mutagenicity study (gene mutation).

5. Was test performed under GLPs (is a quality assurance statement present)? (Y) / N (circle one)

6. CBI appendix attached (Y) / N (circle one)

ACETOCHLOR

TOX R# 008728

Page_____ is not included in this copy.

Pages 93 through 104 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.

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Attachment F

008728

Guideline Series 84: MUTAGENICITY

Reviewed by: John H.S. Chen, D.V.M. [signature] 6/11/91
Section I, Toxicology Branch II (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. [signature] 6/12/91
Section I, Toxicology Branch II (H7509C)

~~008478~~

DATA EVALUATION REPORT

CHEMICAL: Acetochlor **Tox. Chem. No.:** 003B

MRID No.: 415651-23 **EPA File Symbol:**

STUDY TYPE: In vivo micronucleus assay in mouse bone marrow

ACCESSION NUMBER:

SYNONYMS/CAS No.:

SPONSOR: ICI Americas, Inc., Agricultural Products, Wilmington, DL 19897

TESTING FACILITY: ICI Central Toxicology Laboratory
Cheshire, UK

TITLE OF REPORT: Acetochlor: An evaluation in the mouse micronucleus test

AUTHOR(S): V. Randall

STUDY NUMBER(S): SM0339

REPORT ISSUED: 7/31/89

CONCLUSION(S) - Executive Summary:

Acetochlor was not clastogenic in the mouse micronucleus test at the dose levels tested.

Dose levels tested: 898 & 1436 mg/kg (males)
1075 & 1719 mg/kg (females)

Deficiencies found: no information on stability and storage conditions of the test material; and no indication of coded slides prior to scoring.

The study may be upgraded if the above missing information can be provided.

Study: Unacceptable
This study does not satisfy the guideline requirements, 84-3, for a mutagenicity study (chromosomal aberrations)

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MICRONUCLEUS

A. **MATERIALS** Acetochlor

1. **Test Material**: Name:
Description (e.g. technical, nature, color, stability):
Brown liquid
Batch #: SC31/88 Purity: 89.4%
Contaminants: if reported, list in CBI appendix
Solvent used: Corn oil
Other comments: CTL Reference No. Y06341/007/001

2. **Control Materials**:
Negative/Route of administration:
Corn oil/Intragastric route/10 ml/kg

Vehicle/Final concentration/Route of administration:

Positive/Final concentration/Route of administration:

Cyclophosphamide/ Intragastric route / 65 mg/kg (10 ml/kg)

3. **Test compound**:
Route of administration: Intragastric route
(single dose at volume of 10 ml/kg)
Dose levels used:
898 & 1436 mg/kg (males)
1075 & 1719 mg/kg (females)

4. **Test animals**:
a. Species mouse Strain CS7BL/6JFCD-1/A1PK Age 9-14 weeks & 8-11 weeks for Phase 1 & 2, respective
Weight _____ Source:
b. No. animals used per dose: 5 males 5 females
c. Properly maintained? (Y) / N (circle one)

B. **TEST PERFORMANCE**

1. **Treatment and Sampling Times**:
a. **Test compound**
Dosing: X once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
X 24 hr X 48 hr X 72 hr (mark all that are appropriate)
_____ other (describe):

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MICRONUCLEUS

b. Negative and/or vehicle control
Dosing: __X__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
__X__ 24 hr __X__ 48 hr __X__ 72 hr (mark all that are appropriate)
_____ other (describe):

c. Positive control
Dosing: __X__ once _____ twice (24 hr apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hr _____ 12 hr
__X__ 24 hr _____ 48 hr _____ 72 hr (mark all that are appropriate)
_____ other (describe):

2. **Tissues and Cells Examined:**

__X__ bone marrow _____ other (list):

No. of polychromatic erythrocytes (PCE) examined per animal: 1000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: 1000
Other (if other cell types examined, describe):

3. **Details of slide preparation:**

At the end of specified intervals following dosing, bone marrow smears were prepared and stained with polychrome methylene blue and eosin (details of slide preparation were not given).

4. **Preliminary cytotoxicity assay (reported results, e.g. include dose range, signs of toxicity - e.g. MTD considerations, clinical signs; no. animals):**

Acetochlor was initially administered as a single intragastric dose to two groups of 2 female mice at 2000 and 3000 mg/kg. Both animals survived at the 2000 mg/kg dose level whereas both died at the 3000 mg/kg level. Then, two groups of 5 male and 5 female mice were dosed at 2000 and 3000 mg/kg for the cytotoxicity study. Three males and one female died at the 2000 mg/kg level and all animals died at the 3000 mg/kg level. Again, another group of five male and 5 female mice

were dosed at 1000 mg/kg and no deaths were observed in either sex at this dose level. Based on these results, a dose level of 1436 mg/kg or 1719 mg/kg was selected as the highest dose for males or females, respectively, in the micronucleus test.

5. **Micronucleus assay (reported results, e.g. include induction of micronuclei; appropriateness of negative, solvent and positive control micronucleus frequencies; ratio of PCE/NCE; sex differences (if any); appropriateness of dose levels and route; statistical evaluation; include representative table, if appropriate):**

Significant increase ($P<0.05$) in the frequency of MPE was observed in the female mice at 1719 mg/kg dose level (24 hour sampling time) (See results given in Table 2). However, no statistically significant increases in the frequency of MPE were observed at either dose level (50% MLD: 898 mg/kg, Males; 1075 mg/kg, Females; 80% MLD: 1436 mg/kg, Males; 1719 mg/kg, Females) of acetochlor at any of the sampling times (24, 48, or 72 hours) investigated when the sexes were combined (See results given in Table 1). In order to assess the validity of this effect, a further 2000 PE were examined for females at both dose levels of acetochlor (1075 and 1719 mg/kg) and the solvent control at the 24 hour sampling time. Following the extended counts there were no statistically significant difference between the test groups and the solvent control whether the extended counts were analyzed alone (as 2000 PE) (See results given in Table 3), or combined with the original counts (as 3000 PE) (Table 4). The positive control compound (CP) induced significant increase ($P<0.01$) in the frequency of MPE in both male and female mice as expected.

When the sexes were combined, significant reductions ($P<0.01$) in the ratios of PCE:NCE (expressed as % PCE) were observed in the high dose group (i.e., 80% MLD) at the 24 hour sampling time and in both levels (i.e., 50% MLD & 80% MLD) of acetochlor at the 72 hour sampling time (See results given in Table 5). Significant reductions ($P<0.01$) in the ratios of PCE:NCE (expressed as % PCE) were also observed in male mice at 1436 mg/kg dose level at the 24 hour sampling time, in female mice at 1075 mg/kg level and both sexes at the high dose level (i.e., 1436 mg/kg for males; 1719 mg/kg for females) at the 72 hour sampling time (Table 6). These results suggest that acetochlor induced a cytotoxic response in the bone marrow.

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MICRONUCLEUS

6. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions):

(A) The positive control compound, cyclophosphamide, apparently induced significant increases of the PCE with micronuclei ($P<0.01$) in the bone marrows of both males and females, indicating the sensitivity of the assay system to detect a clastogen.

(B) The spontaneous rates of micronuclei in the PCE of the vehicle control were found from 0.26% (females) to 0.1% (males) in this study. These results are within the normal range for performing the mouse micronucleus test as recommended by Heddle et al.(Mutation Res. 123: 61-118, 1983).

(C) The test compound has been tested to cytotoxicity level (1436 mg/kg for males and 1719 mg/kg for females) as evidenced by reducing the ratios of PCE:NCE (expressed as %PCE) in the bone marrows of high dose male and female mice.

(D) Sampling times (i.e., 24, 48, & 72 hour intervals) used were adequate in this mouse micronucleus assay.

(E) However the evaluation of this study cannot be accomplished due to the following deficiencies:

(a) Information on the stability and storage conditions of the test material were missing; and
(b) The report did not indicate whether slides were coded prior to scoring.

Based on the above deficiencies, the study is not fully acceptable in the present form and may be upgraded on resolution of the reported deficiencies. Therefore, this study does not satisfy the guideline requirements, 84-3, for a mutagenicity study (chromosomal aberrations).

7. Was test performed under GLPs (is a quality assurance statement present)? (Y) / N (circle one)

8. CBI appendix attached (Y) / N (circle one)

ACETOCHLOR

Tox R# 008728

Page_____ is not included in this copy.

Pages 110 through 115 are not included.

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X FIFRA registration data.

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(116)

008728

Attachment G

Guideline Series 84: MUTAGENICITY

Reviewed by: John H.S. Chen, D.V.M. John H.S. Chen 6/11/91
Section I, Toxicology Branch II (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. YMI 6/12/91
Section I, Toxicology Branch II (H7509C)

~~008478~~

IA EVALUATION REPORT

CHEMICAL: Acetochlor

Tox. Chem. No.: 003B

EPA File Symbol:

STUDY TYPE: Mammalian cells in culture cytogenetics assay in human lymphocytes

ACCESSION NUMBER:
MRID No.: 415651-22
SYNONYMS/CAS No.:

SPONSOR: ICI Americas Inc., Wilmington, Delaware 19897

TESTING FACILITY: ICI Central Toxicology Laboratory, Cheshire, UK

TITLE OF REPORT: An evaluation in the in-vitro cytogenetic assay with Acetochlor in human lymphocytes

AUTHOR(S): C.A. Howard

STUDY NUMBER(S): SV0336

REPORT ISSUED: July 20, 1989

CONCLUSION(S).- Executive Summary:

Technical acetochlor was clastogenic in cultured human lymphocytes at 100 ug/ml in both the presence and absence of rat S9 mix activation and at 50 ug/ml without metabolic activation .

Dose levels tested: 10, 50, 100 ug/ml

Classification: Acceptable

This study satisfies the Guideline Requirements, 84-3, for a mutagenicity study (chromosomal aberrations)

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IN VITRO MAMMALIAN CYTOGENETICS

A. **MATERIALS** Acetochlor Technical

1. **Test Material**: Name:
Description (e.g. technical, nature, color, stability):
a brown liquid
Batch #: A1016/9 Purity: 89.4%
Contaminants: if reported, list in CBI appendix
Solvent used: DMSO
Other comments:

2. **Control Materials**:
Negative: DMSO
Solvent/final concentration:
Positive: Non-activation (concentrations, solvent):
Mitomycin C/0.5 ug/ml/physiological saline (0.85%)

Activation (concentrations, solvent):
Cyclophosphamide/100 ug/ml /physiological saline (0.85%)

3. **Activation**: S9 derived from Alpk:APFSD albino rat
_X_ Aroclor 1254 ___ induced male rat ___X liver
___ phenobarbital ___ non-induced ___ mouse ___ lung
___ none ___ hamster ___ other
___ other ___ other
If other, describe below
Describe S9 mix composition (if purchased, give details):
Final concentration in S9-mix (mM): Na_2HPO_4 75 mM; KCl 25 mM; Glucose-6-phosphate 4 mM; NADP 3 mM; $MgCl_2$ 6 mM

4. **Test compound concentrations used:**
Non-activated conditions: 10, 50, & 100 ug/ml

Activated conditions: 10, 50, & 100 ug/ml

IN VITRO MAMMALIAN CYTOGENETICS

5. Test Cells: mammalian cells in culture
Describe cell line, cell strain or primary cell culture (if human lymphocytes, describe conditions of subjects) used:

Human blood was drawn aseptically from two healthy donors, donor 1 who is male and donor 2 who is female, both donors having a previously established low incidence of chromosomal damage. Cultures were initiated with phytohemagglutinin (0.1 mg/ml) and maintained in supplemented RPMI 1640 tissue culture medium at 37o C.

