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JAN 27 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Third Peer Review of Acetochlor

FROM: Kerry L. Dearfield, Ph.D. *Kerry Dearfield*
Executive Secretary, Peer Review Committee
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

and

Timothy F. McMahon, Ph.D. *Timothy McMahon*
Toxicologist, Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

TO: Robert Taylor
Product Manager #25
Registration Division (H7505C)

The Health Effects Division Peer Review Committee met on October 16, 1991 to discuss and evaluate the weight-of-the-evidence on Acetochlor with particular reference to its carcinogenic potential. The Peer Review Committee agreed that Acetochlor should be classified as a Group B2 - Probable Human Carcinogen. It was recommended for the purpose of risk characterization that a low dose extrapolation model applied to the experimental animal tumor data should be used for quantification of human risk (Q1*). For quantification, the Committee recommended separate calculations for both sexes of rat using the combined incidence for nasal tumors for each sex. The separate values would then be combined using appropriate statistical methods.

A. Individuals in Attendance:

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

William L. Burnam

Reto Engler

W L Burnam
Reto Engler



Karl Baetcke

Karl D. Baetcke

Marcia Van Gemert

Marcia Van Gemert

Esther Rinde

Esther Rinde

Kerry Dearfield

Kerry Dearfield

Marion Copley

Marion Copley

Hugh Pettigrew

Hugh Pettigrew

Richard Hill

Richard Hill

Lucas Brennecke

Lucas D. Brennecke

Yin-Tak Woo

Yin Tak Woo

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

Timothy F. McMahon

Timothy F. McMahon

Yiannakis M. Ioannou

Y. M. Ioannou

Bernice Fisher

Bernice Fisher

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Penelope A. Fenner-Crisp

Penelope A. Fenner-Crisp

John Quest

John A. Quest

William Sette

William Sette

George Ghali

G. Ghali

Robert Beliles

Robert Beliles

Julie Du

Julie Du

Jean Parker

Jean C. Parker

4. Other Attendees: Gary Burin (HED)

B. Material Reviewed:

The material available for review consisted of DER's, one-liners, and other data summaries prepared by Dr. Timothy McMahon; tables and statistical analysis by Bernice Fisher. The material reviewed is attached to the file copy of this report. The data reviewed are based upon studies submitted to the Agency by ICI Central Toxicology Laboratory.

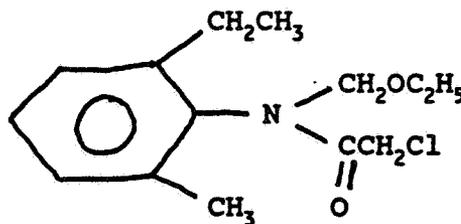
C. Background Information:

Acetochlor, or 2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide, is a 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 Peer Review Committee 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 (from previous two Peer Reviews; reports dated March 30, 1987 and May 31, 1989). These conclusions were based upon data submitted to the Agency by Monsanto Chemical Company.

The current 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 (SC-5676) on corn and ornamental shrubs in commercial nurseries.

The Chemical Abstracts Service (CAS) Registry number for Acetochlor is 34256-82-1 and the Tox Chem Number (or Caswell number) is 3B.

Structure of Acetochlor:



D. Evaluation of Carcinogenicity Evidence for Acetochlor:

1. Summary of Previous Peer Review Committee Evaluations

A previous registrant (Monsanto Chemical Co., St. Louis, MO) had submitted information concerning Acetochlor that the Peer Review Committee considered (reports dated March 30, 1987 and May 31, 1989). Based on this database, the Peer Review Committee classified Acetochlor as a Group B2 - Probable Human Carcinogen. This decision was based on: the incidence of hepatocellular carcinomas (male and female), of thyroid follicular cell adenomas (male), and of papillary adenomas of nose/turbinate (male and female) in Sprague-Dawley rats; and, the incidence of both benign and malignant tumors at multiple sites - hepatocellular carcinomas (male, trend for female), lung carcinomas (female), uterine histiocytic sarcomas (female), benign ovarian tumors (female), and kidney adenomas (trend only for females) - in Swiss Albino CD-1 mice. Furthermore, Acetochlor is structurally related to analogues (particularly Alachlor) that are carcinogenic. There are positive mutagenicity data that show Acetochlor is a genotoxic chemical. The Scientific Advisory Panel (SAP) at its September 28, 1989 meeting concurred with the Peer Review Committee's classification of Acetochlor as a Group B2 Carcinogen.

