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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

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MEMORANDUM

Subject: **Acetochlor**. Report of the Metabolism Assessment Review Committee.

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1. INTRODUCTION

Purpose of the *ad hoc* MARC meeting
Members of the MARC team and the CARC Committee met with the risk assessment team for acetochlor on May 13, 2004 to discuss whether the acetochlor sulfonic acid (ESA) and acetochlor oxanilic acid (OXA) environmental degradates should be included in the dietary drinking water assessment, based on an evaluation of their carcinogenic or toxicity potential.

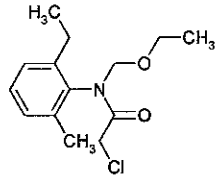
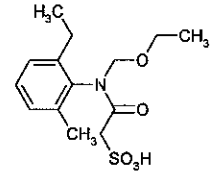
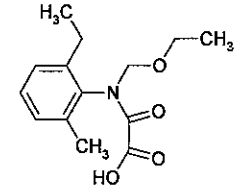
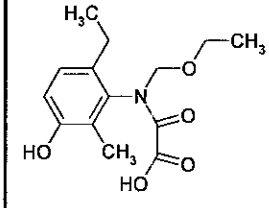
Identification of Chemicals:
The structures of the two chemicals primarily covered in this meeting appear in Table 1, together with related structures. It is noted that:

- ESA differs from the parent acetochlor (in Table 1) in that the chlorine of the parent has been replaced by a sulfonic group (a fully dissociated group at physiological pHs).

- OXA differs from the parent acetochlor in that the **-CH_2Cl** group present in the parent has been de-chlorinated and oxidized to a carboxyl group **(COOH)**, so that now the compound is an amide of oxalic acid.

Two other degradates (ring-hydroxylated forms of OXA, Table 1), Metabolite 57 and 55, and PJ2 (a 3-4:1 mix of Compounds 57 and 55), have mutagenicity data and were briefly discussed.

Table 1. Chemical names and structures of acetochlor and some of its water degradates.

Common Name	Acetochlor (PC 121601)	Acetochlor ethane sulfonic acid (ESA)	Acetochlor oxanilic acid (OXA)	Compound 57[a]
Synonyms	MON 097	Acetochlor ESA MON 52754 R290131	Acetochlor OXA MON 52755 R290130	-
Structure:				

[a] N-(2-ethyl-5-OH-6-methylphenyl) oxamic acid; Compound 55 is the 3-OH isomer of Compound 57.

Issues for the *Ad Hoc* Group:

1. For Acetochlor ESA:
   a. For the risk asse sment: Is acetochlor ESA a potential carcinogen?
   b. Should acetochl r ESA be included in the water risk assessment with the parent?

2. For Acetochlor OXA:
   a. For the risk asse sment: Is acetochlor OXA a potential carcinogen?
   b. Should acetochl r OXA be included in the water risk assessment with the parent.

2. MARC MEETING INFORM ATION

Decision:

Table 2.1. Residues of Concern in Water		
Matrix	olerance Expression	Residues for Risk Assessment
Water	I/A	Parent only

Meeting Date: May 13, 2004

MARC Rationale:

A. Carcinogenicity Risk Assessn ent

Mechanistic data have been revie ed by HED and the Mechanism of Toxicity Committees. It was concluded that neither acetoc lor ESA (ESA) nor acetochlor OXA (OXA) degradates are likely to be carcinogenic, so they s ıould not be included in the carcinogenicity assessment for acetochlor. This conclusion is su ɔorted by the following items:

(a) Limited oral absorption and ɔiotransformation in rats:

- OXA is less absorbed th ı parent (about 34-39% of the dose) and undergoes little biotransformation (about 8 -85% of the dose is untransformed OXA).
- ESA is poorly absorbed (ıbout 10-12% of the dose) and undergoes little biotransformation (76-79% of the dose is untransformed ESA).

(b) ESA and OXA are non muta ;enic.

(c) ESA and OXA are devoid of ctive chlorine. The elimination of active chlorine in these two compounds should also reduc or even eliminate their ability to deplete cells of protective nucleophiles (e.g. GSH). Additio ally, the absence of active chlorine decreases their capacity to

form adducts with cell macromolecules.

(d) Concerning rat nasal turbinate tumors, both ESA and OXA lack the capacity to form a quinoneimine species leading to nasal tumors with parent. Parent acetochlor has been shown :

- to be extensively absorbed and metabolized;
- to undergo extensive metabolism to precursors of a quinoneimine;
- to bind extensively to the nasal olfactory epithelium (autoradiographic and chemical data are available). The structure of the bound material indicates that the bound material came from electrophilic species : a quinoneimine and a CH_2CL- containing precursor.
- to produce a dose related-increase in cell proliferation in the nasal olfactory epithelium.

In contrast neither ESA nor OXA parallel the effects, shown above for parent acetochlor. ESA and OXA are less absorbed than the parent, undergo very little metabolism, do not bind to nasal epithelium and do not produce nasal epithelium cell proliferation in rats.

(e) Concerning rat thyroid follicular cell tumors, parent acetochlor produced (MTARC/CARC 4/2004):

- increased thyroid weights at 1750 and 5000 ppm up to day 90 of dosing;
- slight but statistically significantly reduced levels of T_3 on day 14 at 1750 and 5000 ppm. Levels of T_4 were significantly increased on day 14.
- TSH was significantly increased on days 14 and 28 at 5000 ppm , and at day 56 only at 1750 ppm.
- Increased liver weights and microsomal UDPGT were seen by day 14 at 5000 ppm and day 28 at 1750 ppm

In contrast to parent, ESA:

- did not produce changes in thyroid weights up to 12000 ppm, up to day 90 of dosing;
- neither T_3 nor T_4 were decreased at any dose level;
- produced dose-related but not statistically significant increases in TSH at 6000 and 12000 ppm;
- statistically significant increases in microsomal UDPGT at 12000 ppm in females, and in microsomal UDPGT activity/ total liver in males at 12000 ppm. No changes were seen at 6000 ppm.

In contrast to parent, OXA:

- did not produce changes in thyroid weights up to 12000 ppm, up to day 90 of dosing, although increased thyroid weights were seen in males only in another experiment at 4 weeks at 3000 ppm and higher doses;
- T_4 was not statistically significantly decreased at any dose level in a 4-week study. T_3 was significantly decreased at 12000 ppm but not at 6000 ppm and the decrease was limited to males.

- TSH was decreased (not increased as in parent) in both sexes, but not statistically significantly so in a 4 week study.

- Microsomal UDPGT ac ivity was decreased (not increased as in parent) in females at 12000 ppm at 4 weeks, bu not at 6000 ppm. No statistically significant changes were seen in males up to 12000)pm.

