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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No.: 0052727

MEMORANDUM

DATE: August 31, 2004

SUBJECT: **ACETOCHLOR:** Report of the Cancer Assessment Review Committee (CARC) (Fourth Evaluation)

PC Code: 121601

FROM: Jessica Kidwell, Executive Secretary
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CC: J. Pletcher
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The Cancer Assessment Review Committee met on April 21-22, 2004 to re-evaluate the carcinogenic potential of Acetochlor. Attached please find the Final Cancer Assessment and Mode of Action Assessment Documents.

The Final Document is divided into three parts as follows:

Part 1: Cancer Assessment Document: Evaluation of the Carcinogenic Potential of Acetochlor (4th Evaluation)

Part 2: Mode of Action Assessment Document: Evaluation of the Mode of Action of Acetochlor for Nasal Olfactory Epithelium Tumors in Rats and its Relevance to Human Cancer Risk Assessment

Part 3: Dissenting Views on April 21-22, 2004 CARC Assessment of Acetochlor (Brian Dementi, Ph.D., DABT., May 6, 2004 (Revised May 19, 2004))

EPA VOTING STAFF IN ATTENDANCE (Signature indicates concurrence with the assessment unless otherwise stated.)

Karl Baetcke _____

Lori Brunsman, Statistical Analysis _____

William Burnam, Chair _____

Larry Chitlik _____

Vicki Dellarco _____

Brian Dementi (Presenter) _____

Kit Farwell _____

Linnea J. Hansen (Presenter) _____

Abdallah Khasawinah _____

Jessica Kidwell, Executive Secretary _____

Nancy McCarroll (Presenter) _____

Alberto Protzel (Presenter) _____

Jess Rowland _____

Linda Taylor _____

Doug Wolf, Pathologist _____

Yintak Woo _____

NON-EPA STAFF IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise noted.)

John Pletcher, Consulting Pathologist _____

OTHER ATTENDEES: Lisa Eisenhauer (EFED), Abby Farwell, Bill Hirzy (Union Representative), Yong-Hwa Kim (Visiting Scholar), Susan Makris (HED), Santini Ramasamy (HED), Christina Scheltema (SRRD)

Part 1: Cancer Assessment Document: Evaluation of the Carcinogenic Potential of Acetochlor (4th Evaluation)

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF ***ACETOCHLOR*** (FOURTH EVALUATION)

FINAL REPORT

August 31, 2004

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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EXECUTIVE SUMMARY

The carcinogenicity of acetochlor was reviewed by the Agency on three previous occasions. On October 16, 1991, HED's Cancer Peer Review Committee (CPRC) classified acetochlor as a B2 (probable human) carcinogen according to the Agency's 1986 Guidelines for Cancer Assessment. This classification was based on increased incidence of tumors of the thyroid, liver and nasal epithelium in both sexes of the rat, along with rare benign chondroma of the femur (male and female) and basal cell tumor of the stomach (female) and in the mouse, increased incidence of liver and lung tumors in both sexes and histiocytic sarcoma and tumors of the ovary and kidney in females. A Q_1^* of 0.0169 was assigned, based on the incidence of nasal epithelial tumors in male and female rats. In the previous two evaluations by the CPRC, acetochlor was also classified as a B2 carcinogen. For the second CPRC evaluation (meeting of February 8, 1989), the classification was based on increased incidence of tumors of the nasal epithelium, liver and thyroid of the rat and in the mouse, increased incidence of liver and lung tumors and in females, histiocytic sarcoma and tumors of the ovary and kidney. A Q_1^* of 0.010 was assigned, based on the incidence of nasal epithelial tumors in male and female rats. In the first CPRC evaluation (meeting of September 12, 1985), classification was based on increased incidence of liver and thyroid tumors in rats and in the mouse, increased incidence of liver and lung tumors in both sexes and histiocytic sarcoma, kidney and ovarian tumors in females.

On April 21 and 22, 2004, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to re-evaluate the carcinogenic potential of acetochlor and determine the adequacy of the database to support proposed modes of action for rat nasal and thyroid tumorigenesis. This was the fourth cancer assessment of acetochlor and was conducted to consider the following new information: (1) pathology working group (PWG) reevaluations of many of the tumors in rats and mice that were determined to be treatment-related in previous assessments of acetochlor; (2) mode of action data evaluating thyroid parameters to support an antithyroid mode of action for thyroid carcinogenesis; (3) numerous studies in support of a mode of action for nasal tumor carcinogenesis in the rat and (4) a rat multigeneration reproductive toxicity study in which nasal epithelial tumors in F0 and F1 parental animals were observed. Discussion of the nasal tumor mechanistic data and the conclusions are presented in a separate document (See Mode of Action Assessment Document, Part 2).

The CARC evaluated three dietary chronic toxicity/carcinogenicity studies in the rat, two carcinogenicity studies in the mouse (23-month and 18-month) and a rat two-generation reproductive toxicity study. Acetochlor was administered to male and female Sprague Dawley CD rats as follows: (1) 0, 500, 1500 or 5000 ppm for 27 months (males) or 24 months (females) (equivalent to average daily intakes of 0, 22, 69 or 250 mg/kg/day, males and 0, 30, 93 or 343 mg/kg/day, females); (2) 0, 40, 200 or 1000 ppm for 24 months (average daily intakes of 0, 2.0, 10.0 or 50.0 mg/kg/day, estimated by conversion factor of 0.05); and (3) 0, 18, 175 or 1750 ppm for 24 months (average daily intakes of 0, 0.67, 6.37 or 66.9 mg/kg/day, males and 0, 0.88, 8.53

or 92.1 mg/kg/day, females). In a two-generation reproductive toxicity, acetochlor was administered to Sprague Dawley rats at 0, 200, 600 or 1750 ppm (average daily intakes of 0, 21.2, 65.6 or 196.4 mg/kg/day, males and 0, 22.4, 70.9 or 215.9 mg/kg/day, females).

Acetochlor was administered to male and female CD-1 mice as follows (1) 0, 500, 1500 or 5000 ppm for 23 months (equivalent to average daily intakes of 0, 75, 225 or 750 mg/kg/day, estimated by conversion factor of 0.15) and (2) 0, 10, 100 or 1000 ppm for 18 months (average daily intakes of 0, 1.1, 11 or 116 mg/kg/day, males and 0, 1.4, 13 or 135 mg/kg/day, females).

The CARC concluded that acetochlor was "Likely to Be Carcinogenic to Humans" based on the following:

- In male and female rats, increases in rare nasal olfactory epithelial polypoid tumors were observed at 1000 ppm (50 mg/kg/day) and higher in the two-year studies and at 600 ppm (66-71 mg/kg/day) in the two-generation reproductive toxicity study. Mode of action data were determined to support a cytotoxic (nonmutagenic) mode of action dependent upon formation of reactive quinoneimine intermediates, which is followed by cell proliferation, preneoplastic lesions (hyperplasia/respiratory metaplasia) and eventually tumors (see Mode of Action Assessment Document, Part 2). Nasal tumors in F0 and F1 parental animals in a two-generation reproductive toxicity study were also observed at about 18 weeks of treatment. Data showing nasal cell proliferative lesions after 160 days' treatment with acetochlor (together with published data on the related chloroacetanilide alachlor showing nasal tumors at 5 months of treatment) suggested that the finding of tumors reflected earlier sacrifice times rather than reduced latency in young animals.

- In all three studies conducted on the rat, dosing in males and females was considered adequate in all three studies, based on significantly decreased body weight at 1500 and 1750 ppm (slight at 1000 ppm). The highest dose tested of 5000 ppm in the earliest study was considered excessive in both sexes, based on sharply reduced body weights and increased mortality.

- Statistically significant increases in lung tumors were observed in male mice at 1000 ppm (increased adenoma/combined adenoma and carcinoma, 78-week study only, with a positive trend). An increase in bronchiolar hyperplasia suggested that the incidence might have increased in a two-year study. In female mice (23-month study only), significantly increased lung tumors (adenoma and combined tumors) were observed at all dose groups along with a positive trend, with only adenoma exceeding available historical control values.

- Statistically significant increases in histiocytic sarcoma were observed in female mice at 500 ppm and above in the 23-month study; in the 78-week study, only a positive trend was observed due to an increase at 1000 ppm. Incidence showed a dose-response when the

two mouse studies were combined and exceeded available historical control values at 1500 ppm.

- Dosing in mice was considered adequate in both sexes, based on increased mortality in females at 1500 ppm; males were considered to have been adequately tested despite no toxicity at 1500 ppm because decreased body weight at 1200 ppm in a 6-week range-finding study suggested that an effect level was approached. The 5000 ppm dose was considered excessive in both sexes based on high mortality and sharply reduced body weights. Although a maximum tolerated dose was not achieved in the 78-week mouse study, acetochlor was adequately tested when both studies were considered together.

The CARC concluded that rat thyroid tumors were treatment-related but should not be included in the cancer quantification, based on evidence for disruption of thyroid-pituitary homeostasis secondary to increased clearance of thyroid hormones by increased hepatic UDPGT activity and marginal increases in incidence. Slight increases in thyroid tumors were observed in females at 1000 ppm (NS) and at 1750 ppm ($p<0.05$ ppm). Tumors in males at 5000 ppm were at an excessive dose. Although some criteria required by Agency policy to demonstrate this mode of action were not met (e.g., reversibility of the effect, hyperplasia; EPA, 1998), the CARC determined that when considered together with thyroid mechanistic data on the structural analog alachlor, there was sufficient information to support this mode of action.

Other tumors previously included in the weight-of-the-evidence considerations for acetochlor were excluded at the present time. In reevaluation of the cancer classification, the CARC accepted the rediagnoses performed by the PWG (rat nasal and thyroid and mouse ovarian tumors were not reexamined). Rat liver - increased hepatocellular tumors in males and females at 5000 ppm occurred at an excessive dose (rat study 1). A small but significant increase in liver tumors in females at 1000 ppm (rat study 2) slightly exceeded historical controls, but incidence in concurrent controls was low relative to historical data and tumors were not increased at 1750 ppm (rat study 3). Rat femur - benign chondroma of the femur was rediagnosed as hyperplasia (rat study 3). Rat stomach - rare stomach basal cell tumors at 1750 ppm were rediagnosed as squamous cell carcinomas of the non-glandular stomach (rat study 3). The stomach tumors were not considered relevant to humans because they were most likely secondary to local irritation of the rat forestomach. In mouse study 1, the following tumors were not considered treatment-related. Mouse liver - increased hepatocellular tumors in males and females at 5000 ppm occurred at an excessive dose. Mouse kidney - renal adenomas and sarcomas were observed in females only at an excessive dose of 5000 ppm. Mouse ovary - increased combined benign ovarian tumors at 1500 and 5000 ppm were not considered treatment-related because it was inappropriate to combine the tumors (adenoma, granulosa cell tumor and luteoma); when considered separately, their incidence was low and not considered treatment-related.

The database for acetochlor does not support mutagenicity as the primary mechanism of tumorigenesis. Acetochlor was negative in bacterial gene mutation assays, comet assays using rat

nasal olfactory and respiratory epithelial cells, dominant lethal tests and in mammalian *in vivo* clastogenicity assays. Positive responses observed in mammalian *in vitro* clastogenicity assays and *in vitro* gene mutation assays were associated with significant cytotoxicity. The data are consistent with clastogenicity secondary to cytotoxicity that may be associated with cellular oxidative damage, as evidenced by a positive response in a UDS assay in rat liver that was associated with significant hepatocellular cytotoxicity and depletion of intracellular GSH. Human lymphocytes *in vitro* also appeared to be partially protected from clastogenic effects by the glutathione in whole blood compared to separated lymphocytes.

Acetochlor is structurally related to other chloroacetanilide herbicides, including alachlor, propachlor, butachlor and metolachlor. Alachlor, butachlor and metolachlor also induce nasal epithelial tumors; alachlor and butachlor induce thyroid follicular cell tumors. The FIFRA SAP concluded in 1997 that alachlor, acetochlor and butachlor may be grouped together for common mode of action for induction of nasal and thyroid tumors and the CARC concurred. Alachlor, butachlor and propachlor are classified as "likely to be a human carcinogen." MOE approaches for cancer risk quantitation were selected for alachlor and butachlor.

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July 1999), the CARC classified acetochlor as **"Likely to Be Carcinogenic to Humans"** based on multiple tumors in both sexes of two species. Rare rat nasal tumors were determined to have a mode of action defined in rats that had possible relevance to humans. For the quantitation of human cancer risk, the CARC recommended a linear low-dose extrapolation approach based on the incidence of either lung tumors or histiocytic sarcoma in female mice, depending upon which is the more potent. Although the data do not support mutagenicity of acetochlor as the primary mode of action of tumor formation, in the absence of mechanistic studies on the mouse lung tumors and histiocytic sarcoma, the default position of linear low-dose extrapolation was selected.

A reviewing toxicologist considered the submitted studies insufficient to adequately support the proposed non-mutagenic mechanism of action for nasal tumor carcinogenesis of acetochlor and prepared a minority opinion report addressing his concerns on this and other issues, which are attached to this document as a minority dissenting opinion (See Part 3).

I. INTRODUCTION

The carcinogenic potential of acetochlor has been evaluated in three previous meetings of the HED Cancer Assessment Review Committee (CARC). A brief summary of each of the three previous peer reviews, including the carcinogenicity studies evaluated at each meeting and the weight-of-evidence (WOE) determinations of the Committee, is provided below in Table 1.

The fourth meeting of the CARC on acetochlor was held to reevaluate the cancer classification in light of the following additional information submitted by the Acetochlor Reregistration Partnership (ARP) since the third cancer peer review: (1) numerous studies addressing the mode of action of nasal tumor formation in the rat and relevance to humans (data are presented in a separate Mode of Action Assessment document (Part 2)); (2) mode of action data on thyroid parameters in the rat to determine if thyroid follicular cell tumors result from perturbation of thyroid-pituitary homeostasis; (3) a rat two-generation reproductive toxicity study in which nasal epithelial adenomas and hyperplasia were observed in F0 and F1 generations after four months of treatment; (4) reevaluations by a Pathology Working Group (PWG), at the request of the ARP, of numerous other tumors observed in the rat and mouse that had been determined to be treatment-related (in the rat, liver, femur, stomach; in the mouse, liver, lung, histiocytic sarcoma and kidney); (5) Benign ovarian tumors in the mouse, while not reevaluated by the PWG, are also discussed in this report. The PWG reevaluations were stated to have been performed in accordance with EPA Pesticide Regulation (PR) Notice 94-5 (8/24/94). This CARC document addresses the above items 2 through 5.

TABLE 1: SUMMARY OF THE THREE PREVIOUS CANCER PEER REVIEWS OF ACETOCHLOR

Peer review number/date of memorandum/ date of meeting	**Carcinogenicity studies considered**	**Carcinogenicity classification/WOE determination and tumors used as basis of classification**
First Cancer Peer Review March 30, 1987 Meeting of September 12, 1985 TXR# 007697	(1) Rat 2 year dietary (MRID 00131088) (2) Mouse 2 year dietary (MRID 00131089).	**B2-Probable human carcinogen**/In the rat, hepatocellular carcinomas in males and females, thyroid follicular cell adenoma in males. In the mouse, hepatocellular carcinoma in males and females and in females, lung carcinoma, uterine histiocytic sarcoma, benign ovarian tumors and kidney adenomas. Structural relationship to other compounds such as butachlor, alachlor, metolachlor. Weakly mutagenic in CHO/HGPRT assay, mouse lymphoma assay
Second Cancer Peer Review May 31, 1989 Meeting of February 8, 1989 TXR# 007697	Above studies plus : (1) Second 2 year dietary study in the rat (MRID 40077601) and (2) reevaluation of nasal tissues from MRID 00131088 (MRID 40484801)-nasal tissues not evaluated in original study report.	**Reaffirmed as B2 Carcinogen**. In addition to the weight-of-evidence considerations noted in the first cancer peer review of acetochlor, the increased incidence of nasal adenomas was cited in both rat studies. A quantitative risk assessment (Q_1*) based on the nasal turbinate papillary adenomas in male and female rats was recommended.
Third Cancer Peer Review January 27, 1992 Meeting of October 16, 1991 TXR# 0012828	Above studies plus two additional studies (1) Third two-year dietary study in the rat (MRID 41592004) and (2) Second long-term dietary study in the mouse (78-week)(MRID 41565119).	**Reaffirmed as B2 Carcinogen.** In addition to the weight-of-evidence considerations in the first two cancer peer reviews of acetochlor, the following tumors in the two new studies were also considered treatment-related: (1) in rats, the occurrence of follicular cell adenomas and carcinomas in females as well as males, as well as unusual benign chondroma of the femur in 1 male and basal cell tumors of the stomach in 1 male and female each at the highest dose tested and (2) in mice, pulmonary adenomas in males as well as females. It was concluded that acetochlor had genotoxic potential based on several positive studies. A quantitative risk assessment (Q_1*) was recommended based on the nasal turbinate papillary adenomas in male and females.

II. BACKGROUND INFORMATION

Acetochlor, a chloroacetanilide compound, is a selective preplant incorporated and preemergence herbicide used to control annual grasses and certain broadleaf weeds. Structural similarities are seen with a number of other chloroacetanilide herbicides such as alachlor, butachlor, propachlor and metolachlor (see Figure 1 in the Structure-Activity Relationship section of this document for structures of acetochlor and related chloroacetanilide compounds). As discussed in that section, there is overlap in the types of tumors observed in animals treated with these herbicides, as well as in systemic toxicity. Tolerances are established for acetochlor and its metabolites in or on field corn and sorghum fodder, forage and grain; soybean forage, grain and hay; and wheat forage, grain and straw. The PC Code for acetochlor is 121601 and the CAS Number is 34256-82-1.

III. EVALUATION OF CARCINOGENICITY STUDIES

Data evaluated for this peer review: Three combined chronic toxicity/carcinogenicity dietary studies in the rat and two carcinogenicity dietary studies in the mouse are available for acetochlor. The results of these studies have already been summarized in the previous three cancer peer reviews and the reader is referred to the appropriate CARC document for summaries of the tumor incidence and non-tumor findings for each study. In addition, the Appendix to this document contains tumor incidence summary tables (Tables A-1 through A-17) for all tumor types identified for acetochlor. The purpose of these tables is to compare (1) tumor incidence among different studies for tumors found in more than one study; (2) tumor incidence and statistical analysis from the original study report as presented in previous cancer assessments with those of the PWG report, as well as HED statistical analysis of the findings of the PWG with statistical analysis by HED; (3) nasal tumor incidence in all rat studies with increasing dose.

This CARC document presents the results of the PWG tumor reevaluations, including statistical analyses of the tumor rates in the PWG reevaluations and a statistical evaluation of study mortality rates by L. Brunsman (2004). Dr. John Pletcher, consulting veterinary pathologist to the HED CARC, has also provided his expert opinion of the PWG reevaluations (Pletcher 2002a-f). Details of the PWG conduct may be found below for each study and tumor type. Because thyroid and nasal tumors were not reevaluated by the PWG, the reader is referred to the previous three cancer evaluations of acetochlor for details of tumor incidence and other study findings.

The Acetochlor Reregistration Partnership (ARP) has submitted mechanistic data evaluating thyroid parameters to support the mechanism of disruption of thyroid-pituitary homeostasis in the rat. The findings are evaluated according to the current US EPA policy on thyroid tumors.

The incidence of nasal tumors in the rat was evaluated in the second and third cancer peer

reviews of acetochlor. Mechanistic studies submitted to demonstrate a mode of action for rat nasal tumors are addressed in a separate Mode of Action Assessment document (Part 2). The findings of a recently conducted reproductive toxicity study in which nasal tumors were observed are presented in this CARC document.

1. Combined Chronic Toxicity/Carcinogenicity Study (27 month, male/24 month, female) with Acetochlor in Charles River CD Rats

References: Ahmed F.E. and Seely, J.C. (1983) Acetochlor: Chronic Feeding Toxicity and Oncogenicity Study in the Rat. Pharmacopathics Research Laboratories, Inc., Laurel, MD. Study No. PR-80-006. May 20, 1983. MRID 00131088. Unpublished study.

Ribelin, W.E. (1987) Histopathology Findings in Noses of Rats Administered MON 097 in a Lifetime Feeding Study. Tegeris Laboratories, Laurel, MD and Monsanto Environmental Health Laboratory, St. Louis, MO. Laboratory Project No. ML-86-44/EHL 86027. November 4, 1987. MRID 40484801. Unpublished report (supplement to original study report).

a. Experimental Design

Acetochlor technical (94.5% a.i.) was administered in the diet to Sprague-Dawley CD rats (60/sex/dose) at dose levels of 0, 500, 1500 or 5000 ppm for 115 consecutive weeks (males) or 103 consecutive weeks (females; discontinued earlier due to high mortality). Doses were equivalent to an average daily intake of 0, 22, 69 or 250 mg/kg/day, males and 0, 30, 93 or 343 mg/kg/day, females). Groups of 10/sex/dose additional animals were also treated for 52 weeks for an interim pathological evaluation.

b. Discussion of Liver Tumor Data

A full discussion of the results of the original pathology evaluation of this study are presented in the First Cancer Peer Review Committee document on acetochlor.

Conduct of PWG reevaluation (MRID 44496205): The incidence of hepatocellular tumors in male and female rats was reevaluated by a (1) pathology peer review (conducted by Dr. Peter C. Mann), in which all sections of liver from all rats in the 1983, 1986 and 1988 studies (and all mice in the 78-week and 23-month studies) were reexamined. This was followed by (2) the PWG panel review in which all slides diagnosed with liver neoplasms, either by the study pathologist or the peer review pathologist, were reexamined and a consensus diagnosis (agreement of 3/5 pathologists) was determined. All examinations of slides were blind to dose group. Statistical analysis of the reevaluation is presented below:

Table 2. Acetochlor - 1983 Rat Study

Male Liver Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	500	1500	5000
Adenomas (%)	2/58 (3)	1/60 (2)	1[a]/58 (2)	6/56 (11)
p =	0.0118*	0.4872	0.5000	0.1245
Carcinomas (%)	1/58 (2)	3/60 (5)	3/58 (5)	6[b]/56 (11)
p =	0.0285*	0.3222	0.3092	0.0514
Combined (%)	3/58 (5)	4/60 (7)	4/58 (7)	11/56 (20)
p =	0.0029**	0.5190	0.5000	0.0180*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 56.
[a]First adenoma observed at week 90, dose 1500 ppm.
[b]First carcinoma observed at week 94, dose 5000 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. Acetochlor - 1983 Rat Study

Female Liver Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	500	1500	5000
Adenomas (%)	0/55 (0)	0/42 (0)	1/48 (2)	3[a]/41 (7)
p =	0.0265*	-	0.1241	0.0459*
Carcinomas (%)	0/26 (0)	0/20 (0)	0/27 (0)	2[b]/15 (13)
p =	0.0146*	-	-	0.0713
Combined (%)	0/55 (0)	0/42 (0)	1/48 (2)	5/41 (12)
p =	0.0015**	-	0.1241	0.0085**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 80, dose 5000 ppm.