2. Rat Carcinogenicity Study

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 #41592004.

a. Experimental Design

Acetochlor (technical SC-5676; 91.0% purity) was administered in the diet to groups of 50 male and 50 female Sprague-Dawley CD rats at doses of 0, 18, 175 or 1750 ppm (equivalent to 0, 0.8, 7.9 or 79.6 mg/kg/day active ingredient) for 104 weeks. In addition, an extra 10 animals/sex were treated at 18 and 175 ppm dose levels for 52 weeks as well as an extra 20 animals/sex at the 0 and 1750 ppm dose levels. An additional 10 male and female rats were selected from the total number of rats ordered for the study and were used as veterinary controls to monitor the potential outbreak of disease during the study. No outbreak of disease was evident during the study.

b. Discussion of Tumor Data

Multiple tumor types were observed in both sexes of Sprague-Dawley rats in this study (Tables 1-4). Large, significant increases in adenomas of the nasal epithelium were observed in both male and female rats at the 1750 ppm dose level ($\geq 50\%$ incidences;

Table 1. Acetochlor, Rat Study, Males - Nasal epithelium tumor rates* and Peto's Prevalence test results (p values)

<u>Tumor</u>	<u>Dose level (ppm)</u>			
	<u>0</u>	<u>18</u>	<u>175</u>	<u>1750</u>
Adenoma	0/69 (0)	0/59 (0)	0/59 (0)	35 ^a /70 (50)
p=	0.000**	1.000	1.000	0.000**
Carcinoma	0/69 (0)	0/59 (0)	0/59 (0)	2 ^b /70 (3)
p=	0.073**	1.000	1.000	0.166
Combined	0/69 (0)	0/59 (0)	0/59 (0)	37/70 (53)
p=	0.000**	1.000	1.000	0.000**

* Number of tumor bearing animals/Number of animals examined
(excluding those that died before observation of the first tumor)

** Exact trend test result

() percent

^a First adenoma observed at week 53

^b First carcinoma observed at week 105

Note: Significance of trend denoted at Control
Significance of pair-wise comparison with control denoted
at dose level

if * then $p < 0.05$ and if ** then $p < 0.01$

Table 2. Acetochlor, Rat Study, Males - Thyroid follicular cell tumor rates* and Peto's Prevalence test results (p values)

Tumor	<u>Dose level (ppm)</u>			
	<u>0</u>	<u>18</u>	<u>175</u>	<u>1750</u>
Adenoma	2 ^a /49 (4)	1/50 (2)	2/48 (4)	5/49 (10)
p=	0.042*	0.720(n)	0.680	0.164
Carcinoma	1 ^b /49 (2)	3/50 (6)	0/48 (0)	3/49 (6)
p=	0.178	0.191	0.880(n)	0.149
Combined	3/49 (6)	4/50 (8)	2/48 (4)	8/49 (16)
p=	0.026*	0.427	0.660(n)	0.060

* Number of tumor bearing animals/Number of animals examined
(excluding those that died before observation of the first tumor)

() percent; (n) negative change from control

^a First adenoma observed at week 71

^b First carcinoma observed at week 83

Note: Significance of trend denoted at Control
Significance of pair-wise comparison with control denoted
at dose level

if * then $p < 0.05$ and if ** then $p < 0.01$

Table 3. Acetochlor, Rat Study, Females - Nasal epithelium tumor rates* and Cochran-Armitage trend test and Fisher's Exact test results (p values)

<u>Tumor</u>	<u>Dose level (ppm)</u>			
	<u>0</u>	<u>18</u>	<u>175</u>	<u>1750</u>
Adenoma	0/69 (0)	0/57 (0)	0/58 (0)	36 ^a /63 (57)
p=	0.000**	1.000	1.000	0.000**
Carcinoma	0/69 (0)	0/57 (0)	0/58 (0)	1 ^b /63 (2)
p=	0.255**	1.000	1.000	0.477
Combined	0/69 (0)	0/57 (0)	0/58 (0)	37/63 (59)
p=	0.000**	1.000	1.000	0.000**

* Number of tumor bearing animals/Number of animals examined
(excluding those that died before observation of the first tumor)