(f) **Concerning other tumors wit iout known mode of action (MOA)** (lung tumors and histiocytic sarcomas in mice, and ver at excessive doses in rats), OXA and ESA have the general structural and metabolic c aracteristics, described above, that preclude them from being activated into carcinogens or to ge ierate precursors to carcinogens:

- the compounds are non- autagens;
- they are very polar com; ounds with limited oral absorption and biotransformation.
- they are devoid of an ele :trophilic active chlorine, that would consume protective nucleophiles and are not c:)able of forming a quinoneimine, as the parent does;
- due to their limited biot insformation, their lack of electrophilic chlorine and their inability of forming a quin ne imine they are unlikely to form adducts with macromolecules or produc significant, if any, oxidative damage;
- they are not effective in(icers of microsomal enzymes.

B. Other Dietary Risk Assessme ts:
The committee concluded that nei ier ESA nor OXA degradates should be included in the acute and chronic dietary water assessm nts for acetochlor. This conclusion is supported by the following items:

(a) Limited oral absorption and iiotransformation in rats (less opportunity for formation of toxicologically active species, e.g. hey do not form quinoneimine)

- OXA is less absorbed tha 1 parent (about 34-39% of the dose) and undergoes little biotransformation (about 8 -85% of the dose is untransformed OXA).
- ESA is poorly absorbed (bout 10-12% of the dose) and undergoes little biotransformation (76-79% of the dose is untransformed ESA).

(b) High polarity, facilitating ex retion

(c) Lower subchronic toxicity (9 -day feeding studies) in rats than parent acetochlor:
With **parent acetochlor,** the NO/ EL = 16.1 / 19.1 mg/kg/day [M/F] and the LOAEL = 161 / 191 mg/kg/day based on hematolo y and significant increases in organ weights.

In contrast:

- With **ESA,,** the NOAEI = 225.4 / 259.1 mg/kg/day [M/F] and the LOAEL = 919.4 / 1073.2 mg/kg/day based or reduced body weights, body weight gains and food utilization.
- With **OXA,,** the NOAE = 230.2 / 268.0 mg/kg/day [M/F] and the LOAEL = 955.2 / 1082.7 mg/kg/day based or reduced body weights, body weight gains and food utilization. No thyroid wei ht increases were seen in this study.

(d) No developmental toxicity reported in rats for OXA and the analog alachlor ESA at the highest dose tested.
Parent acetochlor had a maternal and developmental NOAEL = 150 mg/kg/day and a maternal and developmental LOAEL = 600 mg/kg/day. The developmental LOAEL was based on resorptions and decreased fetal weight. In another study, parent acetochlor, had a maternal and developmental NOAEL = 200 mg/kg/day and a maternal and developmental LOAEL = 400 mg/kg/day. The developmental LOAEL was based on decreased fetal weight.

In contrast:

- The analog alachlor ESA (The diethyl analog of ESA) showed in rats a NOAEL (maternal & developmental) greater than or equal to 900 (HDT)mg/kg/day and a LOAEL (maternal & developmental) greater than 900 mg/kg/day.
- OXA showed in rats a NOAEL (maternal) = 500 mg/kg/day and a LOAEL (maternal) = 1000 mg/kg/day, based on maternal mortality. The NOAEL (developmental)was equal or greater than 1000 mg/kg/day and the LOAEL (developmental) was greater than 1000 mg/kg/day.

Members attended:

Abdallah Khasawinah, Christine Olinger, Rick Loranger, Alberto Protzel, John Doherty, P.V. Shah,

Members in Absentia: Bill Wassell , Leung Cheng, Yan Donovan, Norman Birchfield.

Non MARC Members: Michael Barrett, Bill Burnam (**CARC member**), Christina Scheltema, Phil Errico, Nancy McCarroll (**CARC member**), Karl Baetcke (**CARC member**), Susan Makris, Stephen Dapson

3. BRIEFING MATERIALS

Residue Chemistry
Only toxicology issues were covered in this meeting. Any issues related to residue chemistry would have to be subject of a future meeting, if required.

Toxicology
The following paragraphs summarize toxicity data on the analog alachlor ESA and the acetochlor degradates acetochlor ESA and acetochlor OXA.

I. Previous HED actions on the analog alachlor ESA metabolite.

An *ad hoc* HED Metabolism Committee meeting held 1/18/95 discussed the available

toxicity data for the alachlor ethar e sulfonic acid (ESA) metabolite.

The *ad hoc* HED Metabolism Cor mittee concluded the following:

(a) Since alachlor ESA is sulfonated, and highly polar, there is likely to be little absorption via the oral or dermal r outes, and even if absorbed, it is expected to be readily excreted.

(b) Information ha been provided by the Registrant which indicates toxicity of the parent is based in part on form tion of the quinone imine. [HED agrees with the hypothesis.]

(c) Formation of t e potentially carcinogenic quinone-imine from Alachlor ESA is unlikely if the metabolite occur solely in the sulfonated form in the body, or if minimal cleavage to the unsulfonated form occurs.

(d) Because of the easons cited above, alachlor ESA is unlikely to be carcinogenic in a 2-year bioassay.

(e) Alachlor ESA s hould, however, continue to be included in non-cancer dietary exposure estimates (for comparis n to the RfD).

(f) Alachlor ESA w as non-mutagenic.

Available toxicity data for the ala lor ESA degradate have been summarized below in Table 2a and compared with the respective nd-points for its parent alachlor.

TABLE 2a. Comparison of Alachlor and Alachlor ESA

Test	Alachlor	Alachlor ESA
Acute oral LD_{50}	930 mg/kg Toxicity category III	> 6000 mg/kg Toxicity category IV
Subchronic Toxicity[1]	90 day invalidated feeding study	91-day drinking water study NOAEL = 157 mg/kg/day LOAEL = 896 mg/kg/day
Developmental Toxicity	maternal NOAEL = 150 mg/kg/day LOAEL = 400 mg/kg/day developmental NOAEL = 150 mg/kg/day LOAEL = 400 mg/kg/day	maternal NOAEL => 900 mg/kg/day LOAEL > 900 mg/kg/day developmental NOAEL => 900 mg/kg/day LOAEL > 900 mg/kg/day
Mutagenicity	weakly mutagenic - (+) for in vitro but not in vivo Chr. Damage; tested positive in 2 UDS studies. Other alachlor metabolites also found to be weakly mutagenic	no mutagenic activity in four studies
Metabolism[2]	Absorption was essentially complete with alachlor being present in the blood at 24 hours and 5 days post dose. Alachlor excreted approximately equally between urine and feces.	ESA is the major component in both urine and feces. ESA is poorly absorbed, rapidly excreted (71-82% in the feces within 24 hours), and undergoes minor metabolism.

[1] The subchronic data available for comparison of alachlor with the ESA metabolite of alachlor are not by the same route of administration (in the diet for alachlor *per se* and in the drinking water for the ESA metabolite of alachlor). Also, the study with alachlor *per se* is an IBT study which was not validated nor repeated; therefore the data may be suspect. It is important to note that the subchronic and chronic toxicity studies with alachlor were conducted with different strains of rats ("Charles River Albino rats" vs Long-Evans rats) than the 91 day drinking water study (Fisher 344 rats); however, the available metabolism data do not show any major differences in the handling of the compounds in the Long-Evans versus the Fisher rats.