[b]First carcinoma observed at week 103, dose 5000 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control data for liver tumors in rats: Historical control data on the Crl:CD®BR rat have been published by Charles River. Nineteen groups of control animals from 24-month studies conducted at independent contract toxicology laboratories were evaluated between April, 1984 and November, 1986. Animals were supplied by one of three Charles River Facilities in the United Kingdom or North America. The incidence of hepatocellular tumors in males and females is summarized below (combined tumor incidence not available):

TABLE 4: CHARLES RIVER HISTORICAL CONTROL DATA FOR INCIDENCE OF HEPATOCELLULAR TUMORS IN Crl:CD®BR RATS (24 MONTHS)

Tumor/parameter	Males	Females
Hepatocellular adenoma:		
No. examined	1258	1263
No. tumors	53	28
Mean % incidence	4.21	2.2
Range % incidence	1.3-18.2	1.0-5.5
Hepatocellular carcinoma:		
No. examined	1258	1263
No. tumors	33	5
Mean % incidence	2.62	0.40
Range % incidence	1.1-9.1	1.0-4.0

Conclusions of the original study report review and previous CARC determinations: In the first cancer peer review of acetochlor, hepatocellular carcinomas were attributed to treatment and considered as part of the WOE for the cancer classification. The tumors showed an increasing trend with dose in both males and females and were significantly increased in males at 5000 ppm, although that dose was considered in excess of the MTD. Conclusions of the PWG report: The PWG concluded that a statistically significant, treatment-related increase in the incidence of combined hepatocellular adenomas and carcinomas was observed in both males and females at the high dose of 5000 ppm. However, this dose was considered to have exceeded the maximum tolerated dose (MTD), based on markedly decreased body weights and increased mortality compared to concurrent controls.

Statistical analyses of the PWG reevaluation data by HED showed a statistically significant increase in combined adenomas/carcinomas for both males ($p<0.05$) and females ($p<0.01$), along with a significant positive trend ($p<0.01$). In addition, the pair-wise incidence of adenomas was significantly increased at 5000 ppm above controls in females at $p<0.05$, and both males and females had a significantly increased trend for adenomas at $p<0.05$.

Dr. Pletcher's evaluation of the PWG report (Pletcher, 2002f): Dr. Pletcher agreed with the conclusions of the PWG report. He noted that it would be up to the CARC to determine whether the HDT of 5000 ppm was in excess of the MTD.

Conclusions of the CARC: The Committee agreed with the PWG and Dr. Pletcher that liver tumors, while showing a treatment-related increase in both sexes, were only observed at an excessive dose of 5000 ppm and should not be used in the cancer classification of acetochlor (see below for discussion of dose levels).

c. Statistical Analysis of Mortality

Mortality rates in males (control to high dose) were 67%, 67%, 57% and 73%. Mortality rates in females were 58%, 68%, 58% and 82%. The statistical evaluation of mortality indicated no statistically significant incremental changes with increasing doses of acetochlor in male rats. There was a statistically significant ($p<0.01$) increasing trend in mortality with increasing doses of acetochlor in female rats (L. Brunsman, 2004). Pairwise comparison of treated groups to controls showed a significant increase at high dose ($p<0.01$), as well as at low dose ($p<0.05$) in females.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

It was determined in the first cancer peer review of acetochlor that the MTD was exceeded at the high dose of 5000 ppm, based on increased mortality in both sexes. It is noted here that although females showed a statistically significantly increasing trend in mortality, males showed only a slight increase in mortality that was not statistically significant (L. Brunsman, 2004). At 5000 ppm, mean body weights were sharply reduced in both sexes during the second year of the study ($\geq$30% below controls in the last months of the study). The present meeting reaffirmed that the 5000 ppm dose was an excessive dose for assessing the carcinogenic potential of acetochlor in both sexes.

Dosing at 1500 ppm was considered adequate in both sexes, based on decreased body weights during the second year of treatment that were $\geq$10% less than controls (some time points statistically significant). Females also showed significantly increased abs/rel thyroid weights (+33%/+50%); relative thyroid weight in males was increased by 29%.

2. Combined Chronic Toxicity/Carcinogenicity Study (24 months) with Acetochlor in Charles River CD Rats

Reference: Naylor, M.W. and Ribelin, W.E. (1986) Chronic Feeding Study of MON 097 in Albino Rats. Study No. ML-83-200, Report No. MSL-6119; 93-190, Laboratory Project ID EHL-83107. September 25, 1986. MRID 40077601. Unpublished study.

a. Experimental Design

Acetochlor technical (96.1% a.i.) was administered in the diet to Sprague-Dawley rats (60/sex/dose) at dose levels of 0, 40, 200 or 1000 ppm for 24 consecutive months. Doses were equivalent to an average daily intake of 0, 2.0, 10.0 or 50.0 mg/kg/day, calculated using a standard conversion factor of 0.05 for conversion of ppm to mg/kg bw/day in adult rats. Groups of 10/sex/dose additional animals were also treated for 52 weeks for interim evaluation.

b. Discussion of PWG Reevaluation of Liver Tumor Data

A full discussion of the results of the original pathology evaluation of this study are presented in the Second Cancer Peer Review Committee document on acetochlor.

Conduct of the PWG reevaluation: Details of the conduct of the PWG reevaluations are summarized above under the discussion of the1983 rat study (Section III.1.b of this document). Statistical analysis of the reevaluation is presented below:

Table 5. Acetochlor - 1986 Rat Study

Male Liver Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	40	200	1000
Adenomas (%)	0/59 (0)	3[a]/56 (5)	1/59 (2)	2/59 (3)
p =	0.3120	0.1123	0.5000	0.2479
Carcinomas (%)	1[b]/59 (2)	1/56 (2)	1/59 (2)	1/59 (2)
p =	0.5759	0.7390	0.7521	0.7521
Combined (%)	1/59 (2)	4/56 (7)	2/59 (3)	3/59 (5)
p =	0.3554	0.1661	0.5000	0.3093

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 55.
[a]First adenoma observed at week 106, dose 40 ppm.
[b]First carcinoma observed at week 106, dose 0 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6. Acetochlor - 1986 Rat Study

Female Liver Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	40	200	1000
Adenomas (%)	0/59 (0)	1[a]/59 (2)	1/57 (2)	5/57 (9)
p =	0.0042**	0.5000	0.4914	0.0261*
Carcinomas (%)	1[b]/59 (2)	1/59 (2)	0/57 (0)	1/57 (2)
p =	0.5252	0.7521	0.5086	0.7435
Combined (%)	1/59 (2)	2/59 (3)	1/57 (2)	6/57 (11)
p =	0.0105*	0.5000	0.7435	0.0516

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 55.
[a]First adenoma observed at week 106, dose 40 ppm.
[b]First carcinoma observed at week 91, dose 0 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control data for incidence of liver tumors in rats: Historical control data in rats are presented above in the discussion of the 1983 rat study (see Table 4).

Conclusions of the original study report review and previous CARC determinations: A slight increase (not statistically significant) in the incidence of neoplastic nodules in females was reported, but in the second cancer peer review of acetochlor, they were not considered to be of treatment-related significance and were not considered as part of the WOE for cancer classification of acetochlor.

Conclusions of the PWG report: The PWG confirmed some of the original diagnoses for liver neoplasms, but many were changed. The original diagnoses of neoplastic nodule were either reclassified as hepatocellular adenoma or as foci of hepatocellular alteration. The PWG concluded that there was no treatment-related increase in the incidence of combined hepatocellular adenomas and carcinomas and identified no statistically significant increases relative to controls.

Statistical analysis of the PWG reevaluation by HED showed no significant trends or increases in liver tumor incidence with dose in male rats. In females, liver adenomas a statistically significant positive trend ($p<0.01$) and increased incidence at 1000 ppm compared to controls ($p<0.05$) were observed.

Dr. Pletcher's evaluation of the PWG report (Pletcher, 2002f): Dr. Pletcher agreed with the conclusions of the PWG report and recommended that the data be considered valid.

Conclusions of the CARC: The Committee agreed with the PWG and Dr. Pletcher that there was not a treatment-related increase in the incidence of liver tumors in this study. At 1000 ppm, a significant increase in adenomas in females that slightly exceeded historical control values was observed but it was noted that the concurrent control value of 0% was lower than historical controls and that liver tumors were not increased at 1500 or 1750 ppm in the other two rat studies.

c. Statistical Analysis of Mortality

The mortality rates in males were (control to high dose) 53%, 60%, 62% and 58%. The mortality rates in females were 60%, 57%, 57% and 50%. The statistical evaluation of mortality indicated no statistically significant incremental changes with increasing doses of acetochlor in male or female rats (L. Brunsman, 2004).

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

In males, dosing was considered to be adequate for assessing the carcinogenic potential of acetochlor. Mean body weight/weight gain was statistically significantly decreased from days 455 through 678 (at 24 months, -8.5%/-12.1% less than controls). Statistically significant increases in serum GGT at 18 and 24 months (>300% above controls) and cholesterol at 24 months (67% above controls), increased absolute/relative liver weights (+9.9%/+18.3%) and slightly increased hepatocellular alterations and hepatocyte necrosis were also reported. Thyroid weight was not measured. Increased thyroid c cell hyperplasia was observed in terminal sacrifice males, with a smaller, nonsignificant increase in all males on study.

In females, dosing was also considered to be adequate for assessing carcinogenic potential of acetochlor. Body weight/weight gain were slightly and not significantly reduced during the study (about -3%/-4.8%) but decreases up to -7%/-10% were seen in the last weeks of the study. Total bilirubin was increased at 24 months (+363%).

3. Combined Chronic Toxicity/Carcinogenicity Study (24 months) with Acetochlor

in Sprague-Dawley CD Rats

Reference: Virgo, D.M. and Broadmeadow, A. (1988) SC-5676: Combined Oncogenicity and Toxicity Study in Dietary Administration to CD Rats for 104 Weeks. Life Science Research Ltd., Suffolk, England. Study No. 88/SUC017/0348. March 18, 1988. MRID 41592004. Unpublished study.

a. Experimental Design

Acetochlor (91.0% a.i.) was administered in the diet to Sprague-Dawley CD rats (50/sex/dose) at dose levels of 0, 18, 175 or 1750 ppm for 104 consecutive weeks. Dose levels were equivalent to average daily intakes of 0, 0.67, 6.37 or 66.9 mg/kg/day (males) and 0, 0.88, 8.53 or 92.1 mg/kg/day (females). Additional interim sacrifice animals (20/sex at 0 and 1750 ppm and 10/sex at 18 and 175 ppm) were also administered these diets for 52 weeks.

b. Discussion of PWG Reevaluation of Liver Tumor Data

A full discussion of the results of the original pathology evaluation of this study are presented in the Third Cancer Peer Review Committee document on acetochlor.

Conduct of the PWG reevaluation: Details of the conduct of the PWG reevaluations are summarized above under the discussion of the1983 rat study (Section III.1.b of this document). Statistical analysis of the reevaluation is presented below:

Table 7. Acetochlor - 1988 Rat Study

Male Liver Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	18	175	1750
Adenomas (%)	0/43 (0)	0/46 (0)	0/42 (0)	2[a]/48 (4)
p =	0.0454*	-	-	0.1662
Carcinomas (%)	2/43 (5)	3/46 (7)	2[b]/42 (5)	1/48 (2)
p =	0.8209	0.2702	0.4069	0.6955
Combined (%)	2/43 (5)	3/46 (7)	2/42 (5)	3/48 (6)
p =	0.4827	0.2702	0.4069	0.4216

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor. Also excludes interim sacrifice animals.

[a]First adenoma not in an interim sacrifice animal observed at week 106, dose 1750 ppm.

[b]First carcinoma observed at week 73, dose 175 ppm.

Note: Interim sacrifice animals are not included in this analysis. One interim sacrifice animal in the 1750 ppm dose group had an adenoma
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 8. Acetochlor - 1988 Rat Study

Female Liver Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	18	175	1750
Adenomas (%)	0/49 (0)	1/48 (2)	0/47 (0)	2[a]/47 (4)
p =	0.1040	0.4949	1.0000	0.2371

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 55.
[a]First adenoma observed at week 97, dose 1750 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control data for incidence of liver tumors in rats: Historical control data in rats are presented above in the discussion of the 1983 rat study (see Table 4).

Conclusions of the original study report review and previous CARC determinations: Liver neoplasms in this study were not considered to show treatment-related increases in either males or females.

Conclusions of the PWG report: The PWG determined that there were no treatment-related increases in the incidence of hepatocellular adenomas, carcinomas or combined adenomas/carcinomas for either males or females.

Statistical analysis of the PWG reevaluation data by HED showed that there was a positive trend for liver adenoma in males but no increases in treated groups compared to controls.

Dr. Pletcher's evaluation of the PWG report (Pletcher, 2002f): Dr. Pletcher agreed with the conclusions of the PWG report and recommended that the data be considered valid.

Conclusions of the CARC: The Committee concurred that no treatment-related increase in liver tumors was observed based on low incidence in both sexes.

c. Discussion of PWG reevaluation of rare femur and stomach tumors (MRID 45367404)

The PWG also reevaluated slides from the femur and the stomach. For this PWG review, (1) a pathology peer review was first conducted (reviewing pathologist Dr. John Ferrell) in which all sections from the femur and non-glandular stomach of control and high dose male and female rats were examined (interim and terminal sacrifice groups). This was

followed by (2) a PWG review, which consisted of reexamination of all of the primary neoplasms and selected nonneoplastic proliferative lesions of these tissues that had been either identified by the original study pathologist or the reviewing pathologist. The histopathologic criteria and nomenclature for proliferative lesions in these organs described by Long et al. (1993) and Franz et al. (1991) were used to identify observed lesions.

These data were not reanalyzed statistically due to their low incidence. Results of the reevaluation compared to the original diagnoses are presented below:

Table 9: Identification of Proliferative Lesions of the Femur in the Rat - Comparison of original study diagnoses and PWG diagnoses

Group/Sex/Dose	Animal No.	Original diagnosis	Reviewing pathologist's diagnosis	PWG Consensus
2M/18 ppm	0109	epiphyseal fibrosis (minimal)	hyperostosis	hyperostosis
4M/1750 ppm	0215	chondroma	cartilage hyperplasia	cartilage hyperplasia
1F/0 ppm	0328	--	cartilage hyperplasia	cartilage hyperplasia
2F/18 ppm	0370	--	hyperostosis	hyperostosis
4F/1750 ppm	0459	chondroma	cartilage hyperplasia	cartilage hyperplasia
4F/1750 ppm	0473	--	cartilage hyperplasia	cartilage hyperplasia

Data extracted from Table A-1 of MRID 45367404.

The original diagnosis of chondroma was considered to be cartilage hyperplasia by the reviewing pathologist and the PWG group. The consensus was that there were no neoplastic lesions in the femur of the rat in this study.

The results of the reevaluation of the non-glandular stomach are presented below:

Table 10: Identification of Proliferative Lesions of the Non-Glandular Stomach of the Rat-Comparison of the original study diagnoses and the PWG Reevaluation

Group/Sex/Dose	Animal No.	Original diagnosis	Reviewing Pathologist's diagnosis	PWG Consensus
3M/175 ppm	0161	Acanthosis/hyperkeratosis, moderate, diffuse; submucosal inflammation, moderate; cystic glands, slight; keratinized region, ulcer(s); basal cell proliferation of ketatinized portion, moderate	Acanthosis/hyperplasia, moderate	Squamous cell hyperplasia, inflammation
4M/1750 ppm	0227	Acanthosis/hyperkeratosis, moderate; cystic glands, slight; squamous cell papilloma; basal cell tumor; arteritis, moderate	Squamous cell papilloma; poorly differentiated squamous cell carcinoma	Squamous cell papilloma; poorly differentiated squamous cell carcinoma
4F/1750 ppm	0498	Basal cell tumor	Well-differentiated squamous cell carcinoma	Well-differentiated squamous cell carcinoma

Data extracted from MRID 45367404.

The PWG report stated that "basal cell tumor" is not generally used to describe neoplastic lesions of the non-glandular stomach and characterized the two tumors previously identified by the study pathologist as squamous cell carcinomas (one well- and one poorly-differentiated). The report stated that the tumors were not considered to be related to treatment due to lack of other focal proliferative lesions in the non-glandular stomach and the low incidence of the tumors. No historical control values for this type of stomach tumor were available.

Dr. Pletcher's evaluation of the PWG reevaluations (Pletcher, 2002a): Dr. Pletcher agreed with the conclusions of the PWG and felt they were in keeping with current standards of diagnosis/nomenclature.

Conclusions of the CARC: The CARC accepted the rediagnoses of the femur (not a neoplastic lesion) and stomach tumors. The Committee determined that the non-glandular tumors of the stomach were not of significance to human cancer risk due to low incidence and because they were probably secondary to local irritation of the rat forestomach and

therefore not relevant to humans.

d. Discussion of data for thyroid follicular cell tumors (not reevaluated by PWG)

A complete discussion of the original pathology evaluation for this study can be found in the third Cancer Peer Review Committee document on acetochlor.

e. Statistical Evaluation of Mortality

Mortality rates in males were (controls to high dose) 80%, 76%, 82% and 56%. Mortality rates in females were 62%, 62%, 70% and 65%. The statistical evaluation of mortality indicated a statistically significant decreasing trend with increasing doses of acetochlor in male rats and significant pair-wise decrease at 1750 ppm compared to controls. There were no statistically significant incremental changes in mortality with increasing doses of acetochlor in female rats (L. Brunsman, 2004).

f. Adequacy of the Dosing for Assessment of Carcinogenicity

In males, dosing was considered adequate to assess the carcinogenicity of acetochlor based on toxicity observed at the high dose of 1750 ppm. Body weight and weight gain were decreased throughout the study (increasing throughout the first year of treatment) and gain was significantly different than controls for much of the study. Ophthalmologic examinations revealed an increased incidence of foci or plaques in the vitreous or posterior capsule of lens beginning at week 76. Increased serum GGT (throughout study) and cholesterol (22 weeks only) were observed, with a slight but statistically significant increase in relative liver weight seen at 52-weeks. No microscopic effects were reported for the liver. Nonneoplastic microscopic findings observed in males included nasal epithelial hyperplasia (also increased at the interim sacrifice). Quantitative presentation of these data are shown in the previous section.

In females, dosing was also considered adequate to assess the carcinogenicity of acetochlor, based on toxicity observed at 1750 ppm. Body weight and weight gain were decreased throughout the study and gain was significantly different than controls and was more pronounced than in males. Hyperreflection was reported at an increased incidence at 76 and 101 weeks. Significant increases in nasal epithelial hyperplasia (also increased at week 52), renal pelvic epithelial hyperplasia and degeneration of the outer nuclear layer of the retina were observed.

4. Two-Generation Reproductive Toxicity Study in the Rat

Reference: Milburn, G.M. (2001) Acetochlor: Multigeneration Reproduction Toxicity Study in Rats. Central Toxicology Laboratory, Alderley Park Macclesfield, Cheshire, UK.

Study No. RR0818, Report No. CTL/RR0818, ARP Submission No. 852-544. February 16, 2001. MRID 45357503. Unpublished study.

a. Experimental Design

Acetochlor was administered continuously in the diet to CD (SD) IGS BR (Sprague-Dawley) rats (26/sex/dose) at nominal dose levels of 0, 200, 600, or 1750 ppm (equivalent to 0, 21.2, 65.6, and 196.4 mg/kg/day in F1 males and 0, 22.4, 70.9, and 215.9 mg/kg/day in F1 females). F0 animals were given test article diet formulations for 10 weeks prior to mating to produce the F1 litters. On postnatal day (PND) 29, F1 animals (26/sex/dose) were selected to become the F1 parents of the F2 generation and were given the same concentration test formulation as their dams. F1 animals were given test formulations for 10 weeks prior to mating to produce the F2 litters.

b. Discussion of Tumor Data

Nasal epithelial tumor rates in males and females of the F0 and F1 parental groups with statistical analysis are presented below:

Table 11. Acetochlor - 2-Generation Rat Reproduction Study F0 Generation

Male Nasal Epithelium Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	200	600	1750
Polypoid Adenomas (%)	0/26 (0)	0/26 (0)	0/26 (0)	4[a]/26 (15)
p =	0.0033**	1.0000	1.0000	0.0552
Hyperplasia of Olfactory Epithelium (%)	0/26 (0)	0/26 (0)	0/26 (0)	3[b]/26 (12)
p =	0.0143*	1.0000	1.0000	0.1176
Hyperplasia of Respiratory Epithelium (%)	0/26 (0)	0/26 (0)	0/26 (0)	2[c]/26 (8)
p =	0.0607	1.0000	1.0000	0.2451

+Number of tumor bearing animals/Number of animals examined.
[a]First polypoid adenoma observed at week 21, dose 1750 ppm.
[b]First hyperplasia of olfactory epithelium observed at week 19, dose 1750 ppm.
[c]First hyperplasia of respiratory epithelium observed at week 21, dose 1750 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 12. Acetochlor - 2-Generation Rat Reproduction Study F1 Generation

Male Nasal Epithelium Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	200	600	1750
Polypoid Adenomas (%)	0/26 (0)	0/26 (0)	3/26 (12)	8[a]/26 (31)
p =	0.0001**	1.0000	0.1176	0.0021**
Hyperplasia of Olfactory Epithelium (%)	0/26 (0)	0/26 (0)	0/26 (0)	7[b]/26 (27)
p =	0.0000**	1.0000	1.0000	0.0049**
Hyperplasia of Respiratory Epithelium (%)	0/26 (0)	0/26 (0)	0/26 (0)	1[c]/26 (4)
p =	0.2500	1.0000	1.0000	0.5000

+Number of tumor bearing animals/Number of animals examined.
[a]First polypoid adenoma observed at week 19, dose 1750 ppm.
[b]First hyperplasia of olfactory epithelium observed at week 19, dose 1750 ppm.
[c]First hyperplasia of respiratory epithelium observed at week 20, dose 1750 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 13. Acetochlor - 2-Generation Rat Reproduction Study F0 Generation

Female Nasal Epithelium Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	200	600	1750
Polypoid Adenomas (%)	0/26 (0)	0/26 (0)	0/26 (0)	6[a]/26 (21)
p =	0.0002**	1.0000	1.0000	0.0113*
Hyperplasia of Olfactory Epithelium (%)	0/26 (0)	0/26 (0)	0/26 (0)	7[b]/26 (27)
p =	0.0000*	1.0000	1.0000	0.0049**
Hyperplasia of Respiratory Epithelium (%)	0/26 (0)	0/26 (0)	0/26 (0)	2[c]/26 (8)
p =	0.0607	1.0000	1.0000	0.2451

+Number of tumor bearing animals/Number of animals examined.
[a]First polypoid adenoma observed at week 19, dose 1750 ppm.
[b]First hyperplasia of olfactory epithelium observed at week 19, dose 1750 ppm.
[c]First hyperplasia of respiratory epithelium observed at week 19, dose 1750 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 14. Acetochlor - 2-Generation Rat Reproduction Study F1 Generation

Female Nasal Epithelium Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	200	600	1750
Polypoid Adenomas (%)	0/26 (0)	0/26 (0)	1/26 (4)	17[a]/23 (74)
p =	0.0000**	-	0.1587	0.0000**
Hyperplasia of Olfactory Epithelium (%)	0/26 (0)	0/26 (0)	4/26 (15)	14[b]/24 (58)
p =	0.0000**	-	0.0196*	0.0000**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First polypoid adenoma observed at week 19, dose 1750 ppm.

[b]First hyperplasia of olfactory epithelium observed at week 14, dose 1750 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

In the F0 parental males, a statistically significant trend ($p<0.01$) was observed for polypoid adenomas of the nasal epithelium (from controls to high dose, 0%, 0%, 0% and 15%). A positive trend was also observed for hyperplasia of the olfactory epithelium (12% at high dose vs. 0% all other groups). In the F0 parental females, nasal polypoid adenomas showed both a positive trend ($p<0.01$) and significance by pairwise comparison of the 1750 ppm group to controls (21% vs. 0%, all other groups). Hyperplasia of the olfactory epithelium also showed a positive trend ($p<0.05$) and significant increase at 1750 ppm at $p<0.01$ (27% vs. 0%, all other groups).