** Exact trend test result

() percent

^a First adenoma observed at week 52

^b First carcinoma observed at week 90

Note: Significance of trend denoted at Control
Significance of pair-wise comparison with control denoted
at dose level

if * then $p < 0.05$ and if ** then $p < 0.01$

Table 4. Acetochlor, Rat Study, Females - Thyroid follicular cell tumor rates* and Cochran-Armitage trend test and Fisher's Exact test results (p values)

Tumor	Dose level (ppm)			
	0	18	175	1750
Adenoma	1/69 (1)	1/58 (2)	3 ^a /59 (5)	6/63 (10)
p=	0.008**	0.707	0.253	0.044*
Carcinoma	0/69 (0)	0/58 (0)	0/59 (0)	1 ^b /63 (2)
p=	0.253**	1.000	1.000	0.477
Combined	1/69 (1)	1/58 (1)	3/59 (5)	7/63 (11)
p=	0.003**	0.707	0.253	0.022*

* Number of tumor bearing animals/Number of animals examined
(excluding those that died before observation of the first tumor)

** Exact Trend test result

() percent

^a First adenoma observed at week 53

^b Carcinoma observed at week 105

Note: Significance of trend denoted at Control
Significance of pair-wise comparison with control denoted
at dose level

if * then $p < 0.05$ and if ** then $p < 0.01$

p < 0.01 for trend and pair-wise statistical comparisons; Tables 1 and 3). This increase in nasal epithelial adenomas was also significant when decedent and surviving rats were considered separately.

Follicular cell adenomas of the thyroid were observed in both sexes (Tables 2 and 4). The trends for increased thyroid follicular cell adenomas and combined adenomas/carcinomas were statistically significant for female and male rats. There was pair-wise statistical significance compared to concurrent controls for adenomas and combined adenomas/carcinomas for females at the top dose, 1750 ppm, but none for males at any dose level; it is noted however that the percentage of rats with this tumor was equivalent between sexes at the 1750 ppm dose level (10%) - the males had a control level of 4% and the females had a control level of 1%. The incidence of thyroid follicular cell adenoma at the 1750 ppm dose level in females and males was outside the historical control range for this tumor type (data supplied by registrant, five studies with range females, 0-6%; males, 2-6%).

Two rare tumor types were also observed in this study, all found at the top dose, 1750 ppm. 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.

c. Non-Neoplastic Lesions

Treatment of male and female rats with Acetochlor, especially at the 1750 ppm dose level, resulted in several non-neoplastic lesions in both sexes. These included nasal epithelial hyperplasia (0% in control males and females to 8% and 11% in 1750 ppm males and females, respectively), epithelial hyperplasia of the kidney pelvis (6% in control males to 22% in 1750 ppm males), stromal fatty infiltration of the pancreas (1750 ppm both sexes), focal hyperplasia of the adrenal cortex (1750 ppm females), and degeneration of the outer nuclear layer of the retina (1750 ppm females). The appearance of nasal epithelial hyperplasia was particularly relevant as tumors occurred in the same site.

d. Considerations of Adequate Dosing for Assessment of Carcinogenic Potential

The highest dose of test article examined in this study was 1750 ppm in both male and female rats. The statistical evaluation of mortality in the rats indicated that there was no evidence of increase in either sex with incremental doses of Acetochlor. The top dose caused a body weight gain 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 (from 0-52 weeks, decreased by 17% and 24% for males and females, respectively, versus control; for the entire study, 0-104 weeks, decreased by 14% and 33% for males and females, respectively, versus control); there was little effect on body weight gain with lower doses (only 8% decrease with mid-dose males at end of study noted). 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 the 1750 ppm dose level. In light of these effects and the observance of tumors, the high dose level of 1750 ppm is considered to be an adequate dose for assessing the carcinogenic potential of Acetochlor in rats.

3. Mouse Carcinogenicity Study

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 #41565119.

a. Experimental Design

Acetochlor (technical SC-5676; 90.5% purity) was administered in the diet to 50 male and 50 female CD-1 mice at doses 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), or 1000 ppm (116 mg/kg/day active ingredient - males; 135 mg/kg/day active ingredient - females) for 78 weeks. An additional 10 animals/sex were used for a 52 week interim sacrifice for each of the four dose groups.

b. Discussion of Tumor Data

An increased incidence of pulmonary adenomas was observed in male and female mice (Tables 5 and 6). A statistically significant trend of increase for combined pulmonary adenomas/carcinomas was observed in both male and female mice. A statistically significant trend of increase for pulmonary adenomas was observed for male mice. In addition, the incidence of pulmonary adenomas in male and female mice from the 1000 ppm dose group was statistically significantly different from control (males 23% incidence versus 8% control; females 12% incidence versus 2% control). A statistically significant increase in combined pulmonary adenomas/carcinomas was found only for males at 1000 ppm (30% incidence versus 17% control).