[2] The available *in vivo* metabolism data indicate that in comparison to alachlor, the ESA metabolite is poorly absorbed and metabolized to only a minor degree. The products of alachlor ESA metabolism were not identified. The available autoradiography data indicate that in comparison to alachlor, the ESA metabolite does not show any significant localization to the nasal cavity, thyroid and glandular stomach (gastric mucosa). The available cell proliferation data indicate that in comparison to alachlor, ESA does not induce cell proliferation.

Overall, the data provided indicate that alachlor's ESA metabolite has less toxic potential

than the parent alachlor.

II. Evaluation of Acetochlor ES. . metabolite.

a. Summary of Acetochlor ESA metabolite toxicity.

Acute Oral Toxicity.

In an acute oral toxicity study in r: ts (MRID 44632704), the LD_{50} was greater than 2000 mg/kg. No animals died, and there were n) treatment-related clinical signs, necropsy findings or significant changes in body weigh during the 14-day observation period.

Metabolism

In a metabolism study (MRID 45: 00504), radiolabeled acetochlor ethane sulfonate (acetochlor ESA) was administered to the fol)wing dose groups:

(1) 300 mg/kg to 3 males a id 2 females, followed by whole body autoradiography after 24 hrs and
(2) 300 mg/kg/day to 3 ani nals/sex, with daily urine and feces collection for 5 consecutive days' post-dos ng, followed by whole body autoradiography on Day 5.

As summarized in Table 2b, oral : bsorption of ESA was limited to 10-12% of the dose, as indicated by urinary excretion. Al hough there was extensive excretion in feces (80% and 76.7% of administered dose, male: and females, respectively), this material is likely to be unabsorbed test material since it w is largely un-metabolized test material. Furthermore, sulfonic acid compounds are known to hav limited oral absorption due to their polarity.

As summarized in Table 3, ESA u dergoes very little biotransformation. Some dealkylation is seen (3-5%) of the dose and 2 unic entified metabolites amounting to 1-1.1% of the dose. None of the identified metabolites is a pl usible precursor of a quinoneimine, that will result in binding to the nasal turbinates.

TABLE 2b. Recovery of radioactivity in excreta of rats after administration of [^{14}C, ^{13}C]- Acetochlor ESA[a].

	Percent of radioactive dose recovered	
	Single Oral Dose (300 mg/kg)	
	Male	Female
Exhaled air	ND[b]	ND
Tissues	ND	ND
Carcass	ND	ND
Cage wash	0.11±0.026	0.93±0.341
Urine	10.23±0.103	12.74±1.077
Feces	80.00±0.912	76.68±1.382
Total	90.34±0.788	90.36±0.411

a Data extracted from Table 6, p. 31of MRID 45300504. N = 3, both sexes.
b ND = Not determined.

TABLE 3. Metabolite profile in excreta of rats dosed with [^{14}C, ^{13}C]-Acetochlor ESA[a].

	Percent of administered dose					
Dose	300 mg/kg Oral Dose					
Compound	Male			Female		
	Urine	Feces	Total	Urine	Feces	Total
Acetochlor ESA (parent)	3.18	72.44	75.61	9.54	69.63	79.17
Acetochlor Sec ESA	3.95	1.27	5.22	2.34	0.85	3.19
Unidentified Metabolite 1	1.00	ND[b]	1.00	ND	ND	ND
Unidentified Metabolite 2	0.61	0.51	1.12	0.05	ND	0.05
Total unidentif.	1.61	0.51	2.12	0.05	ND	0.05
Total accounted for [c]	8.74	74.21	82.95	11.93	70.48	82.41
Lost/unaccounted for [d]	–[e]	--	17.05	--	--	17.59
Total	--	--	100	--	--	100

a Data extracted from Table 5, p. 77 of MRID 45300504. N = 3, both sexes.
b ND = Not detected.
c Total accounted for = (Total identified) + (Total unidentified).
d Lost or unaccounted for = 100 - (Total accounted for).
e -- cannot be determined.

Whole-body autoradiography 24 hrs post-dosing showed radioactivity localized to the intestinal contents, with lower radioactivity identified in the kidney, stomach and liver. At 5 days post-

dosing, the autoradiograms showe l relatively low activity only in a small proportion of the intestinal contents. More importa itly, no evidence was seen of radioactivity accumulation in the nasal turbinate region, as seen wit i the parent acetochlor.

Subchronic Toxicity

1. 4-Week range finding study.

In this non-guideline 28-day range finding subchronic study (MRID 45300503), acetochlor ESA metabolite (97.6% w/w a.i., Lot # P3) was administered continuously in the diet for 28 days to 5 Sprague-Dawley Crl:CD (SD) IG BR rats/sex/dose at nominal doses of 0, 3000, 6000 or 12,000 ppm (equivalent to 0, 370.3, 766.6 and 1578.7 mg/kg/day in males and 0, 374.6, 762.3 and 1607.4 mg/kg/day in females).

No treatment-related effects were observed on mortality, clinical signs, food consumption, hematology, clinical chemistry (ot ier than thyroid hormones) or organ weight.

There was an initial minor reducti n in body weight gain observed in both sexes at 6000 and 12,000 ppm and females at 3000 p om; however, body weights were similar to the concurrent controls by Day 6. Minor decreas s ($p \leq 0.05$ or 0.01) in adjusted body weights (covariate is Day 1 body weight) were observed as f llows: (i) 12,000 ppm males at Days 2-5 (↓3-6%); (ii) 6000 ppm males at Days 2-3 (↓2%, eac); (iii) 12,000 ppm females at Days 2-4 (↓5-6%); (iv) 6000 ppm females at Days 2-4 (↓4-5%) and (v) 3000 ppm females at Days 2-3 (↓3%, each). Overall body weight gains (calculated by t e reviewers) were decreased (statistical analysis not performed) in females at 3000 (↓4 %), 6000 (↓5%) and 12,000 ppm (↓7%); however, a dose-dependent decrease was not obser ed in the males.

In the 12,000 ppm males, increase (not statistically significant [NS]) plasma thyroid stimulating hormone (↑61%), plasma free trii dothyronine (↑28%), plasma total thyroxine (↑15%) and free thyroxine (↑14%) were observed See Table 4, below.

Table 4. Plasma thyroid hormones (mean ± SD) in male rats fed acetochlor ESA metabolite for up to 28 days.[a]

Parameter	Dose (ppm)			
	0	3000	6000	12,000
TSH (ng/mL)	9.81±5.53	11.23±4.64 (↑14)	13.62±4.54 (↑39)	15.77±10.44 (↑61)
Total T3 (nmol/l)	1.27±0.05	1.38±0.19	1.59±0.17** (↑25)	1.32±0.18 (↑4)
Free T3 (pmol/l)	1.47±0.37	1.50±0.28	1.70±0.38 (↑16)	1.88±0.67 (↑28)
Total T4 (nmol/l)	68.0±9.5	64.9±6.0	71.6±6.9 (↑5)	78.0±8.9 (↑15)
Free T4 (pmol/l)	14.54±3.85	13.24±2.15	14.16±0.83	16.51±2.59 (↑14)

a Data were obtained from this study report, Table 9 on page 53; n = 5.
* or ** Significantly different from controls, p≤0.05 or 0.01, respectively.