In the F1 parental animals, statistically significant increases ($p<0.01$) in polypoid adenomas of the nasal epithelium were observed at 1750 ppm in males and females by pairwise comparison with controls, and a positive trend was also observed. From control to high dose, incidence in males was 0%, 0%, 12% and 31% and in females was 0%, 0%, 4% and 74%, respectively. The incidence of olfactory epithelial hyperplasia showed the same statistical pattern for both sexes (males 27% at high dose vs. 0%, all other groups and females 0%, 0%, 15% and 58%, with additional significance of $p<0.05$ at 600 ppm).

The first tumors were reported at week 19 at 1750 ppm except for F0 males, where the first tumor was observed at week 21. The CARC determined that these tumors were related to treatment based on the rarity of spontaneous nasal tumors in rats and occurrence in the rat chronic toxicity/carcinogenicity studies.

c. Non-Neoplastic Lesions

Non-neoplastic findings in F0 and F1 parental animals are shown below:

Table 15: Non-neoplastic histopathology findings in the nasal cavity [a]

Finding and severity	Sex	Dietary Concentration (ppm)							
		F0				F1			
		Control	200	600	1750	Control	200	600	1750
Nasal Cavity Examined	M	26	26	26	25	26	26	26	26
No Abnormalities Detected		5	12	3	0	14	4	2	0
Nasal Cavity Examined	F	25	25	25	25	26	26	26	22
No Abnormalities Detected		10	4	0	0	15	7	0	0
Nasal cavity - Hyperplasia of the olfactory epithelium (Minimal to slight)	M	0	0	0	3	0	0	0	7
	F	0	0	0	7	0	0	4	14
Nasal cavity - Hyperplasia of the respiratory epithelium (Minimal)	M	0	0	0	2	0	0	0	1
	F	0	0	0	2	0	0	0	0
Nasal cavity - Increased lipofuscin of the olfactory mucosa (Minimal to slight)	M	0	0	21	25	0	0	15	26
	F	0	11	25	25	0	9	25	22
Nasal cavity - Chronic inflammation, nasolacrimal duct (Minimal to slight [e])	M	12	13	8	8	10	17	14	11
	F	14	18	9	9	10	15	20	12
Nasal cavity - Rhinitis (Minimal to slight)	M	12	4	4	12	4	8	7	2
	F	5	2	3	7	3	1	0	0
Nasal cavity - Hyperplasia, squamous epithelium (Minimal)	M	0	0	0	0	0	0	0	0
	F	0	0	0	1	0	0	0	0

a Data extracted from Tables 63 and 64 of the test report (pages 223, 227, and 231).
b Includes 4 animals with single and 2 with multiple lesions.
c Includes 5 animals with single and 3 with multiple lesions.
d Includes 7 animals with single and 9 with multiple lesions.
e Minimal to moderate severity for F1 findings.

At 1750 ppm in males and females (and 600 ppm in females), hyperplasia of the olfactory epithelium (minimal to slight) was observed for F0 and F1 parental animals. The incidence was higher in F1 animals by about 2-fold (at 1750 ppm, males 12% vs. 27% and females 28% vs. 64%). Hyperplasia of the respiratory epithelium affected 2/25 F0 males, 2/25 F0 females and 1/26F1 male at 1750 ppm. Increased lipofuscin of the olfactory mucosa was

observed in all dose groups of females and in males at 600 and 1750 ppm (almost all animals of both sexes were affected at 600 and 1750 ppm); this finding was considered treatment-related. It was noted in the review of this study that many of the animals across the dosing groups were affected by rhinitis or chronic inflammation of the nasolacrimal duct, which could indicate a confounding factor in the study.

d. Other Treatment-Related Toxicity

There was no effect on mortality in males or females. Treatment-related toxicity was observed in parental animals at 1750 ppm and included statistically significantly decreased body weight/weight gain during premating (males up to 8%/10% below controls; females up to 10%/19%), as well as significant decreases in F0 and F1 females during gestation and lactation. Food consumption also tended to be decreased in F0 animals (6-11% below controls) during premating and in the first weeks of gestation. Food consumption in F1 animals was reduced in F1 males throughout premating but only in the first week for F1 females. Although thyroid, liver and kidney weights showed slight increases, there were no associated gross or microscopic lesions associated with these changes.

Offspring showed toxicity at 1750 ppm. Reductions in the number of implantations in F0 and F1 generations, reduced number of live pups on postnatal day 1 in F1 and F2 litters and significantly decreased F1 and F2 pup body weights at day 1 were reported. Pup weight/weight gain was also significantly reduced in late lactation.

5. Carcinogenicity Study (23-Month) in Mice

Reference: Ahmed., F.E., Tegeris, A.S. and Seely, J.C. (1983) MON-097: 24-Month Oncogenicity Study in the Mouse. Pharmacopathics Research Laboratories, Inc., Laurel, MD. Report No. PR-80-007. May 4, 1983. MRID 00131089. Unpublished study.

a. Experimental Design

Acetochlor was administered in the diet to 50 Swiss-bred CD-1 albino mice/sex/dose for up to 23 months at dose levels of 0, 500, 1500 or 5000 ppm. Dose levels were equivalent to an estimated average daily intake of 0, 75, 225 or 750 mg/kg/day (calculated using a dietary ppm-to-mg/kg/day conversion factor of 0.15). Additional groups of 10 mice/sex/dose were administered these diets for 12 months and sacrificed for a one-year interim evaluation.

b. Discussion of Reanalyses of Liver Tumor Data

A full discussion of the results of the original pathology evaluation of this study are presented in the First Cancer Peer Review Committee document on acetochlor.

Conduct of PWG Reevaluation: Details of the conduct of the PWG reevaluations are summarized above under the discussion of the1983 rat study (Section III.1.b of this document). The results of the PWG reevaluation of liver tumors in this study are presented below, with statistical evaluation performed by HED:

Table 16. Acetochlor - 23-month Mouse Study

Male Liver Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	500	1500	5000
Adenomas (%)	8/49 (16)	7/39 (18)	10/45 (22)	19[a]/40 (48)
p =	0.0000**	0.3688	0.1647	0.0001**
Carcinomas (%)	4/47 (9)	4[b]/36 (11)	4/45 (9)	9/39 (23)
p =	0.0475*	0.1962	0.4594	0.0390*
Combined (%)	12/49 (24)	10[c]/39 (26)	14/45 (31)	26[d]/40 (65)
p =	0.0000**	0.3242	0.1960	0.0000**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor. Also excludes interim sacrifice animals.
[a]First adenoma observed at week 69, dose 5000 ppm.
[b]First carcinoma not in an interim sacrifice animal observed at week 74, dose 500 ppm.
[c]One animal in the 500 ppm dose group had both an adenoma and a carcinoma.
[d]Two animals in the 5000 ppm dose group had both an adenoma and a carcinoma.
Note:: Interim sacrifice animals are not included in this analysis. One interim sacrifice animal in the control group had a carcinoma.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 17. Acetochlor - 23-month Mouse Study

Female Liver Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	500	1500	5000
Adenomas (%)	2/41 (5)	0/38 (0)	1[a]/34 (3)	5/24 (21)
p =	0.0007**	-	-	0.0188*
Carcinomas (%)	0/40 (0)	0/35 (0)	0/30 (0)	2[b]/22 (9)
p =	0.0075**	-	-	0.0787
Combined (%)	2/41 (5)	0/38 (0)	1/34 (3)	7/24 (29)
p =	0.0000**	-	-	0.0052**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor. Also excludes interim sacrifice animals.
[a]First adenoma observed at week 87, dose 1500 ppm.
[b]First carcinoma observed at week 90, dose 5000 ppm.
Note:: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control data for liver tumors in mice: Historical control data on the CD-1® mouse have been published by Charles River. Ten groups of animals from 24-month studies conducted at independent contract toxicology laboratories were evaluated between July, 1983 and October, 1990. Animals were supplied by Charles River Facilities in the United Kingdom or North America. The incidence of hepatocellular tumors in males and females is summarized below:

TABLE 18: HISTORICAL CONTROL DATA FOR INCIDENCE OF HEPATOCELLULAR TUMORS IN CD-1® MICE (24 MONTHS)

Tumor/parameter	Males	Females
Hepatocellular adenoma:		
No. examined	521	571
No. tumors	97	18
Mean % incidence	18.62	3.15
Range % incidence	4.08-37.5	0-11.27
Hepatocellular carcinoma:		
No. examined	521	521
No. tumors	68	9
Mean % incidence	13.05	1.58
Range % incidence	0.0-28.00	0-4.00

Conclusions of the original study report review and previous CARC determinations: In the first cancer peer review of acetochlor it was concluded that although a statistically significant, treatment-related increase in hepatocellular carcinoma in males and females (and combined liver tumors; statistical significance not identified) was observed at 5000 ppm, the MTD was exceeded at that dose level. However, it was noted as part of the weight-of-evidence considerations for cancer classification of acetochlor.

Conclusions of the PWG report: The PWG consensus confirmed many of the original diagnoses, but there were also many that were changed (e.g., carcinomas reclassified as adenoma and some adenomas reclassified as carcinomas). The PWG determined that a statistically significant, treatment-related increase in the incidence of combined hepatocellular adenomas and carcinomas was observed in both males and females at 5000 ppm. However, the PWG report also noted that the toxicity observed at 5000 ppm was excessive, based on mortality, marked reductions in body weight and liver and kidney toxicity and concluded that the tumors were not of relevance to humans based on occurrence only at an excessive dose level.

Dr. Pletcher's evaluation of the PWG report (Pletcher, 2002f): Dr. Pletcher agreed with the conclusions of the PWG report. He noted that the CARC will need to determine whether these tumors were observed at an excessive dose.

Conclusions of the CARC: The Committee agreed with the PWG and Dr. Pletcher that although the liver tumors in both sexes this study were related to treatment, they were only observed at an excessive dose of 5000 ppm and determined that they should not be included as part of the weight-of-evidence determination for cancer classification (see discussion of dosing adequacy, below).

c. Discussion of Reanalysis of Lung Tumors

A full discussion of the results of the original pathology evaluation of this study are presented in the First Cancer Peer Review Committee document on acetochlor.

Conduct of the PWG (MRID 44496206): The incidence of lung tumors in male and female mice was reevaluated by a (1) pathology peer review (conducted by Dr. Peter C. Mann), in which all sections of lung from all animals in the study were reexamined. This was followed by the PWG panel review in which all slides diagnosed with pulmonary neoplasms, either by the study pathologist or the peer review pathologist, were reexamined and a consensus diagnosis (agreement of 3/5 pathologists) was determined. All examinations of slides were blind to dose group. The findings of the PWG consensus, with statistical analyses performed by HED, are presented below (only data for females are presented because there was no increase observed in male mice):

Table 19. Acetochlor - 23-month Mouse Study

Female Lung Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	500	1500	5000
Adenomas (%)	1/43 (2)	7/42 (17)	9/40 (22)	7[a]/31 (23)
p =	0.0394*	0.0204*	0.0055**	0.0007**
Carcinomas (%)	0/43 (0)	4/43 (9)	1/40 (2)	6[b]/33 (18)
p =	0.0014**	0.0211*	0.0885	0.0017**
Combined (%)	1/43 (2)	10[c]/43 (23)	10/40 (25)	11[d]/33 (33)
p =	0.0041**	0.0026**	0.0019**	0.0001**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor. Also excludes interim sacrifice animals.

[a]First adenoma not in an interim sacrifice animal observed at week 76, dose 5000 ppm.

[b]First carcinoma observed at week 75, dose 5000 ppm.

[c]One animal in the 500 ppm dose group had both an adenoma and a carcinoma.

[d]Two animals in the 5000 ppm dose group had both an adenoma and a carcinoma.

Note:: Interim sacrifice animals are not included in this analysis. One interim sacrifice animal in the 1500 ppm dose group had an adenoma.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control incidence for lung tumors in mice: Historical control values for lung alveolar/bronchiolar in CD-1 mice were provided in the PWG report from three published reports, including published Charles River Laboratory data. In 24-month studies the average incidence of total primary lung tumors in females ranged from 13.8% to 26.6% (21.8% to 33.5%, males). For adenoma alone, the rates in females ranged from 9.6% to 14.5% (males 14.6% to 19.3%). For carcinoma alone, rates in females ranged from 1.5% to 12.1% (males 2.5% to 18.9%).

Conclusions of the original study report review and previous CARC determinations: In the first cancer peer review of acetochlor, it was determined that a treatment-related increase in the incidence of lung carcinoma and combined lung tumors in female mice was observed at all dose levels, although the high dose of 5000 ppm was considered to exceed the MTD.

This was part of the WOE consideration in determining the cancer classification of acetochlor.

Conclusions of the PWG report: With a few exceptions, the original diagnoses were confirmed by the reevaluation. Statistically significant increases in lung tumor increase were observed in the female mice treated with acetochlor. Single findings of lung adenomas were also observed in a mid dose male and mid dose female from the interim sacrifice group. The PWG report concluded that although statistically significant increases in pulmonary tumors were observed in treated animals, the tumors were not treatment-related. The PWG report noted lack of a linear dose-response, lack of increase in precursor proliferative lesions, absence of a dose-dependent increase in tumor multiplicity for lung tumors in mice and the common finding of these tumors in older mice. The available historical control data suggest that the tumor rates observed in these studies in treated animals fall within spontaneous incidence range.

Dr. Pletcher's evaluation of the PWG report (Pletcher, 2002e): Dr. Pletcher agreed with the conclusions of the PWG report and recommended that they be considered valid.

Conclusions of the CARC: The Committee determined that the lung tumors in this study were treatment-related in females, based on significant increases in adenoma and combined adenoma/carcinoma at several dose levels and incidence exceeding historical control values for adenomas.

d. Discussion of Reanalysis of Histiocytic Sarcoma in Females

A full discussion of the results of the original pathology evaluation of this study are presented in the first Cancer Peer Review Committee document on acetochlor.

Conduct of PWG reevaluation (MRID 44496204): The incidence of histiocytic tumors in female mice was reevaluated by a (1) pathology peer review (conducted by Dr. Peter C. Mann), in which all sections of liver and uterus (the most commonly affected organs for this type of tumor) from all female mice on the study were reexamined, along with all tissues with an initial diagnosis of histiocytic sarcoma in the original study report. This was followed by the PWG panel review in which all slides diagnosed with histiocytic sarcoma, either by the study pathologist or the peer review pathologist, were reexamined and a consensus diagnosis (agreement of 3/5 pathologists) was determined. All examinations of slides were blind to dose group. The findings of the PWG consensus with statistical analyses performed by L. Brunsman (2004) are shown below:

Table 20. Acetochlor - 23-month Mouse Study

Female Histiocytic Sarcoma Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	500	1500	5000
All Sites (%)	0/47 (0)	3/44 (7)	7/47 (15)	6[a]/41 (15)
p =	0.0555	0.0442*	0.0087**	0.0167*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor. Also excludes interim sacrifice animals.

[a]First histiocytic sarcoma observed at week 61, dose 5000 ppm.

Note:: Interim sacrifice animals are not included in this analysis. One interim sacrifice animal in the 1500 ppm dose group had a histiocytic sarcoma.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control values for histiocytic sarcoma in females: Values are available for 24-month studies in CD-1 female mice. Incidence has been reported to range from 0-10% (average 3.76%)(Charles River Laboratories data). Data from Inveresk Research International gives similar values of 0-10% (average 4.6%). The PWG report states that these tumors are rare in the first year, are more common in females and increase steeply with age after 18 months.

Conclusions of the original study report review and previous CARC determinations: It was concluded that the incidence of histiocytic sarcoma in the uterus of female mice was increased with treatment at all dose levels and the increase was included as part of the WOE for cancer classification of acetochlor.

Conclusions of the PWG report: The PWG did not confirm the presence of histiocytic sarcoma in 3 females from the 500 ppm group (rediagnosed as undifferentiated sarcomas). The report concluded that the increase in tumor incidence at 1500 and 5000 ppm was equivocal, represented normal variation and was probably not related to dietary exposure to acetochlor. This conclusion was based on (1) low control incidence of 0% (was 4% in 78-week mouse study; 3% including interim sacrifice animals), (2) variable spontaneous incidence observed, (3) lack of linear dose-response and (4) absence of precursor lesions.

Statistical analysis of the PWG reevaluation findings by HED indicate statistically significant increases in the incidence of histiocytic sarcoma in female mice at all dose levels ($p<0.05$, low and high dose and $p<0.01$, mid dose) with no positive trend identified.

Dr. Pletcher's evaluation of the PWG report (Pletcher, 2002g): Although Dr. Pletcher found no fault with the performance of the PWG reevaluation, he did not entirely agree with the conclusions of the PWG report. He prepared a table combining the data from the two studies, shown below (interim sacrifice animals excluded from calculations):

Table 21: Evaluation of histiocytic sarcoma incidence in mice studies on acetochlor

	0 ppm #/+	10 ppm +	100 ppm+	500 ppm#	1000 ppm+	1500 ppm#	5000 ppm#
#Animals with tumor	2/100	1/50	0/50	3/50	5/50	7/50	6/50
Percentage	2	2	0	6	10	14	12

Monsanto's 23-month study + Zeneca's 78-week (approximately 18-month) study

Based on the above percentages, Dr. Pletcher concluded that the data indicated a slight increase with treatment and that the incidence at the highest two doses exceeded historical control range (0-10%), but noted that if 1000 ppm is determined to be the MTD, then the tumors would not be considered of relevance to humans. He also stated that he was unaware of any type of precursor lesions for histiocytic sarcoma. Dr. Pletcher stated that "...although I have a "gut feeling" that the incidence of histiocytic sarcoma is probably not directly related to Acetochlor (the data suggesting otherwise possibly being an unfortunate distribution of the incidental occurrence of this neoplasm among the test animals), I find it difficult to argue my inclination on the points used by Dr. Hardisty. I am not inclined to assign much significance to a slight increase in this neoplasm when seen only in female mice. If the MTD of Acetochlor in mice is 1000 ppm, then any perceived carcinogenic effect is a moot issue."

Conclusion of the CARC: The CARC concluded that histiocytic sarcomas in female mice were related to treatment and should be included in the cancer classification for acetochlor, based on significant increases in treated groups (exceeding historical control values at 1500 ppm).

e. Discussion of Reanalysis of Renal Tumors in Females

A full discussion of the results of the original pathology evaluation of this study are presented in the first Cancer Peer Review Committee document on acetochlor.

Conduct of PWG reevaluation (MRIDs 45367402 and -03): The incidence of renal tumors in female mice was reevaluated by a (1) pathology peer review (conducted by Dr. Gordon C. Hard), in which all sections of kidney from all female mice on the study were reexamined. This was followed by the PWG panel review in which all slides diagnosed with renal neoplasms and hyperplastic lesions, either by the study pathologist or the peer

review pathologist, were reexamined and a consensus diagnosis (agreement of 3/5 pathologists) was determined. Criteria for diagnosis of tubule hyperplasia and tubule tumors were as described for rats and mice by the Society of Toxicologic pathologists and the International Agency for Research on Cancer (Hard *et al.*, 1995, 1999 and 2000). All examinations of slides were blind to dose group. The findings of the PWG consensus with statistical analyses performed by HED are presented below:

Table 22. Acetochlor - 23-month Mouse Study

Female Kidney Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results#

	Dose (ppm)			
	0	500	1500	5000
Adenomas (%)	0/33 (0)	0/25 (0)	0/20 (0)	2[a]/14 (14)
p =	0.0217*	1.0000	1.0000	0.0842

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 100. Also excludes interim sacrifice animals.
[a]First adenoma observed at week 100, dose 5000 ppm.
#Although a Peto's Prevalence Test would have been the preferred test for this analysis, the lack of several time intervals made the Peto's Test impossible to compute.

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Female Kidney Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	500	1500	5000
Sarcomas (%)	0/41 (0)	0/39 (0)	0/38 (0)	2[b]/29 (7)
p =	0.0109*	-	-	0.1269

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor. Also excludes interim sacrifice animals.
[b]First sarcoma observed at week 84, dose 5000 ppm.
Note:: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control data for renal adenomas in mice: The "Expert Report on Renal Histopathological Changes in a Mouse Study (Monsanto Study PR-80-007) with

Acetochlor" submitted with the PWG report states that the typical range for spontaneous incidence of renal adenomas would be 10-15%. Data from Charles River give a lower rate of 0-4%.

Conclusions of the original study report review and previous CARC determinations: The increased incidence of renal tumors in female mice was considered to be related to dietary exposure to acetochlor and was used as part of the weight-of-evidence for the cancer classification of acetochlor.

Conclusions of the PWG report: The PWG did not confirm one diagnosis of renal adenoma from the original report (rediagnosed as an undifferentiated sarcoma, possibly lymphoreticular origin) and the PWG report concluded that the tumors at 5000 ppm were not related to treatment due to lack of statistical significance of the increase by their analysis (no animals excluded) and lack of atypical hyperplastic tubular lesions (defined as "internal proliferation of the epithelial lining of a tubule beyond the normal single-cell-layer but essentially retaining the integrity of the tubule lining") in that dose group.

Statistical analysis by HED of the PWG findings indicated a positive trend ($p<0.05$) for each type of renal tumor (adenomas and sarcomas) but no significant increases at any dose level.

Dr. Pletcher's evaluation of the PWG report (Pletcher, 2002b,c): Dr. Pletcher found the conclusions of the PWG report to be reasonable and felt that "...the data from the above named study is greatly strengthened by the inclusion of the findings of this Peer Review/PWG."

Conclusions of the CARC: The CARC agreed with the conclusions of the PWG and Dr. Pletcher and determined that the kidney tumors were not related to treatment based on low incidence and occurrence only at an excessive dose of 5000 ppm.

f. Discussion of Benign Ovarian Tumors - Response of ARP (data not evaluated by PWG)

A PWG reevaluation of sections of ovarian tissue was not performed. In the original study report, the following benign ovarian tumor incidence was reported (original statistical analysis from the first cancer peer review):

TABLE 23: INCIDENCE OF BENIGN OVARIAN TUMORS IN 23 MONTH MOUSE STUDY

Tumor type	0 ppm	500 ppm	1500 ppm	5000 ppm
adenoma	0/59	0/60	1/60 (1.67%)	0/58
granulosa cell tumor	0/59	0/60	3/60 (5.0%)	2/58 (3.44%)
luteoma	0/59	0/60	1/60 (1.67%)	1/58 (1.72%)
total benign tumors	0/59**	0/60	5/60* (8.33%)	3/58 (5.17%)

* $p \leq 0.05$ (pairwise to controls) ** at control group $p \leq 0.01$ for trend

Based on these findings, the tumors were attributed to acetochlor exposure in the first cancer peer review. It is noted that the high dose of 5000 ppm was considered to have exceeded the MTD.

In response to this determination, the ARP has proposed that the ovarian tumors are not related to treatment, based on the following: (1) lack of precursor lesions in this study or the other rodent studies on acetochlor; (2) lack of tumor multiplicity or bilateral tumors and (3) lack of linear dose-response.