Properly maintained? (Y) / N (circle one)

Cell line or strain periodically checked for Mycoplasma contamination? Y / N (circle one) Not applicable

Cell line or strain periodically checked for karyotype stability? Y / N (circle one) Not applicable

B. TEST PERFORMANCE

1. Cell treatment:
a. Cells exposed to test compound for:
2.5-~~3.5~~ hours (non-activated) 2.5-~~3.5~~ hours (activated)

b. Cells exposed to positive controls for:
2.5-~~3.5~~ hours (non-activated) 2.5-~~3.5~~ hours (activated)

c. Cells exposed to negative and/or solvent controls for:
2.5-~~3.5~~ hours (non-activated) 2.5-~~3.5~~ hours (activated)

2. Protocol (brief description, or attach copy to appendix, if appropriate; include e.g. number of cell cultures; medium; incubation times; if lymphocytes, nature of mitogen and when added; cell density during treatment; harvest times; spindle inhibitor and when used; chromosome preparation and analysis; number of cells/culture analyzed; statistics used):

The test protocol used was based on the criteria established by Scott et al. (In-vitro chromosome aberration assays: In: Brian J. Dean (Ed) Report of UKEMS Sub-Committee onguidelines for mutagenicity testing, United Kingdom Environmental Mutagen Society (Page 19-22).

3. Preliminary cytotoxicity assay (include concentration ranges, activation and nonactivation; reported results, e.g. cytotoxicity and solubility; rationale for determining harvest times (e.g. alterations in cell cycle) and concentration levels, if reported):

At 44 hours after culture initiation, the test sample of acetochlor was administered to duplicate cultures from donors 1 and 2 at concentrations ranging from 3-900 ug/ml growth media, from which an appropriate dose range range was selected for the main study. The top dose was determined by the toxicity of this solution to reduce the mitotic index. In the absence of metabolic activation, the mitotic index in cultures (donors 1 & 2) treated with 100 ug/ml of acetochlor was reduced to 35.1-40.8% of the concurrent control values (See results given in Table 1). Therefore, a range of dose levels (100, 50, & 10 ug/ml) was selected for the cytogenetic test with 100 ug/ml as the highest concentration.

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IN VITRO MAMMALIAN CYTOGENETICS

4. Cytogenetics assay (reported results, e.g. induction of aberration frequency; types of aberrations, e.g. whether gaps are included in analysis or not, chromatid vs. chromosomal events, complex aberrations; positive and background aberration frequencies; number of cultures per concentration; levels of cytotoxicity obtained, e.g. effect on mitotic index or cell survival, if examined; include representative table, if appropriate):

Technical acetochlor was found to induce significant increases($P<0.05$) in the incidences of chromosomal damage at dose level of 100 ug/ml in both the presence or absence of metabolic activation (See results provided in Tables 1 & 2). In the absence of metabolic activation, acetochlor also demonstrated significant increases in the incidences of chromosomal damage at 50 ug/ml. The positive control compounds (0.5 ug/ml Mitomycin C and 100 ug/ml cyclophosphamide) induced significant positive responses ($P<0.01$) in both the presence and absence of metabolic activation as expected (See also results given in Tables 1 & 2).

The study author concluded that"under the conditions of this assay acetochlor is clastogenetic to human lymphocytes in vitro."

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IN VITRO MAMMALIAN CYTOGENETICS

5. Reviewer's discussion/conclusions (include e.g. rationale for acceptability or not; necessity for repeat, if appropriate; address any discrepancies with author conclusions; remember, do not include gaps in final aberration frequency analysis):

- o The positive control compounds (Mitomycin C & Cyclophosphamide) adequately demonstrated the sensitivity of the cultured human lymphocytes with or without metabolic activation to detect a clastogenic agent.
- o The number of cells with chromosomal aberrations in the negative (solvent) control group (less than 0.5% metaphases observed) was found within the acceptable range established by the testing laboratory.
- o The test compound, acetochlor, was tested at cytotoxicity level (100 ug/ml).
- o Although the preliminary assessment of cell cycle delay was not conducted in this study, the single harvest time (22.5 hrs posttreatment) for cells exposed to acetochlor in the presence or absence of metabolic activation appeared adequate for the detection of chromosomal aberrations in the cultured human lymphocytes.
- o This study was conducted in a manner to generate valid results. We agree with the study Author's conclusion that acetochlor is clastogenic to human lymphocytes in-vitro at 100 ug/ml in both the presence or absence of metabolic activation and at 50 ug/ml without metabolic activation. This study satisfies the guideline requirements, 84-3, for a mutagencity study (chromsomal aberrations).

6. Was test performed under GLPs (is a quality assurance statement present)? (Y) / N (circle one)

7. CBI appendix attached (Y) / N (circle one)

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ACETOCHLOR: AN EVALUATION IN THE IN VITRO CYTOGENETIC ASSAY IN HUMAN LYMPHOCYTES

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TABLE 2

CHROMOSOMAL ABNORMALITIES, AND MITOTIC INDEX SHOWN AS A MEAN PERCENTAGE OF THE TOTAL NUMBER OF CELLS ANALYSED PER DOSE LEVEL WITH AUXILIARY METABOLIC ACTIVATION

Treatment Atmosphere Concentration	Mean % Abnormal Cells Excluding Gaps	No. of Aberrations per Cell Excluding Gaps	Mean Mitotic Index (%)
Donor 1			
Dimethylsulphoxide 1µl/ml	1.00	0.010	16.15
Cyclophosphamide - 100µg/ml	44.00**	0.720	5.20Δ
Acetochlor - 100µg/ml	12.67**	0.400	5.10
- 50µg/ml	1.00*	0.010	11.10
- 10µg/ml	0.00	0.000	12.00
Donor 2			
Dimethylsulphoxide 1µl/ml	0.00	0.000	8.15
Cyclophosphamide - 100µg/ml	32.00**	0.320	1.30Δ
Acetochlor - 100µg/ml	16.67**	0.493	5.20
- 50µg/ml	2.00	0.020	9.60
- 10µg/ml	0.00	0.000	9.45

** Statistically significant increase in chromosomal damage at $p<0.01$ using Fisher's Exact Test (one-sided).

Δ Positive control mitotic index is determined from a single culture.

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Attachment H

Guideline Series 84: MUTAGENICITY

Reviewed by: John H.S. Chen, D.V.M. John H.S. Chen 5/15/91 008728
Section I, Toxicology Branch II (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. JM.I. 6/6/91
Section I, Toxicology Branch II (H7509C)

~~003478~~

DATA EVALUATION REPORT

CHEMICAL: Acetochlor **Tox. Chem. No.:** 003B

MRID No.: 415651-24 **EPA File Symbol:**

STUDY TYPE: Unscheduled DNA synthesis in rat hepatocytes in-vivo

ACCESSION NUMBER:

SYNONYMS/CAS No.:

SPONSOR: ICI Americas Inc., Agricultural Products
Wilmington, Delaware 19897

TESTING FACILITY: ICI Central Toxicology Laboratory, Alderley Park
Macclesfield, Cheshire, UK

TITLE OF REPORT: Acetochlor: Assessment for the induction of unscheduled DNA synthesis in rat hepatocytes in vivo

AUTHOR(S): R. W. Trueman

STUDY NUMBER(S): SR0357

REPORT ISSUED: August 8, 1989

CONCLUSION(S) - Executive Summary:

Acetochlor induced a weak DNA repair (as measured by UDS) in rat hepatocytes derived from animals exposed in vivo at 2000 mg/kg (at 20-hour time point).

Dose levels tested: 500, 1000, & 2000 mg/kg

Study: Acceptable

This study satisfies the guideline requirements for a genotoxic effect, 84-4 (other genotoxic effects)

I. Test Materials and Methods

1. Test Material

The test compound, acetochlor, a brown liquid was received from ICI Americas, Inc., Western Research Center, California, in November, 1988. The test sample was analyzed and found to be 84.4% pure. Two lots of this compound were used in these experiments, lot 002 for experiment 266, lot 004 for the remainder. There is no difference between these lots as they are merely aliquots of the original material received.

2. Animals

Male Alderley Park (Alpk-APFSD specific pathogen free) albino rats were used in this study. At the beginning of each experiment, the rats were young adults and weighed between 200 and 299 g.

3. Animal Treatment

Test and control substances were administered by gavage in value of 10 ml/kg b.w. Positive control animals were dosed with 6-P-dimethyl-aminophenyl-azobenzthiazole (40 mg/kg) in corn oil. Negative control animals were dosed with corn oil only.

4. Exposure Period

Two exposure periods were used: 4 hours and 12 hours.

5. Liver Perfusion and Hepatocyte Preparation

Suspension of hepatocytes were prepared from animals dosed with acetochlor, corn oil, or positive control compound by a collagenase perfusion technique (See detailed procedures provided in Appendix A). The suspension of hepatocytes was then diluted in order to give a concentration of 1.5 x 10^5 viable cells per ml and these cells were allowed to attached to a series of plastic coverslips. Monolayer cultures were established on coverslips for data analysis.

6. Cytotoxicity Test

The observation of treated animals confirmed that 2000 mg/kg body weight of acetochlor was an appropriate top dose level, as three of the five animals thus tested at the twelve hour time point showed over signs of toxicity, manifest as piloerection and a subdued attitude. This top dose selection for this study was consistent with the ASTM guideline for in-vivo UDS assays (Rutterworth et al., Mutation Res. 189: 123-133, 1987).

7. UDS Assay

Hepatocytes were prepared from rats four hours and twelve hours following administration of 500, 1000, or 2000 mg/kg b.w. of acetochlor. When the cells were attached (1.5-2 hrs), the supernatant medium was removed and replaced with medium containing 3H-thymidine (100 uCi/10 ml). The hepatocyte cultures were then incubated in a 5% CO2/air atmosphere for 4 hours. After incubation, the cultures were rinsed with WMF and then incubated overnight with medium containing unlabeled thymidine. The cells were washed with medium and fixed with glacial acetic acid: absolute alcohol (1:3). The coverslips, which have been mounted on slides, were dipped into Ilford K2 emulsion and stored in toal darkness at 4 C for 14 days to expose the emulsion. The slides were then developed and stained with aqueous eosin Y phloxine (1%).

8. Grain Counting

The slides were then analyzed (100 cells/animal) using an automated image analyzer linked to a computer to determine the mean net grain count (nuclear-cytoplasmic count) and the percentage of cells in repair (mean net grain count 5 or greater).

9. Evaluation Criteria

If an individual dose level has a mean net grain count of 5 or greater and has a percentage of cells in repair of 20 or greater, that is regarded as a positive response. If an individual dose level has a mean net grain count of zero or less that is regarded as a negative response. Such responses must be reproduced in an independent experiment.

II. Reported Results

1. As shown in Table 1, the mean net nuclear grain counts for the 1000- and 2000- mg/kg dose groups were similar to the solvent control at the 4-hour time point. However, at the 12-hour time point, the hepatocyte cultures derived from animals treated with 2000 mg/kg acetochlor displayed a mean net grain count of greater than zero (3.42+2.90; such a value did not reach 5 net grains which is required to define an unequivocal positive response), and were reproducible in Experiment I (See results given in Table 2) and Experiment 2 (Table 3).

2. At both time points (4-hour; or 12-hour), the positive and negative controls produced the expected responses, thus confirming the sensitivity of the assay system.

3. Based on these findings, the study author concluded that "acetochlor induced DNA repair albeit weakly (as measured by unscheduled DNA synthesis) in rat hepatocytes derived from animals exposed in vivo, at a limit dose for this assay of 2000 mg/kg."

III. Reviewer's Discussion and Conclusion

1. The positive control compound (6-BT) induced significant increases in the net nuclear grains per nucleus when compared to that of the corresponding negative controls (Tables 1, 2, & 3). These results demonstrated the sensitivity of the in-vivo rat hepatocyte assay system to detect a DNA-damaging response.

2. The nuclear labeling in the negative (solvent) control was found within the normal range of net nuclear grain count per nucleus (less than 1) for performing the rat hepatocyte UDS assay as recommended by Mitchell et al. (Mutation Res. 123: 364-410, 1983).