The Peer Review Committee considered this pulmonary tumor type relevant as it was apparent Acetochlor had a disposition to the lung. This was also noted from studies considered in previous Peer Review Committee meetings on Acetochlor. Historical control data

Table 5. Acetochlor, Mouse Study, Males - Pulmonary tumor rates* and Peto's Prevalence test results (p values)

<u>Tumor</u>	<u>Dose level (ppm)</u>			
	<u>0</u>	<u>10</u>	<u>100</u>	<u>1000</u>
Adenoma	5/60 (8)	3/60 (5)	11/59 (19)	13 ^a /57 (23)
p=	0.007**	0.770(n)	0.080	0.010*
Carcinoma	5/60 (8)	4/60 (7)	3 ^b /59 (5)	4/57 (7)
p=	0.479	0.640(n)	0.750(n)	0.570(n)
Combined	10/60 (17)	7/60 (12)	14/59 (24)	17/57 (30)
p=	0.018*	0.790(n)	0.188	0.049*

* Number of tumor bearing animals/Number of animals examined
(excluding those that died before observation of the first tumor)

() percent; (n) negative change from control

^a First adenoma observed at week 53

^b First carcinoma observed at week 41

Note: Significance of trend denoted at Control
Significance of pair-wise comparison with control denoted
at dose level

if * then $p < 0.05$ and if ** then $p < 0.01$

Table 6. Acetochlor, Mouse Study, Females - Pulmonary tumor rates* and Cochran-Armitage trend test and Fisher's Exact test results (p values)

<u>Tumor</u>	<u>Dose level (ppm)</u>			
	<u>0</u>	<u>10</u>	<u>100</u>	<u>1000</u>
Adenoma	1/58 (2)	4 ^a /59 (7)	6/58 (10)	7/60 (12)
p=	0.067	0.187	0.057	0.034*
Carcinoma	4/58 (7)	0/59 (0)	2 ^b /58 (3)	4/60 (7)
p=	0.146	0.057 (n)	0.340 (n)	0.622
Combined	5/58 (9)	4/59 (7)	8/58 (14)	11/60 (18)
p=	0.029*	0.489 (n)	0.279	0.101

* Number of tumor bearing animals/Number of animals examined
(excluding those that died before observation of the first tumor)

() percent; (n) negative change from control

^a First adenoma observed at week 46

^b First carcinoma observed at week 70

Note: Significance of trend denoted at Control
Significance of pair-wise comparison with control denoted
at dose level

if * then $p < 0.05$ and if ** then $p < 0.01$

from the testing laboratory was presented during the meeting and the high dose incidences of pulmonary tumors for both sexes were found to be above the historical control range.

In males only, a statistically significant increase in the incidence of combined hepatocytic adenomas/carcinomas compared to concurrent control was observed at 1000 ppm (8/54 or 15% versus 3/56 or 5% for control; $p = 0.031$). No increases in trend or significant increase in tumor incidence were observed for any other dose or adenomas or carcinomas alone. The Peer Review Committee noted this tumor type.

c. Non-Neoplastic Lesions

At the 52-week interim sacrifice, nephropathy was observed in 44% (4/9) of high dose males, compared to 0% controls. Of the high dose females, 20% were affected versus 10% of controls. Statistically significant increases in the incidences of interstitial fibrosis, hyaline cysts, and cortical mineralization were observed in high dose males when compared to 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.

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. There was a similar incidence in bronchiolar hyperplasia between control and high dose females, but a slightly higher incidence was observed in low- and mid-dose females.

d. Considerations of Adequate Dosing for Assessment of Carcinogenic Potential

The highest dose of test article examined in this study was 1000 ppm in both male and female mice. The statistical evaluation of mortality in the mouse study indicated a significant increasing trend in males and no differences in females with incremental doses of Acetochlor. There were no statistically significant reductions in body weight or body weight gain during the 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. While the Peer Review Committee agreed that the dosing in the carcinogenicity study may not be totally adequate for the assessment of carcinogenicity of Acetochlor, especially for female mice, the observance of tumors in both sexes reduces the concern for higher dosing.