Increased thyroxine-uridine diphosphoglucuronosyl transferase (T4-UDPGT) activities were observed as follows: (i) pmol/hour/g liver at 12,000 ppm in males (↑35%; NS) and females (↑74%; p≤0.01); (ii) pmol/hour/ total liver at 12,000 ppm in males (↑36%; p≤0.05) and females (↑61%; p≤0.01); (iii) pmol/hour/mg protein in males at 12,000 ppm (↑28%; NS) and females (↑75%; p≤0.01) [See Table 5, below].

The LOAEL is 6,000 ppm for males and females (equivalent to 766.6/762.3 mg/kg/day [M/F]) based on decreased body weights and body weight gain and increased TSH and free T3 in males. The NOAEL is 3000 ppm (equivalent to 370.3/374.6 mg/kg/day [M/F]).

Table 5. T4-UDPGT enzyme activity in rats fed acetochlor ESA metabolite for up to 28 days.[a]

Parameter	Dose (ppm)			
	0	3000	6000	12,000
Males				
pmol/hour/g liver	181.7±55.9	190.0±69.6	179.2±46.4	245.4±46.2 (↑35)
pmol/hour/total liver	2580±922	2641±674	2233±599	3519±600* (↑36)
pmol/hour/mg protein	14.5±4.6	13.6±3.9	15.5±5.6	18.5±2.9 (↑28)
Females				
pmol/hour/g liver	214.0±67.0	207.3±63.4	244.0±45.2	373.1±57.8** (↑74)
pmol/hour/total liver	2137±707	1975±779	2114±283	3436±250** (↑61)
pmol/hour/mg protein	19.1±6.3	19.4±7.5	18.7±4.3	33.4±6.3** (↑75)

a Data were obtained from this study report, Table 10 on page 55; n = 5.
* or ** Significantly different from controls, p≤0.05 or 0.01, respectively.

2. 90-Day Subchronic toxicity (ats).

In this subchronic study (MRIDs 45313801 and 45300503), acetochlor ESA metabolite (97.6% w/w a.i., Lot #: P3; GLP-9902-94 0-T) was administered continuously in the diet for 13 weeks to 20 Sprague-Dawley CD (SD) I S BR rats/sex/dose at nominal doses of 0, 1000, 3000 or 12,000 ppm (equivalent to 0, 75.0 225.4 and 919.4 mg/kg/day in males and 0, 85.2, 259.1 and 1073.2 mg/kg/day in females). O these rats, 8 rats/sex/dose were used exclusively for the measurement of cellular replicatio in the nasal passages.

No treatment-related effects were bserved on mortality, clinical signs, the functional observational battery, motor activi y, ophthalmoscopic abnormalities, hematology, urinalyses, organ weights or gross pathology. Interval motor activity indicated that habituation was normal. No treatment-related effects were oted in the 1000 or 3000 ppm treatment groups. Despite findings in a range-finding study (**MRID 45300503, Document 4 of the Package)** which suggested effects on the thyroid h rmones, no follow-up assessment was performed in this study.

Decreased food utilization, as wel as decreased food consumption in males, contributed to the decreased body weights and body veight gains in the 12,000 ppm treatment group. The following treatment-related decrea es ($p \leq 0.05$ or 0.01) were observed at 12,000 ppm: (i) adjusted body weight (covariate is Day 1 body weight) in males at Weeks 2-14 (↓3-9%) and females at Weeks 3-13 (↓2-3%); ( ) overall body weight gains (calculated by reviewers, statistical analysis not performed) n males (↓17%) and females (↓7%); (iii) food consumption in males during Weeks 1 and 3-13 (5-9%); and (iv) food utilization in males at Weeks 1-4 (↓12%) and 1-13 (↓11%) and in females a Weeks 1-4 (↓10%) and 1-13 (↓8%).

Other evidence of toxicity in the 1 ,000 ppm groups included increased plasma total bilirubin in males (↑27%; $p \leq 0.05$) In additio , decreased cellular proliferation was observed at 6 of 6 levels in the nasal passage of males (↓15 44%) and 5 of 6 levels in females (↓10-63%), but significant differences from the concurrent cc ntrols were not observed due to considerable inter-animal variability. In the absence of histc ical control data, however, this assessment cannot be completed and historical control d ta are required. Increased incidences (# affected/12 in treated vs controls) of the followir g abnormalities were observed microscopically: (i) kidney cyst(s) in males (2 vs 0 controls); i) minimal mononuclear cell infiltration of the liver in males (6 vs 3 controls); and (iii) minimal demyelination in the sciatic nerve in males (4 vs 1 control). Furthermore, the study investigato s stated that the abnormalities were within the range of historical controls but failed to inc ude these data..

The tentative LOAEL was 12,00 ppm (equivalent to 919.4 mg/kg/day in males and 1073.2 mg/kg/day in females) based on educed body weights, body weight gains and food utilization in both sexes and foo consumption in males. The tentative NOAEL for this study is 3000 ppm (equivalent to 225.4 mg/kg/day in males and 259.1 mg/kg/day in females).

However, the submitted study is N **OT** classified as **acceptable/guideline (§82-1a)** and **does not**

currently satisfy the requirements for a subchronic oral toxicity study in rat. Historical control data for cellular proliferation in the nasal passages, histopathology data for liver and sciatic nerve and functional observational battery and motor activity must be submitted. It is also noted that despite thyroid hormone changes in the range-finding study, no assessment was performed in this study. If dose related histopathology are confirmed at the high dose level, examination of lower dose tissues is required.

Developmental Toxicity

No developmental toxicity data were submitted for the Acetochlor ESA metabolite. The Registrant requested a waiver of this requirement, since the developmental toxicity data for the analog **alachlor ESA** can be considered applicable in this case. As summarized in the DER for alachlor ESA (MRID 43908101), female Sprague-Dawley Crl:CD BR rats from Charles River Breeding Labs. were gavaged with Alachlor ESA metabolite in Mazola corn oil at actual doses of 0, 135, 360 or 900 mg/kg/day (based on 90% ai) for days 0 through 15 of gestation. No maternal toxicity was noted in any measured parameter at the dose levels tested. The Maternal Toxicity NOAEL is equal or greater than 900 mg/kg/day, and the Maternal Toxicity LOAEL is greater than 900 mg/kg/day.

No developmental toxicity was noted in any measured parameter at the dose levels tested. The Developmental Toxicity NOAEL is equal or greater than 900 mg/kg/day, and the Developmental Toxicity LOAEL is greater than 900 mg/kg/day.

Mutagenicity.

- Water metabolites of acetochlor **(ESA, OXA, or PJ 2)** were not mutagenic in plate incorporation or preincubation microbial reverse gene mutation assays performed with *Salmonella typhimurium* or *Escherichia coli* (MRID Nos. 44632706, 44632705 or 42713118).