3. The test material, acetochlor, has been tested to cytotoxicity level (i.e., 2000 mg/kg).

4. The statements of GLP and QA for this study were provided.

5. Since the hepatocyte cultures derived from animals treated with 2000 mg/kg b.w. of acetochlor demonstrated a reproducible dose-related increase in the mean net grain count with a significant increase in the percentage of cells in repair at the 12-hour time point, we agree with the study author's conclusion that acetochlor induced a weak DNA-repair (as measured by UDS) in the rat hepatocytes, which were derived from the treated animals at 2000 mg/kg. This study satisfies the guideline requirements for a genotoxic effect (84-4: other genotoxic effects).

ACETOCHLOR

Tox R # 008728

Page_____ is not included in this copy.

Pages 128 through 146 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.

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Attachment I

Reviewed by: Timothy F. McMahon, Ph.D. 7/3/91
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. 7/10/91
Section I, Toxicology Branch II (HFAS) (H7509C)

008478

Data Evaluation Report

Study type: Metabolism (85-1) Tox. Chem. No.: 003B

EPA identification numbers: EPA MRID numbers: 415651-25; 415920-07; 415920-08; 415651-26; 415651-27
Caswell number: 003B
HED project numbers: 0-1920, 0-1999

Laboratory Project numbers: HRC/STR 18/88502; HRC/STR 18/89184; HRC/STR 18/89487; HRC/STR 18/89603; CTL/P/2809

Test material: [U-^{14}C]-Acetochlor

Synonyms: 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide

Testing Facilities: Huntingdon Research Centre Ltd.
Huntingdon, Cambridgeshire, England

ICI Central Toxicology Laboratory
Alderly Park, Cheshire, UK

Sponsor: ICI Central Toxicology Laboratory
Alderly Park, Cheshire, UK

Title of reports:
[1]: Laboratory Project No. HRC/STR 18/88502, "The Biokinetics of 14-C Acetochlor After Oral Administration to Rats at a Nominal Level of 10 mg/kg."

[2]: Laboratory Project No. HRC/STR 18/89184, "The Biokinetics of 14-C Acetochlor After Oral Administration to Rats at a Nominal Level of 200 mg/kg."

[3]: Laboratory Project No. HRC/STR 18/89487, "The Distribution and Excretion of Radioactivity after Oral Administration of 14-C Acetochlor at 10 mg/kg to Rats Pre-treated with Non-Radiolabelled Acetochlor."

[4]: Laboratory Project No. HRC/STR 18/89603, "The Metabolism of 14-C Acetochlor in the Rat after Oral Administration."

[5]: Laboratory Project No. CTL/P/2809, "Acetochlor: Biotransformation Study in the Rat."

Author(s): D.R. Hawkins, D. Kirkpatrick, G. Dean [1-4];
B.K. Jones [5]

Reports issued: [1-3]: February, April, June 1987; [4], June 1989; [5], March 1990

Conclusions:

In studies [1], [2], and [3], the the disposition and metabolism of ^{14}C-acetochlor was investigated in male and female rats at a low oral dose (10 mg/kg), repeated low oral doses (10mg/kg x 14 days), and a high dose (200 mg/kg). Comparison of disposition data in bile duct cannulated and non-cannulated rats demonstrated that acetochlor was well absorbed after oral administration. Excretion was relatively rapid at the low dose, with a majority of radioactivity eliminated in the urine by 24 hours. At 200 mg/kg, urinary elimination of ^{14}C-acetochlor derived radioactivity was decreased in male and female rats, while fecal (biliary) elimination was increased. At both 10 and 200 mg/kg, female rats eliminated a greater percentage of ^{14}C acetochlor derived radioactivity in urine than male rats. No effect was observed from repeated low oral dosing on the disposition of ^{14}C-acetochlor in male or female rats.

Fecal elimination of ^{14}C-acetochlor derived radioactivity was due to elimination *via* the bile, and was consistently less in female rats vs male rats at both the 10 and 200 mg/kg dose of acetochlor.

Residual ^{14}C-acetochlor derived radioactivity was minimal in all dose groups, except in those tissues well-perfused with blood (heart, spleen, kidney, lungs, liver). This apparent accumulation of ^{14}C-acetochlor derived radioactivity was due to binding of acetochlor and/or a metabolite to red blood cells, with a blood:plasma ratio of approximately 100 observed at 5 days post-dosing.

Urinary, biliary, and fecal metabolites of ^{14}C-acetochlor were isolated and identified in studies [4]and [5] by TLC, HPLC, and LC/MS. The major biotransformation product in urine was the mercapturic acid conjugate of acetochlor after removal of the ethoxymethyl side chain. The percentage of this metabolite was decreased by approximately 50% in urine from rats dosed at 200 mg/kg; no other major alterations were observed in metabolite profile at the 200 mg/kg dose. Both glucuronide and glutathione conjugates of acetochlor were identified in bile, with no significant quantitative change in biliary metabolite profile with increasing dose. Fecal metabolites were difficult to identify. Enterohepatic recirculation of ^{14}C-acetochlor derived radioactivity is suggested from these studies, but the nature of the reactive species which binds to red blood cells was not identified in the present studies.

Core Classification: supplementary

This study does not satisfy the guideline requirements (85-1) for a metabolism study in rats. Justification for the omission of intravenous data is requested. In addition, percentage recovery from tissues and feces during processing is requested. As acetochlor is proposed for food use, the determination of the species bound to red blood cells may be toxicologically relevant.

Purity of unlabeled acetochlor was also not stated in studies 1 through 3 and is requested.

I. MATERIALS

A. Test Material

Metabolism Studies

[1]: ^{14}C-Acetochlor
code no: CFQ 4196 (10 mg/kg dose)
batch analysis sheet: C/7499
Radiochemical Purity: 97.6% (3.0 mg/kg dose)
Specific Activity: 8.5 µCi/mg (10.0 mg/kg dose)

Unlabelled Acetochlor
batch 1+3
Chemical Purity: not stated

[2]: ^{14}C-Acetochlor
code no: CFQ 4196 (200 mg/kg dose)
batch analysis sheet: C/7499
Radiochemical Purity: 98.9% (200 mg/kg dose)
Specific Activity: 0.5 µCi/mg (200 mg/kg dose)

Unlabelled Acetochlor
batch 1+3
Chemical Purity: not stated

[3]: ^{14}C-Acetochlor
code no: CFQ 4196 (10 mg/kg x 14 days dose)
batch analysis sheet: C/7499
Radiochemical Purity: >98% (10 mg/kg x 14 days dose)
Specific Activity: 7.9 µCi/mg (10 mg/kg x 14 days dose)

Unlabelled Acetochlor
batch 1+3
Chemical Purity: not stated

Metabolite Characterization and Identification Studies [4, 5]

Study [4] utilized samples of excreta obtained from the above listed metabolism studies [1-3]. In study [5], a separate group of rats was dosed for bulk collection and identification of acetochlor metabolites. Unlabelled acetochlor with a purity of 99.5% was used, while radiolabelled acetochlor (purity not stated; specific activity 0.6 GBq/mmol) was used.

B. Vehicles: Polyethylene glycol (PEG) 400 (Studies 1, 2, and 3)
PEG 600 (Study 4)

C. Test Animals: Species: rat
Strain: CD Sprague-Dawley
Source: Charles River, Margate, U.K.
Weights: study [1]: males, 199-264g; females, 190-224g.
study [2]: males, 189-211g; females, 205-224g
study [3]: males, 152-160g; females, 164-175g.
study [4]: males, 240-320g; females, 210-240g.

II. METHODS

A. Study Design

1) Metabolism Studies [1-3]

The bioavailability and disposition of ^{14}C-Acetochlor was assessed in male and female rats following oral administration of the test compound. Rats received either a single oral dose of 10 or 200 mg/kg ^{14}C-Acetochlor or 14 repeated daily doses of unlabelled test material at 10 mg/kg followed by a single radiolabelled dose of 10 mg/kg. Plasma concentrations following single oral doses of ^{14}C-Acetochlor at 10 and 200 mg/kg were also determined from 0.25-360 hours post dosing. Dose groups were as follows:

Study#	Dose (mg/kg)	Dose Route	Number of Animals Male	Female
1	10	Oral[a]	5	5
2	200	Oral[b]	5	5
3	10	Oral[a]	5	5

[a]single dose

[b]14 daily unlabelled doses followed by one radiolabelled dose on day 15.

2) Metabolite Characterization and Identification Studies [4,5]

Metabolites of acetochlor in urine and feces from rats dosed orally with 10 and 200 mg/kg ^{14}C-Acetochlor and from rats given repeated oral doses of 10 mg/kg acetochlor were characterized by TLC in study [4]. In study [5], male and female rats were orally dosed with either 10 or 200 mg/kg ^{14}C-Acetochlor for pooling of urine, feces, and bile samples and characterization of

metabolite profiles in these samples by TLC, with subsequent identification of metabolite structures by LC/MS or GC/MS. A proposed pathway for acetochlor biotransformation was proposed based on identification of metabolites in the various excreta (Study 5).

C. Experimental

1) Metabolism Studies [1-3]

a. Animal Husbandry

Animals were acclimated to the laboratory environment for at least 14 days before dosing. Male rats were approximately 9 weeks old and female rats 12 weeks old at the time of dosing. Animals were given food (LAD 1 pellets, Labsure, Croydon, U.K.) and water *ad libitum*. Conditions of animal housing were not provided.

b. Dosing

For the single oral doses of 10 and 200 mg/kg ^{14}C-Acetochlor, appropriate amounts of labelled and unlabelled acetochlor were dissolved in polyethylene glycol (PEG) 400 to give final solution concentrations of 4mg/ml or 84 mg/ml for the 10 and 200 mg/kg dose groups, respectively. Dose volume was 2.5 ml/kg. For the repeated low dose oral study (10 mg/kg x 14 days), 2 batches of non-radiolabelled acetochlor were prepared, each sufficient for 7 days dosing. Stability of acetochlor had been demonstrated in the dose solution for up to 17 days (page 16 of Study [3]), but data were not provided.

The quantity of radioactivity received by each rat was determined from liquid scintillation counting in triplicate of duplicate aliquots of dose solution equivalent to the volume received by the rats which was diluted in 200ml acetonitrile.

A pilot study was conducted at the 10 mg/kg dose level to determine if a five-day collection period was sufficient for excretion studies, and if significant amounts of ^{14}C-acetochlor were eliminated via expired air. Results (Table 1, page 22 of Study [1]) showed that the five day period was sufficient and that negligible amounts of radiolabel were excreted in expired air.

Rats were housed individually in glass metabolism cages during the single dose studies. In the repeat dose studies, rats were housed in groups of 5 for the first 12 days of the study, until 2 days before administration of the radiolabelled dose, when they were transferred to individual glass metabolism cages.

c. Sample Collection and Analysis

Studies [1-3]:

Urine was collected on solid CO_2 at 6, 12, and 24 hours following dosing, and after 24 hours was collected at 24 hour intervals up to 5 days. Feces were collected every 24 hours for 5 days. Cages of treated animals were rinsed with tap water following sacrifice of the animals. Following sacrifice by cervical dislocation, the liver, kidneys, heart, lungs, brain, testes or ovaries, spleen, uterus, g.i. tract plus contents, and samples of bone marrow, muscle, and fat

and the entire skin and fur were removed and stored with the carcass at -15 °C until analysis. Blood was also removed by cardiac puncture at sacrifice, and part of the sample was retained as whole blood, while plasma was obtained from the remainder.

Samples of feces homogenates, tissues, plasma, and whole blood were mixed with cellulose powder and analyzed for radioactivity by combustion in a sample oxidizer followed by liquid scintillation counting. Aliquots of urine, cagewash, carcass digests, and skin digests (1ml for each) were mixed with MI-31 special scintillator cocktail and counted by liquid scintillation counting.

In plasma experiments, samples were withdrawn into heparinized tubes and centrifuged for immediate analysis of plasma radioactivity.

d. Statistics

No statistical analysis was reported in Studies [1-3].