E. Additional Toxicology Data on:

1. Metabolism

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

a) 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.

b) 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.

c) 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.

d) Laboratory Project No. HRC/STR 18/89603, The Metabolism of 14-C Acetochlor in the Rat after Oral Administration.

e) Laboratory Project No. CTL/P/2809, Acetochlor: Biotransformation Study in the Rat.

The 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 after 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 the 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.

2. Mutagenicity

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 # 41565121.

Acetochlor induced a reproducible, positive, mutagenic response in strain TA1538 of Salmonella typhimurium with metabolic activation at 1000 µg/plate (however, this was less than 2X background mutation, but was 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 # 41565123.

Acetochlor was not clastogenic in a 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 # 41565122.

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 # 41565124.

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

Based on the total weight of the evidence, Acetochlor presents a mutagenicity concern. According to the ICI submitted studies, Acetochlor is clastogenic in vitro, induces DNA repair in response to DNA damage in vivo (UDS), and has suggestive activity in a Salmonella assay. Earlier submitted studies from Monsanto also demonstrated Acetochlor is mutagenic in the Chinese hamster ovary (CHO) and mouse lymphoma gene mutation assays. Results from ICI and Monsanto do not show clastogenic activity in vivo (mouse micronucleus and rat bone marrow aberration assays). The positive UDS result is particularly significant as relatively few compounds that the Peer Review Committee has considered are positive in this

assay, it is an in vivo result, and the primary analogue, Alachlor, is also positive in this assay (MRID #00141061; Monsanto). The overall mutagenicity concern would support a concern for carcinogenicity.

It is recommended from this total weight of evidence that Acetochlor be tested in a dominant lethal assay and an alkaline elution or UDS assay in germ cell tissue (in vivo). At the Peer Review meeting, it was learned that ICI has submitted a dominant lethal study in rats (MRID #41963309). This study will be reviewed and the above recommendation for a dominant lethal study taken into account.

3. Developmental and Reproductive Effects

a) 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 # 41592005.

The developmental toxicity of Acetochlor was assessed by oral administration 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, reduced body weight gain, and food consumption. Additional data are needed in order to assign a Maternal and a Developmental NOEL and LEL.

b) Reference: Brooker, 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 # 41592006.

The developmental toxicity of Acetochlor was assessed by oral administration to pregnant New Zealand White Rabbits on 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 a Maternal and a Developmental NOEL and LEL.

c) 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 # 41565120.

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 and LEL = 1750 ppm.
Reproductive toxicity NOEL = 175 ppm and LEL = 1750 ppm:

4. Chronic Toxicity in Dogs

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 # 41565118.

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 also were 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.

5. Structure-Activity Correlations

Acetochlor is structurally related to Alachlor, Butachlor, Metolachlor, Allidochlor, Propachlor, and SAN 582H (Figure 1).

Alachlor is carcinogenic in two species (rats and mice). In a dietary administration study in rats, nasal turbinate tumors were found at 15 and 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 lung tumors was observed at 260 mg/kg in females. In submitted studies (Monsanto), Alachlor gave a negative response in the Salmonella assay, but was positive in an in vivo/in vitro DNA damage/repair (UDS) assay. Negative findings were reported from other bacterial assays, in vivo cytogenetics, and a CHO/hgpRT gene mutation assay. Some metabolites of Alachlor were positive in the Salmonella assay while other metabolites were not. Published

reports suggest that Alachlor is clastogenic in vitro and in vivo. The Peer Review Committee has classified Alachlor as a Group B2 Carcinogen.

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 the Salmonella assay, to induce aberrations in CHO cells, and to be negative in a bacterial rec assay and an E. coli reverse mutation assay. The Peer Review Committee has not evaluated this chemical.

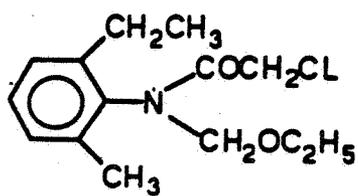
Metolachlor, in a dietary administration study in rats, was found to cause a significantly elevated incidence of proliferative liver lesions in females (neoplastic nodules and carcinomas, combined) at 150 mg/kg. There was also a suggestive, although not statistically significant, increase in nasal turbinate tumors in female rats. Mutagenicity data from Salmonella, mouse lymphoma and micronucleus assays were negative. The Peer Review Committee has classified Metolachlor as a Group C Carcinogen.