- Acetochlor **ESA** was also not mutagenic in the mouse lymphoma mammalian cell gene mutation assay (MRID 45313803) or clastogenic in the *in vitro* human lymphocyte chromosome aberration assay (MRID 45313804). It was also not clastogenic or aneugenic in the *in vivo* bone marrow micronucleus assay in mice (MRID 45313802).

- On the other hand, acetochlor **OXA** induced a significant and reproducible increase in the mutation frequency (MF) of mouse lymphoma cells at two S9-activated concentrations (MRID 45313809). The positive response seen in the mouse lymphoma assay was primarily caused by the induction of small colony mutants which are generally associated with chromosomal damage rather than gene mutations. This finding was, however, not confirmed in either the *in vitro* chromosomal aberration assay of human lymphocytes using comparable concentrations (MRID 45313810) or in the *in vivo* mouse micronucleus assay up to the limit dose of 2000 mg/kg (MRID 45313808). The relevance, if any, of this positive finding is unclear but is of doubtful significance since the parent compound is clastogenic in cultured human lymphocytes but the response was not expressed in whole animal models.

- Another water metabo ite, **Compound 57** was neither clastogenic in cultured human lymphocytes (MRID 437 5702) nor induced unscheduled DNA synthesis (UDS) in the hepatocytes of treated rats (MRID 43785701).

Studies performed with ESA and (XA satisfy FIFRA guideline requirements for mutagenicity. At this time, there is no concern for m tagenicity of the evaluated water metabolites of acetochlor. The mutagenicity of the water degrada es is summarized below:

Table 6. MUTAGENICI Y STUDIES WITH ACETOCHLOR WATER DEGRADATES

Test System	MRID (year) Purity Batch No.	Result
Acetochlor ethane sulfonic acid (ESA)		
870.5100 Bacterial Gene Mutation Assay(plate or preincubation) *Salmonella typhimurium, Escherichia coli*	4632706 (1997) 97.6% 290131 cceptable/guideline 00-5000 µg/plate - /+ S9 **plate** or **reincubation**	**Negative** up to the limit dose (5000 µg/plate -/+ S9) **plate** or **preincubation**
870.5300 *In vitro* mammalian cell gene mutation L5178Y mouse lymphoma cells	5313803 (2000) 97.6% 290131 cceptable/guideline 50- 3010 µg/mL (equiv to 10mM) +/- 9; two trials	**Negative** up cytotoxic levels (3010 µg/mL -/+ S9).
870.5375 Cytogenetics *In vitro* mammalian cell chromosomal aberration assay human lymphocytes	5313804 (2000) 97.6% 290131 cceptable/guideline 50-3010 µg/mL (equiv to 10mM) +/- 9; two trials	**Negative** up to the limit dose, 3010 µg/mL (equiv to 10mM) +/-S9
870.5395 Mammalian Erythrocyte Micronucleus Test CD-1 Mice	5313802 (2000) 97.6% 290131 cceptable/guideline 500, 1000, 2000 mg/kg oral gavage X	**Negative** up to a the limit dose (2000 mg/kg).
Acetochlor oxanilic acid (OXA)		
870.5100 Bacterial Gene Mutation Assay(plate or preincubation) *Salmonella typhimurium, Escherichia coli*	4632705 (1998) 97% 290130 cceptable/guideline 00-5000 µg/plate - /+ S9 **plate** or **reincubation**	**Negative** up to the limit dose (5000 µg/plate -/+ S9) **plate** or **preincubation**

870.5300 *In vitro* mammalian cell gene mutation L5178Y mouse lymphoma cells	45313809 (2000) 93.15% R290130 Acceptable/guideline 250- 2650 µg/mL (equiv to 10mM) +/- S9; two trials	**Negative** up cytotoxic levels or the limit dose (2650 µg/mL). **Positive:** S ↑in MF at 2000 & 2650 µg/mL +S9 both trials--outside of historical control range. Predominantly small colony mutants
MUTAGENICITY STUDIES WITH ACETOCHLOR WATER DEGRADATES		
Test System	MRID (year) Purity Batch No.	Result
Acetochlor oxanilic acid (OXA)		
870.5375 Cytogenetics *In vitro* mammalian cell chromosomal aberration assay human lymphocytes	45313810 (2000) 93.15% R290130 Acceptable/guideline 250-2650 µg/mL (equiv to 10mM) +/- S9; two trials	**Negative** up to the limit dose (2650 µg/mL -/+ S9).
870.5395 Mammalian Erythrocyte Micronucleus Test CD-1 Mice	45313808 (2000) 93.15% R290130 Acceptable/guideline 0, 500, 1000, 2000 mg/kg oral gavage 1X	**Negative** up to a the limit dose (2000 mg/kg).
Compound 57		
870.5375 Cytogenetics *In vitro* mammalian cell chromosomal aberration assay human lymphocytes	43785702 (1995) 99% P3 Acceptable/guideline 500-2500 µg/mL (Donor 1) 200- 2500 µg/mL (Donor 2) +/-S9	**Negative** up to cytotoxicity (2500 µg/mL- S9) or the highest tested level w S9 (2500 µg/mL) .
In vivo/in vitro unscheduled DNA synthesis Alderley Park (Alpk:APfSD) rats	43785701 (1995) 99% P3 Acceptable/nonguideline 1250 or 2000 mg/kg oral gavage 1X	**Negative** up to a the limit dose (2000 mg/kg).
PJ 2		
870.5100 Bacterial Gene Mutation Assay(plate or preincubation) *Salmonella typhimurium, Escherichia coli*	42713118 (1991) 96% ASW-1351-R Acceptable/guideline 100-5000 µg/plate - /+ S9 **plate** or **preincubation**	**Negative** up to the limit dose (5000 µg/plate -/+ S9) **plate** or **preincubation**

S = Significant ($p<0.01$)
NS= Nonsignificant
MF = Mutation frequency

b. Comparison with Parent Acetochlor

The toxicities of Acetochlor and its ESA degradate are compared below in Table 7.

As summarized in Table 7, in contrast to the parent acetochlor, Acetochlor ESA is poorly absorbed , undergoes very little metabolism, does not bind to nasal epithelium and does not produce nasal epithelium cell proliferation (Docs 3, 4, & 5). Acetochlor ESA produces statistically significant

increases in TSH and in microsom l UDPGT at high doses.

In a rat 90-day feeding study, the N OAEL = 225.4 / 259.1 mg/kg/day [M/F] and LOAEL = 919.4 / 1073.2 mg/kg/day [M/F], based on educed body weights, body weight gains and food utilization in both sexes. Decreased cell prolifer tion in nasal passages was seen at the LOAEL, but it was not statistically significantly diff. with ontrols because of variability.