2) Metabolite Characterization and Identification Studies [4,5]

a. Animal Husbandry

Husbandry data apply only to study [5], as study [4] utilized samples of excreta from studies [1-3]. In study [5], animals were housed in groups in stock rat cages prior to dosing. Rats were provided with food (pelleted PCD diet, Special Diet Services Ltd, Essex) and water *ad libitum*. Animals were acclimated to the room conditions for at least 3 days prior to use in temperature and humidity controlled rooms.

Note: The diet given these rats may not be identical to the diet given the rats in studies [1-3]. This may or may not influence metabolism of acetochlor, depending upon the relative similarity of the 2 diet formulations.

b. Dosing:

As in (a) above, dosing applies only in study [5]. Four dose solutions (I-IV) were prepared by mixing the appropriate amounts of labelled and unlabelled acetochlor in PEG 600 for the following purposes:

I: 200 mg/kg acetochlor, for urinary metabolite collection in 8 male rats.
II: 200 mg/kg acetochlor, for urinary metabolite collection in 6 female rats.
III: 10 mg/kg acetochlor, for biliary metabolite collection in 2 male rats.
IV: 200 mg/kg acetochlor, for biliary metabolite collection in 2 male rats.

Rats were transferred to stainless steel group metabolism cages upon dosing, except bile duct cannulated rats, which were housed in individual glass metabolism cages.

c. Sample Collection and Analysis:

Urine and feces were collected for up to 3 days over solid CO_2. Bile from bile duct cannulated rats was collected for up to 3 days at room temperature. Urine and bile samples were either diluted or weighed for counting by LSC in duplicate. Fecal samples were homogenized in a similar weight of magnesium sulphate for sample oxidation and subsequent counting by LSC.

Samples of urine or bile were pooled by sex and dose for analysis by TLC. Radioactivity from portions of pooled urine was extracted by elution from Bond Elut C8 (study 5) or C18 (study 4) columns. Samples of urine subjected to β-glucuronidase/sulfatase enzyme hydrolysis were also applied to Bond Elut columns for extraction of radioactivity.

Feces (pooled over 24 hours from each sub-group of five animals of the same sex) were extracted sequentially with ethyl acetate, acetonitrile, and acetonitrile:water (7:3, v/v). The ethyl acetate extract was evaporated to near dryness under a stream of nitrogen. Acetonitrile in the final 2 extraction solvents was extracted and the aqueous residue processed by sorbent extraction as described for urine above. Normal phase TLC was then carried out using a variety of solvent systems described in both study [4] (page 12) and study [5] (page 17).

Chromatographic correspondence between reference compounds and radioactive metabolites in excreta samples was achieved by co-chromatography of reference compound with sample extract.

Solvent extract samples of urine and bile were also subjected to HPLC with both UV and radiochemical detection. Three different separation procedures were employed for complete metabolite separation and identification, as listed on pages 19-20 of study [5]. Resolved metabolites from HPLC were subjected to thermospray MS, using a VG LC-MS thermospray/plasmaspray interface. GC-derived mass spectra were obtained using electron impact MS. Standards of synthesized acetochlor metabolites (pages 47-49 of study [5]) were used for comparison to biological samples for mass spectral analysis.

D. Compliance

A signed statement of no data confidentiality claims was provided with all studies.

A signed statement of GLP compliance (40 CFR 160.35) was provided with all studies.

A signed statement of quality assurance was provided with all studies.

A signed statement of EPA flagging criteria was provided with studies 1,2,3, and 5.

III. RESULTS

1) Metabolism Studies

The stability of the dose solution for the repeated low-dose study was stated to be 17 days in study [3] (page 16 of report). However, there were no confirmatory data to support this statement. Verification of dose for rats in the 10 and 200 mg/kg dose groups (studies 1, 2, and 3) was presented by liquid scintillation counting of dose solution aliquots. In study [5], the dosing syringe was weighed prior to and following dosing of each rat to obtain the precise dose received by each rat (page 41 of study [5]. Recovery of radiolabel from urine after sorbent extraction and enzyme incubation was reported in study [4]; this recovery was between 98-99%. No data were presented on recovery of radiolabel from feces or tissues in any study.

a. Absorption

In male rats dosed orally with a single dose of 10 mg/kg ^{14}C-acetochlor, 59.6% of the dose was excreted in urine by 24 hours, while the total percentage of the dose excreted in urine was 70.6% at 120hr. In female rats at this dose level, 66.5% was excreted in urine in 24 hours, while a total of 77% of the dose was excreted in 120hr. Thus, urinary excretion of ^{14}C-acetochlor derived radioactivity was slightly higher in females at the 10 mg/kg dose level. This is reflected in the lower fecal excretion of ^{14}C acetochlor derived radioactivity in female rats (Table 2, below). Repeated oral dosing at the 10 mg/kg dose level produced a similar pattern of urinary and fecal elimination in male and female rats.

At the 200 mg/kg ^{14}C acetochlor dose level, 43.1% of the total dose was eliminated in urine by 24 hours in male rats, and a total of 51.6% was eliminated by this route in 120hr. In female rats at this dose level, 51.6% of the total dose was eliminated in 24 hours in urine, and 64.7% was found in urine at 120hr. This decreased urinary elimination at the 200 mg/kg dose as compared to the pattern seen at 10 mg/kg was accompanied by an increase in fecal elimination of ^{14}C-acetochlor derived radioactivity.

Although a significant amount of radioactivity was observed in feces at both doses of ^{14}-C acetochlor, this radioactivity was determined to be of biliary origin in experiments conducted in study [5], where bile duct cannulated rats were administered similar oral doses of acetochlor. Thus, absorption of acetochlor at both 10 and 200 mg/kg was apparently complete.

b. Distribution

Tissue levels of ^{14}C-acetochlor derived radioactivity were negligible (between 0.01-0.05% of the total dose) at all dose levels, with the exception of the following, as summarized in the table below (Table 1):

Table 1

Distribution of ^{14}C-Acetochlor Derived Radioactivity in Male and Female Rats[a]

	LDM	LDF	PCM	PCF	HDM	HDF
g.i. tract	0.49(0.55)[b]	0.27(0.19)	0.36(0.41)	0.18(0.15)	3.3(0.19)	3.6(0.15)
liver	0.50(0.3)	0.58(0.22)	0.45(0.23)	0.38(0.18)	8.7(0.25)	7.6(0.16)
blood	4.58(1.79)	6.18(2.01)	4.41(1.46)	4.72(0.91)	112(2.05)	105(1.87)
spleen	0.77(0.02)	0.92(0.02)	0.85((0.02)	0.74(0.02)	19.7(0.02)	19.3(0.02)
lungs	0.65(0.03)	1.02(0.05)	0.73(0.03)	0.75(0.03)	15.7(0.04)	16.7(0.04)
kidneys	0.40(0.04)	0.53(0.04)	0.43(0.04)	0.41(0.03)	8.6(0.04)	9.1(0.03)
heart	0.94(0.04)	0.84(0.03)	1.11(0.04)	0.83(0.03)	23.3(0.04)	19.6(0.03)
bone marrow[c]	0.22	0.26	0.18	0.26	4.6	4.9

Abbreviations are: LD, low dose (10 mg/kg); PC, pre-conditioned dose (10mg/kg x 14days); HD, high dose (200 mg/kg).

[a]data represent the mean concentration (µg equivalents acetochlor/g) found at 120 hours post-dosing.

[b]percent total dose

[c]percentage for bone marrow not provided

As shown in **Table 1**, the concentration of radioactivity in tissues from administration of ^{14}-C acetochlor was highest in those tissues well-perfused with blood. Few sex-dependent differences were seen. The concentration of ^{14}-C acetochlor derived radioactivity was higher in male rats vs female rats at the 10 mg/kg single and repeated dose levels. Blood levels of ^{14}-C acetochlor derived radioactivity were higher in female rats at the 10 mg/kg dose level vs male rats at this dose. Lung levels of ^{14}-C acetochlor derived radioactivity were higher in female rats at the 10 mg/kg dose level vs male rats at this dose.

While the heart, spleen, lungs, and kidneys were all observed with significant amounts of ^{14}-C acetochlor derived radioactivity on a µg/g tissue basis, the total percentage of a dose of ^{14}-C acetochlor found in these tissues was less than 0.05% at all dose levels(**Table 1**). This is likely due to the presence of significant amounts of blood in these tissues, which as an organ showed the highest amount of radioactivity on a per gram tissue basis as well as percentage of total

radioactivity in pre-perfused tissues.

No apparent differences were observed in the distribution of ^{14}-C acetochlor derived radioactivity between dose groups which would indicate accumulation of ^{14}-C acetochlor derived radioactivity upon repeated dosing at 10 mg/kg, or altered distribution at the 200 mg/kg dose level.

c. Excretion

The excretion of ^{14}C-acetochlor in urine and feces at both 3 and 200 mg/kg is summarized in the following Table:

Table 2

Excretion of ^{14}C-acetochlor Derived Radioactivity in Male and Female Rats[a]

	LDM	LDF	PCM	PCF	HDM	HDF
urine (+cage wash)	70.6± 3.6	77.0± 7.1	65.0± 2.8	74.6± 1.8	51.6± 8.3	64.7± 5.5
feces	22.8± 2.8	13.2± 2.7	26.1± 2.5	16.5± 1.1	37.2± 5.7	26.9± 5.0
carcass (mean)	0.68	0.71	0.62	0.60	0.88	0.65
Total (urine+ feces+ tissues)	97.3	94.0	94.4	94.6	92.9	95.1

Abbreviations are: LD, low dose (3 mg/kg); PC, pre-conditioned dose (10mg/kg x 14days); HD, high dose (200 mg/kg).

[a]data represent the mean percent dose excreted at 120 hours post-dosing.

In all dose groups, >90% of a given dose of ^{14}C-acetochlor was excreted within 5 days. Urinary excretion in males and females was largely complete in all dose groups by 24 hours. No apparent delay was observed in the high dose groups. However, as noted in Table 2, female rats in all dose groups showed higher percentages of urinary ^{14}-C acetochlor derived radioactivity than males.

Fecal elimination of ^{14}C-acetochlor derived radioactivity was a significant route of excretion, representing between 13 and 37% of a given dose in all dose groups. Male rats showed a higher percentage of fecal excretion than female rats in the single low dose groups and repeated low dose groups (Table 2), reflective of the decreased urinary elimination in male rats.

Fecal elimination of ^{14}C acetochlor derived radioactivity was increased at the 200 mg/kg dose level by 63% in male rats, and by 103% in female rats relative to that observed at the 10 mg/kg dose level. However, the percentage of a given dose of ^{14}C acetochlor eliminated by the urinary route was decreased by only 26% and 15% in males and females at the 200 mg/kg dose, indicating the possibility of enterohepatic recirculation of ^{14}C acetochlor derived radioactivity. This is supported by results from study [5], in which excretion of ^{14}C acetochlor derived radioactivity by the biliary route was examined in male rats given a single oral dose of 10 or 200 mg/kg ^{14}C acetochlor. Results (pages 45 and 46 of study [5]), indicated that greater than 80% of a given dose of ^{14}C acetochlor was eliminated *via* this route at both doses, while excretion in urine from non-bile duct cannulated male rats was approximately 70%.

d. Plasma Levels of 14-C Acetochlor derived Radioactivity

In studies [1], [2], and [3], the concentration of ^{14}-C acetochlor derived radioactivity was measured from 0.25hr until 240 hours post-dosing. Peak plasma levels of ^{14}-C acetochlor derived radioactivity occurred at 7 hours post-dosing in both male and female rats at the 10 mg/kg dose level. Plasma levels at 72 hours in both sexes were approximately 1/10 of peak plasma levels. A biphasic pattern of decline was observed in plasma levels of ^{14}-C acetochlor derived radioactivity over the time course of blood radioactivity measurement, with plasma levels of ^{14}-C acetochlor derived radioactivity consistently higher in female rats over the time course of radioactivity measurment. Estimated half life of elimination from inspection of Figure 3, page 33 of study [1] was 20 hours for both male and female rats.