Historical control data on benign ovarian tumors in mice: Data on spontaneous neoplastic lesions in CD-1 mice for 24-month studies are available from Charles River (1995, on website). In general, ovarian tumors are not common in this strain of mouse. The type of adenoma observed at 1500 ppm was not indicated. Tubular adenomas are uncommon and show an incidence of 0-2.9% (mean 0.35%). Cystadenomas show a slightly higher incidence of 0-6.12% (mean 1.94%). Granulosa cell/theca cell tumors show a range of 0-6.0% (mean 1.94%). Values for luteomas or for combined tumors were not listed in the Charles River database. Based on these data, the incidences for the above individual tumors do not exceed historical control values unless the granulosa cell tumors and luteomas were combined (6.67%). Data from control groups in studies from Inveresk Research International (1993-study dates not provided in available data) give a similar incidence profile.

Conclusion of the CARC: During the meeting, Dr. Pletcher noted that it was not appropriate to combine the different types of benign ovarian tumors, as has been done in the previous HED analysis. He agreed with the Registrant that the tumors were incidental. The CARC determined that the ovarian tumors were not related to treatment due to low incidence of each individual type of tumor.

g. Statistical Analysis of Mortality

Mortality rates in males were (control to high dose) 40%, 50%, 48% and 74%. Mortality rates in females were 38%, 50%, 66% and 74%. A statistically significant increasing trend for increased mortality was observed in both males and females ($p<0.01$). Pairwise comparisons in males showed significant increases at high dose ($p<0.01$), with significance

also identified at low dose ($p<0.05$). Pairwise comparisons in females showed significant increases at mid and high dose ($p<0.01$).

h. Adequacy of Dosing for Assessment of Carcinogenicity

It was determined at the first cancer peer review of acetochlor that dosing at 5000 ppm was excessive for both males and females. This determination was made based on increased mortality rates in males and females and significantly reduced mean body weights (up to about 20% by study termination). Females had reduced RBC/Hct/Hgb values of 21-23% below controls. Significantly increased absolute and relative liver and kidney weights were seen in males at all dose levels (females increased only at interim sacrifice). The incidence of interstitial nephritis was significantly increased at 5000 ppm in both sexes (83% and 76.3%, males and females) but incidence at lower doses was relatively high (>50% both sexes). At 1500 ppm, females showed significantly increased mortality but no other effects. Males at 1500 ppm showed no signs of toxicity; however, a 6-week range-finding study found statistically significantly decreased body weights (-9%) in males at 1200 ppm.

6. Carcinogenicity Study (78-Week) in Mice

Reference: Amyes, S.J. (1989) SC-5676: 78-Week Feeding Study in CD-1 Mice. Life Science Research Ltd., Suffolk, England. Study No. 87/SUC0012/0702. June 9, 1989. MRID 41565119. Unpublished report.

a. Experimental Design

Acetochlor (tech., 90.5% a.i.) was administered in the diet to 50 CD-1 mice/sex/dose for 78 weeks at dose levels of 0, 10, 100 or 1000 ppm. Dosing was equivalent to an average daily intake of 0, 1.1, 11 or 116 mg/kg/day (males) and 0, 1.4, 13 or 135 mg/kg/day (females). Additional groups of 10 CD-1 mice/sex/dose were administered the test material at the same dose levels for 52 weeks for interim evaluation at one year.

b. Discussion of PWG Reevaluation of Lung Tumors)

A full discussion of the results of the original pathology evaluation of this study are presented in the third Cancer Peer Review Committee document on acetochlor.

Conduct of PWG reevaluation: Details of the conduct of the PWG reevaluations are summarized above under the discussion of the1983 mouse study (Section III.5.c. of this document). Results of the PWG reevaluation are presented below, with statistical evaluations performed by HED:

Table 24. Acetochlor - 78-week Mouse Study

Male Lung Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	10	100	1000
Adenomas (%)	9/60 (15)	5/60 (8)	11/58 (19)	17[a]/57 (30)
p =	0.0025**	0.8685	0.3089	0.0337*
Carcinomas (%)	3/60 (5)	3/60 (5)	3[b]/59 (5)	4/57 (7)
p =	0.3060	0.4872	0.5094	0.3337
Combined (%)	11[c]/60 (18)	8/60 (13)	13[d]/59 (22)	19[e]/57 (33)
p =	0.0064**	0.7648	0.3400	0.0295*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 52, dose 1000 ppm.

[b]First carcinoma observed at week 41, dose 100 ppm.

[c]One animal in the control group had both an adenoma and a carcinoma.

[d]One animal in the 100 ppm dose group had both an adenoma and a carcinoma.

[e]Two animals in the 1000 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 25. Acetochlor - 78-week Mouse Study

Female Lung Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	10	100	1000
Adenomas (%) p =	4/58 (7) 0.0719	5[a]/59 (8) 0.5109	6/58 (10) 0.3713	9/60 (15) 0.1330
Carcinomas (%) p =	1/58 (2) 0.1763	0/59 (0) 1.0000	2[b]/58 (3) 0.5000	2/60 (3) 0.5128
Combined (%) p =	5/58 (9) 0.0429*	5/59 (8) 0.6395	8/58 (14) 0.2788	11/60 (18) 0.1011

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 46.
[a]First adenoma observed at week 46, dose 10 ppm.
[b]First carcinoma observed at week 69, dose 100 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control data for lung tumors in mice: Historical control data for lung tumors in 78/80-week studies on CD-1 mice were provided in the PWG report. Data from LSR show a range of 4.0% to 17.3% for pulmonary adenoma in males and 0% to 9.6% in females. Carcinoma ranged from 3.3% to 13.5% in males and 0 to 9.6% in females. Combined tumor incidence for males was 15%-28.9% and for females was 3.8% to 19.2%.

Conclusions of the original study report review and previous CARC determinations: At the third cancer peer review of acetochlor, it was determined that at 1000 ppm (HDT), significant increases in pulmonary adenoma at 1000 ppm were observed for males and females and a significant increase in combined tumors was observed for males. A positive trend was noted for adenomas in males and for combined adenomas/carcinomas in both sexes. These data were used to support the WOE for the cancer classification of acetochlor.

Conclusions of the PWG report: With a few exceptions, the original diagnoses were confirmed by the reevaluation. Statistically significant increases in lung tumors were observed in male and female mice treated with acetochlor. Single findings of lung adenomas were also observed in a mid dose male and mid dose female from the interim sacrifice group. The statistical analyses conducted by the PWG indicated that there were

no significant increases in lung tumors, unlike the HED analysis. The PWG report concluded that lung tumors observed in this study were not treatment-related. Although adenomas in males and combined tumor incidences in both sexes at 1000 ppm slightly exceeded historical control values, the report cited the lack of statistical significance in this increase. The PWG report also noted lack of a linear dose-response, lack of increase in precursor proliferative lesions, absence of a dose-dependent increase in tumor multiplicity for lung tumors in mice and the high frequency of these tumors in older mice.

Statistical analysis of the PWG findings by HED indicated a significantly increased incidence of both adenomas and combined adenomas/carcinomas of the lung in males at the high dose (both $p<0.05$) along with a significant positive trend ($p<0.01$). There was also a significant positive trend for combined lung tumors in females at $p<0.05$.

Dr. Pletcher's evaluation of the PWG report (Pletcher, 2002e): Dr. Pletcher agreed with the conclusions of the PWG report and recommended that the data be considered valid.

Conclusions of the CARC: The Committee determined that the increased lung adenomas and combined tumors observed in males and positive trend in females were related to treatment, based on positive findings in the 23-month study in females and findings of precursor proliferative lesions in males in this study.

c. Discussion of PWG Reevaluation of Liver Tumor Data

A full discussion of the results of the original pathology evaluation of this study are presented in the third Cancer Peer Review Committee document on acetochlor.

Conduct of PWG: Details of the conduct of the PWG reevaluations are summarized above under the discussion of the1983 rat study (Section III.1.b of this document). Result of the PWG reevaluation are presented below with statistical analyses performed by HED:

Table 26. Acetochlor - 78-week Mouse Study

Male Liver Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	10	100	1000
Adenomas (%)	6/43 (14)	9/42 (21)	5/41 (12)	6[a]/37 (16)
p =	0.4887	0.2010	0.6321	0.4487
Carcinomas (%)	0/43 (0)	2/42 (5)	3[b]/41 (7)	3/37 (8)
p =	0.1628	0.0747	0.0505	0.0284*
Combined (%)	6/43 (14)	11/42 (26)	8/41 (20)	9/37 (24)
p =	0.2708	0.0897	0.2958	0.1481

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor. Also excludes interim sacrifice animals.

[a]First adenoma not in an interim sacrifice animal observed at week 73, dose 1000 ppm.

[b]First carcinoma observed at week 71, dose 100 ppm.

Note: Interim sacrifice animals are not included in this analysis. One interim sacrifice animal in the 10 ppm dose group had an adenoma.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control data for incidence of liver tumors in mice: Historical control data on the CD-1® mouse have been published by Charles River. Twelve groups of animals from 18-month studies conducted at independent contract toxicology laboratories were evaluated between December, 1984 and March, 1991. Animals were supplied by Charles River Facilities in the United Kingdom, Portage, MI, Kingston, NY or Wilmington, MA. The incidence of hepatocellular tumors in males and females is summarized below:

TABLE 27: HISTORICAL CONTROL DATA FOR INCIDENCE OF HEPATOCELLULAR TUMORS IN CD-1® MICE (18-MONTH STUDIES)

Tumor/parameter	Males	Females
Hepatocellular adenoma:		
No. examined	770	769
No. tumors	83	5
Mean % incidence	10.78	0.65
Range % incidence	0-19.3	0-2.00
Hepatocellular carcinoma:		
No. examined	770	769
No. tumors	38	3
Mean % incidence	4.94	0.39
Range % incidence	1.25-11.54	0-2.00

Conclusions of the original study report review and previous CARC determinations: In the third cancer peer review of acetochlor it was determined that the statistically significantly increased combined incidence of hepatocellular adenomas/carcinomas in male mice at 1000 ppm was treatment-related and that toxicity at the high dose of 1000 ppm was minimal and animals could have been tested at a higher dose. The finding of liver tumors in males was part of the WOE considerations in determining the cancer classification of acetochlor.

Conclusions of the PWG report: Although the majority of findings of neoplasms from the original study report were confirmed, there were several instances of differences (e.g., hyperplasia reclassified as adenoma, a few carcinomas reclassified as benign or proliferative lesions or carcinomas reclassified as adenomas). The PWG concluded that there were no treatment-related increases in hepatocellular neoplasms in either sex in this study and did not identify any statistically significant increases in any tumor type.

Statistical analysis of the PWG findings by HED indicated a significant increase in liver carcinomas in males at the high dose ($p<0.05$). No significant trend was identified.

Dr. Pletcher's evaluation of the PWG report (Pletcher, 2002f): Dr. Pletcher agreed with the conclusions of the PWG report and recommended that the data be considered valid.

Conclusions of the CARC: The Committee concluded that the liver tumor incidence did not show a treatment-related effect. Although a significant increase in carcinoma was identified in males at 1000 ppm, it was within historical control range.

d. Discussion of Reanalysis of Histiocytic Tumors in Females

A full discussion of the results of the original pathology evaluation of this study are presented in the third Cancer Peer Review Committee document on acetochlor.

Conduct of PWG: Details of the conduct of the PWG reevaluations are summarized above under the discussion of the1983 mouse study (Section III.5.d of this document). The findings of the PWG consensus with statistical analyses performed by HED are shown below:

Table 28. Acetochlor - 78-week Mouse Study

Female Histiocytic Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	10	100	1000
Sarcomas (%)	2/59 (3)	1[a]/60 (2)	0/60 (0)	5/60 (8)
p =	0.0248*	0.8813	1.0000	0.2264

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 41.
[a]First sarcoma observed at week 41, dose 10 ppm.
Note: Significance of trend denoted at control.

Historical control values for histiocytic sarcoma in females: Values are available for 24-month studies in CD-1 female mice. Incidence has been reported to range from 0-10% (average 3.76%) (Charles River Laboratories data). Data from Inveresk Research International gives similar values of 0-10% (average 4.6%). The PWG report states that these tumors are rare in the first year, are more common in females and increase steeply with age after 18 months.

Conclusions of the original study report review and previous CARC determinations: The incidence of histiocytic sarcoma in the 78-week mouse study was not considered to be increased by dietary exposure to acetochlor.

Conclusions of the PWG report: Most of the original pathologist's diagnoses were confirmed by the PWG. The PWG determined that acetochlor did not cause a treatment-related increase in the incidence of histiocytic sarcoma in female mice in this study. The incidence observed was considered to be within the normal variation of spontaneous tumor incidence.

In the statistical analyses performed by HED, a positive trend with dose was reported, but no statistical significance by pairwise comparison of treated animals with controls was observed.

Dr. Pletcher's evaluation of the PWG report (Pletcher, 2002g): The discussion of this reevaluation by Dr. Pletcher is summarized above for the 23-month mouse study.

Conclusion of the CARC: Although a statistically significant increase in histiocytic sarcoma was not observed in treated females in this study (by pairwise comparison), the CARC concluded that the incidence was increased by treatment with acetochlor, based on comparison with the incidence in the 23-month mouse study. Comparison of the two mouse studies showed a positive dose-response for this tumor.

e. Statistical Analysis of Mortality Rates

Mortality rates in males were (control to high dose) 22%, 20%, 28% and 33%. Mortality rates in females were 34%, 22%, 24% and 28%. A significant increasing trend was observed for males ($p<0.05$) but not in females and no significant increases in mortality were observed by pairwise comparisons to controls (L. Brunsman, 2004).

f. Adequacy of Dosing for Assessment of Carcinogenicity

No evidence of systemic toxicity was observed in either male or female mice. Increased incidence of nephropathy in interim sacrifice males, along with increased kidney weights, and increased incidence of tubular basophilia were observed at all dose groups. An increased incidence of bronchiolar hyperplasia was also observed in males at 100 and 1000 ppm (13%, 10%, 39% and 38%, control to high dose). In the second cancer peer review of acetochlor, the Committee determined that the limited toxicity observed at the high dose of 1000 ppm indicated that although "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."

IV. TOXICOLOGY

1. Metabolism

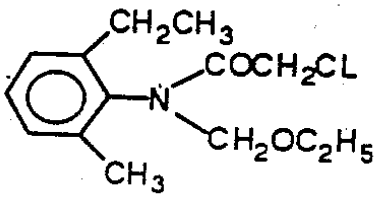
The available data on the metabolism of acetochlor is discussed in the Mode of Action Assessment Document document (Part 2) as well as the third Carcinogenicity Peer Review of Acetochlor document.

2. Mutagenicity

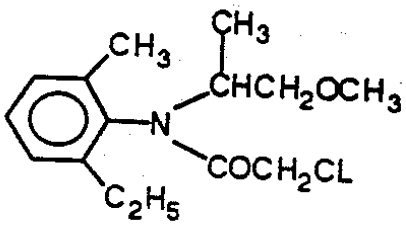
A detailed discussion of the mutagenicity of acetochlor is provided in the Mode of Action Assessment Document document (Part 2).

3. Structure-Activity Relationship

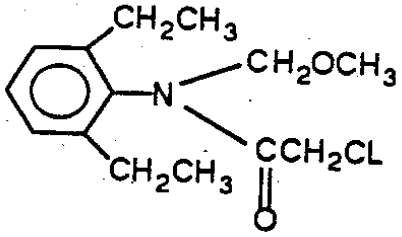
Acetochlor is a member of the chloroacetanilide group of herbicides. Structural similarities exist for acetochlor to alachlor, propachor, butachlor and metolachlor. Structures for these chemicals are shown below in Figure 1, along with structures for allidochlor and SAN 582H. In addition, this class of compounds has been reviewed as part of the Common Mechanism of Toxicity (FIFRA SAP, March 19, 1997 meeting).



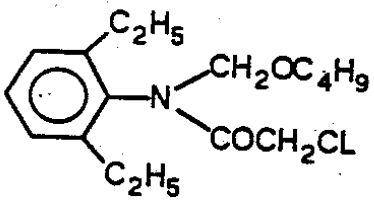
Acetochlor



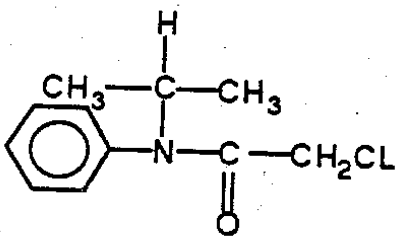
Metolachlor



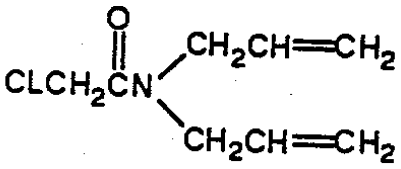
Alachlor



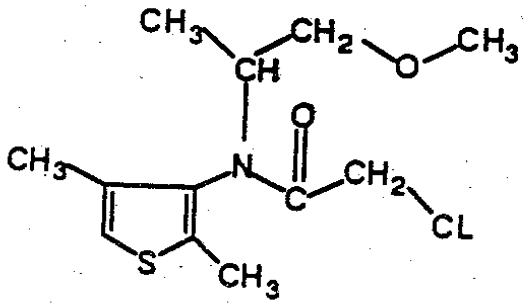
Butachlor



Propachlor



Allidochlor



SAN 582H

Figure 1

Alachlor [2-chloro-2'6'-diethyl-N-(methoxymethyl)-acetanilide); PC Code 090501; CAS No. 15972-60-8] is currently classified as **"likely to be a human carcinogen at high doses, but not likely at low doses**," in accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996). A non-linear margin-of-exposure (MOE) approach was selected for the purpose of human risk assessment. This conclusion of the fourth and most recent carcinogenicity peer review of alachlor was determined with consideration of comments by the FIFRA Scientific Advisory Panel on the mode of action data on alachlor (meeting of October 30, 1996). Classification is based on treatment-related increases in several tumor types in studies on the Long Evans rat: (1) nasal epithelial cell adenomas and adenocarcinomas, (2) thyroid follicular cell adenomas and carcinomas and (3) a rare mixed gastric tumor. Alachlor induced unscheduled DNA synthesis *in vivo* in rats, some of its metabolites are weakly genotoxic in the Ames assay and it was clastogenic *in vitro* but not *in vivo*. Overall, alachlor is considered to have genotoxic potential but possibly only at higher dose levels that may cause depletion of GSH or saturation of protein binding.

The Committee, in agreement with the FIFRA SAP, determined that (1) thyroid tumors in the rat were observed only at an excessive dose and were produced by a hormonal mode of action; (2) nasal tumors were observed only at dose levels in excess of anticipated human exposures. Mechanistic data suggested that tumors resulted from toxicity of a diethylbenzoquinone imine metabolite of alachlor that may be of greater significance in the rat than human, but there was insufficient evidence to consider them not of relevance to humans and (3) the gastric tumors were considered to be a direct-contact effect resulting from a nongenotoxic mechanism secondary to change in pH that may also be relevant to humans. Lung tumors in mice were discussed but discounted as not related to treatment.

Butachlor [N-(butoxymethyl)-2-chloro-N-(2,6-diethylphenyl)-acetamide; PC Code 112301; CAS NO. 23184-66-9] is currently classified as **"likely to be a human carcinogen"**, in accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996). A combination of linear low-dose and non-linear approaches was selected for human risk assessment. Classification of butachlor is based on treatment-related increases in several tumor types in studies on the Sprague-Dawley rat: (1) thyroid follicular cell adenomas and carcinomas in males and females, (2) nasal mucosal adenomas and carcinomas in males and females, (3) rare tumors of the glandular stomach in females (carcinoids of the fundus, as determined by a reevaluation of stomach slides) and (4) renal cortical tumors in males and females. Butachlor was negative in genotoxicity studies submitted to the Agency but published reports indicate some *in vitro* clastogenicity-it was concluded that it was not the primary cause of thyroid, nasal or stomach tumors but may contribute at higher dose levels. In addition, the findings of tumors induced at one or more of the same sites by three structurally related chloroacetanilide compounds (acetochlor, alachlor and propachlor) were noted. The Committee concluded that the relevance of the tumors to humans could not be completely ruled out.

The Committee determined that (1) thyroid follicular tumors produced by butachlor were produced by a hormonal mode of action (2) nasal tumors were due to a mechanism of toxicity based on the formation of a quinone-imine intermediate of 2,6-diethylaniline (DEIQ) but that there was insufficient evidence to consider them not of relevance to humans and (3) the gastric tumors were considered to be a direct-contact effect resulting from a nongenotoxic mechanism secondary to change in pH that may also be relevant to humans. A non-linear margin of exposure (MOE) approach was selected for these tumors based on mode of action data. A linear low-dose approach was selected for the renal cortical tumors, which are rare and observed in both males and females.

Alveolar/bronchiolar adenomas and carcinomas were observed in male and female CD-1 mice exposed to butachlor but were not considered treatment-related because pairwise significance was not observed in females, no increases in carcinomas were observed in males or females and significant increases were observed only in males at an excessive dose. Hepatocellular adenomas/carcinomas in rats were not considered treatment-related due to lack of statistical significance of the increase, lack of evidence of progression and incidences not exceeding historical controls.

Propachlor (2-chloro-N-isopropylacetanilide; PC Code 019101; CAS No. 1918-16-7) is currently classified as **"likely to be a human carcinogen"** in accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996). A combination of linear low-dose and non-linear approaches was selected for human risk assessment. Classification of propachlor was based on treatment-related increases in thyroid C-cell adenomas and carcinomas in males and female Sprague Dawley rats, combined ovarian granulosa/theca cell benign and malignant tumors in female Sprague Dawley rats, a single rare stomach carcinoma in a male F344 rat and hepatocellular adenomas and carcinomas in male CD-1 mice. Propachlor did not induce nasal tumors in the rat. Propachlor showed *in vitro*, but not *in vivo* clastogenicity. A linear low-dose approach was selected for human risk characterization based on the ovarian tumors in female rats and liver tumors in male mice, along with liver hypertrophy in mice.

Metolachlor [(S)-2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide; PC Code 108801; CAS No. 51218-45-2] is currently classified as a **Group C (possible human) carcinogen**, in accordance with the previous EPA Guidelines for Carcinogen Risk Assessment of 1986 and has not been reevaluated under current guidelines. Classification was based on increased incidence of liver adenomas and carcinomas in female Charles River rats and trend for these tumors in males. Metolachlor is also of concern for nasal turbinate tumors. Metolachlor was negative in available genotoxicity tests but induced cell proliferation in hepatocytes of treated rats. A MOE approach was recommended for estimation of human risk, based on the increased incidence of liver tumors in female rats.

Dimethenamid or SAN 582H [N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-(S)-acetamide; PC Code 120051; CAS No. 87675-68-8] is currently classified as a **Group**

C (possible human) carcinogen, in accordance with the previous EPA Guidelines for Carcinogen Risk Assessment of 1986. Classification was based on increased incidence of hepatocellular adenomas and carcinomas in male Sprague Dawley rats. In addition, mutagenicity concerns (positive UDS and CHO chromosomal aberration assays) and unanswered questions regarding nasal tumors were cited.

4. Subchronic and Chronic Toxicity

See summary in Third Carcinogenicity Peer Review of Acetochlor.

5. Mode of Action Studies

a. Nasal tumors: Numerous studies have been submitted in support of proposed mode of action for toxicity and carcinogenicity of acetochlor to the nasal olfactory mucosa of the rat. Data pertinent to this discussion is presented in the Mode of Action Assessment Document (Part 2).

b. Thyroid follicular cell tumors: A mechanistic study was submitted to evaluate effects in the liver and thyroid following exposure to acetochlor (MRID 44496208, with the thyroid evaluation portion of MRID 44496207 included in report).

