UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

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OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

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

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

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

### A. Introduction

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

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

b. positive mutagenic effects and

c. structurally related known oncogens.

B. Assessment of Oncogenicity

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

C. Assessment of Mutagenicity

Several mutagenicity studies were conducted with Acetochlor:

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

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

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

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

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

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

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

## D. Assessment of Structure-Activity Relationship

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

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

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

Metolachlor in a dietary administration study in rats was found to cause a significantly elevated incidence of proliferative liver lesions (neoplastic nodules and carcinomas, combined) at 150 mg/kg in females. Further, examinations of the nasal turbinates found nasal malignancies (adenocarcinoma and fibrosarcoma) in 3/69 in the 150 mg/kg/day dose group vs. 0/67 in control. The mouse study (dietary administration) was negative for proliferative lesions. The PRC has tentatively classified Metolachlor as a Category C oncogen.

## E. Previous Peer Review Committee Assessment

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

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

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

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

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

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

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