Plasma levels of ^{14}-C acetochlor derived radioactivity in rats subjected to repeated oral dosing at the 10 mg/kg dose level were not determined.

At the 200 mg/kg dose level, peak plasma levels of ^{14}-C acetochlor derived radioactivity occurred at 12 hours post-dosing in both male and female rats. Peak concentration in female rats was considerably higher (41.9±9.6 µg equivalents/ml) than in male rats (25.0±4.6 µg equivalents/ml). Plasma concentrations in female rats remained higher for the duration of the plasma measurements. As with the 10 mg/kg dose level, plasma levels of ^{14}-C acetochlor derived radioactivity fell significantly between 12- 48 hours. Estimated half life of elimination from examination of Figure 3, page 31 of study [2] was approximately 22 hours in males, and 30 hours in females.

2) Metabolite Characterization and Identification Studies ([4], [5])

a. Preparative TLC of urine

Urinary metabolites of acetochlor at 10 and 200 mg/kg were isolated from 0-24 hour urine in male and female rats in study [4], while pooled urine from 0-72 hours in male and female rats

given a single oral dose of 200 mg/kg ^{14}-C Acetochlor. Metabolites in both studies were resolved by thin layer chromotography, and tentative identification made based upon co-chromatography with authentic synthesized standards of acetochlor metabolites. Urine was also subjected to treatment with ß-glucuronidase/sulfatase to determine the presence of glucuronide and/or sulfate conjugates.

In study [4], chromatographic analysis showed at least 15 radiolabelled compounds and no unchanged acetochlor. Rats in the repeated low dose groups (10 mg/kg x 14 days) showed no apparent change in the profile of urinary metabolites compared to the single dose groups at this dose level, but quantitative differences in urinary metabolites were observed in urine from rats at the 200 mg/kg dose level. In study [5], at least 12 radiolabelled components were identified from tlc chromatograms (page 30 of study [5]).

The major urinary metabolite identified in both study [4] and [5] was confirmed by mass spectrometry following resolution by hplc as the mercapturic acid conjugate of N-de-ethylated acetochlor (see Figure 1, attached, and page 23 of study [4]). This metabolite was given the designation **H1and J9** in study [4] (based on the use of two tlc systems to resolve urinary metabolites), and **U9** in study [5]. A significant percentage of urinary radioactivity was identified as polar compounds. Information on the relative percentage of the metabolite H1 (J9) and polar compounds in urine at the various doses is summarized as follows (Table 3):

TABLE 3
Proportion of H1 (J9) and Polar Compounds in Urine of Male and Female Rats Treated with 10 or 200 mg/kg Acetochlor[a]

	10mg/kg		10mg/kg x 14 days		200mg/kg	
component	males	females	males	females	males	females
polars	21.1	19.3	20.2	21.9	16.2	15.8
H1	22.1	29.8	22.1	28.3	8.9	10.5
J9	24.8	32.3	27.3	37.2	12.1	15.9
polars (after enzyme incubation)	12.8[b]					

[a]data from Table 5, page 25 of study [4]. Results are expressed as % dose.

[b]enzyme incubation performed only on male rats at the 10 mg/kg dose level.

As shown above, metabolite H1(J9) accounted for between 22-32% of a dose of acetochlor in the urine at 10 mg/kg. The level of this metabolite was not significantly affected by repeated oral

dosing at 10 mg/kg. However, at the 200 mg/kg dose, the percentage of H1(J9) in the urine was decreased in male rats by 60%, and in and female rats by 65%. There was no other obvious increase in the percentage of other urinary metabolites to account for this decrease, with the exception of the appearance of a metabolite of acetochlor in which the terminal chlorine atom was replaced by a hydroxyl group. This metabolite represented 4% and 12% of the dose of acetochlor in urine at the 200 mg/kg dose level.

Incubation of urine with glucuronidase/sulfatase resulted in a decreased percentage of polar metabolites in urine (from 22% to 13%), indicating the presence of minor amounts of glucuronide conjugates in urine.

Remaining urinary metabolites of acetochlor were minor, constituting between 1-5% of the total dose (Table 5, page 25 of report [4]). Comparison of the total percentage urinary metabolites with urinary recovery of radioactivity showed that there was no significant amount of urinary radioactivity unaccounted for in metabolite analysis.

b. TLC of bile

The profile of biliary metabolites in male rats given a single oral dose of 10 or 200 mg/kg ^{14}C acetochlor is summarized below:

TABLE 4

Biliary Metabolites of Acetochlor[a]

	10mg/kg		10mg/kg x 14 days		200mg/kg	
component	males	females	males	females	males	females
B5	3.8	-	-	-	3.7	-
B7	8.9	-	-	-	13.8	-
B9	30.2	-	-	-	41.1	-
B15	5.7	-	-	-	3.6	-

[a]data from Table 1, page 39 of study [5].

As shown, four metabolites were isolated from bile of male rats. Two metabolites, B9 and B7, appeared to constitute the major biliary metabolites at both doses of acetochlor, while metabolites B5 and B15 constituted a smaller percentage of biliary radioactivity. Remaining biliary metabolites were resolved into at least 10 minor components, each of which represented less than 5% of the total dose.

The major metabolite, B9, was confirmed as the O-glucuronide of de-ethylated acetochlor following derivatization with diazomethane, purification of this derivative by hplc, and confirmation by mass spectrometry (figure 7, page 36 of study [5]). Metabolites B7 and B5 were identified by mass spectrometry following purification as the glutathione conjugate of acetochlor (B7) and the de-acetylated glutathione conjugate of acetochlor (B5).

Metabolite B15 was identified by LC-MS as the mercapturic acid conjugate of acetochlor (Figure 1, attached).

c. TLC of fecal extracts

TLC quantitation of fecal extracts of acetochlor dosed rats at 200 mg/kg showed complex patterns of components which were difficult to identify even when co-chromatographed. In summary, ethyl acetate extracts contained mainly non-polar compoments, while acetonitrile:water extracts contained a high percentage of polar material.

Five bands (A-E) were distinguished in fecal metabolite analysis by tlc. Band A co-chromatographed with acetochlor, although two reference compounds (the sulphonylmethyl and thiomethyl derivatives of acetochlor) also co-chromatographed with band A. However, the total radioactivity of band A accounted for no more than 2% of the dose in 0-24 hour fecally excreted radioactivity.

Band B was not identified, and accounted for 3% and 4% of a dose of acetochlor in males and females, respectively.

Band C contained approximately 2% (male) or 1% (female) of a dose of acetochlor, and co-chromatographed with the sulphoxymethyl derivative (reference compound 13, page 23 of study [4].

Band D co-chromatographed with the cysteine conjugate of acetochlor formed after removal of the ethoxymethyl side chain (compound pictured above U9, Figure 1, attached).

Band E co-chromatographed with reference compound 16, the mercapturic acid conjugate of acetochlor and the major urinary metabolite, as described above for urine.

IV. DISCUSSION

In this study, the disposition and metabolism of acetochlor was investigated in male and female rats. Data were presented in studies HRC/STR 88502 (study [1]), 89104 (study [2]), and 89407 (study [3]) demonstrating the absorption, distribution, and excretion of ^{14}C- acetochlor in male and female rats at a single low oral dose (10 mg/kg), repeated low oral doses (10mg/kg x 14 days) and a single high oral dose (200 mg/kg). In studies HRC/STR 18/89603 (study [4]) and

CTL/P/2809 (study [5]), the biotransformation and identification of acetochlor metabolites in urine, bile, and feces was investigated in excreta samples from rats used in studies [1-3], or in rats administered single oral doses of 10 and 200 mg/kg (study [5]).

Absorption of a dose of ^{14}C-acetochlor appeared complete at both 10 and 200 mg/kg by comparison of excretion data in bile duct cannulated and non-cannulated rats (studies [1-3] and [5]). While a significant percentage of ^{14}C-acetochlor derived radioactivity was found in feces of rats (between 13-26% at the 10 mg/kg dose level, and between 26-37% at the 200 mg/kg dose level), it was demonstrated in bile duct cannulated rats that fecal excretion of ^{14}C-acetochlor derived radioactivity was greatly diminished (between 3-7%, pages 45 and 46 of study [5]). Thus, fecal radioactivity was of biliary origin and did not represent unabsorbed acetochlor.

Excretion of a dose of ^{14}C acetochlor in urine was relatively rapid at the 10mg/kg dose level, with the majority of radioactivity (59-67%) eliminated in the urine by 24 hours. At the 200 mg/kg dose level, a lower percentage of ^{14}C-acetochlor was eliminated in urine by 24 hours (43-51%), but a greater percentage was found in feces at this same time point. Although this altered pattern of elimination may be the result of enterohepatic recirculation of radioactivity eliminated in bile at the high dose, there was no apparent difference in the percentage of ^{14}C-acetochlor derived biliary radioactivity eliminated at both doses (pages 45-46 of study [5]). In addition, examination of the time course of urinary elimination (Figure 1, page 31 of study [1] and page 29 of study [2]) shows that a much smaller percentage of ^{14}C acetochlor derived radioactivity was eliminated in urine between 0-12 hours. Thus, some delay in urinary elimination of ^{14}C acetochlor derived radioactivity is apparent. At all doses examined, the percentage of ^{14}C acetochlor derived radioactivity eliminated in urine of female rats was somewhat higher than that of male rats, with a corresponding decrease in fecal elimination (Table 2 of this review).

The concentration of ^{14}C-acetochlor derived radioactivity in tissues examined at 120 hours showed that radioactivity was concentrated in those tissues receiving the greatest amount of cardiac output (heart, lungs, liver, kidneys, and spleen; Table 1). Few sex- or dose-dependent differences were noted, but at each dose level, the greatest concentration of ^{14}C-acetochlor derived radioactivity was found in whole blood. This is reflected in the ratio of whole blood to plasma radioactivity, which exceeded 100 at all doses in both sexes. Thus, binding of acetochlor or a metabolite to red blood cells is extremely likely from review of these data. Identification of the chemical species responsible for binding was not performed in the present studies. However, it should be noted that conjugation with glutathione (see Table 3, above) is a major biotransformation pathway for acetochlor as shown by the profile of urinary metabolites, indicating the formation of a potentially reactive electrophilic species capable of such binding.

Metabolites of acetochlor in urine, feces, and bile were characterized and identified in studies [4] and [5]. From the results of these studies, the ethoxymethyl side chain and the chlorine atom of acetochlor were identified as major reaction sites for biotransformation. It is proposed, from analysis of acetochlor metabolites in bile and urine, that initial conjugation with glucuronic acid and glutathione occurs in liver, with subsequent excretion of the glucuronide conjugate (B9), the mercapturic acid conjugate (B15), and the glutathione conjugate of N-dealkylated acetochlor

(B5) in bile. The two glutathione conjugates are reabsorbed, with further metabolism to the mercapturic acid conjugate which is excreted in urine. Since the amount of mercapturic acid metabolite in urine exceeded the combined amounts of glutathione conjugates in bile, the additional marcapturic acid found in urine is proposed to come from hydrolysis of the glucuronide conjugate in the gut, with subsequent reabsorption and metabolism to the glutathione conjugate which is then excreted in urine as the mercapturic acid conjugate.

The limited data presented on *in vivo* plasma kinetics of acetochlor demonstrated that the elimination of ^{14}C-acetochlor derived radioactivity in plasma was at least biexponential (page 33 of study [1]), page 31 of study [2]). While it is possible to approximate the half life of elimination for ^{14}C-acetochlor by inspection of the graphical data, the observation that acetochlor and/or a metabolite binds to red blood cells does not give much meaning to an evaluation, as $t_{1/2}$ would likely be different for data on whole blood levels of ^{14}C-acetochlor derived radioactivity over time. The lack of intravenous data does not make it possible to distinguish which chemical species might be responsible for binding to red cells, as binding likely occurs prior to the peak plasma levels reported in studies [1] and [2]. The avid binding of ^{14}C-acetochlor derived radioactivity to red blood cells makes it likely that volume of distribution for acetochlor is small (limited to total body water).