Acetochlor (tech., 95.2% a.i.) was administered in the diet to male Sprague Dawley rats according to the following dosing schedules: (1) (MRID 44496208) 20 rats/dose group at 0, 1750 or 5000 ppm (equivalent to 0, 100.6 or 280.9 mg/kg/day, average daily intake) for 14, 28 or 56 days and (2)(MRID 44496207) 15 rats/dose group for 90 days and 10 rats/dose group for 160 days at 0, 200, 1750 or 5000 ppm (equivalent to 0, 10.4, 91.9 or 270.3 mg/kg/day, average daily intake). Animals were evaluated for clinical signs of toxicity, mortality, body weight and food consumption. Blood samples were collected at terminal sacrifice for measurement of thyroid stimulating hormone (TSH), thyroxine (T4) and triiodothyronine (T3) via radioimmunoassay (all groups except the 200 ppm animals and the 160 days' groups). At sacrifice, liver and thyroid glands were weighed. Thyroids were processed for light microscopy and liver microsomal fractions were prepared from the livers for T4-UDPGT activity on 5 animals/group/time point (except 200 ppm animals and the160 days' group) using [^{125}I]-T4 as substrate. There were no recovery groups to evaluate reversibility of the effects.

Mortality and clinical signs were unaffected by treatment. At 5000 ppm, mean body weight was significantly decreased relative to controls at all time points (range -6% to -12% to day 56; -11%, day 90 and -17%, day 160).

Effects on thyroid hormone levels: The results of analyses of serum levels of TSH, T4 and T3 throughout the studies are shown below:

TABLE 29. Thyroid function studies of rats fed acetochlor up to 90 days[a]

Study day	Dose (ppm)		
	0	1750	5000
TSH (ng/mL) - MRID 4496208			
14	4.18 ± 1.79	4.42 ± 1.73	5.41** ± 2.42
28	2.50 ± 1.11	3.32 ± 1.70	4.56** ± 1.66
56	3.22 ± 1.45	4.52* ± 2.59	4.35 ± 1.91
TSH (ng/mL) - MRID 44496207			
90	1.45 ± 0.91	1.70 ± 1.46	1.42 ± 1.15
T_4 (ug/dL) - MRID 4496208			
14	2.98 ± 0.54	4.18** ± 0.58	4.22** ± 0.75
28	3.16 ± 0.84	3.30 ± 1.08	3.11 ± 0.87
56	2.13 ± 0.71	2.60 ± 0.74	2.57 ± 0.66
T_4 (ug/dL) - MRID 44496207			
90	3.60 ± 1.04	3.85 ± 0.65	3.44 ± 0.72
T_3 (ng/dL) - MRID 4496208			
14	60.81 ± 12.48	53.69 ± 7.04	52.30* ± 10.66
28	56.33 ± 15.17	52.04 ± 12.43	53.21 ± 11.89
56	55.94 ± 14.78	57.27 ± 14.40	60.36 ± 12.44
T_3 (ng/dL) - MRID 44496207			
90	60.99 ± 17.30	72.14 ± 5.81	62.46 ± 12.56

[a] Data extracted from Table 4, page 25 of MRID 4496208
*$p \leq 0.05$; $p \leq 0.01$

At 1750 ppm, TSH showed statistically a significant increases at day 56 (+40% above controls), but was also non-significantly increased at day 28 (+33%). At 5000 ppm, TSH was significantly increased at days 14 and 28 (+29% and +82%, respectively), with a non-significant increase of +35% at day 56. T3 levels showed significant reduction only at day 14 in the 5000 ppm group (-14% less than controls), with the 1750 ppm group showing a slight, non-significant reduction of about 12%. T4 levels showed a statistically significant increase of about 40% in both the 1750 and 5000 ppm groups at day 14 only.

The effects of acetochlor on thyroid and liver weights are presented below:

TABLE 30. Absolute and relative liver and thyroid weights of rats fed acetochlor up to 160 days[a]

Study day	Dose (ppm)			
	0	200	1750	5000
Liver weight (g) - MRID 4496208				
14	20.4 (4.2)[b]	-	21.5 (4.6**)	23.8** (5.4**)
28	21.2 (3.9)	-	23.6* (4.5**)	25.2** (5.1**)
56	21.7 (3.7)	-	25.2** (4.2**)	27.6** (5.0**)
Liver weight (g)- MRID 44496207				
90	23.7 (3.8)	23.9 (3.7)	26.9* (4.2**)	27.7** (4.9**)
160	23.5 (3.4)	24.3 (3.5)	27.0 (3.9**)	25.8 (4.4**)
Thyroid weights (mg) - MRID 4496208				
14	20.3 (4.2)[c]	-	22.4 (4.8*)	23.8** (5.4**)
28	23.3 (4.3)	-	26.4* (5.1**)	26.2* (5.3**)
56	24.4 (4.1)	-	25.8 (4.3)	27.~~2~~ 1 (4.9**)
Thyroid weights (mg)- MRID 44496207				
90	24.5 (3.9)	27.4 (4.3)	27.2 (4.3)	28.1 (5.0**)
160	30.4 (4.4)	30.0 (4.4)	35.3 (5.2)	30.0 (5.2)

[a] Data extracted from Tables 2 and 3, page 23-24, of MRID 4496208
[b] The number in parentheses is the relative organ weight in %.
[c] relative thyroid weight in % x 10^3

Statistically significant, dose-dependent increases in mean absolute and relative liver weights were observed at all time points for animals dosed at 1750 ppm (+10 to 15% above controls, relative weight) and 5000 ppm (+29 to 30%, relative weight). Mean relative thyroid weights were statistically significantly increased at all time points except day 160 at 5000 ppm (+19 to 29% above controls), with significant increases at 1750 ppm occurring only at days 14 and 28 (+14 to 19%). At 200 ppm, no increases in mean liver or thyroid weight were observed at days 90 or 160 (earlier time points not tested at that dose level).

The effects of acetochlor treatment on levels of hepatic T4-UDPGT activity are shown below:

TABLE 31. Hepatic T_4-UDPGT Activity of rats fed acetochlor up to 90 days[a]

Study Day	Dose (ppm)			
	0	200	1750	5000
MRID 4496208				
14	0.49[b] 5.81[c] 117.4[d]	- - -	0.57 7.81 167.2*	0.78** 10.81** 256.1**
28	0.46 5.04 118.4	- - -	0.63** 6.65* 149.9**	0.82** 9.55** 237.1**
56	0.51 6.68 125.7	- - -	0.69* 8.58 226.0**	0.78** 8.98 237.2**
MRID 44496207				
90	0.38 7.59 189.0	0.46 8.56 181.2	0.45 7.77 210.6	0.63** 13.72** 361.1**

[a] Data were extracted from Table 5, page 26 of MRID 4496208
[b] Activity expressed as pmol/min/mg protein
[c] Activity expressed as pmol/min/gram liver
[d] Activity expressed as pmol/min/total liver
* $p \leq 0.05$; $p \leq 0.01$.

At 5000 ppm, statistically significant increases in hepatic T4-UDPGT activity (approximately +50 to 75% above controls when expressed per mg protein) were observed at 14, 28, 56 and 90 days (not examined at day 160). Statistically significant increases were also observed at 1750 ppm at day 28 and 56, although values were nonsignificantly increased at days 14 and 90. Animals in the 90-day group were also evaluated at 200 ppm and showed no activity increases compared to controls.

Although animals were not tested for recovery and the low dose group of 200 ppm was not evaluated for the thyroid hormone endpoints, the data are suggestive of perturbation of thyroid-pituitary homeostasis which may result in thyroid hyperplasia and tumor formation.

The CARC concluded that the thyroid tumors in rat were secondary to disruption of pituitary-thyroid homeostasis, as discussed in detail below in Section V.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity:

When considering the carcinogenicity data for acetochlor, the Committee determined that the tumor reevaluations performed by the PWG were acceptable. The diagnoses prepared

in the PWG reports were, therefore, used as the basis for the HED statistical analysis of these tumors. HED analyses from previous cancer assessments were used for tumors that were not reevaluated by the PWG (rat nasal, thyroid; mouse ovary).

A. Sprague Dawley Rats: **The CARC concluded that acetochlor was carcinogenic to male and female rats based on increased incidence of nasal epithelial cell tumors in both sexes.**

- Adequacy of dosing: The Committee considered three two-year dietary chronic toxicity/carcinogenicity studies in the rat. Dosing was considered adequate in all three studies, based on decreased body weight at 1500 ppm (study 1), decreased body weight at 1000 ppm (study 2) and decreased body weight at 1750 ppm (study 3). The highest dose tested, 5000 ppm (study 1), was determined to cause excessive toxicity based on sharply reduced body weights and in females, increased mortality.

- Nasal olfactory epithelium (these lesions were not reevaluated by the PWG): Nasal olfactory epithelial tumors observed in male and female rats were determined to be treatment-related and were considered as part of the cancer classification of acetochlor. Statistically significant increases in rare olfactory epithelial papillary adenomas were observed at 1000 ppm and above (with a positive trend also identified), and tumors were observed in all three two-year dietary studies, although the incidence in study 1 was unexplainedly low relative to the other studies. In males, a single tumor at 500 ppm (study 1) was also observed which was considered treatment-related due to its rare spontaneous occurrence. At 500 ppm (study 1), 1000 ppm (study 2), 1500 ppm (study 1) and 1750 ppm (study 3), incidence in males was 1%, 20%, 9% and 50%, respectively. Incidence in females at these dose levels was 0%, 28%, 3% and 57%, respectively. Adenocarcinoma was only observed in study 3 at 1750 ppm (2 males and 1 female). Although nasal tumors were also reported at the high dose of 5000 ppm in study 1 (papillary adenomas 26%, males and 1%, females; also 2% papillary adenocarcinomas in males and 0% in females), these findings were not considered as part of the cancer weight of the evidence assessment because this dose was associated with excessive toxicity in both sexes.

  Nasal tumors were also observed in a two-generation rat reproductive toxicity study at 1750 ppm in parental F0 males (15%) and females (21%) and at 600 and 1750 ppm in parental F1 males (12% and 31%) and females (4% and 74%). Olfactory epithelial hyperplasia was also observed in both sexes at 1750 ppm (and in F1 females at 600 ppm). One or two incidences of respiratory epithelial hyperplasia were also observed in the F0 animals and F1 males.

- Nasal tumors were identified at 19 weeks of treatment. The rapid development of tumors was noted, but determined to be consistent with nasal olfactory cell proliferation observed after 160 days and with nasal tumor formation after 5 months of treatment with the closely related compound alachlor. Tumor incidence did not exceed that of comparable dose levels in the chronic studies (compared on a mg/kg/day basis).

- Thyroid (these lesions were not reevaluated by the PWG): Thyroid follicular cell tumors were considered to be related to treatment but were not considered as part of the cancer quantification, based on relatively low incidence and evidence for disruption of thyroid hormonal homeostasis as the mode of action. Statistically significant increases in thyroid adenoma and combined adenoma/carcinoma were observed in female rats at 1750 ppm in study 3 (adenoma 1%, 2%, 5% and 10%; combined 1%, 1%, 5% and 11% at 0, 18, 175 and 1750 ppm) and a positive trend only was identified in females in study 2 (3%, 5%, 6% and 11% at 0, 10, 100 and 1000 ppm). Males in study 1 showed significant increases only at 5000 ppm, an excessive dose. However, a positive trend only for adenoma was identified in study 3 (4%, 2%, 4% and 10% at 0, 18, 175 and 1750 ppm); combined tumors also were increased but not significantly (6%, 8%, 4% and 16%).

- Liver (these lesions were reevaluated by the PWG): Liver tumors were not considered as part of the cancer classification, based on the following factors: statistically significant increases in males and females for combined adenoma/carcinoma of the liver (and adenoma in females) were observed and exceeded historical control values, but only at 5000 ppm, an excessive dose. At 1000 ppm, a significant increase in adenomas in females that slightly exceeded historical control values was noted but it was noted that the concurrent control value of 0% was lower than historical controls and that liver tumors were not increased at 1500 or 1750 ppm in the other two studies.

- Femur (these lesions were reevaluated by the PWG): A rare chondroma of the femur was reported at 1750 ppm (study 3) in one male and one female but was rediagnosed by the PWG as cartilage hyperplasia. Accordingly, it was not considered as part of the cancer classification.

- Stomach (these lesions were reevaluated by the PWG): A rare basal cell tumor of the stomach was reported in one female at 1750 ppm (study 3). The PWG reevaluation rediagnosed this tumor as a well-differentiated squamous cell carcinoma of the nonglandular stomach. A squamous cell papilloma with poorly differentiated squamous cell carcinoma in one male at 1750 ppm was identified. The CARC did not include this tumor in the weight-of-evidence considerations for cancer classification because it was of low incidence and probably was secondary

to local irritation of the rat forestomach and, therefore, not considered relevant to human cancer risk.

B. CD-1 Mice

- Adequacy of dosing: The Committee evaluated two carcinogenicity studies in the mouse. Dosing was considered adequate in mice. In study 1 (23-month) the high dose of 5000 ppm was determined to be excessive in both sexes based on high mortality and sharply reduced body weights. The mid dose of 1500 ppm was considered adequate in females based on decreased body weights in females. In males at 1500 ppm no toxicity was reported, but the dose was considered acceptable because decreased body weight was observed at 1200 ppm in a 6-week range-finding study. Although no toxicity was reported in study 2 (up to 1000 ppm for 78 weeks), it was considered adequate when both studies were considered together to assess carcinogenicity, particularly since increased incidence of lung tumors and histiocytic sarcoma were observed.

4. Lung (these lesions were reevaluated by the PWG): Statistically significant increases in the incidence of lung tumors with a positive trend were observed in female mice in the 23-month study (0, 500, 1500 and 5000 ppm) at all dose levels: adenomas 2%, 17%, 22% and 23%; carcinomas 0%, 9%, 2% (NS) and 18%; and combined adenomas/carcinomas 2%, 23%, 25% and 33% (high dose of 5000 ppm considered excessive). There were no increases observed in males. Incidence exceeded available historical control range for adenoma (9.6% to 14.5%) at 500 and 1500 ppm and were within the high normal range for combined tumors (13.8 to 26.6%).

   In the 78-week study (0, 10, 100 and 1000 ppm), the incidence of adenoma and combined tumors was significantly increased in males at 1000 ppm with a positive trend (adenoma 15%, 8%, 19% and 30%; combined 18%, 13%, 22% and 33%). Females showed only a positive trend for increased incidence of combined tumors only (9%, 8%, 14% and 18%); the incidence of adenomas was increased but showed no trend or pairwise increases (7%, 8%, 10% and 15%). Incidence exceeded in-laboratory historical control values for adenoma and combined tumors at 1000 ppm in males (adenoma 4.0 to 17.3%, combined 15% to 28.9%) and females (adenoma 0% to 9.6% and combined 0% to 9.6%). An increased incidence of bronchiolar hyperplasia was also observed at 100 and 1000 ppm (13%, 10%, 39% and 38%). Based on these findings, the Committee concluded that lung tumors were related to treatment and considered them as part of the cancer classification for acetochlor.

5. Histiocytes (these lesions were reevaluated by the PWG): Statistically significant increases in the incidence of histiocytic sarcoma (using liver and uterus for each animal) were observed in female mice at 500, 1500 and 5000 ppm in study 1 (0%, 7%, 15% and 15%; high dose of 5000 ppm considered excessive). A positive trend only was observed in study 2 due to increases at the high dose of 1000 ppm (3%, 2%, 0% and 8%). The incidence at 1500 ppm exceeded the available historical control data range of 0 to 10%. Combination of the animals from both studies showed a positive dose-response. Based on these findings, the Committee concluded that histiocytic sarcomas were related to treatment and considered them as part of the weight-of-evidence for cancer classification.

6. Liver (these lesions were reevaluated by the PWG): Liver tumors were not considered as part of the cancer classification. Statistically significant increases in the incidence of liver adenoma and combined adenoma/carcinoma in both sexes (and carcinoma in males) were observed at 5000 ppm (study 1) and exceeded historical control values, but toxicity at this dose was considered excessive.

7. Kidney (PWG): Renal adenomas and sarcomas diagnosed by the PWG were not considered as part of the cancer classification because they were only observed at the excessive dose of 5000 ppm (study 1). Additionally, an expert on renal pathology who provided his opinion to the PWG did not consider them to be related to treatment.

8. Ovary (no PWG): Benign ovarian tumors (combined adenoma, granulosa cell and luteoma) were not considered as part of the cancer classification. Small but statistically significant increases at 1500 and 5000 ppm in study 1 (not reevaluated by the PWG) were observed. However, the consulting pathologist (J. Pletcher) noted that it was not appropriate to combine these tumors and due to the low incidence of each, they were not considered treatment-related.

2. Mutagenicity

The CARC concluded that available genetic toxicology studies indicate that genotoxicity is not likely to be a primary cause of tumor induction for acetochlor. Positive findings in *in vitro* clastogenicity assays appear to result from cytotoxicity secondary to oxidative damage (depletion of GSH). The conclusions are based on the following:

- Gene mutation studies do not provide clear evidence of mutagenic potential in either bacterial or mammalian cell *in vitro* test systems. Acetochlor is not mutagenic in several bacteria (*Salmonella typhimurium)* gene mutation assays. A weak positive response was observed in a CHO gene mutation assay at cytotoxic concentrations. A positive response in a mouse lymphoma assay was also

accompanied by cytotoxicity. Because colony size distribution was not evaluated, it could not be determined whether the response was due to gene mutation or clastogenicity.

- *In vitro* chromosomal aberration studies in human lymphocytes are positive, showing breaks, fragments and minutes and accompanied by reduction in mitotic indices. Comparison of lymphocytes from separated blood showed a sharply increased frequency of aberrations compared to whole blood lymphocytes, which was attributed to the absence of GSH in the isolated lymphocytes. Clastogenicity was observed only at cytotoxic doses and the types of cytogenetic aberrations observed were consistent with cytotoxicity. *In vivo* assays (rat bone marrow cytogenetic assay, mouse micronucleus assay) were negative up to cytotoxic/systemic toxic doses.

- Rat dominant lethal assays and comet assays on rat nasal olfactory and respiratory cells were negative. A sister chromatid exchange assay gave a weak positive response. An *in vivo* UDS assay gave a weak positive response but only at a dose that caused significant hepatic necrosis and a sharp reduction in hepatocellular GSH.

- This conclusion is consistent with the available data for the related chloroacetanilide, alachlor.

3. Structure-Activity Relationships

- Acetochlor is structurally related to other chloroacetanilide herbicides, including alachlor, butachlor, propachlor and metolachlor. These chemicals have overlapping, but not identical, tumor profiles as compared below. The FIFRA SAP concluded in 1997 that acetochlor, alachlor and butachlor share common modes of action for induction of nasal epithelial tumors and thyroid follicular cell tumors and the CARC affirmed this conclusion.

TABLE 32: SUMMARY OF TUMOR FINDINGS FOR RELATED CHLOROACETANILIDES

CHEMICAL	TUMORS	CANCER CLASSIFICATION
Alachlor	Rat nasal epithelial, thyroid follicular, rare mixed gastric	Likely to be a human carcinogen at high doses but not low doses. MOE approach.
Butachlor	Rat nasal epithelial cell, thyroid follicular cell, rare stomach tumors, renal cortical tumors	Likely to be a human carcinogen. MOE approach for all tumors except renal-use linear low-dose approach for renal tumors
Propachlor	Rat thyroid c-cell, ovarian granulosa/theca cell Mouse hepatocellular	Likely to be a human carcinogen. Linear low-dose extrapolation for ovarian tumors.
Metolachlor	Rat hepatocellular, nasal epithelial	Group C (probable human carcinogen). MOE approach for liver tumors.
SAN H582	Rat hepatocellular (males)	Group C (possible human carcinogen). Linear low-dose extrapolation.

4. Mode of Action:

- **Thyroid follicular cell tumors of the rat** - The Committee concluded that the available data on acetochlor, together with SAR on alachlor, are adequate to support an antithyroid mode of action for thyroid follicular cell tumor formation. According to this model, perturbation of thyroid-pituitary homeostasis, e.g. chronically reduced circulating thyroid hormone levels due to increased hepatic clearance by UDPGT or decreased thyroid hormone synthesis, may result in an increased level of circulating TSH, followed by increased follicular cell division, thyroid hyperplasia and enlargement and eventually an increase in thyroid tumors. The available data on acetochlor, which include a mechanistic study examining circulating thyroid hormone and liver UDPGT levels, indicate thyroid enlargement (hyperplasia was not demonstrated), and increased liver UDPGT activity.

  According to Agency guidelines (see Section V.5, below), the data on acetochlor *per se* do not completely meet the criteria assigned by the Agency for demonstration of the mechanism; for example, hyperplasia of the thyroid and reversibility of the treatment-related thyroid effects upon cessation of treatment were not demonstrated. However, the CARC determined that the data are

adequate for supporting this mode of action when considered together with the more complete data available for thyroid tumorigenesis by the closely related chloroacetanilide, alachlor.

- **Nasal epithelial cell tumors of the rat** - Mechanistic studies, together with the genotoxicity profile of acetochlor and mode of action data previously submitted for the structurally related compound alachlor, were considered adequate by the Committee to support a non-mutagenic mode of action for nasal tumor formation (see Mode of Action Assessment document for details). Based on this information, nasal tumor induction is dependent upon the following events: (1) a quinone imine intermediate forms during metabolism of acetochlor (from acetochlor sulfoxide or possibly ethylmethyl aniline (EMA); (2) the quinone imine moiety causes oxidative damage to the nasal olfactory cells; (3) oxidative damage results in cytotoxicity; (4) a proliferative response to cellular injury results in hyperplasia and respiratory metaplasia; (5) spontaneous mutations are fixed, resulting in tumors as a result of the cytotoxicity-driven cell proliferative response. The Committee recognized that rats are likely to be more sensitive than humans by this mode of action but determined that the mechanism may still have relevance to humans particularly via the alternate EMA pathway for quinoneimine formation.

5. Considerations of the Use of the Non-linear Extrapolation Approach for Thyroid Tumors Induced by Acetochlor:

- Background: When evaluating acetochlor, the CARC considered whether use of a non-linear extrapolation approach for thyroid neoplasms was appropriate. The quotations which follow are taken from the Agency's Policy Document entitled "Assessment of Thyroid Follicular Cell Tumors", March 1998 (EPA/630/R-97/002):

"...Treatments of rodents that cause *thyroid-pituitary disruption* result in chronic reduction in circulating thyroid hormone levels, increase in TSH levels and the development of increased cell division, increased size and numbers of thyroid cells, increased thyroid gland weight and, finally, tumors of the thyroid. In some cases, there is also an increase in tumors of the pituitary cells that produce TSH. Cessation of treatment early in the process before tumor development results in reversal of processes back towards normal."

When assessing tumors of the thyroid, "For those cases where thyroid tumors arise from chemically induced disturbances in thyroid-pituitary functioning, tumors are considered to be secondary to the adverse effects on the thyroid gland function that precede them. As exposures to such agents decrease, the likelihood of cancer decreases; risks may be seen as minimal at doses where there is no effect on thyroid-pituitary homeostasis. Generally,

homeostasis is considered to apply when serum T4, T3 and TSH levels and thyroid and pituitary morphology and growth are within their normal limits."