Table 7. Comparison of toxicity of Acetochlor and Acetochlor ESA

Test	Acetochlor	Acetochlor ESA	Doc. # for ESA
Acute oral LD_{50}	LD_{50}(M&F) = 4124 (3557-4691) mg/kg	LD_{50}(M&F) > 2000 mg/kg	-
Metabolism	>80% oral absorption, extensive biotransformation, precursors of quinonone imine, binding to nasal turbinates	Poorly absorbed by the oral route (about 10-12% of the dose). Limited biotransformation (75-79%) excreted untransformed. Does not bind to nasal turbinates.	(Doc 3)
4-Week feeding range finding (rats)	No Data	NOAEL = 370.3 / 374.6 mg/kg/day [M/F] LOAEL = 766.6/762.3 mg/kg/day [M/F], based on decreased body weights and body weight gain and increased TSH and free T_3 in males. At 1578.7 mg/kg/day (HDT) there was a statistically significant increase in T_4-UDPGT (microsomal enzyme)	(Doc. 4)
90-Day feeding (rats)	Study 1: NOAEL = 16.1 / 19.1 mg/kg/day [M/F] LOAEL = 161 / 191 mg/kg/day [M/F] based on hematology, small but significant increases relative liver, kidney & brain weights. Supplementary based on absence of purity information Study 2: NOAEL = 800 ppm LOAEL = 2000 ppm, based on body weight loss & food consumption. Minimum	NOAEL = 225.4 / 259.1 mg/kg/day [M/F] LOAEL = 919.4 / 1073.2 mg/kg/day [M/F], based on reduced body weights, body weight gains and food utilization in both sexes. Decreased cell proliferation in nasal passages was seen at the LOAEL, but not statistically significantly diff. with controls because of variability.	(Doc 5)
Developmental toxicity	Study 1(rats): NOAEL(maternal & developmental) = 150 mg/kg/day, LOAEL (ma & de) = 600 mg/kg/day; **Maternal**: based on clinical signs & b.wt. gain↓. Developmental: based on resorptions↑ & fetal body weights ↓ Study 2 (rats): NOAEL(maternal & developmental) =200 mg/kg/day, LOAEL (m & d) = 400 mg/kg/day; **Maternal**: based on clinical signs & b.wt. gain↓. Developmental: based on fetal body weights↓	**No data for Acetochlor ESA.** Data are for Alachlor ESA. Rats: NOAEL (maternal & developmental): greater than or equal to 900 (HDT)mg/kg/day LOAEL (maternal & developmental): greater than 900 mg/kg/day	(Doc 2)
Mutagenicity	No Concern	No Concern	

III. Evaluation of Acetochlor OXA metabolite toxicity

a. Summary of Acetochlor OXA metabolite toxicity.

Acute Oral Toxicity

In an acute oral toxicity study (MRID 44632703) in , Alpk:APfSD (Wistar-derived) rats the LD_{50} was greater than2000 mg/kg test article. There were no deaths and none of the animals manifested or showed clinical macroscopically adverse findings; all showed overall weight gain during the study.

Metabolism

In a metabolism study (MRID 45 00507), Radiolabeled acetochlor oxanilic acid (acetochlor OXA), was administered to the following dose groups:

(1) 300 mg/kg; sacrificed and processed for whole body autoradiography at 24 hrs postdosing and
(2) 300 mg/kg/day; urine and feces collection every 24 hr postdosing for 5 consecutive days', then sacrificed and processed on Day 5 for whole body autoradiography. Exhaled air was collected at 6, 12 and 24 hr post-dosing.

As summarized in Table 8, absorption of the OXA degradate in rats amounted to 38.6-39.5% of the dose. About 56% of the dose was excreted in feces. Presumably, excretion in feces reflects unabsorbed material, due to the polarity of the compound. Radiolabel was rapidly excreted following dosing, with most excreted during the first 24 hr (92.4%, males and 83%, females).

TABLE 8. Recovery of radioactivity in exhaled air and excreta of rats (Group 2) after administration of [^{14}C, ^{13}C]-labeled Acetochlor OXA[a].

	Percent of radioactive dose recovered	
	Single Oral Dose (300 mg/kg)	
	Male	Female
Exhaled air[b]	0.02±0.001	0.02±0.001
Tissues	ND[c]	ND
Carcass	ND	ND
Cage wash	0.50±0.190	1.36±0.369
Urine	38.63±1.931	33.95±2.348
Feces	56.13±1.938	56.12±3.364
Total	95.27±0.225	91.44±0.695

a Data extracted from Table 6, p. 33 of MRID 45300507. N = 3, both sexes.
b Collected only for the first 24 hr postdosing due to low amounts recovered.
c ND = Not determined.

As summarized in Table 9, OXA undergoes very little biotransformation. Only two unidentified metabolites, amounting to 4.8% (metabolite 1) and 2.2% metabolite (metabolite 2) were seen in males. The rest was unmetabolized OXA. In females no metabolite 1 was seen; and metabolite 2 amounted to 0.7% of the dose.

TABLE 9. Metabolite profile in excreta of rats dosed with [^{14}C, ^{13}C]-labeled Acetochlor OXA[a].

Dose		Percent of administered dose					
		300 mg/kg Oral Dose					
Compound		Male			Female		
	Urine	Feces	Total		Urine	Feces	Total
Acetochlor OXA	31	3	51.41	81.44	31.85	53.05	84.90
Unidentified Metabolite 1	3.	6	0.89	4.75	ND[b]	ND	ND
Unidentified Metabolite 2	1.	6	0.28	2.24	0.72	ND	0.72
Total unidentif.	5.	2	1.17	6.99	0.72	ND	0.72
Total accounted for [c]	35	35	52.58	88.43	32.58	53.05	85.62
Lost/unaccounted for [d]	-		--	11.57	--	--	14.38
Total	-		--	100	--	--	100

a Data extracted from Table 5, p. 76 of MRID 45300507. N = 3, both sexes.
b ND = Not detected.
c Total accounted for = (Total identified) - (Total unidentified).
d Lost/unaccounted for = 100 - (Total accounted for).
e -- cannot be determined.

Whole-body autoradiography of th Group 2 animals sacrificed at 24 hrs post-dosing showed radioactivity in the stomach content and lining, intestinal contents and cecum. Lower levels were detected, to a variable extent, in the idney, liver, skin, blood lungs, heart and lining of the tongue and esophagus. By 5 days post-dos ng, the autoradiograms were stated to show no radioactivity (photographs of the autoradiograms were not included in the study report because exposed films showed no detectable darkening). Although autoradiography did not provide a quantitative analysis of tissue retention of radioa ivity, it was considered sufficient to demonstrate that neither the test material nor its metabolites ere retained at significant levels. No clinical signs of toxicity were reported.

Subchronic Toxicity

1. 4-Week Range-Finding Study

In this non-guideline 28-day rang -finding subchronic study **(MRID 45300506)**, acetochlor oxanilate metabolite (93.15% w/w .i., Lot #: P6) was administered continuously in the diet for 28 days to 5 Sprague-Dawley Crl:C D (SD) IGS BR rats/sex/dose in nominal doses of 0, 3000, 6000 or 12,000 ppm (equivalent to 372.6, 768.5, and 1467.9 mg/kg/day in males and 0, 367.2, 737.3 and 1506.5 mg/kg/day in fem ales).
Mortality, clinical signs, food co sumption, hematology and clinical chemistry (with the exception of the thyroid hormones) were unaffected by the test substance.