Core Classification: supplementary

This study does not satisfy the guideline requirements (85-1) for a metabolism study in rats. Justification for the omission of intravenous data is requested. In addition, percentage recovery from tissues and feces during processing is requested. As acetochlor is proposed for food use, the determination of the species bound to red blood cells may be toxicologically relevant. Purity of unlabeled acetochlor was also not stated in studies 1 through 3 and is requested.

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TOX ONELINERS

TOXCHEM NO. 0038- Acetochlor FILE LAST PRINTED: 09/23/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-1(a) and 83-2(b) Feeding/oncogenic-2 year Species: mice Pharmacopathics Res. Lab PR-80-007; 5/4/83	Acetochlor Tech. 94.5%	071966 071967 071968	Doses: 500, 1500, 5000 ppm. Oncogenic NOEL < 500 ppm. Incr. incidence of Liver carcinomas - high dose males. Total lung tumors all doses (Female) (Lung carcinomas - low & high dose females. Uterine histiocytic sarcoma all doses. Positive trends for: liver carcinomas-both sexes. Lung carcinomas (F). Benign ovarian tumors & kidney adenomas-females. Systemic NOEL < 500 ppm (LDT) based on organ weight changes.		Minimum 004586
83-1(a) and 83-2(a) Feeding/oncogenic-2 year Species: rat Pharmacopathics Res. Lab 80-006; 5/20/83	Acetochlor Tech. 94.5% Lot# NRP-1737874	071962 071963 071964 071965 404848-01	Doses: 500, 1500, 5000 ppm in Sprague Dawley str. Onco NOEL = 1500 ppm. Onco LEL = 5000 ppm. Increased incidence of: liver carcinomas-high dose males. Thyroid adenomas-males. Sys NEL < 500 ppm based on body wt. change Non neoplastic lesions-polyarteritis general & in the testes. Peripheral nerve neuropathy. Reanalysis of histopath. indicated dose-related increase in nasal papillary adenomas in male rats (statistically sig; $p < 0.05$, 0.01 in 1500 and 5000 ppm dose groups); papillary adenomas present in two dose males; small number of papillary adenomas are also present in mid & high dose females; the lack of dose-related findings for female adenomas may relate to the significantly lower survival rate observed in these groups. Results of this report should be considered in the context of acetochlor's oncogenic classification.		Minimum 004586 006764
83-1(a) and 83-2(a) Feeding/oncogenic-2 year Species: rat Monsanto EHL-83107; 9/25/86	Acetochlor, Lot dayton RDNT 08001	400770601	Sys NOEL = 200 ppm. Sys LEL = 1000 ppm (Decr. body wt. & body wt. gains M & F, accompanied by incr. in serum gamma glutamyl transpeptidase activity and cholesterol levels in 1000 ppm males, incr. total bilirubin levels in 1000 ppm females, incr. absolute & relative kidney & liver wts. & incr testicular weights in 1000 ppm males. Several increases in non-neoplastic findings were noted in 1000 ppm males & females. Oncogenic NOEL = 200 ppm. Oncogenic LOEL = 1000 ppm. (Neoplastic findings of neoplastic nodules of the liver, follicular adenoma/ cystadenoma of the thyroids & papillary adenoma of the mucosa of the nose/turbinates were noted in 1000 ppm males and females. Levels tested: 40, 200 & 1000 ppm in the diet. Animal used: M & F Sprague Dawley albino rats from Charles River.		Supplementary 006571 Minimum 007002
83-1(a) and 83-2(a) Oncogenic risk assessment Species: rat	Acetochlor		Two year chronic/oncogenic study in Sprague Dawley rats - - Quant. Risk Assessment - Q*1 = 10 exp -2 (mg/kg/day)-1 in human equivalents Based upon papillary adenomas (nasal turbinates) in Sprague Dawley rats fed 0, 40, 200, & 1000 ppm: for males - Q*1 = 1.08 X 10 exp-2; for females - Q*1 = 9.20 X 10 exp-3 both in (mg/kg/day)-1 human equivalents. Category B2		007513

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008728

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TOXCHEM NO. 0038- Acetochlor FILE LAST PRINTED: 09/23/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-1(a) and 83-2(a) Feeding/oncogenic-2 year Species: rat Life Science Research 88/SUC017/0348; 3/18/88 ICI	SC-5676 (Acetochlor) 91.0% a.i.	415920-04	SC-5676 technical was administered to male & female Sprague-Dawley rats in the diet for 104 weeks at dose levels of 0, 18, 175 and 1750 ppm (0, 0.8, 7.9, and 79.6 mg/kg/day). Systemic toxicity in the form of reduced body weight gain, reduced food efficiency, ophthalmologic abnormalities increased organ:body weight ratios, & elevated plasma GGT and cholesterol was observed in both sexes at the 1750 ppm dose level. Tumorigenic responses included a significant increase in nasal epithelial adenomas & thyroid follicular cell adenomas in both sexes at the 1750 ppm dose. Nasal carcinoma was present in 2 males and 1 female rats at this dose. Rare tumors in the form of benign chondroma of the femur and basal cell tumor of the stomach were also observed at 1750 ppm test article. Non-neoplastic pathology in the kidney, retina, pancreas, and nasal epithelium was increased at the 1750 ppm dose level. Systemic NOEL = 175 ppm. Systemic LOEL = 1750 ppm (both sexes; decr. body weight gain, decr. food efficiency, incr. organ:body weight ratios incr. plasma GGT and cholesterol). MTD = 1750 ppm (both sexes; decr. body weight gain).		Minimum 008478
83-1(a) and 83-2(b) Oncogenic ICI Species: mice Life Science Research 87/SUC0012/0702; 6/9/89	SC-5676 (acetochlor) 90.5% a.i.	415651-19	Technical SC-5676 was administered in the diet to male & female CD-1 mice for 78 weeks at dietary levels of 0, 10 ppm (1.1 mg/kg/day a.i. (M); 1.4 mg/kg/d a.i. (F), 100 ppm (11 mg/kg/d (M); 13 mg/kg/d (F), & 1000 ppm (116 mg/kg/d (M); 135 mg/kg/d (F)). A dose related increases in absolute and relative (kidney:whole body) kidney weight was observed in male mice, as was an increase in tubular basophilia at all dietary levels of SC-5676 In females, the only compound-related finding was a significant increase in anterior polar vacuoles in the lens at the 1000 ppm dose level. Dietary exposure to SC-5676 resulted in a significant increase in pulmonary adenomas in female mice, and significant positive trends toward the development of pulmonary adenomas in both males and females. However, definitive assessment of the carcinogenic potential of SC-5676 was not possible because laboratory historical control data and definitive characterization of pulmonary tumors, i.e., site & type were not provided		Supplementary 008478
83-1(b) Feeding-1 year Species: dog Pharmacopathics Res. Lab PR-80-008, PRL8; 10/14/81	Acetochlor Tech. 94.5%	070134 248618 248619	Doses: 4, 12, & 40 mg/kg/day. NOEL = 12 mg/kg/day. LOEL = 40 mg/kg/day. Testicular atrophy, decr. testes wt., incr. adrenal wt.(F). decr. body wt. gain - males; decr. body wt. (F) and incr. in liver weight of both sexes.		Minimum 004586 005943

008728

TOXCHEM NO. 003B- Acetochlor FILE LAST PRINTED: 09/23/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-1(b) Feeding-1 year ICI Species: dog Life Science Research 88/SUC018/0136; 12/2/88	SC-5676 (acetochlor) 91.0% (a.i.)	415651-18	NOEL = 2 mg/kg/day. LOEL = 10 mg/kg/day, with salivation, increased salivation, ornithine carbamyl transferase, and triglyceride values accompanied by decreased blood glucose levels and liver glycogen levels. Interstitial nephritis, tubular degeneration of the testes and hypospermia were reported. Neurological effects were apparent after 39 weeks of treatment at the 50 mg/kg level by changes in posture, gait, reflexes and ataxia. Two males and four females were killed between weeks 39 and 51 due to the severity of the neurological effects. Doses levels: 2, 10 & 50 mg/kg/day by gelatin capsule to beagles (5/sex/ group).		Guideline 008478
83-3(a) Developmental Toxicity Study Species: rat Interntl. Res. and Develop. Co 401-066; 10/15/80	Acetochlor CP-55097	099811	Teratogenic NOEL > 400 mg/kg/day. Fetotoxic NOEL = 200 mg/kg/day Maternal NOEL = 200 mg/kg/day Levels tested by gavage in Charles River str.: 0, 50, 200 & 400 mg/kg.		Minimum 005865 005113
83-3(a) ICI Developmental Toxicity Study Species: rat Huntingdon Res. Centre, Eng. ISN204/89369RR0431; 8/14/89	Acetochlor, 90.5% w/w; batch P2	415920-05	Additional data are required before Maternal NOEL & LOEL, & Developmental Toxicity NOEL and LOEL can be determined. The study may be upgraded if the requested information is submitted and accepted by the Agency. Vehicle: Corn oil; strain: CrL; CD (SD) BR VAF/Plus; Source: Charles River, Portage, Mich. Doses: 0, 40, 150 & 600 mg/kg/day.		Supplementary 008478
83-3(b) Developmental Tox. - pilot Species: rabbit Interntl. Res. and Develop. Co 401-103; 11/24/81	Acetochlor Tech. 94.5%	248620	Pilot study: Dose levels: 30, 60, 125, 250 and 500 mg/kg/day by gavage		Invalid 004586
83-3(b) Developmental Tox. - pilot Species: rabbit Interntl. Res. and Develop. Co 401-103a; 11/24/81	Acetochlor Tech. 94.5%	248620	Pilot study: Dose levels: 30, 60, 125, 250 and 500 mg/kg/day by gavage		Invalid 004586
83-3(b) Developmental Tox. - pilot Species: rabbit Interntl. Res. and Develop. Co 401-103b; 11/24/81	Acetochlor Tech. 94.5%	248620	Pilot study: dose levels: 50, 100, and 150 mg/kg/day by gavage. Decreased litter size, and increased pre and post implantation loss observed at 50 and 150 mg/kg/day. Maternal NOEL < 50 mg/kg.		Supplementary 004586