- Determination of whether neoplasms are due to thyroid-pituitary imbalance

The Science Policy Guidance discusses the types of information necessary to characterize the mechanism of thyroid carcinogenesis. These are addressed as they apply to acetochlor, as follows:

Consideration of whether the thyroid tumors associated with administration of acetochlor can be attributed to disruption of the thyroid-pituitary hormonal balance (demonstration of antithyroid activity). In addressing this point, the policy lists eight areas of inquiry for evidence demonstrating antithyroid activity (for additional details on the results described below, see individual study summaries presented earlier in this document or attached DERs for carcinogenicity and the mechanistic study):

a. Increases in cellular growth *in vivo* (evidence required):

Relative thyroid weights were statistically significantly increased in the 1983 rat dietary study at 1500 ppm and 5000 ppm in both males and females and at 500 ppm in females (also absolute in females at 500, 1500 and 5000 ppm). Thyroid weight was not evaluated in the 1988 rat study, although follicular cell tumors were observed at 1750 ppm in males and females. Follicular cell hyperplasia was not reported in this study, nor in the other two rat chronic toxicity/carcinogenicity studies. In the 1986 study which tested up to 1000 ppm, no effects on thyroid were observed. Although increased thyroid weights were reported in the thyroid mechanistic study at 1750 and 5000 ppm (up to day 90), microscopic evaluation of the tissues was not performed to evaluate proliferative events. There was no effect on thyroid weight at 200 ppm in the mechanistic study. Thyroid weights were not examined in two 90-day rat subchronic toxicity studies on acetochlor.

b. Hormonal changes (e.g., reduced thyroid hormones T3, T4 and increased TSH; evidence required):

In the thyroid mechanistic study (rats evaluated up to 90 days), circulating levels of T3 were slightly but statistically significantly reduced on day 14 at 1750 and 5000 ppm. Levels of T4 were significantly increased on day 14. TSH was significantly increased on day 14 and 28 at 5000 ppm; at 1750 ppm, at day 56 only. Levels of circulating thyroid hormones were not evaluated in the chronic rat studies.

c. Site of action (intra-thyroidal, peripheral tissues, liver or other sites; evidence required):

The thyroid mechanistic study provided evidence that liver is a site of action, as demonstrated by increased liver weights and levels of UDPGT by day 14 at 5000 ppm and day 28 at 1750 ppm. Evidence of the thyroid as a direct site of action was not provided.

d. Dose correlations (evidence required):

The available rat chronic studies and the thyroid mechanistic study indicate that the effects on liver and thyroid weights, liver UDPGT and thyroid hormones occur at dose levels at which thyroid follicular cell tumors have also been observed.

e. Reversibility (evidence required):

The available studies on acetochlor did not evaluate the reversibility of the increase in liver microsomal enzymes, thyroid peroxidase or circulating TSH, levels of circulating T3/T4, thyroid weight or follicular cell hypertrophy/hyperplasia.

f. Lesion progression (evidence desirable):

Only limited and suggestive evidence exists for lesion progression. Thyroid enlargement is observed within two weeks of treatment, as shown in the mechanistic thyroid study, but no microscopic data are available from that study that indicate a correlation with hyperplasia of the follicular cells. Treatment-related increases in hyperplasia of the thyroid were not reported in the chronic toxicity/carcinogenicity studies. Thyroid tumors were observed in the terminal sacrifice group, but no increase was observed in the interim sacrifice animals. No thyroid lesions were reported in two 90-day dietary studies in the rat, one testing up to 2000 ppm and one up to 6000 ppm. Thyroids were not weighed in the subchronic studies, nor in the 1988 rat study.

g. Structure-activity analysis (evidence desirable):

The closely related chloroacetanilide compound alachlor has also been shown to induce thyroid follicular cell tumors. Mechanistic studies on thyroid effects and UDPGT were performed. The data was determined by the CARC to support disturbance by alachlor of pituitary-thyroid homeostasis, but only at an excessive dose level. Butachlor, another related chloroacetanilide, also was

determined to cause thyroid tumors by this mechanism. Tumor profiles are compared above in Table 32.

h. Other studies (evidence desirable):

No additional data were submitted.

Consideration of the extent to which genotoxicity may account for the observed tumor effects. Acetochlor showed weak evidence of mutagenic potential in several assays, including the Ames assay and in mammalian systems. Although a positive response was reported in a mouse lymphoma L5178 assay, colony size distribution was not evaluated and it could not be determined whether the increase was due to mutagenic or clastogenic activity. Acetochlor showed clear evidence of clastogenicity *in vitro* (cultured human lymphocytes) but not *in vivo* (micronucleus assays and dominant lethal assays). UDS data on primary rat hepatocyte cultures are negative in one study and weakly positive in another at doses where significant hepatotoxicity (including necrosis and depletion of GSH) were observed. Available data suggest that clastogenic activity may be associated with cytotoxicity and oxidative stress (depletion of intracellular GSH). Comet assays using rat nasal olfactory and respiratory epithelial cells were negative.

Consideration of the occurrence of tumors in other tissues in addition to the thyroid. Other tumors attributed to treatment with acetochlor included nasal epithelial tumors in rats and in mice, lung tumors and histiocytic sarcoma. A mode of tumor induction in which cytotoxicity from a quinoneimine metabolic intermediate lead to increased cell proliferation was accepted. A mode of action for lung and histiocytic sarcoma induction is not available, although the genotoxicity data suggest that the clastogenicity of acetochlor is due to cytotoxicity from oxidative damage.

Consideration of the dose-response. Effects on thyroid hormone levels and thyroid weight were observed at the same dose levels (1750 and 5000 ppm) at which increases in thyroid tumors were observed (males only at 5000 ppm). Liver hypertrophy was also observed at these dose levels, along with increased UDPGT. Statistically significant increases in hepatocellular tumors were observed in both males and females, but only at 5000 ppm, a dose considered to exceed the MTD.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

The majority of CARC members concluded that acetochlor was likely to be carcinogenic to humans based on the following weight-of-the-evidence considerations:

(1) A dose-related increase was observed in the incidence of lung tumors in males and female mice in two mouse carcinogenicity studies, sometimes exceeding historical

controls. An increased incidence of bronchiolar hyperplasia in males in the 23-month study was also observed;

(2) A dose-related increase in the incidence of histiocytic sarcoma in female mice was observed in two carcinogenicity studies, exceeding historical controls at 1500 ppm;

(3) Dose-related increases in the incidence of rare nasal epithelial cell papillary adenomas and carcinomas were observed in male and female rats in three combined chronic toxicity/carcinogenicity studies, and in F0 and F1 parental animals in a rat multigeneration reproductive toxicity study;

(4) Treatment-related but marginal increases in thyroid follicular cell adenomas were observed in male and female rats but were not included in the cancer quantification because an antithyroid mode of action was accepted;

(5) The available genotoxicity data on acetochlor do not support a genotoxic mode of action for tumor induction. Gene mutation data are inconclusive. Tests for dominant lethality and unscheduled DNA synthesis in rodents and comet assays using rat nasal olfactory and respiratory epithelial cells are negative. Acetochlor is clastogenic *in vitro* in cultured mammalian cells at cytotoxic levels, but not *in vivo*. Clastogenicity is most likely secondary to cytotoxicity resulting from oxidative damage by a reactive quinoneimine intermediate formed during the metabolism of acetochlor.

(6) The structurally related chloroacetanilides alachlor and propachlor are also associated with an increased incidence of nasal epithelial tumors. The FIFRA SAP determined in 1997 that these compounds, together with acetochlor, could be combined for risk assessment purposes based on a common mode of action for induction of nasal tumors.

(7) The mechanistic data on nasal tumorigenesis of acetochlor in the rat, when considered together with the mutagenicity data on acetochlor and the mechanistic data on the closely related compound alachlor, are considered adequate to demonstrate a non-mutagenic mode of tumor induction involving cytotoxicity from oxidative damage by a reactive quinoneimine intermediate.

(8) The relevance of the tumors to humans cannot be discounted. In the case of the rat nasal tumors, although the rat is considered to be more sensitive than humans, there is still the potential for humans to metabolize acetochlor to reactive intermediates with carcinogenic potential secondary to oxidative damage and induction of cell proliferation.

Based on the majority opinion and in accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified acetochlor as "**Likely to Be Carcinogenic to**

Humans." The other tumor types identified in the mouse and rat studies were not considered as part of the weight-of-the-evidence for the reasons identified above.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The CARC determined that the carcinogenicity of acetochlor should be quantified by linear low dose extrapolation and that either lung tumors or histiocytic sarcomas of the mouse should be used to calculate the Q_1^*, depending upon which is more potent.

Two members of the CARC considered a non-linear (MOE) approach more appropriate for the quantitation of carcinogenic potential of acetochlor, based on the lack of convincing genotoxicity data on acetochlor and the identification of a mode of action for nasal tumors. A similar mode of action to that of nasal tumorigenesis should therefore be operative in tumorigenesis in other tissues. However, the majority of the committee concluded that in the absence of mechanistic data specifically demonstrating an alternative mechanism of carcinogenicity in these tissues (lung and histiocytic sarcoma of the mouse), the default approach of linear low-dose extrapolation approach should be used to quantitate the carcinogenic potential of acetochlor.

VIII. MINORITY DISSENTING OPINION

One reviewing toxicologist considered the submitted data to be insufficient to support the proposed non- mutagenic mechanism of action for nasal tumor carcinogenesis of acetochlor and prepared a summary of his concerns on this and other issues, which are attached as a minority dissenting opinion to this document (See Part 3).

IX. BIBLIOGRAPHY

SUBMITTED STUDIES

MRID No.	CITATION
00131088	Ahmed F.E. and Seely, J.C. (1983) Acetochlor: Chronic Feeding Toxicity and Oncogenicity Study in the Rat. Pharmacopathics Research Laboratories, Inc., Laurel, MD. Study No. PR-80-006. May 20, 1983. Unpublished report.
00131089	Ahmed., F.E., Tegeris, A.S. and Seely, J.C. (1983) MON-097: 24-Month Oncogenicity Study in the Mouse. Pharmacopathics Research Laboratories, Inc., Laurel, MD. Report No. PR-80-007. May 4, 1983. Unpublished report.
40077601	Naylor, M.W. and Ribelin, W.E. (1986) Chronic Feeding Study of MON 097 in Albino Rats. Study No. ML-83-200, Report No. MSL-6119; 93-190, Laboratory Project ID EHL-83107. September 25, 1986. Unpublished report.
40484801	Ribelin, W.E. (1987) Histopathology Findings in Noses of Rats Administered MON 097 in a Lifetime Feeding Study. Tegeris Laboratories, Laurel, MD and Monsanto Environmental Health Laboratory, St. Louis, MO. Laboratory Project No. ML-86-44/EHL 86027. November 4, 1987. Unpublished report (supplement to original study report).
41565119	Amyes, S.J. (1989) SC-5676: 78-Week Feeding Study in CD-1 Mice. Life Science Research Ltd., Suffolk, England. Study No. 87/SUC0012/0702. June 9, 1989. Unpublished report.
41592004	Virgo, D.M. and Broadmeadow, A. (1988) SC-5676: Combined Oncogenicity and Toxicity Study in Dietary Administration to CD Rats for 104 Weeks. Life Science Research Ltd., Suffolk, England. Study No. 88/SUC017/0348. March 18, 1988. Unpublished report.
44496204	Hardisty, J. (1997) Pathology Working Group Peer Review of Histiocytic Sarcoma in Female Mice from Two Long-Term Studies with Acetochlor. Experimental Pathology Laboratories, Inc., Research Triangle Park, NC. Laboratory Project CTL/C/3196. February 11, 1997. Unpublished report.
44496205	Hardisty, J. (1997) Pathology Working Group Peer Review of Hepatocellular Neoplasms in the Liver of Rats and Mice from Five Long-Term Studies with Acetochlor. Experimental Pathology Laboratories, Inc., Research Triangle Park,

NC. Laboratory Project ID CTL/C/3197. February 11, 1997. Unpublished report.

44496206 Hardisty, J. (1997) Pathology Working Group Peer Review of Neoplastic Lesions in the Lung of Male and Female Mice from Two Long-Term Studies with Acetochlor. Experimental Pathology Laboratories, Inc., Research Triangle Park, NC. Laboratory Project ID CTL/C/3198. February 11, 1997. Unpublished report.

44496207 Hotz, K.J., and Wilson, A.G.E. (1996). A study of the effects of acetochlor on rat nasal cell proliferation. Ceregen (EHL). Report No. MSL-14528. March 4, 1996. Unpublished report.

44496208 Hotz, K.J. and Wilson, A.G.E. (1996). Effects of dietary exposure of acetochlor on thyroid toxicity in male Sprague Dawley rats: Time course. Ceregen, A Unit of Monsanto Company, Environmental Health Laboratory (EHL), 645 S. Newstead, St. Louis, MO. Report No.: EHL95059 (with additional data from EHL94096). February 5, 1996. Unpublished report.

44496209 Hotz, K.J. and Wilson, A.G.E. (1998) A Study on the Effects of Acetochlor on Mouse Nasal Cell Proliferation. Ceregen (EHL), St. Louis, MO. Report No. MSL-14914. December 12, 1996. Unpublished report.

45357503 Milburn, G.M. (2001) Acetochlor: Multigeneration Reproduction Toxicity Study in Rats. Central Toxicology Laboratory, Alderley Park Macclesfield, Cheshire, UK. Study No. RR0818, Report No. CTL/RR0818, ARP Submission No. 852-544. February 16, 2001. Unpublished report.

45367401 Kronenberg, J. (2001) Summary of the Acetochlor Registration Partnership Position Regarding the Oncogenic Effects of Acetochlor in Rats and Mice. Acetochlor Registration Partnership. Report No. ARP/852/556. March 29, 2001. Unpublished report.

45367402 Hard, G. (2000) Expert Report on Renal Histopathological Changes in a Mouse Study (Monsanto Study PR-80-007) with Acetochlor. Gordon Hard, Chappaqua, NY. Study No. XX-2001-31. February 21, 2000. Unpublished report.

45367403 Hardisty, J. (2001) Pathology Working Group Peer Review of Proliferative Lesions in the Kidney of Female Mice from a 24-Month Oncogenicity Study in the Mouse with Acetochlor. Experimental Pathology Laboratories, Inc., Research Triangle Park, NC. Project IDs EPL 550-001, EP-2000-227. January 3, 2001. Unpublished report.

45367404 Hardisty, J. (2001) Pathology Working Group Peer Review of Neoplastic Lesions in the Femur and Non-Glandular Stomach of Male and Female Rats from a Combined Oncogenicity and Toxicity Study in Dietary Administration to CD Rats for 104 Weeks with Acetochlor: Final PWG Report. Experimental Pathology Laboratories, Inc., Research Triangle Park, NC. EPL Project No. 550-003. January 3, 2001. Unpublished report.

MEMORANDA AND HED TXR# DOCUMENTS

Brunsman, L. (2004) Acetochlor Qualitative Risk Assessment Based On Two Carcinogenicity Studies in CD-1 Mice and Three Carcinogenicity Studies in Sprague-Dawley Rats and a Two-Generation Reproduction Study in Sprague-Dawley Rats. Memorandum to L. Hansen through J. Rowland, dated March 9, 2004. TXR No. 0052265.

Pletcher, J. (2002a) Pathology Working Group (PWG) Peer Review of Neoplastic Lesions in the Femur and Non-glandular Stomach of Male and Female Rats from a Combined Oncogenicity and Toxicity Study in Dietary Administration to CD Rats for 104 Weeks with Acetochlor. Memorandum to J. Stewart dated June 7, 2002.

Pletcher, J. (2002b) Pathology Working Group (PWG) Peer Review of Proliferative Lesions in the Kidney of Female Mice from a 24-Month Oncogenicity Study in the Mouse with Acetochlor (Monsanto Report No: PR-80-007). Memorandum to J. Stewart dated June 7, 2002.

Pletcher, J. (2002c) Expert Report on Renal Histopathological Changes in a Mouse Study (Monsanto Study PR-80-007) with Acetochlor. Memorandum to J. Stewart dated June 7, 2002.

Pletcher, J. (2002d) Summary of the Acetochlor Registration Partnership (ARP) Position Regarding the Oncogenic Effects of Acetochlor in Rats and Mice. Memorandum to J. Stewart, dated June 11, 2002.

Pletcher, J. (2002e) Pathology Working Group Peer Review of Neoplastic Lesions in the Lung of Male and Female Mice from Two Long-Term Studies with Acetochlor. Memorandum to J. Stewart dated June 14, 2002.

Pletcher, J. (2002f) Pathology Working Group Peer Review of Hepatocellular Neoplasms in the Liver of Rats and Mice from Five long-Term Studies with Acetochlor. Memorandum to J. Stewart dated June 18, 2002.

Pletcher, J. (2002g) Pathology Working Group Peer Review of Histiocytic Sarcoma in Female Mice from Two long-Term Studies with Acetochlor. Memorandum to J. Stewart dated June 18, 2002.

Engler, R. (1987) Peer Review of Acetochlor. Cancer Peer Review Document for the meeting of September 12, 1985 to R. Taylor, dated March 30, 1987. HED TXR# 007697.

Sette, W. (1989) Second Peer Review of Acetochlor: Nasal Tumors. Cancer Peer Review Document for the meeting of February 8, 1989 to R. Taylor, dated May 31, 1989. HED TXR # 007697.

Dearfield, K. and McMahon, T. (1992) Third Peer Review of Acetochlor. Cancer (1992) Peer Review Document for the meeting of October 6, 1991, dated January 27, 1992. HED TXR# 0012828.

OTHER PUBLICATIONS

Charles River Laboratories, prepared by Patricia L. Lang, Ph.D., Consulting Toxicologist (1992a) Spontaneous Neoplastic Lesions in the Crl:CD-1 BR Mouse.

Charles River Laboratories, prepared by Patricia L. Lang, Ph.D., Consulting Toxicologist (1992b) Spontaneous Neoplastic Lesions and Selected Non-neoplastic Lesions in the Crl:CD®BR Rat.

Franz, J.D., Betton, G., Cartwright, M.E., Crissman, J.W., Macklin, A.W. and Maronpot, R.R. (1991 Proliferative Lesions of the Non-glandular and Glandular Stomach in Rats. In: *Guides for Toxicologic Pathology*, STP/ARP/AFIP, G1-3, Washington, D.C.

Genter, M.B., Burman, D.W. and Bolon, B. (2002) Progression of Alachlor-Induced Olfactory Mucosal Tumours. *Int. J. Exp. Path* 83: 303-307.

Hard, G.C., Alden, C.L., Stula, E.F. and Trump, B.F. (1995) Proliferative Lesions of the Kidney in Rats. In: *Guides for Toxicologic Pathology. STP/ARP/AFIP, Washington, D.C.*

Hard, G.C., Alden, C.L., Bruner, R.H., Frith, C.H., Lewis, R.M., Owen, R.A., Krieg, K. and Durchfeld-Meyer, B. (1999) Non-proliferative Lesions of the Kidney and Lower Urinary Tract in Rats. In: *Guides for Toxicologic Pathology*, pp. 1-32. STP/ARP/AFIP, Washington, D.C.

Hard, G.C., Durchfeld-Meyer, B., Short, B., Krieg, K., Creasy, D. *et al.* (2000) Urinary System. In: *International Classification of Rodent Tumors, Part II. The Mouse.* International Agency for Research on Cancer.

Long, P.H., Leininger, J.R., Nold, J.B. and Lieualen, W.G. (1993) Proliferative Lesions of Bone, Cartilage, Tooth and Synovium in Rats. In: *Guides for Toxicologic Pathology*, STP/ARP/AFIP, MST-2, Washington, D.C.

USEPA (1997) SAP REPORT, April 28, 1997. Report of the FIFRA Scientific Advisory Panel Meeting, March 19-20, 1997, held at the Crystal Gateway Marriott, 1700 Jefferson Davis Highway, Arlington, VA 22202.

USEPA (1998) Assessment of Thyroid Follicular Cell Tumors. Risk Assessment Forum. Environmental Protection Agency, Washington, D.C.

APPENDIX-SUPPLEMENT TO ACETOCHLOR CARC DOCUMENT FOR MEETING OF 4/21-4/22/04: TUMOR INCIDENCE SUMMARY TABLES FOR RAT AND MOUSE CHRONIC TOXICITY/CARCINOGENICITY STUDIES ON ACETOCHLOR

The following tables provide a summary of the tumor findings for acetochlor discussed at the Acetochlor CARC Meeting (Fourth Evaluation) from 3 rat chronic toxicity studies, 2 mouse carcinogenicity studies and a rat 2-generation reproductive toxicity study.

Where a Pathology Working Group (PWG) Review was performed, incidence data are compared for (1) the findings of the original study reports, as analyzed statistically by HED and presented in the previous cancer peer review documents (where available); (2) the findings of the PWG, as analyzed statistically and presented in the PWG report and (3) the findings of the PWG as analyzed statistically by HED for this CARC meeting.

