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

9/25/91

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

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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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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 (IC 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.

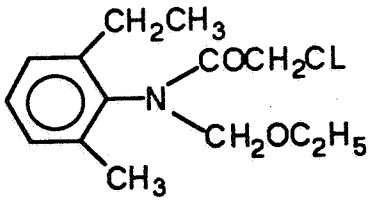
SC-5676 was administered to groups of male and female CD rats in the diet at dose levels of 18 ppm (1.6 mg/kg/day), 175 ppm (21 mg/kg/day) and 1750 ppm (160 mg/kg/day). Systemic toxicity was observed in high dose parental males and females, and consisted of reductions in body weight, food consumption,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

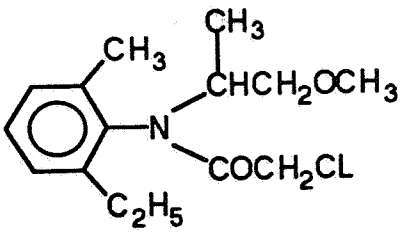
Parental toxicity NOEL = 175 ppm; Parental toxicity LEL = 1750 ppm.
Reproductive toxicity NOEL = 175 ppm; Reproductive toxicity LEL = 1750 ppm.

D. Structure-Activity Considerations

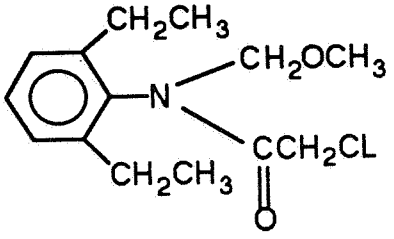
Acetochlor is structurally related to Metolachlor, Alachlor, Allidochlor, Butachlor, Propachlor, and SAN 582H, as illustrated below:



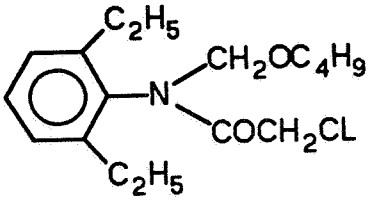
Acetochlor



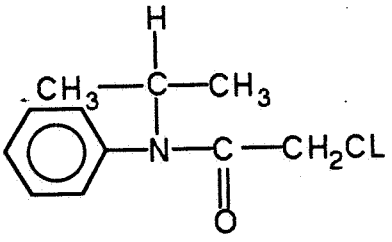
Metolachlor



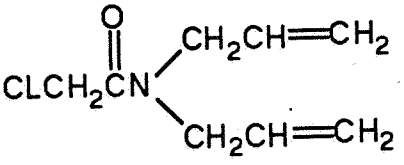
Alachlor



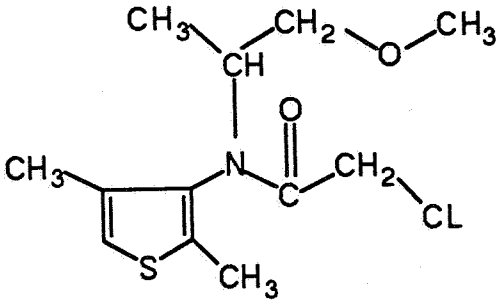
Butachlor



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.