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

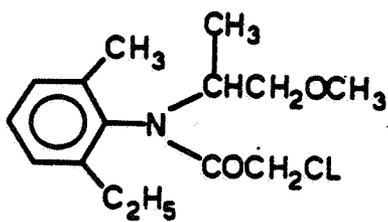
Propachlor, in a two year chronic toxicity/carcinogenicity study in rats, showed evidence of an increased incidence of thyroid and ovarian neoplasia; however, the study did not use high enough dose levels to adequately assess the carcinogenic potential of Propachlor. A carcinogenicity study in mice also used doses below those necessary to adequately assess the carcinogenicity of Propachlor. Propachlor was positive for aberrations in CHO cells, suggestive for gene mutations in CHO cells, and negative in an UDS assay. Evidence for in vivo cytogenetic effects is unclear. A dominant lethal study is now required.

SAN 582H, in a chronic toxicity/carcinogenicity study in rats, was found to cause an 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 with an 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.

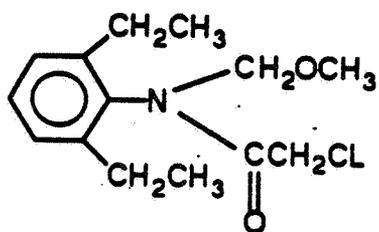
SAN 582H was not mutagenic in the Salmonella assay, but induced positive UDS activity at dose levels well below the cytotoxic level in one study. A second UDS study showed that SAN 582H did not induce any significant increase in net nuclear grain counts. Other studies on the mutagenicity of SAN 582H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



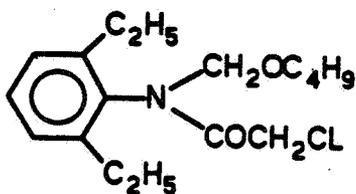
Acetochlor



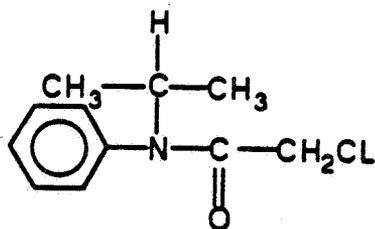
Metolachlor



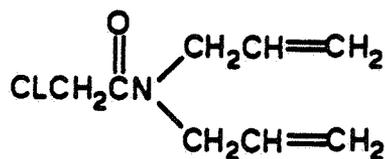
Alachlor



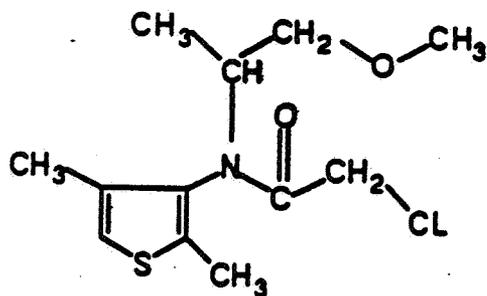
Butachlor



Propachlor



Allidochlor



SAN 582H

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Acetochlor to be of importance in a weight-of-the-evidence determination of carcinogenic potential.

1. In the chronic toxicity/carcinogenicity CD rat study, Acetochlor was associated with:

a) a large, significant increase for adenomas of the nasal epithelium at a dose of 1750 ppm (79.6 mg/kg/day a.i.) in both male and female rats ($\geq 50\%$ incidence versus 0% concurrent controls). Carcinoma of the nasal epithelium was also observed in 2 male and 1 female rats at the 1750 ppm dose level, but not at lower doses or controls;

b) a statistically significant positive trend for the incidence of thyroid follicular cell adenomas in female and male rats with a pair-wise statistically significant increase compared to concurrent control in female rats at the 1750 ppm dose level. The thyroid follicular cell adenoma incidences for females and males were outside the historical control range; and,

c) the appearance of two rare tumor types, benign chondroma of the femur and basal cell tumors of the stomach in both sexes at the top dose of 1750 ppm.