The following legend applies to all tables:

() Values in parentheses indicate the percent incidence.
* statistically significant, $p<0.05$
** statistically significant, $p<0.01$
Significant trends are denoted at the control; significant pairwise comparisons at the appropriate dose group.
nd Not determined

TABLE 1-A: COMPARATIVE INCIDENCE OF NASAL EPITHELIAL CELL TUMORS IN MALE RATS TREATED WITH ACETOCHLOR IN THREE TWO YEAR STUDIES (ORIGINAL REPORT FINDINGS-NOT REEVALUATED BY PWG)

1983 STUDY (MRIDs 00131088, 40484801)[1]				
DIETARY DOSE, PPM	0	500	1500	5000
PAPILLARY ADENOMA	0/69**	1/70 (1)	6/69 (9)*	18/69 (26)**
PAPILLARY ADENOCARCINOMA	0/69*	0/70	0/69	2/69 (2)
COMBINED	0/69**	1/70 (1)	6/69 (9)*	20/69 (29)**
1986 STUDY (MRID 40077601)[2]				
DIETARY DOSE, PPM	0	40	200	1000
PAPILLARY ADENOMA	1/58 (2)**	0/54	0/58	12/59 (20)**
1988 STUDY (MRID 41592004)				
DIETARY DOSE, PPM	0	18	175	1750
ADENOMA	0/69**	0/59	0/59	35/70 (50)**
ADENOCARCINOMA	0/69**	0/59	0/59	2/70 (3)
COMBINED	0/69**	0/59	0/59	37/70 (53)**

TABLE 2-A: COMPARATIVE INCIDENCE OF NASAL EPITHELIAL CELL TUMORS IN FEMALE RATS TREATED WITH ACETOCHLOR IN THREE TWO-YEAR STUDIES (ORIGINAL REPORT FINDINGS-NOT REEVALUATED BY PWG)				
1983 STUDY (MRIDs 00131088, 40484801)[1]				
DIETARY DOSE, PPM	0	500	1500	5000
PAPILLARY ADENOMA	0/69	0/68	2/70 (3)	1/69 (1)
PAPILLARY ADENOCARCINOMA	0/69	0/68	0/70	0/69
1986 STUDY (MRID 40077601)[2]				
DIETARY DOSE, PPM	0	40	200	1000
PAPILLARY ADENOMA	0/69**	0/69	0/67	19/68 (28)**
1988 STUDY (MRID 41592004)				
DIETARY DOSE, PPM	0	18	175	1750
ADENOMA	0/69**	0/57	0/58	36/63 (57)**
CARCINOMA	0/69**	0/57	0/58	1/63 (2)
COMBINED	0/69	0/59	0/58	37/63 (59)**

TABLE 3-A: COMPARATIVE INCIDENCE OF NASAL EPITHELIAL CELL TUMORS AND HYPERPLASIA IN PARENTAL MALE AND FEMALE RATS TREATED WITH ACETOCHLOR IN A TWO-GENERATION REPRODUCTIVE TOXICITY STUDY (NEW REPORT, MRID 45357503-NOT REEVALUATED BY PWG)

DIETARY DOSE, PPM	0	200	600	1750
F0 MALES				
POLYPOID ADENOMAS	0/26**	0/26	0/26	4/26 (15)
OLFACTORY EPITHELIAL HYPERPLASIA	0/26*	0/26	0/26	3/26 (12)
RESPIRATORY EPITHELIAL HYPERPLASIA	0/26	0/26	0/26	2/26 (8)
F1 MALES				
POLYPOID ADENOMAS	0/26**	0/26	3/26 (12)	8/26 (31)**
OLFACTORY EPITHELIAL HYPERPLASIA	0/26**	0/26	0/26	7/26 (27)**
RESPIRATORY EPITHELIAL HYPERPLASIA	0/26	0/26	0/26	1/26 (4)
F0 FEMALES				
POLYPOID ADENOMAS	0/26**	0/26	0/26	6/26 (21)*
OLFACTORY EPITHELIAL HYPERPLASIA	0/26*	0/26	0/26	7/26 (27)**
RESPIRATORY EPITHELIAL HYPERPLASIA	0/26	0/26	0/26	2/26 (8)
F1 FEMALES				
POLYPOID ADENOMAS	0/26**	0/26	1/26 (4)	17/23 (74)**
OLFACTORY EPITHELIAL HYPERPLASIA	0/26**	0/26	4/26 (15)*	14/24 (58)**

TABLE 4-A: COMPARATIVE INCIDENCE OF THYROID FOLLICULAR CELL TUMORS IN MALE RATS IN THREE TWO-YEAR CHRONIC TOXICITY/CARCINOGENICITY STUDIES ON ACETOCHLOR (ORIGINAL REPORTS-NOT REEVALUATED BY PWG)				
1983 (MRIDs 00131088, 40484801)				
DIETARY DOSE, PPM	0	500	1500	5000
ADENOMA	0/69*	0/69	3/70 (4)	5/70 (7)*
CARCINOMA	0/69	0/69	0/70	0/70
COMBINED	0/69	0/69	3/70 (4)	5/70 (7)*
1986 STUDY (MRID 40077601)				
DIETARY DOSE, PPM	0	40	200	1000
NO TREATMENT-RELATED INCREASES IN TUMORS OBSERVED IN THIS STUDY				
1988 STUDY (MRID 41592004)				
	0	18	175	1750
ADENOMA	2/49 (4)*	1/50 (2)	2/48 (4)	5/49 (10)
CARCINOMA	1/49 (2)	3/50 (6)	0/48	3/49 (6)
COMBINED	3/49 (6)	4/50 (8)	2/48 (4)	8/49 (16)

TABLE 5-A: COMPARATIVE INCIDENCE OF THYROID FOLLICULAR CELL TUMORS IN FEMALE RATS IN THREE TWO-YEAR CHRONIC TOXICITY/CARCINOGENICITY STUDIES ON ACETOCHLOR (ORIGINAL REPORTS-NOT REEVALUATED BY PWG)				
1983 STUDY (MRIDs 00131088, 40484801)				
DIETARY DOSE, PPM	0	500	1500	5000
ADENOMA	2/69 (3)	0/69	0/69	3/69 (4)
CARCINOMA	0/69	0/69	0/69	0/70
COMBINED	2/69 (3)	0/69	0/69	3/69 (4)
1986 STUDY (MRID 40077601)				
DIETARY DOSE, PPM	0	40	200	1000
ADENOMA	1/39 (3)	2/44 (5)	2/36 (6)	4/46 (9)
CARCINOMA	0/30	0/35	0/28	1/36 (3)
COMBINED	1/39 (3)*	2/44 (5)	2/36 (6)	5/46 (11)
1988 STUDY (MRID 41592004)				
	0	18	175	1750
ADENOMA	1/60** (1)	1/58 (2)	3/59 (5)	6/63 (10)*
CARCINOMA	0/69**	0/58	0/59	1/63 (2)
COMBINED	1/69 (1)*	1/58 (1)	3/59 (5)	7/63 (11)*

TABLE 6-A: INCIDENCE OF LIVER TUMORS IN MALE RATS TREATED WITH ACETOCHLOR FOR TWO YEARS-COMPARISON OF ORIGINAL PATHOLOGY REPORTS WITH THE PWG REEVALUATION[1]					
1983 STUDY (MRIDs 00131088, 40484801)					
DIETARY DOSE, PPM		0	500	1500	5000
ADENOMA	ORIGINAL REPORT	6/70 (9)	2/70 (3)	5/70 (7)	7/70 (10)
	PWG REEVALUATION	2/60 (3)	1/60 (2)	1/60 (2)	6/60 (10)
	HED ANALYSIS OF PWG DATA	2/58 (3)*	1/60 (2)	1/58 (2)	6/56 (11)
CARCINOMA	ORIGINAL REPORT	0/70*	2/70 (2)	3/70 (4)	6/70 (9)*
	PWG REEVALUATION	1/60 (2)	3/60 (5)	3/60 (5)	6/60 (10)
	HED ANALYSIS OF PWG DATA	1/58 (2)*	3/60 (5)	3/58 (5)	6/56 (11)
COMBINED	ORIGINAL REPORT	6/70 (9)	4/70 (6)	8/70 (11)	13/70 (19)
	PWG REEVALUATION	3/60 (5)	4/60 (7)	4/60 (7)	11/60 (18)**
	HED ANALYSIS OF PWG DATA	3/58 (5)**	4/60 (7)	4/58 (7)	11/56 (20)*
1986 STUDY (MRID 40077601)[2]					
DIETARY DOSE, PPM		0	40	200	1000
ADENOMA	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	0/60	3/60 (5)	1/60 (2)	2/60 (3)
	HED ANALYSIS OF PWG DATA	0/59	3/56 (5)	1/59 (2)	2/59 (3)
CARCINOMA	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	1/60 (2)	1/60 (2)	1/60 (2)	1/60 (2)
	HED ANALYSIS OF PWG DATA	1/59 (2)	1/56 (2)	1/59 (2)	1/59 (2)
COMBINED	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	1/60 (2)	4/60 (7)	2/60 (3)	3/60 (5)
	HED ANALYSIS OF PWG DATA	1/59 (2)	4/56 (7)	2/59 (3)	3/59 (5)
1988 STUDY (MRID 41592004)					
DIETARY DOSE, PPM		0	18	175	1750
ADENOMA	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	0/50	0/50	0/50	2/50 (4)
	HED ANALYSIS OF PWG DATA	0/43*	0/46	0/42	2/48 (4)
CARCINOMA	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	2/50 (4)	3/50 (6)	2/50 (4)	1/50 (2)
	HED ANALYSIS OF PWG DATA	2/43 (5)	3/46 (7)	2/42 (5)	1/48 (2)
COMBINED	ORIGINAL REPORT	nd	nd	nd	nd

	PWG REEVALUATION	2/50 (4)	3/50 (6)	2/50 (40	3/50 (6)
	HED ANALYSIS OF PWG DATA	2/43 (5)	3/46 (7)	2/42 (5)	3/48 (6)

1 The only interim sacrifice group with a tumor was adenoma in 1/10 high dose male, 1988 study (PWG reevaluation).

TABLE 7-A: INCIDENCE OF LIVER TUMORS IN FEMALE RATS TREATED WITH ACETOCHLOR FOR TWO YEARS-COMPARISON OF ORIGINAL PATHOLOGY REPORTS WITH THE PWG REEVALUATION					
1983 STUDY (MRIDs 00131088, 40484801)[1]					
DIETARY DOSE, PPM		0	500	1500	5000
ADENOMA	ORIGINAL REPORT	0/70	2/70 (3)	2/70 (3)	2/70 (3)
	PWG REEVALUATION	0/60	1/60 (2)	1/60 (2)	3/60 (5)
	HED ANALYSIS OF PWG DATA	0/55*	0/42	1/48 (2)	3/41 (7)*
CARCINOMA	ORIGINAL REPORT	1/70 (1)*	1/70 (1)	1/70 (1)	5/70 (7)
	PWG REEVALUATION	0/60	0/60	0/60	2/60
	HED ANALYSIS OF PWG DATA	0/26*	0/20	0/27	2/15 (13)
COMBINED	ORIGINAL REPORT	1/70 (1)	3/70 (4)	3/70 (4)	7/70 (10)
	PWG REEVALUATION	0/60	1/60 (2)	1/60 (2)	5/60 (8)**
	HED ANALYSIS OF PWG DATA	0/55**	0/42	1/48 (2)	5/41 (12)**
1986 STUDY (MRID 40077601)					
DIETARY DOSE, PPM		0	40	200	1000
ADENOMA	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	0/60	1/60 (2)	1/60 (2)	5/60 (8)
	HED ANALYSIS OF PWG DATA	0/59**	1/59 (2)	1/57 (2)	5/57 (9)*
CARCINOMA	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	1/60 (2)	1/60 (2)	0/60	1/60 (2)
	HED ANALYSIS OF PWG DATA	1/59 (2)	1/59 (2)	0/57	1/57 (2)
COMBINED	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	1/60 (2)	2/60 (3)	1/60 (2)	6/60 (10)
	HED ANALYSIS OF PWG DATA	1/59 (2)*	2/59 (3)	1/57 (2)	6/57 (11)
1988 STUDY (MRID 41592004)					
DIETARY DOSE, PPM		0	18	175	1750
ADENOMA	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	0/50	1/50 (2)	0/50	2/50 (4)
	HED ANALYSIS OF PWG DATA	0/49	1/48 (2)	0/47	2/47 (4)
CARCINOMA	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	0/50	0/50	0/50	0/50
	HED ANALYSIS OF PWG DATA	nd	nd	nd	nd
COMBINED	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	0/50 (2)	1/50 (2)	0/50	2/50 (4)

	HED ANALYSIS OF PWG DATA	nd	nd	nd	nd

1 At interim sacrifice no tumors were observed (PWG reevaluation).

TABLE 8-A: IDENTIFICATION OF PROLIFERATIVE LESIONS OF THE FEMUR IN THE RAT - COMPARISON OF ORIGINAL STUDY DIAGNOSES AND PWG DIAGNOSES IN THE 1988 RAT TWO-YEAR STUDY (MRID 41592004)

Group/Sex/Dose	Animal No.	Original diagnosis	Reviewing pathologist's diagnosis	PWG Consensus
2M/18 ppm	0109	epiphyseal fibrosis (minimal)	hyperostosis	hyperostosis
4M/1750 ppm	0215	chondroma	cartilage hyperplasia	cartilage hyperplasia
1F/0 ppm	0328	--	cartilage hyperplasia	cartilage hyperplasia
2F/18 ppm	0370	--	hyperostosis	hyperostosis
4F/1750 ppm	0459	chondroma	cartilage hyperplasia	cartilage hyperplasia
4F/1750 ppm	0473	--	cartilage hyperplasia	cartilage hyperplasia

Table extracted from Table A-1 of MRID 45367404.

TABLE 9-A: IDENTIFICATION OF PROLIFERATIVE LESIONS OF THE NON-GLANDULAR STOMACH OF THE RAT-COMPARISON OF THE ORIGINAL STUDY DIAGNOSES AND THE PWG REEVALUATION IN THE 1988 RAT TWO-YEAR STUDY (MRID 41592004)

Group/Sex/Dose	Animal No.	Original diagnosis	Reviewing Pathologist's diagnosis	PWG Consensus
3M/175 ppm	0161	Acanthosis/hyperkeratosis, moderate, diffuse; submucosal inflammation, moderate; cystic glands, slight; keratinized region, ulcer(s); basal cell proliferation of ketatinized portion, moderate	Acanthosis/hyperplasia, moderate	Squamous cell hyperplasia, inflammation
4M/1750 ppm	0227	Acanthosis/hyperkeratosis, moderate; cystic glands, slight; squamous cell papilloma; basal cell tumor; arteritis, moderate	Squamous cell papilloma; poorly differentiated squamous cell carcinoma	Squamous cell papilloma; poorly differentiated squamous cell carcinoma
4F/1750 ppm	0498	Basal cell tumor	Well-differentiated squamous cell carcinoma	Well-differentiated squamous cell carcinoma

Table extracted from MRID 45367404.

TABLE 10-A: INCIDENCE OF PULMONARY TUMORS IN MALE MICE-COMPARISON OF ORIGINAL PATHOLOGY REPORTS WITH THE PWG REEVALUATION					
23-MONTH STUDY (MRID 00131089)					
DIETARY DOSE, PPM		0	500	1500	5000
ADENOMA	ORIGINAL REPORT	6/60 (10)	10/60 (17)	12/60 (20)	5/60 (9)
	PWG REEVALUATION[1]	7/50 (14)	10/50 (20)	11/50 (22)	5/50 (10)
	HED ANALYSIS OF PWG DATA	nd	nd	nd	nd
CARCINOMA	ORIGINAL REPORT	0/60**	5/60 (9)*	3/60 (5)	7/59 (12)**
	PWG REEVALUATION	6/50 (12)	3/50 (6)	3/50 (6)	3/50 (6)
	HED ANALYSIS OF PWG DATA	nd	nd	nd	nd
COMBINED	ORIGINAL REPORT	13/60 (22)	13/60 (22)	16/60 (27)	8/60 (13)
	PWG REEVALUATION	12/50 (24)	13/50 (26)	14/50 (28)	8/50 (16)
	HED ANALYSIS OF PWG DATA	nd	nd	nd	nd
78-WEEK STUDY (MRID 41565119)					
DIETARY DOSE, PPM		0	10	100	1000
ADENOMA	ORIGINAL REPORT	5/60 (8)**	3/60 (5)	11/59 (19)	13/57 (23)*
	PWG REEVALUATION[2]	9/50 (18)	5/50 (10)	11/50 (22)	16/50 (32)
	HED ANALYSIS OF PWG DATA	9/60 (15)**	5/60 (8)	11/58 (19)	17/57 (30)*
CARCINOMA	ORIGINAL REPORT	5/60 (8)	4/60 (7)	3/59 (5)	4/57 (7)
	PWG REEVALUATION	3/50 (6)	3/50 (6)	3/50 (6)	4/50 (8)
	HED ANALYSIS OF PWG DATA	3/60 (5)	3/60 (50	3/59 (50	4/57 (7)
COMBINED	ORIGINAL REPORT	10/60 (17)*	7/60 (12)	14/59 (24)	17/57 (30)*
	PWG REEVALUATION	11/50 (22)	8/50 (16)	13/50 (26)	18/50 (36)
	HED ANALYSIS OF PWG DATA	11/60 (18)**	8/60 (13)	13/59 (22)	19/57 (33)*

1 At interim sacrifice there was 1/10 mid dose male w/adenoma in the 23-month study (PWG evaluation).
2 At interim sacrifice there was 1/10 high dose male w/adenoma in the 78-week study (PWGevaluation)

TABLE 11-A: INCIDENCE OF PULMONARY TUMORS IN FEMALE MICE-COMPARISON OF ORIGINAL PATHOLOGY REPORTS WITH THE PWG REEVALUATION

23-MONTH STUDY (MRID 00131089)					
DIETARY DOSE, PPM		0	500	1500	5000
ADENOMA	ORIGINAL REPORT	2/60 (3)	6/60 (10)	8/60 (13)*	4/59 (7)
	PWG REEVALUATION[1]	1/50 (2)	7/50 (14)*	9/50 (18)**	7/50 (14)*
	HED ANALYSIS OF PWG DATA	1/43 (2)*	7/42 (17)*	9/40 (22)**	7/31 (23)**
CARCINOMA	ORIGINAL REPORT	0/60	5/60 (8)*	3/60 (5)	7/59 (12)**
	PWG REEVALUATION	0/50**	4/50 (8)	1/50 (2)	6/50 (12)**
	HED ANALYSIS OF PWG DATA	0/43**	4/43 (9)*	1/40 (2)	6/33 (18)**
COMBINED	ORIGINAL REPORT	2/60 (3)*	11/60 (18)**	12/60 (20)**	11/59 (19)**
	PWG REEVALUATION	1/50 (2)	10/50 (20)**	10/50 (20)**	11/50 (22)**
	HED ANALYSIS OF PWG DATA	1/43 (2)**	10/43 (23)**	10/40 (25)**	11/33 (33)**
78-WEEK STUDY (MRID 41565119)					
DIETARY DOSE, PPM		0	10	100	1000
ADENOMA	ORIGINAL REPORT	1/58 (2)	4/59 (7)	6/58 (10)	7/60 (12)*
	PWG REEVALUATION[2]	4/50 (8)	4/50 (8)	5/50 (10)	9/50 (18)
	HED ANALYSIS OF PWG DATA	4/58 (7)	5/59 (8)	6/58 (10)	9/60 (15)
CARCINOMA	ORIGINAL REPORT	4/58 (7)	0/59	2/58 (3)	4/60 (7)
	PWG REEVALUATION	1/50 (2)	0/50	2/50 (4)	2/50 (4)
	HED ANALYSIS OF PWG DATA	1/58 (2)	0/59	2/58 (3)	2/60 (3)
COMBINED	ORIGINAL REPORT	5/58 (9)*	4/59 (7)	8/58 (14)	11/60 (18)
	PWG REEVALUATION	5/50 (10)	4/50 (8)	7/50 (14)	11/50 (22)
	HED ANALYSIS OF PWG DATA	5/58 (9)*	5/59 (8)	8/58 (14)	11/60 (18)

1 At interim sacrifice, 1/10 mid dose female had an adenoma in the 23-month study (PWG reevaluation).
2 At interim sacrifice, 1/10 low and mid dose females each had an adenoma in the 78-week study (PWG reevaluation).

TABLE 12-A: INCIDENCE OF LIVER TUMORS IN MALE MICE TREATED WITH ACETOCHLOR-COMPARISON OF ORIGINAL PATHOLOGY REPORTS WITH THE PWG REEVALUATION					
23-MONTH STUDY (MRID 00131089)					
DIETARY DOSE, PPM		0	500	1500	5000
ADENOMA	ORIGINAL REPORT	8/60 (13)	4/59 (7)	9/60 (15)	7/59 (12)
	PWG REEVALUATION	8/50 (16)	7/50 (14)	10/50 (20)	19/50 (38)*
	HED ANALYSIS OF PWG DATA	8/49 (16)**	7/39 (18)	10/45 (22)	19/40 (48)**
CARCINOMA	ORIGINAL REPORT	6/60 (10)**	7/59 (12)	10/60 (17)	22/59 (37)*
	PWG REEVALUATION[1]	4/50 (8)	4/50 (8)	4/50 (8)	9/50 (18)
	HED ANALYSIS OF PWG DATA	4/47 (9)*	4/36 (11)	4/45 (9)	9/39 (23)*
COMBINED	ORIGINAL REPORT	14/60 (23)**	11/59 (19)	19/60 (32)	29/59 (49)**
	PWG REEVALUATION	12/50 (24)	10/50 (20)	14/50 (28)	26/50 (52)**
	HED ANALYSIS OF PWG DATA	12/49 (24)**	10/39 (26)	14/45 (31)	26/40 (65)**
78-WEEK STUDY (MRID 41565119)					
DIETARY DOSE, PPM		0	10	100	1000
ADENOMA	ORIGINAL REPORT	2/56 (4)	5/59 (8)	3/57 (5)	5/54 (9)
	PWG REEVALUATION[2]	6/50 (12)	9/50 (18)	5/50 (10)	6/50 (12)
	HED ANALYSIS OF PWG DATA	6/43 (14)	9/42 (21)	5/41 (12)	6/37 (16)
CARCINOMA	ORIGINAL REPORT	1/56 (2)	3/59 (5)	2/57 (4)	3/54 (6)
	PWG REEVALUATION	0/50	2/50 (4)	3/50 (6)	3/50 (6)
	HED ANALYSIS OF PWG DATA	0/43	2/42 (5)	3/41 (7)	3/37 (8)*
COMBINED	ORIGINAL REPORT	3/56 (5)	8/59 (14)	5/57 (9)	8/54 (15)*
	PWG REEVALUATION	6/50 (12)	11/50 (22)	8/50 (16)	9/50 (18)
	HED ANALYSIS OF PWG DATA	6/43 (14)	11/42 (26)	8/41 (20)	9/37 (24)

1 At interim sacrifice 1/10 low dose male had a carcinoma in the 23-month study (PWG reevaluation).
2 At interim sacrifice, 1/10 low dose male had an adenoma in the 78-week study (PWG reevaluation).

TABLE 13-A: INCIDENCE OF LIVER TUMORS IN FEMALE MICE TREATED WITH ACETOCHLOR-COMPARISON OF ORIGINAL PATHOLOGY REPORTS WITH THE PWG REEVALUATION					
23-MONTH STUDY (MRID 00131089)					
DIETARY DOSE, PPM		0	500	1500	5000
ADENOMA	ORIGINAL REPORT	2/60 (3)	0/60	0/60	4/58 (7)
	PWG REEVALUATION	0/50	1/50 (2)	0/50	2/50 (4)
	HED ANALYSIS OF PWG DATA	2/41 (5)**	0/38	1/34 (3)	5/24 (21)*
CARCINOMA	ORIGINAL REPORT	1/60 (2)**	0/60	0/60	4/58 (7)
	PWG REEVALUATION	0/50	0/50	0/50	0/50
	HED ANALYSIS OF PWG DATA	0/40**	0/35	0/30	2/22 (9)
COMBINED	ORIGINAL REPORT	3/60 (5)	0/60	0/60	8/58 (14)
	PWG REEVALUATION[1]	0/50	1/50 (2)	0/50	2/50 (4)
	HED ANALYSIS OF PWG DATA	2/41 (5)**	0/38	1/34 (3)	7/24 (29)**
78-WEEK STUDY (MRID 41565119)					
DIETARY DOSE, PPM		0	10	100	1000
ADENOMA	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	0/50	2/50 (4)	0/50	1/50 (2)
	HED ANALYSIS OF PWG DATA	nd	nd	nd	nd
CARCINOMA	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION	1/50 (2)	0/50	0/50	0/50
	HED ANALYSIS OF PWG DATA	nd	nd	nd	nd
COMBINED	ORIGINAL REPORT	nd	nd	nd	nd
	PWG REEVALUATION[2]	1/50 (2)	2/50 (4)	0/50	1/50 (2)
	HED ANALYSIS OF PWG DATA	nd	nd	nd	nd

1 No tumors were identified at the interim sacrifice in the 23-month study (PWG reevaluation).
2 No tumors were identified at the interim sacrifice in the 78-week study (PWG reevaluation).

TABLE 14-A: INCIDENCE OF HISTIOCYTIC SARCOMA IN FEMALE MICE TREATED WITH ACETOCHLOR-COMPARISON OF ORIGINAL PATHOLOGY REPORT WITH PWG REEVALUATION				
23-MONTH STUDY (MRID 00131089)[1]				
DIETARY DOSE, PPM	0	500	1500	5000
ORIGINAL REPORT	0/59	6/57 (11)**	6/60 (10)**	5/59 (8)*
PWG REEVALUATION[1]	0/50	3/50 (6)	7/50 (14)**	6/50 (12)*
HED ANALYSIS OF PWG DATA	0/47	3/44 (7)*	7/47 (15)**	6/41 (15)*
78-WEEK STUDY (MRID 41565119)[2]				
DIETARY DOSE, PPM	0	10	100	1000
ORIGINAL REPORT	nd	nd	nd	nd
PWG REEVALUATION[2]	2/50 (4)	1/50 (2)	0/50	5/50 (10)
HED ANALYSIS OF PWG DATA	2/59 (3)*	1/60 (2)	0/60	5/60 (8)

1 At interim sacrifice, 1/10 mid-dose female had a histiocytic sarcoma in the 23-month study (PWG evaluation).
2 No histiocytic sarcoma were observed at interim sacrifice in the 78-week study (10/dose group)(PWG evaluation).