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-3(b) Developmental Toxicity Study Species: rabbit Interntl. Res. and Develop. Co 401-104; 11/24/81	Acetochlor Tech 94.5%	248620	Dose levels: 15, 50, and 175 mg/kg/day by gavage. Teratogenic potential , maternal NOEL, and fetal toxicity can't be determined due to limited number of dams observed at necropsy.		Supplementary 004586
83-3(b) Developmental Toxicity Study Species: rabbit Wil Research Lab 50009; WI-86-4; 9/5/86	Mon 097 (Acetochlor); 94.2%	40134101	Maternal NOEL = 50 mg/kg. Maternal LEL = 190 mg/kg (body wt. loss during dosing). Developmental NOEL > 190 mg/kg. A/D ratio = 50/190 = 0.2 Levels tested: 0, 15, 50 & 190 mg/kg by gavage in N.Z. White rabbits.		Minimum 005968
83-3(b) ICI Developmental Toxicity Study Species: rabbit Huntingdon Res. Centre, Eng. ISN205/89432RB0432; 8/9/89	Acetochlor, 90.5% w/w; Lot: P2	415920-06	Additional data are required before Maternal NOEL & LOEL, or Developmental Toxicity NOEL and LOEL can be determined. The study may be upgraded if the requested information is submitted and accepted by the Agency; however, it must be noted that if no maternal or developmental toxicity LOEL's are established, a new study may be required. Vehicle: Corn oil; strain: NZW; Source: Interfauna UK Ltd., Huntingdon, Cambridgeshire; Doses: 0, 30, 100 and 300 mg/kg/day.		Supplementary 008478
83-4 Reproduction-2 generation Species: rat Interntl. Res. and Develop. Co IR-80-053; 12/16/82	Acetochlor 94.2% Lot# NBP1992024	071969	Doses: 0, 500, 1500, 5000 ppm (diet) Reprod. NOEL = 500 ppm. Reprod. LEL = 1500 ppm (based on decreased body wt. gain of F2b pups). Systemic NOEL < 500 ppm based on absolute & relative organ weight: Decr. for ovary wt. in F1; decr. for pit. wt. for F1 & F2b males. Incr. thyroid wts in F1b & F2b pups.		Minimum 004586 005353
83-4 ICI Reproduction-2 generation Species: rat Life Science Research 89/0414; 8/16/89	SC-5676 tech (Acetochlor) 90.8%	415651-20	Dietary levels: 0, 18, 175 and 1750 ppm (approx. 1.6, 21, & 160 mg/kg/d). Systemic toxicity in high-dose parents included reductions (10-14% in 2nd gen.) in body weight, accompanied by slight reductions in food consumption and significant increases in relative organ weights. Reprod. performance was not affected. Reprod. Tox. was observed at the high dose as significant reductions in pup weight at lactational day 21 & total body weight gain during lactation. Parental NOEL and LOEL 175 and 1750 ppm, respectively. Reproductive NOEL & LOEL: 175 & 1750 ppm, respectively.		Minimum 008478
82-1(a) Feeding-3 month Species: rat Pharmacopathics Res. Lab 7914; 10/10/80	Acetochlor CP-55097	099808 099809	NOEL = 800 ppm LEL = 2000 ppm (loss of body weight, food consumption Doses tested: 0, 800, 2000 & 6000 ppm in Sprague-Dawley rats.		Minimum 005865 005113

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
82-1(a) ICI Feeding- 13 week oral Species: rat Life Science Research 86/SUC011/0051; 6/23/86	SC-5676 (acetochlor) purity not stated	415651-15	Male & female CD Sprague-dawley rats (10/sex) were given acetochlor (SC5676) in the diet at doses of 0, 20, 200 and 2000 ppm for 13 weeks. Toxic effects were observed in both sexes at the 2000 ppm dose level. These included hematological effects, increases in organ:body wt. ratios decreases in plasma acetyl & butyrylcholinesterase activity, & increased plasma urea and cholesterol. No significant test article related effects were observed at other doses. Sys. NOEL = 200 ppm. Sys. LEL = 2000 ppm.		Supplementary 008478
82-1(b) Feeding-4 month Species: dog Pharmacopathics Res. Lab 7920; 10/10/80	Acetochlor CP-55097	099809 099810	NOEL =< 25 mg/kg/day. (death or morbidity, decreased body weight, abnormal urinalysis findings & histopathological findins in (M&F) beagles		Minimum 005865 005113
82-1(b) ICI 90 day oral Species: dog Life Science Research 86/SUC010/0059; 6/23/86	SC-5676 (Acetochlor) purity not reported	415651-16	NOEL = 10 mg/kg/d. LOEL = 60 mg/kg/d with mucous diarrhea, decreased body weight, decreased RBC and hematocrit values, increased alanine aminotransferase, decr. blood glucose, and incr. relative liver weight. Dose levels: 2, 10 & 60 mg/kg/day orally in capsule to beagle dogs.		Supplementary 008478
82-2 Dermal-3 week Species: rabbit Interntl. Res. and Develop. Co IR-80-356; 12/11/81	Acetochlor Tech. 94.5% Lot# NBP-1737-874	248620	Systemic LOEL = 1200 mg/kg (Mortality and decreased body weight) Dermal NOEL < 100 mg/kg based on irritation. Levels tested in New Zealand White strain -0, 100, 400 and 1200 mg/kg		Minimum 004586
82-2 ICI Dermal-3 week Species: rat ICI Central Tox. Lab. LR0531; 10/15/89	Acetochlor 89.4% a.i.	415651-17	Male & female SPF Wistar rats (5/sex) were given dermal doses of 0.1, 1.0, 10 or 100 mg/kg/day acetochlor in olive oil 5 days/week for 3 weeks. Minimal to mild skin irritation was observed in male & female rats after 21 days. Signs of systemic toxicity were not apparent at any dose level. Higher doses were apparently not possible due to the severe dermal toxicity of acetochlor at higher doses. Systemic NOEL => 100 mg/kg/day.		Minimum 008478
84-2(a) ICI Mutagenic-Ames Species: salmonella ICI Central Tox. Lab. YV2370/VV2423; 7/19/89	Acetochlor, batch # B2993 15; 89.9% pure	415651-21	Acetochlor induced reproducible, positive, mutagenic response to TA1538 strains of Salmonella typh. with metabolic activation system at 1000 ug/plate (less than 2X background mutation rates but P<0.05). The compound did not induce any significant increases in numbers of revertant colonies in strains TA1535, TA1537, TA98 and TA100 under the conditions tested. Therefore, Acetochlor gave a weak & positive response in this study. Doses tested: 1.6, 8, 40, 200, 1000 & 5000 ug/plate.		Acceptable 008478

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
84-2(b) Mutagenic-in vivo cytogenetic Species: rat Hazleton HL83-006; 5/24/83	Acetochlor 96.3% a.i.	071970	Doses tested: 40, 150, 500 mg/kg ip. Negative for chromosome aberration at HDT. Toxicity was demonstrated at high dose (500 mg/kg) by evid. of a stat. sig. body wt. loss (M & F) at 48 hr. See assay in mice.		Unacceptable 004586 Acceptable 005977
84-2(b) Mutagenic-DNA repair test Species: rat hepatocytes Pharmakon Lab; Scranton Pa. PK-82-151; 2/17/83	Acetochlor Lot # NBP-1737813 99.7% a.i.	071970	doses: 0.032 - 320 ug/well. Negative for unscheduled DNA syn/repair at HDT. Unaccep.; purity of comp. method of dose calculation, and criteria for test assessing cytotoxicity not disclosed.		Unacceptable 004586 Acceptable 005374
84-2(b) ICI Mutagenic- in vitro cytogen. Species: human lymphocytes ICI Central Tox. Lab. SV0336; 7/20/89	Acetochlor batch # A1016/9; 89.4% pure	415651-22	Acetochlor was clastogenic in cultured human lymphocytes at 100 ug/ml in both the presence & absence of S9 mix activation & at 50 ug/ml without metabolic activ. Doses: 10, 50 & 100 ug/ml.		Acceptable 008478
84-4 Mutagenic-Ames Species: Salmonella Monsanto MRC-DA838; 12/5/78	Acetochlor CP-55087	099811	Negative		Acceptable 005865 005113
84-4 Mutagenic-reverse mutation Species: mice Stanford Research Inst. SR-81-150; 8/82	Acetochlor Lot# NBP-1924845	071970	Doses: 20-400 ul/L w/o S-9; 5-250ul/L w S-9. Test system L5178Y mouse lymphoma. Mutagenic only in presence of S-9 metabolic activation		Acceptable 004586 005113
84-4 Mutagenic-(HGPRT) Species: CHO cells Monsanto ML-82-281; 6/9/83	Acetochlor 96.3% sample# T830020	071970	Dose: 25-150 ug/ml without & 25-125 ug/ml with S-9 activation. Weakly mutagenic both in the presence & absence of S-9 metabolic activation		Acceptable 004586 005113
84-4 Mutagenic-micronucleus assay Species: mice Hazleton Biotechnologies Corp. HL-84-405; 6/2/86	MON 097 (Acetochlor) Dayton Batch 18 (BA-18) purity = 96.7%.		Doses tested: 200, 660 or 20000 mg/kg administered by oral gavage. Positive control: Cyclophosphamide at 40 mg/kg. Vehicle control: corn oil Tested on Male & Female CD-1 mice under conditions of this test, the high dose level of MON 097 (2000 mg/kg) exhibited mortality and signs of clinical toxicity. There was no evidence of an incr. in micronucleated polychromatic erythrocytes at dose levels tested in this study.		Acceptable 005977

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
84-4 Mutagenic-dominant lethal test Species: rat 1/11/87 EHL-86008	MON-097; 94.3%; Lot XLF-396	403893-01	Unacceptable low pregnancy rates among control and low dose animals with low mating activities in control males limit the sensitivity of this study to adequately detect dominant lethality/reproductive toxicity.		Unacceptable 006769
84-4 ICI Mutagenic-micronucleus assay Species: mice ICI Central Tox. Lab. SH0339; 7/31/89	Acetochlor, batch # 8031/88; 89.4% pure	415651-23	Acetochlor was not clastogenic in the mouse micronucleus test at the dose levels tested. Doses: 898 & 1436 mg/kg (M); 1075 & 1719 mg/kg (F). Deficiencies found: no information on stability and storage conditions of the test material; and no indication of coded slides prior to scoring.		Unacceptable 008478
84-4 ICI Mutagenic-unscheduled DNA synt Species: rat hepatocytes ICI Central Tox. Lab. SR0357; 8/8/89	Acetochlor, batch 002 & 004; 84.4% pure	415651-24	Acetochlor induced a weak DNA repair (as measured by UDS) in rat hepatocytes derived from animals exposed in vivo at 2000 mg/kg (20 hr. time point). Dose levels: 500, 1000, & 2000 mg/kg.		Acceptable 008478
Risk assessment Species: EPA	Acetochlor		Q* is estimated to be 10exp-2 (mg/kg/day)exp-1 for humans & 10exp-3 for mice and 10exp-4 for rats.		005580
Risk assessment-chronic Species: rat Monsanto Environ. Health Lab EHL-83107; 9/25/86	Acetochlor (96.1% pure)	400706-01	Qual. risk assessment. Doses: 0, 40, 200 & 1000 ppm. Female rat: No survival problems. Nose papillary adenomas in 1000 ppm was significantly greater than controls & there was a significant dose trend. Thyroid follicular adenomas and/or carcinomas. Sig. dose trend. Male rat: No survival problems. Nose papillary adenomas in 1000 ppm group was significantly greater than controls. There was a significant dose trend.		006952
85-1 Metabolism Species: rat F. Hoffman-LaRoche & Co. Ltd. MSL-2824; 6/83	Acetochlor 98% a.i. mix of C12, C13, C14	071971 071972	Doses: 10 & 400 mg/kg single oral dose for low & high doses, respectively; 10 mg/kg repetitive oral dose for 14 consecutive days followed by same single labeled dose. Effects - Little (0.5%) elimination via lungs 70% excreted within 48 hrs; preferentially in the urine Biphasic elimination: T1/2 (fast) =< 10 hrs. T1/2 (slow) = 128-286 hrs early metabolites mainly mercapturates, later ones were sulfoxides, sulfones & sulfates. 20 metabolites were identified. < 1% parent compound excreted unchanged in feces-retention (2-2.5% of dose) in RBC due to covalent bonding to hemoglobin.		Acceptable 005113 Acceptable 004486