Treatment-related decreases ($p \leq 0.0$ or 0.01) in adjusted body weight (covariate is Day 1 body

weight) were observed in the 12,000 ppm males at Days 2, 4, 5 and 15-29. Overall body weight gain was decreased (statistical analysis was not performed) in males at 12,000 ppm.

Limited data suggests that the thyroid might be a target organ. In the 12,000 ppm males, decreases ($p \leq 0.05$ or 0.01) were observed in plasma total T3 and free T3. Decreases (not statistically significant [NS, but N was only 5/sex and statistical assessment is of limited utility) were observed in the following plasma thyroid hormones: (i) TSH in males at 6000 and 12,000 ppm and in females at 3000, 6000 and 12,000 ppm; (ii) total T3 in males at 3000 and 6000 ppm and in females at 6000 and 12,000 ppm; (iii) total T4 in males at 12,000 ppm; (iv) free T4 in males at 12,000 ppm; and (v) free T3 in females at 12,000 ppm.. A condition where T3, T4 and TSH are decreased might be indicative of secondary hypothyroidism and might be suggestive of abnormalities in the anterior pituitary and/or hypothalamus. Decreased ($p \leq 0.01$) T4-UDPGT activity was observed in 12,000 ppm females as pmol/hour/g liver, pmol/hour/total liver and pmol/hour/mg protein (NS). Increased (NS) T4-UDPGT activity was also observed in the 12,000 ppm males. Absolute thyroid weight was increased ($p \leq 0.05$ or 0.01) in males at 6000 and 12,000 ppm and adjusted thyroid weight (covariate as terminal body weight) was increased at 3000, 6000 and 12,000 ppm. Thyroid weight was similar to the concurrent controls in females. Apparently, the nasal pharynx was not examined in this range finding study.

The following increased incidences (# affected/5 treated vs 0/5 controls) of macroscopic lesions were observed in males: reduced epididymis at 6000 (1 vs 0 controls) and 12,000 ppm (1 vs 0 controls), unilateral pelvic dilatation in the kidney at 12,000 ppm (3 vs 0 controls), enlarged testis at 12,000 ppm (1 vs 0 controls) and reduced testis at 6000 (1 vs 0 controls) and 12,000 ppm (1 vs 0 controls). No abnormalities were observed in the 12,000 ppm females. The investigators stated that the abnormalities were within the range of historical controls but the investigators failed to include these data in the submitted report. Increased incidences (# affected/5, treated vs controls) of microscopic lesions were observed at 12,000 ppm: (i) unilateral hydronephrosis (minimal to slight) in males (3 vs 0 controls); (ii) microlithiasis (minimal to moderate) in the kidney of males (2 vs 0 controls); (iii) renal tubule basophilia (minimal to slight) in females (5 vs 3 controls); (iv) mononuclear cell infiltration (minimal) in the liver of males (3 vs 1 controls) and females (4 vs 2 controls); (iv) fibrosis (slight) in the spleen of females (1 vs 0 controls); and (v) hyperplasia (slight) in the spleen of females (1 vs 0 controls) . Despite a suggestion of dose related histopathology at the high dose level, lower dose levels were apparently not examined. The study investigators stated that the abnormalities were incidental or within the range of historical controls but these data were not included in the report. Mononuclear cell infiltration in the liver of both sexes was also observed in the 90-day oral toxicity study (MRID 45313805).

The LOAEL is 6,000 ppm (equivalent to 768.5 mg/kg/day) in males based on decreased TSH, T3 and increased absolute and adjusted thyroid weights. In females, the LOAEL was also 6,000 ppm (equivalent to 737.3 mg/kg/day) based on decreased TSH and T3. The NOAEL is 3000 ppm (equivalent to 372.6 mg/kg/day) in males and 3,000 ppm (equivalent to 367.2 mg/kg/day) in females.

2. 90-Day Subchronic Toxicity

In this subchronic study **(MRIDs 45313805 and 45300506)** acetochlor oxanilate metabolite

(93.15% w/w a.i., Lot #: P6) was administered continuously in the diet for 13 weeks to 20 Sprague-Dawley CD (SD) IGS BR r ıts/sex/dose at nominal doses of 0, 1000, 3000 or 12,000 ppm (equivalent to 0, 77.2, 230.2, and 955.2 mg/kg/day in males and 0, 86.5, 268.0 and 1082.7 mg/kg/day in females). Of these rat , 8 rats/sex/dose were used exclusively for the measurement of cellular replication in the nasal p ıssages.

No treatment-related effects were o served on mortality, clinical signs, functional observational battery, food consumption, ophtha mic examination, hematology, clinical chemistry, cellular replication in the nasal passages, rgan weights or gross pathology. Interval motor activity indicated that habituation was norm al.

Treatment-related indications of g neral toxicity were observed in the 12,000 ppm treatment groups. Decreased ($p \leq 0.05$ or 0.01) ıdjusted body weights (covariate is Day 1 body weight) were observed in the males at Weeks 2- 4 (↓2-7%) and females at Weeks 2, 5-12 and 14 (↓2-5%). Overall body weight gains (calculat d by reviewers) were decreased in both sexes (↓11%, each). Decreased ($p \leq 0.05$ or 0.01) food uti ization was observed in the males at Weeks 1-4 (↓10%) and 1-13 (↓9%) and females at Weeks l-4 (↓12%) and 1-13 (↓7%). Furthermore, decreases (not statistically significant [NS]) were lso observed in the males at Weeks 5-8 (↓10%) and 9-13 (↓2%) and females at Weeks 5-8 (↓ %) and 9-13 (↓16%).

Motor activity in the 12,000 ppm m les were increased (↑96%; $p \leq 0.01$). However, the Sponsor reported that the motor activity of th 12,000 ppm males in this study (403.6±186.8) was similar to the controls in a concurrent study n the same strain of rat (342±118). Increased incidences (# affected/12, treated vs controls) of ıe following microscopic lesions were observed at 12,000 ppm in the kidney: unilateral hy ronephrosis (minimal to moderate) in females (2 vs 0), intratubular microlithiasis (slight to marked) in females (9 vs 5 controls), and transitional cell hyperplasia (minimal) in males (4 v 2 controls). Additionally, urine volume increased (NS) in 12,000 ppm females (↑26%) may al) be indicative of renal toxicity. Minimal mononuclear cell infiltration was observed (# affected 12, treated vs controls) in the liver of males (7 vs 4 controls) and females (8 vs 4 controls), the pa ıcreas of males (3 vs 0 controls) and the thyroid of females (2 vs 0 controls). Other microsco ic lesions observed at 12,000 ppm occurred at a similar incidence in the concurrent controls. The investigators stated that all microscopic abnormalities were "consistent with background h stopathological changes for this age and strain of rat" (but the investigators failed to include h storical data in the submitted report).