2. In the 78 week carcinogenicity study in CD-1 mice, a statistically significant increase for the incidence of pulmonary adenomas was observed in male and female mice at the 1000 ppm dose level, as well as a statistically significant increase for combined adenomas/carcinomas in males. There was a positive trend for the increase for adenomas in males and for the increase for combined adenomas/carcinomas in males and females. It was noted that there was a statistically significant increase compared to controls for combined hepatocytic adenomas/carcinomas in males at the top dose of 1000 ppm. Since the pulmonary tumors were seen at doses the Committee felt may not have been at maximal dosing, especially for females, the association of tumors and Acetochlor exposure is strengthened.

3. Significant increases in the incidence of nasal epithelial hyperplasia in rats (both sexes) and bronchiolar hyperplasia in mice (males; slight increase in low- and mid-dose females) indicate the relevance of tumors seen at these target sites.

4. Based on the total weight of the evidence, Acetochlor presents a mutagenicity concern. According to the ICI submitted studies, Acetochlor is clastogenic in vitro, induces DNA repair in response to DNA damage in vivo (UDS), and has suggestive activity in a Salmonella assay. Earlier submitted studies from Monsanto also demonstrated Acetochlor is mutagenic in the Chinese hamster ovary (CHO) and mouse lymphoma gene mutation assays. Results from ICI and Monsanto do not show clastogenic activity in vivo (mouse micronucleus and rat bone marrow aberration assays). The positive

UDS result is particularly significant as relatively few compounds that the Peer Review Committee has considered are positive in this assay, it is an in vivo result, and the primary analogue, Alachlor, is also positive in this assay. The overall mutagenicity concern would support a concern for carcinogenicity.

It is recommended from this total weight of evidence that Acetochlor be tested in a dominant lethal assay and an alkaline elution or UDS assay in germ cell tissue (in vivo). At the Peer Review meeting, it was learned that ICI has submitted a dominant lethal study in rats (MRID #41963309). This study will be reviewed and the above recommendation for a dominant lethal study taken into account. The UDS assay in germ cell tissue needs to be performed.

5. Acetochlor has been previously classified by the Peer Review Committee as a Group B2 - Probable Human Carcinogen. In addition, the structurally related compound Alachlor has also been given a Group B2 classification for carcinogenicity. The classification of Acetochlor as a Group B2 carcinogen was based upon data submitted by Monsanto Chemical Company (see previous Peer Reviews dated March 30, 1987 and May 31, 1989), whose results from carcinogenicity testing of Acetochlor are similar to those results obtained by ICI.

6. The analogue data provide additional support for Acetochlor carcinogenicity and the carcinogenicity of this class of chloroalkylacetamide compounds. Alachlor also induced nasal turbinate tumors and thyroid tumors in rats and lung tumors in mice, just as did Acetochlor. There is suggestive evidence of induced nasal turbinate tumors with Metolachlor and thyroid tumors with Propachlor. Also, stomach tumors were noted in rat carcinogenicity studies with Acetochlor, Alachlor and Butachlor.

G. Classification of Carcinogenic Potential:

Criteria contained in the USEPA Cancer Risk Assessment Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

The Peer Review Committee unanimously agreed that the classification for Acetochlor should remain as a Group B2 - Probable Human Carcinogen. This was based on the appearance of multiple tumors in both sexes of CD rats (nasal epithelium adenoma, thyroid follicular cell adenoma, benign chondroma of femur, and basal cell tumor of stomach) and of pulmonary adenomas in both sexes of CD-1 mice due to Acetochlor exposure; hepatocytic adenomas/carcinomas combined were also noted in male mice. Acetochlor is a genotoxic compound and structural analogues, particularly Alachlor, provide additional support for Acetochlor carcinogenicity. This decision is based upon the ICI submitted database.

This current decision is consistent with the earlier decisions by the Peer Review Committee when an additional Acetochlor database from another registrant was considered. The combined data only strengthen the Group B2 classification. Furthermore, the Committee noted that the two databases on Acetochlor from two different registrants were in close agreement with each other concerning the major tumor types. The duplication of carcinogenicity data provides solid, clear evidence for the carcinogenicity of Acetochlor.

For the purpose of risk characterization for Acetochlor, a low dose extrapolation model applied to the experimental animal tumor data should be used for quantification of human risk (Q1*). For quantification, the Committee recommended separate calculations for both sexes of rat using the combined incidence for nasal tumors for each sex. The separate values would then be combined using appropriate statistical methods.

In light of Acetochlor's potential for neurotoxic effects, the Peer Review Committee recommends that an acute and subchronic neurotoxicity battery be performed in rats.