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
85-1 Metabolism Species: rat Huntingdon Res. Centre, Eng. HRC/STR 18/89603; 3/90 ICI	Acetochlor C-14, purity; study 1: 97.6%, study 2 98.9%; study 3: > 98%; study4: N/A;study 5: 99.5	415651-25 415920-08	Disposition of C14-Acetochlor was examined in CD Sprague-Dawley rats at single oral doses of 10 & 200 mg/kg, and at 10 mg/kg x 14 days. metabolites were characterized & identified in urine, feces & bile. Acetochlor was well absorbed after oral administration at both 10 & 200 mg/kg. The majority of a radioactive dose (50-60%) was eliminated in male & female rats in urine after 24 hrs, with a significant percentage (13-22%) in feces. The percentage in urine was decreased at 200 mg/kg after 24 hrs (40-50%), with an increase in the percentage in feces (26-37%). Repeated oral dosing at 10 mg/kg had no significant effect on disposition. Tissue concentrations after 5 days were highest in those tissues well-perfused with blood, due apparently to the avid binding of C14 acetochlor derived radioactivity to red blood cells (blood/plasma ratio => 100). The major biotransformation product in the urine at 10 & 200 mg/kg was mercapturic acid conjugate of acetochlor after removal of the ethoxymethyl side chain. Glucuronide & glutathione conjugates of acetochlor were identified in bile, with the glucuronide conjugate as the major metabolite in bile. Fecal metabolites were complex and difficult to identify. Enterohepatic recirculation of acetochlor was suggested from these studies.		Supplementary 008478
85-2 ICI Metabolism - dermal absorption Species: rat# ICI Central Tox. Lab. CTL/P/3088; 10/12/90	C14-Acetochlor	417783-03	Acetochlor was absorbed in a dose & time related manner. Material was relatively easily washed from the skin with little residue remaining. Percent of dose absorbed with time and dose are as follows; Dose / Duration of exposure (hours): 0.5, 1, 2, 4, 10, 24 Formulation Concentrate (707.32 mg/gm): 1.4, 5.2, 3.4, 2.7, 4.5, 12.6 1/10 aqueous dilutation (11.772 mg/g): 4.8, 5.6, 3.0, 4.8, 9.3, 17.5 1/1000 aqueous dilutation (0.763 mg/g): 3.8, 4.3, 7.3, 9.9, 19.5, 31.4 Evidence of bioaccumulation was observed in carcass & erythrocytes. Volatilization from the application site was significant at the lowest dose.		Acceptable 008478
Peer Reviews Species: rat & mice 5/31/89	Acetochlor		Current Agency Decision: SAP classification B2; Q* = 1 x 10exp-2 in albino rats (M&F) (HED) and CD-1 mice (SAP). Peer Review documented 05/31/89		007697

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-1 Acute oral LD50 Species: rat Environmental Health Labs 80-49; 10/15/80	Acetochlor MON 097	099807	LD50 (both sexes) = 2953 (2136-4761) mg/kg LD50 (M) = 3712(2794-5297) mg/kg. LD50 (F) = 2018 mg/kg	3	Minimum 005113 005865
81-1 Acute oral LD50 Species: rat Environmental Health Labs R-80-49; 10/15/80	2-Chloro-N-ethoxymethyl-N-(2-ethyl-6-methylphenyl acetamide 86.4%	243802	LD50 > 2500 mg/kg (M); LD50 > 3536 (F) Symptoms: convulsion, prostration, ataxia and body tremors.	3	Guideline 000899
81-1 Acute oral LD50 Species: rat Bio/dynamics Inc. 6067-85; 12/6/85	Acetochlor MON 8449 (EC formulation)	260748	LD50 both sexes = 1900 (1094-2760) mg/kg LD50 (males) = 2400 mg/kg. LD50 (females) = 1550 mg/kg.	3	Minimum 005113
81-1 Acute oral LD50 Species: rat Food and Drug Research Lab 88-2053-015; 7/18/88	Harness-PC; 82.8% acetochlor	409988-01	LD50 (M&F) = 1488 +- 666 mg/kg. LD50 (M) = 2381 +- 1290 mg/kg. LD50 (F) = 1101 +- 668 mg/kg. Doses: 1000, 3000 & 5000 mg/kg (gavage).	3	Minimum 007957
81-1 Acute oral LD50 ICI Species: rat Life Science Research 86/SUC013/039; 2/24/86	SC-5676 Tech (Acetochlor) purity not stated	41565[illegible]-04	LD50 (M) = 4238 (3384-5902) mg/kg. LD50 (F) = 4015 (3258-4772) mg/kg. LD50 (M&F) = 4124 (3557-4691) mg/kg.	3	Supplementary 008478
81-1 Acute oral LD50 Species: rat ICI ICI Central Tox. Lab. AR5002; 4/10/90	Acetochlor 760 g/L EC formulation, 71.6% a.i.	415651-09	The acute oral LD50 of acetochlor 760 g/L EC formulation was 1942 mg/kg in male rats (1363-2519), and 1426 (726-2831) mg/kg (F).	3	Guideline 008478
81-2 Acute Dermal LD50 Species: rabbit Environmental Health Labs 80-48; 10/15/80	Acetochlor MON 097	099807	LD50 (both sexes) = 3667 (3017-4458) mg/kg. LD50 (M) = 3999 (1796-8907) mg/kg LD50 (F) = 5631 (3054-4156) mg/kg	3	Minimum 005865

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81-2 Acute Dermal LD50 Species: rabbit Environmental Health Labs R-80-48; 10/15/80	2-Chloro-N-ethoxymethyl-N-(2-ethyl-6-methyl-phenyl)acetamide 86.4%	243802	LD50 = 3999 mg/kg (M) LD50 = 3563 (F)	3	Guideline 000899
81-2 Acute Dermal LD50 Species: rabbit Bio/dynamics Inc. 6068-85; 11/7/85	Acetochlor MON 8448 (EC formulation)	260748	LD50 (both sexes) > 2000 < 5000 mg/kg	3	Minimum 005113
81-2 Acute Dermal LD50 Species: rabbit Food and Drug Research Lab 88-2053-016; 7/18/88	Harness-PC; 82.8% acetochlor	409988-02	LD50 > 2000 mg/kg (ODT).	3	Minimum 007957
81-2 Acute Dermal LD50 ICI Species: rat Life Science Research 86/SUC014/30; 2/24/86	SC-5676 (Acetochlor) purity not stated	415651-05	LD50 > 2.0 g/kg (M&F).	3	Supplementary 008478
81-2 Acute Dermal LD50 ICI Species: rat ICI Central Tox. Lab. CR2724; 2/12/90	Acetochlor 760 g/L EC formulation, 71.6% a.i.	415651-10	LD50 > 2.0 g/kg (M&F) for acetochlor 760 g/L EC formulation.	3	Guideline 008478
81-3 Acute Inhalation LC50 Species: rat Bio/dynamics Inc. 88-8072; 10/31/88	Harness-PC; 82.8% acetochlor	409988-05	Three groups of five males & five females rats exposed to concentrations of 0.94, 3.2 and 5.3 mg/L of test material for 4 hrs. LC50 = 5.0 mg/L (M) (0.63 to 39). LC50 (F) = 4.7 mg/L (2.6 to 8.3 mg/L) in Sprague-Dawley.		Supplementary 007957 Minimum 008439
81-3 Acute Inhalation LC50 Species: rat Environmental Sci Corp. 88097; 10/7/88	MON 097; 92.5% acetochlor	409944-01	LC50 > 3.0 mg/L (ODT).	3	Minimum 007957

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-3 Acute inhalation LC50 ICI Species: rat ICI Central Tox. Lab. HR0864; 6/30/89	Acetochlor 89.4% a.i.	415651-06	LC50 (M) > 4.46 mg/L. LC50 (F) = 3.99 mg/L.	3	Minimum 008478
81-3 Acute inhalation LC50 ICI Species: rat ICI Central Tox. Lab. HR1976; 2/26/90	Acetochlor 760 g/L EC formulation, 71.6% a.i.	415651-11	LC50 > 2.10 mg/L (M&F) for acetochlor 760 g/L EC formulation.	3	Minimum 008478
81-4 Primary eye irritation Species: rabbit Environmental Health Labs 80-51; 10/15/80	Acetochlor MON 097	099807	Unwashed 18.8/110. Washed 1.2/110	2	Minimum
81-4 Primary eye irritation Species: rabbit Environmental Health Labs R-80-51; 10/15/80	2-Chloro-N-ethoxymethyl-N-(2-ethyl-6-methyl-phenyl)acetamide 86.4%	243802	corneal opacity at 24 hrs; all irritation clear by day 7.	2	Guideline 000899
81-4 Primary eye irritation Species: rabbit Bio/dynamics Inc. 6070-85; 11/7/85	Acetochlor MON 8449 (EC formulation)	260748	6/6 showed corneal opacity and ulceration, iris effects & conjunctival redness & chemosis. Opacity persisted through 7 days in 3/6.	1	Minimum 005113
81-4 Primary eye irritation Species: rabbit Food and Drug Research Lab 88.2053-017; 7/8/88	Harness-PC; 82.8% acetochlor	409988-03	Corneal opacity & conjunctival irritation after test administration and disappeared by day 7. At day 14 all eyes were normal. Test material of 0.1 ml was instilled into one eye of NZW rabbits.	3	Minimum 007957
81-4 Primary eye irritation Species: rabbit ICI Central Tox. Lab. FB4198; 9/29/89	Acetochlor 89.4% a.i.	415920-03	Acetochlor was minimally irritating to the eyes of white rabbits.	3	Minimum 008478

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81-4 ICI Primary eye irritation Species: rabbit ICI Central Tox. Lab. FB4344; 4/9/90	Acetochlor 760 g/L EC formulation	415651-12	Acetochlor 760 g/L EC formulation was mildly irritating to the eyes of white rabbits.	3	Minimum 008478
81-5 Primary dermal irritation Species: rabbit Environmental Health Labs 80-50; 10/15/80	Acetochlor MON 097	099807	PIS = 0.6/8.0	4	Minimum 005865
81-5 Primary dermal irritation Species: rabbit Environmental Health Labs R-80-51; 10/15/80	2-Chloro-N-ethoxymethyl-N-(2-ethyl-6-methyl-phenyl)acetamide 86.4%	243802	PIS = 0.6/8.0	4	Guideline 000899
81-5 Primary dermal irritation Species: rabbit Bio/dynamics Inc. 6069-85; 12/7/85	Acetochlor MON 8449 (EC formulation)	260748	6/6 showed slight to mild erythema or edema through 72 hrs.	4	Minimum 005113
81-5 Primary dermal irritation Species: rabbit Food and Drug Research Lab 88.2053-018; 7/8/88	Harness-PC; 82.8% acetochlor	409988-04	Erythema 6/6; slight edema 2/6; dermal irritation not observed on day 10. A 0.5 ml of test material was administered to two sites.	3	Minimum 007957
81-5 Primary dermal irritation Species: rabbit ICI ICI Central Tox. Lab. EB3655; 9/14/89	Acetochlor 89.4% a.i.	415651-07	Acetochlor was severely irritating to the skin of male rabbits.	2	Guideline 008478
81-5 ICI Primary dermal irritation Species: rabbit ICI Central Tox. Lab. EB3763; 3/8/90	Acetochlor 760 g/L EC formulation	415651-13	Acetochlor 760 g/L EC formulation was severely irritating to the skin of male rabbits.	2	Guiddeline 008478

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-6 Dermal sensitization Species: guinea pig Bio/dynamics Inc. BD 82-204; 4/13/83	Acetochlor Tech. 96.3% a.i. Lot # nbp1992053	071970	positive dermal sensitizer		Minimum 004586
81-6 Dermal sensitization Species: guinea pig Bio/dynamics Inc. BD 82-205; 4/13/83	Acetochlor E.C. 86.5% a.i Lot #XLC-3	071970	Positive dermal sensitizer		Minimum 004586
81-6 Dermal sensitization Species: guinea pig Bio/dynamics Inc. 6071-85; 11/7/85	Acetochlor MON 8449 (EC formulation)	260748	10/10 had a positive challenge response, thus this is a positive sensitizer in guinea pigs.		Guideline 005113
81-6 ICI Dermal sensitization Species: guinea pig ICI Central Tox. Lab. GG4647, GG4553; 7/19/89	Acetochlor 89.4% a.i.	415651-08	Extreme sensitization was observed in response to dermal application of undiluted acetochlor in female guinea pigs. A 30% dilution of acetochlor in corn oil produced strong sensitization in these same animals.		Minimum 008478
81-6 Dermal sensitization ICI Species: guinea pig Safepharm Lab Limited GG4856; 12/29/89	Acetochlor 760 g/L EC formulation	415651-14	No sensitization was observed in response to a challenge dermal application of 100% or 30% acetochlor EC formulation in female guinea pigs.		Supplementary 008478