The tentative LOAEL was 12,000 ppm (equivalent to 955.2 mg/kg/day in males and 1082.7 mg/kg/day in females) based on reduced body weights, body weight gains and food utilization in both sexes. The ten ıtive NOAEL for this study is 3000 ppm (equivalent to 230.2 mg/kg/day in males and 268 0 mg/kg/day in females).

The submitted study is **NOT** classifi d as **acceptable (§82-1a)** at this time and **does not** satisfy the requirements for a subchronic or l toxicity study in rat. Historical control data of "cellular replication in the nasal passages" nd "motor activity" are required in order to complete assessment of the submitted study.

Developmental Toxicity

In a developmental toxicity study (**MRID number 45313807, Document 9 of the Package**), MON 52755 (tech., 93.15% a.i.) was administered once daily to 25 presumed pregnant female Crl:CD®(SD)IGS BR rats/dose by gavage in distilled water vehicle (5 mL/kg body weight) at dose levels of 0, 250, 500 or 1000 mg/kg bw/day from days 6 through 19 of gestation, inclusive.

At 1000 mg/kg bw/day, two dams died within an hour of dosing, one on gestation day 8 and the second on gestation day 9, with no evidence of gavage error observed at necropsy. Eight dams showed clear matting of the fur on a total of 17 occasions within 1 hr after dosing. There were no treatment-related, biologically significant effects on body weight/weight gain, food consumption or cesarean parameters. **The maternal toxicity LOAEL is 1000 mg/kg bw/day, based on maternal mortality. The maternal toxicity NOAEL is 500 mg/kg bw/day.**

No treatment-related decreases in fetal survival (increased early/late resorptions or fetal death), litter size, weight, sex ratio or increases in external, visceral or skeletal variations/malformations were reported at the doses tested. **The developmental toxicity LOAEL is >1000 mg/kg bw/day (limit dose). The developmental toxicity NOAEL is ≥1000 mg/kg bw/day.**

Mutagenicity

- Acetochlor **OXA** was not mutagenic in plate incorporation or preincubation microbial reverse gene mutation assays performed with *Salmonella typhimurium* or *Escherichia coli* (MRID Nos. 44632706, 44632705 or 42713118).

- On the other hand, acetochlor **OXA** induced a significant and reproducible increase in the mutation frequency (MF) of mouse lymphoma cells at two S9-activated concentrations (MRID 45313809). The positive response seen in the mouse lymphoma assay was primarily caused by the induction of small colony mutants which are generally associated with chromosomal damage rather than gene mutations. This finding was, however, not confirmed in either the *in vitro* chromosomal aberration assay of human lymphocytes using comparable concentrations (MRID 45313810) or in the *in vivo* mouse micronucleus assay up to the limit dose of 2000 mg/kg (MRID 45313808). The relevance, if any, of this positive finding is unclear but is of doubtful significance since the parent compound is clastogenic in cultured human lymphocytes but the response was not expressed in whole animal models.

Studies performed with OXA satisfy FIFRA guideline requirements for mutagenicity. At this time, there is no concern for mutagenicity of the evaluated water metabolites of acetochlor. The mutagenicity of acetochlor OXA and other acetochlor water degradates is summarized in Table 6 and compared to parent in Table 10.

b. Comparison with Parent Acetochlor.

The toxicity of acetochlor OXA is compared with that of parent acetochlor in Table 10. Although about 34-39% of the dose of OXA is absorbed, the compound undergoes very little metabolism (about 81-85%) and is excreted untransformed. OXA does not bind to nasal epithelium and does not produce nasal epithelium cell proliferation (Docs 6,7 & 8).

Although acetochlor OXA produced increased thyroid weight in a 28-day feeding study, TSH

Table 10. Comparison of toxicity of Acetochlor and Acetochlor OXA

Test	Acetochlor	Acetochlor OXA	Doc. # for OXA
Acute oral LD_{50}	LD_{50}(M&F) = 4124 (3557-4691) mg/kg	LD_{50}(M&F) > 2000 mg/kg	-
Metabolism	Rats: >80% oral absorption, extensive biotransformation, precursors of quinone imine, binding to nasal turbinates	Rats: About 33.9-38.6% of the dose absorbed by the oral route (as seen in urine) Limited biotransformation: (81.4-84.9% excreted untransformed. Two unidentified metabolites (5 & 2% of dose, resp.). Does not bind to nasal turbinates. No data for mouse.	(Doc. 6)
4-Week feeding range finding (rats)	No Data	NOAEL = 372.6 / 367.2 mg/kg/day [M/F] LOAEL = 768.5 / 737.3 mg/kg/day [M/F], based on TSH & T_3 ↓ and absolute & relative thyroid weights ↑in males and TSH & T_3 ↓ in females.	(Doc.7)
90-Day feeding (rats)	Study 1: NOAEL = 16.1 / 19.1 mg/kg/day [M/F] LOAEL = 161 / 191 mg/kg/day [M/F] based on hematology, small but significant increases relative liver, kidney & brain weights. Supplementary based on absence of purity information Study 2: NOAEL = *800 ppm* LOAEL = 2000 ppm, based on body weight loss & food consumption. Minimum	NOAEL = 230.2 / 268.0 mg/kg/day [M/F] LOAEL = 955.2/1082.7 mg/kg/day [M/F], based on reduced body weights, body weight gains and food utilization in both sexes. Thyroid weight increases were not seen in this study. T_4-UDPGT activity was slightly, but not significantly, increased in high-dose males and was statistically significantly decreased in high-dose females	(Doc 8)
Developmental toxicity	Study 1(rats): NOAEL(maternal & developmental) = 150 mg/kg/day, LOAEL (ma & de) = 600 mg/kg/day; **Maternal**: based on clinical signs & b.wt. gain↓. Developmental: based on resorptions↑ & fetal body weights ↓ Study 2 (rats): NOAEL(maternal & developmental) =200 mg/kg/day, LOAEL (m & d) = 400 mg/kg/day; **Maternal**: based on clinical signs & b.wt. gain↓. Developmental: based on fetal body weights↓	Rats: NOAEL (maternal) = 500 mg/kg/day, LOAEL (maternal) = 1000 mg/kg/day, based on maternal mortality. NOAEL (developmental) is equal or greater than 1000 mg/kg/day (limit dose). LOAEL (developmental) greater than 1000 mg/kg/day	(Doc 9)
Mutagenicity	No Concern	No Concern	

IV. Data for other degradate

As summarized in Table 6, (p.15) compounds 57 and PJ2 were negative for cytogenetics and UDS (Compound 57) and for the Ames Assay (PJ2). The structures of these compounds are shown in Table 1 (they are ring-hydroxylated versions of OXA).

13544

R102258

Chemical:	**Acetochlor**
PC Code:	**121601**
HED File Code	**13000 Tox Reviews**
Memo Date:	**08/31/2004**
File ID:	**00000000**
Accession Number:	**412-05-0037**

HED Records Reference Center
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