TABLE 15-A: INCIDENCE OF RENAL TUMORS IN FEMALE MICE-COMPARISON OF ORIGINAL PATHOLOGY REPORT WITH PWG REEVALUATION					
23-MONTH STUDY (MRID 00131089)					
DIETARY DOSE, PPM		0	500	1500	5000
ADENOMA	ORIGINAL REPORT	0/60*	0/60	0/60	3/59 (5)
	PWG REEVALUATION	0/50	0/50	0/50	2/50 (4)
	HED ANALYSIS OF PWG DATA	0/33*	0/25	0/20	2/14 (14)
SARCOMA	ORIGINAL REPORT	0/60	0/60	0/60	1/60 (2)
	PWG REEVALUATION	0/50	0/50	0/50	2/50 (4)
	HED ANALYSIS OF PWG DATA	0/41*	0/39	0/38	2/29 (7)
78-WEEK STUDY (MRID 41565119) - NO INCREASE OBSERVED UP TO 1750 PPM					

There were no tumors observed in the interim sacrifice animals (10/sex/dose group)(PWG reevaluation)

TABLE 16-A: INCIDENCE OF BENIGN OVARIAN TUMORS IN 23 MONTH MOUSE STUDY (MRID 00131089)-NOT REEVALUATED BY PWG

Tumor type	0 ppm	500 ppm	1500 ppm	5000 ppm
adenoma	0/59	0/60	1/60 (2)	0/58
granulosa cell tumor	0/59	0/60	3/60 (5)	2/58 (3)
luteoma	0/59	0/60	1/60 (2)	1/58 (2)
total benign tumors	0/59**	0/60	5/60* (8)	3/58 (5)

TABLE 17-A: INCIDENCE OF RAT NASAL EPITHELIAL TUMORS WITH INCREASING DOSE (COMPARISON OF ALL RAT STUDIES)													
DOSE IN PPM [STUDY]	0 [All]	18 [3]	40 [2]	175 [3]	200 [2]	200 [4]	500 [1]	600 [4]	1000 [2]	1500 [1]	1750 [3]	1750 [4]	5000 [1]
MALES													
DOSE, MG/KG/DAY	0	0.67	2.0	6.4	20.0	21.2	22	65.6	50.0	69	66.9	196.4	250
Adenoma	0	0/59	0/54	0/59	0/58	0/26	1/70 (1)	3/26 (12)	12/59 (20)	6/69 (9)	35/70 (50)	8/26 (31)	18/69 (26)
Carcinoma	0	0/59	0/54	0/59	0/58	0/26	0/70	0/26	0/59	0/69	2/70 (3)	0/26	2/69 (3)
Combined	0	0/59	0/54	0/59	0/58	0/26	1/70 (1)	3/26 (12)	12/59 (20)	6/69 (9)	37/70 (53)	8/26 (31)	20/69 (29)
FEMALES													
DOSE, MG/KG/DAY	0	0.88	2.0	8.5	20.0	22.4	30	70.9	50.0	93	92.1	215.9	343
Adenoma	0	0/57	0/69	0/58	0/67	0/26	0/68	1/26 (4)	19/68 (28)	2/70 (3)	36/63 (57)	17/23 (74)	1/69 (1)
Carcinoma	0	0/57	0/69	0/58	0/67	0/26	0/68	0/26	0/68	0/70	1/63 (2)	0/23	0/60
Combined	0	0/57	0/69	0/58	0/67	0/26	0/68	1/26 (4)	19/68 (28)	2/70 (3)	37/63 (59)	17/23 (74)	1/60 (1)

1 1983 rat chronic toxicity/carcinogenicity study (MRIDs 00131088, 40484801)
2 1986 rat chronic toxicity/carcinogenicity study (MRID 40077601)
3 1988 rat chronic toxicity/carcinogenicity study (MRID 41592004)
4 2001 rat 2-generation reproductive toxicity study (MRID 45357503)
All nasal tumor data shown are presented according to the incidence calculations prepared by HED cancer assessments of acetochlor. Statistical analyses not presented.

Part 2: Mode of Action Assessment Document: Evaluation of the Mode of Action of Acetochlor for Nasal Olfactory Epithelium Tumors in Rats and its Relevance to Human Cancer Risk Assessment

MODE OF ACTION ASSESSMENT DOCUMENT

EVALUATION OF THE MODE OF ACTION OF

ACETOCHLOR

FOR

NASAL OLFACTORY EPITHELIUM TUMORS IN RATS

AND ITS

RELEVANCE TO HUMAN CANCER RISK ASSESSMENT

FINAL REPORT

August 31, 2004

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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EXECUTIVE SUMMARY

On April 21 and 22, 2004, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to:

1. Reevaluate the carcinogenic potential of acetochlor
2. Determine the adequacy of the database to support a proposed mode of action for thyroid tumorigenesis
3. Determine the adequacy of the database to support a proposed mode of action for nasal tumors in rats and the relevance of such tumors to human cancer risk assessment.

This document covers the conclusions of the CARC meeting concerning Item No. 3: the adequacy of the acetochlor database to support a proposed mode of action for nasal tumors in rats and the relevance of such tumors to human cancer risk assessment. Items Nos. 1 and 2 are covered in a separate document: Evaluation of the Carcinogenic Potential of Acetochlor (Fourth Evaluation, June 2004).

Acetochlor is structurally related to other chloroacetanilide herbicides, including alachlor, propachlor, butachlor and metolachlor. Alachlor and butachlor also induce nasal epithelial tumors and thyroid follicular cell tumors. On the basis of preliminary reviews of mechanistic data for acetochlor and butachlor, the FIFRA SAP concluded on March 19, 1997 that alachlor, acetochlor and butachlor may be grouped together for common mode of action (MOA) for induction of nasal and thyroid tumors (USEPA 1997, 2001).

The mechanistic data for acetochlor have been now fully reviewed. Based on these data the committee concluded:

1. MOA for nasal tumors.

The data supporting the mechanism of action for nasal olfactory epithelium tumors in rats by acetochlor have been evaluated. It is concluded that the non-genotoxic MOA for nasal olfactory epithelium tumors in rats, discussed in this document, is supported by the data.

The MOA includes the he following steps:

i) Acetochlor conjugates with glutathione (GSH) and is excreted in the bile.
ii) The conjugate is biotransformed to a series of sulfur-containing products. Enterohepatic circulation of these products creates a pool of metabolites that are delivered to the nose.
iii) Biotransformation to tissue-reactive and toxic metabolites. Metabolism by nasal enzymes results in formation of a benzoquinoneimine, an electrophile and redox-active molecule.
iv) Binding to cellular proteins plus possible generation of oxidative stress.

v) Cytotoxicity (respiratory metaplasia)
vi) Regenerative cell proliferation.
vii) Sustained cytotoxicity and cell proliferation that results in neoplasia.

The following data are consistent with the MOA:

a. The absence of a demonstrated positive mutagenic effect of the chemical.

b. Acetochlor administration results in dose related increases in the binding of the quinone imine metabolite in the target tissue. This metabolite is considered to be the putative active species.

c. There is respiratory metaplasia of the nasal olfactory epithelium, an indication of cytotoxicity to the original olfactory tissue and its being replaced by respiratory epithelium, which originates from undifferentiated cells in the epithelium.

d. Lipofucsin pigment was observed to increase in a dose related manner in the nasal olfactory epithelium of rats that show nasal olfactory tumors at the high dose. Lipofucsin pigment is associated with oxidative damage to lipids and lipoproteins, which is consistent with the redox alterations known to be produced by quinones and quinone imines.

e. Acetochlor administration results in dose related increases in cell proliferation in cells of the target tissue.

f. The absence of nasal epithelial tumors in mice correlated with their inability to form adducts of the quinone imine at the target site. This evidence of no quinone imine binding was confirmed autoradiographically.

g. Rats administered the sulfoxide metabolite of acetochlor (a proximate precursor of the toxic metabolite, the quinone imine) show nasal olfactory mucosa adenomas after 26 weeks of treatment (MRID 46081801).

The data on the non-genotoxic MOA for acetochlor are supported by the entire database for the analog alachlor, in particular:

i. Reversibility of cell proliferation in rats treated with alachlor for 60 days at a tumorigenic dose, after placement on basal diet for 60 days (MRID 42852102)

ii. Rats treated with the analog alachlor for 1 month at a tumorigenic dose (126 mg/kg/day) did not have detectable neoplasms when examined after a 5-month holding period on basal diet. No detectable olfactory mucosal lesions were observed in any of the “stop study” rats (Genter et al. 2002b)

The weight of the evidence in support for the mode of action evaluated in this document is high. The evidence would have been strengthened if corroborative experiments, such as prevention or reversal of a precursor event (e.g. cell proliferation) by appropriate administration of a chemical (e.g. N-acetylcysteine) known to interfere with a key step (e.g. formation of quinone imine), had been available. Although dimethylaniline (DMA) and diethylaniline (DEA) [analogs of ethylmethyl aniline (EMA)] have been found to form *in vivo* DNA adducts in rat nasal mucosa, concerns about a genotoxic mechanism for acetochlor are mitigated by several factors. These include absence of formation of DNA adducts in nasal mucosa in parallel experiments in rats using the analog alachlor and the reversibility of cell proliferation of olfactory epithelium observed with alachlor.

2. Relevance of rat nasal olfactory epithelium tumors to human health risk assessment.

The Registrant's data in support of the idea that rat nasal olfactory epithelium tumors have no relevance to human health risk assessment has been evaluated. It is concluded that the Registrant's argument that there is no relevance to humans cannot be sustained.

This conclusion is supported by:

- The realization that production of a metabolite (EMA) with the capacity of undergoing biotransformation to a quinone imine is possible for humans (Coleman et al. 2000)**.**

- *In vitro* studies of p-hydroxylation of EMA using olfactory epithelium enzymes indicate that rat-to-monkey ratios of activities are not as large as 23.7 but could be as small as 7 or 8.

- In *in vitro* studies, the ratio of rat to monkey for p-hydroxylation of the sulfoxide metabolite of acetochlor may be not astronomically large, as initially postulated, but as small as 88.

- Although nasal tissue was not included in the Coleman et al. (2000) study, the data indicate that human liver has the potential to produce EMA (Figure 13), a plausibly carcinogenic metabolite of acetochlor, which would then be available to all organs via the circulatory system.

Comments by one of the presenters (B. Dementi OPP/HED/TOX) are discussed as a minority dissenting opinion in Part 3 of the final document.

I. INTRODUCTION

On April 21-22, 2004, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs reviewed the recommendations of the toxicology reviewer for acetochlor with regard to the proposed mechanism of action of

acetochlor in the production of nasal olfactory epithelium tumors. The conclusions drawn at this meeting are presented in this report.

On October 30 of 1996 the FIFRA SAP was presented a postulated mode of action (MOA) for alachlor for nasal epithelium tumors in rats similar to the one to be discussed in this document. Concerning the relevance of the presented mechanism for human cancer risk assessment, the SAP concluded that " .. the argument that limitation in the metabolism of alachlor in the human precludes alachlor being considered as a human carcinogen can not be supported."

On March 19 of 1997, on the basis of preliminary reviews of mechanistic data for acetochlor and butachlor, the FIFRA SAP concluded that alachlor, acetochlor, and butachlor share a common MOA for nasal epithelium tumors and for thyroid follicular tumors in rats (USEPA 1997, 2001).

II. BACKGROUND INFORMATION

1. Introduction: Acetochlor is a herbicide used both pre- and post-emergence for killing grasses and some broad leafed weeds in corn. Joint USA registration was granted to the Acetochlor Registration Partnership (ARP) in March 1994. Acetochlor is sold in the USA as *Surpass* (Zeneca) and *Harness* (Monsanto).

2. Chemical Identification:

Table 1 summarizes the identification data for acetochlor. Table 2 summarizes the physicochemical properties of acetochlor.

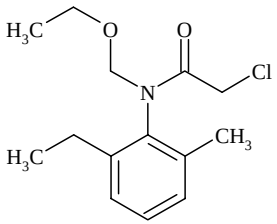
Table 1. Acetochlor Nomenclature	
Chemical structure	
Common name	Acetochlor
Molecular Formula	$C_{14}H_{20}ClNO_2$
Molecular Weight	269.8
IUPAC name	2-chloro-N-ethoxymethyl-6'-ethylacet-o-toluidide
CAS name	2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide

Table 1. Acetochlor Nomenclature	
CAS #	34256-82-1
PC Code	121601

TABLE 2. Physicochemical Properties of Acetochlor.		
Parameter	Value	Reference
Boiling point/range	163 °C at 10 mm Hg; decomposition occurs before the boiling point at atmospheric pressure; (calculated by extrapolation of vapor pressure at lower temperature)	DEB 7474, 2/6/91, M. Flood
pH	4.41, 1% solution in acetone:water (1:1, v:v)	DEB 7474, 2/6/91, M. Flood
Density at 20 °C	1.123 g/mL	DEB 7474, 2/6/91, M. Flood
Water solubility at 25 °C	223 mg/L	2001 Farm Chem Handbook
Solvent solubility at 25 °C	Infinitely soluble in acetone, benzene, carbon tetrachloride, ethanol, chloroform, and toluene	HED Memo, 1/21/94, M. Flood
Vapor pressure at 25 °C	0.045 µ Hg (4.5×10^{-5} mm Hg)	DEB 7474, 2/6/91, M. Flood
Dissociation constant, pK_a	Not applicable because acetochlor is neither an acid nor a base.	DEB 7474, 2/6/91, M. Flood
Octanol/water partition coefficient	970 or 1082	DEB 7474, 2/6/91, M. Flood
UV/visible absorption spectrum	Not available	

III. DATA PRESENTATION

1. Listing of Available Data

Data from the following documents were examined by the Committee in its deliberations:

Table 3. List of Documents used in the Cancer Assessment Review Committee Meeting for evaluation of Acetochlor MOA and Carcinogenicity status.

No.	Type of Study	Comments	Reference
1	Chronic Rat	1983 Sprague-Dawley Rat Study	MRID 00131089 ✻
2	Chronic Rat	1986 Sprague-Dawley Rat Study	MRID 400770601 ✻
3	Chronic Rat	1988 Sprague-Dawley Rat Study	MRID 41592004 ✻
4	2-Gen Repro Rat.	2001 Sprague-Dawley Rat Study	MRID 45357503 ✻
5	Chronic Mouse.	1983 CD-1 Mouse Study (24-months)	MRID 00131088 ✻
6	Chronic Mouse.	1989 CD-1 Mouse Study (18-months)	MRID 41565119 ✻
7	CARC Doc.	Details MOA for Alachlor nasal tumors	
8	CARC Doc.	Pathology Report-Rats & Mice J. Pletcher	✻
9	Mechanism Rat	1998 SD Rat quinone imine-protein binding; autoradiography	MRID 44496210
10	Mechanism Mouse	1998 CD-1 Mouse quinone imine-protein binding	MRID 44496211
11	Mechanism Rat; (aceto.sec.sulfide)	1998 SD Rat quinone imine-protein binding; autoradiography	MRID 44496212
12	Mechanism Monkey	1998 Rhesus Monkey quinone imine-protein binding	MRID 44496213
13	Mechanism Rat	1996 SD Rat nasal cell proliferation	MRID 44496207
14	Mechanism Mouse	1996 CD Mouse nasal cell proliferation	MRID 44496209
15	Mechanism Rat	1996 SD Rat thyroid parameter effects	MRID 44496208 ✻
16	Metabolism	1998 Comparative metabolism Rat & Mouse	MRID 44496203
17	Metabolism	1998 Protein Adducts Rats	MRID 46009402
18	Review	"Acetochlor Mechanism of Nasal Tissue Carcinogenicity" (Dementi, 1/26/04)	

Table 3 (Continued) List of Documents used in the Cancer Assessment Review Committee Meeting for evaluation of Acetochlor MOA and Carcinogenicity status.

No.	Type of Study	Comments	Reference
19	Review/concerns	2/4/04 Email B. Dementi (BD)/Colleagues expressing Acetochlor issues of concern	
20	Review/concerns	3/2/04 Email BD/Colleagues expressing additional Acetochlor issues of concern	
21	Comments	3/9/04 Email BD/Colleagues comments on SD Rat Repro. Study DER	
22	Correspondence	12/19/03 Email J. Kronenberg (JK)/BD; nasal cytotoxicity	
23	Comments	1/14/04 Email BD/Gentlepersons; comments on Monsanto's 1996 assessment of alachlor for SAP	
24	Review	10/1/96 Monsanto: Executive Summary from review of info. on carcinogenicity of alachlor for SAP 1996.	
25	Review	1998 Clapp et al., Monsanto document assessing carcinogenic potential	MRID 44496201
26	Review	1992 Pathology: rat nasal tissue mapping	MRID 44496214
27	Review	2003 *In Vitro* Metabolism; Multiple Species Plus Humans	MRID 46081802
28	Review	2003 *In vitro* metabolism; rat, mouse, primate	MRID 44530002
29	Review	1998 *In vitro* metabolism (sulfoxide metabolite), rat and mouse	MRID 44530001
30	Review	1996 Mouse thyroid study *(mechanism)*	MRID 44496208
31	Review	9/5/03 Monsanto Justification for Reclassification of Carcinogenic Potential. "White Paper"	MRID 46081801
32	Comments	10/24/03 Dementi on "White Paper"	
33	Correspondance	5/28/03 Email: BD/JK; comments on Clapp et al.	
34	Correspondance	10/8/03 Email: JK/BD;	
35	Correspondance	10/20/03 Email: JK/BD	

Table 3 (Continued). List of Documents used in the Cancer Assessment Review Committee Meeting for evaluation of Acetochlor MOA and Carcinogenicity status.

No.	Type of Study	Comments	Reference
36	Correspondence	11/17/03 Memorandum BD/Jim Jones; concerns about PWGs	
37	SAP	12/6/96 report of 10/30/96 SAP mtg.	✻
38	Review	6/27/97 Report of 2/5/97 CARC meeting addressing 10/30/96 SAP/SAB	
39	SAP	4/28/97 report of 3/19-20 SAP meeting	
40	Phone call/notes	10/20/03 Email BD/L.Hansen (LH) & A. Protzel (AP); record of 10/20/03 conference with Dr. Genter	
41	Phone call/notes	10/8/03 Email BD/JK	
42	Phone call/notes	12/17/03 Email BD/JK	
43	Phone call/notes	12/17/03 Email BD/Colleagues; comments on reproduction study	
44	Phone call/notes	1/14/04 Email BD/AP; phenacetin cytotoxicity	
45	Phone call/notes	1/14/04 Email BD/W.Burnam; CARC report - are nasal tumors respiratory as so recorded?	
46	Phone call/notes	1/21/04 Email LH/BD; lung tumors in both alachlor and acetochlor mouse studies	
47	Phone call/notes	5/21/03 Email BD/BD record of May 20 request of JK for “White Paper”	
48	Review	3/16/96 Cancer Peer review Alachlor 3th	✻
49	Review	2/5/97 Cancer Peer review Alachlor 4th	✻
50	Review	3/30/87 Peer Review Acetochlor	✻

Table 3 (continued). List of Documents used in the Cancer Assessment Review Committee Meeting for evaluation of Acetochlor MOA and Carcinogenicity status.

No.	Type of Study	Comments	Reference
51	Review	5/31/89 2nd Peer review- Acetochlor	✻
52	Review	1/27/92 3rd Peer review- Acetochlor	✻
53	Correspondence	2/25/04 D Wolf to A Protzel	
54	Correspondence	3/15/04 D Wolf to A Protzel	
55	Article	Coleman et al., 2000	
56	Review	Common Mechanism Document	
57	Review	ACETOCHLOR QUALITATIVE RISK ASSESSMENT	✻
58	Correspondence	3/17/04 J Pletcher to N McCarroll	
59	Correspondence	3/18/04 J Pletcher to N McCarroll	

✻ Document for CARC

2. Presentation of Available Data

a. Introduction

The Acetochlor data presented below were submitted by the Registrant in support of a non genotoxic mode of action (MOA) for the production of tumors of the nasal olfactory epithelium in rats. The genotoxic data are discussed below under **Mutagenicity Results**, and are summarized in Table 5.

The postulated MOA for the induction of nasal tumors by acetochlor in rats (See Figure 1) proposes that acetochlor conjugates with glutathione (GSH) and is excreted in the bile. Subsequent biotransformation of the conjugate to a series of sulfur-containing products, followed by enterohepatic circulation of these products creates a pool of metabolites that are delivered to the nose where they undergo further biotransformation to tissue-reactive and toxic metabolites. Metabolism by nasal enzymes results in formation of benzoquinoneimine, an electrophile, which binds to cellular proteins and produces oxidative damage, producing cytotoxicity and regenerative cell proliferation. If cytotoxicity and cell proliferation is sustained, neoplasia eventually results.

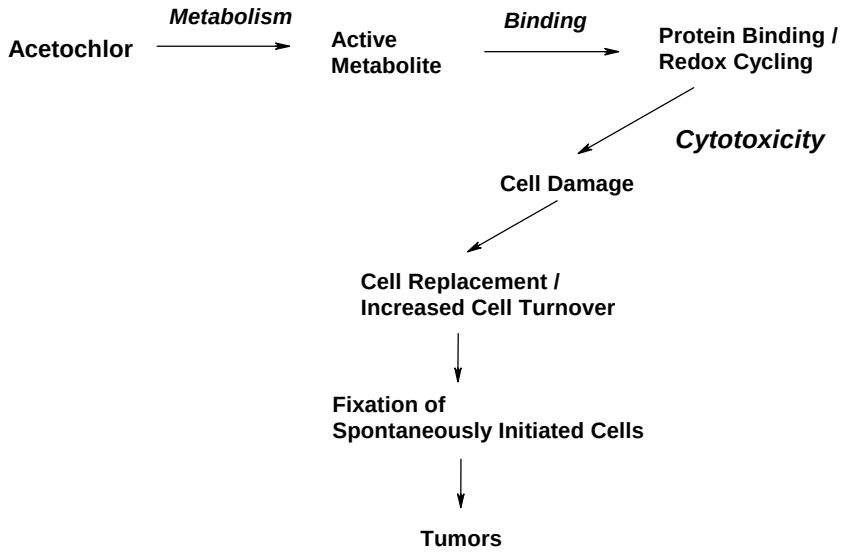


Figure 1. Postulated mode of action of acetochlor in the induction of nasal tumors in rats

Because the MOA database for acetochlor is very large, the data have been briefly summarized, as an overview, in Table 4 and keyed to the MOA steps shown in Figure 1.

Additionally, because the chloroacetanilides have been studied as a common mechanism grouping, data are given in Table 4 for four additional chloroacetanilides. These include alachlor and butachlor, which have been grouped together with acetochlor, as a common mechanism group based on nasal turbinate tumors in rats by the FIFRA SAP in a meeting dated March 19 of 1997.

Table 4. Summary of data supporting the MOA for nasal tumors in rats.

MOA Step	Data for Acetochlor and Analogs				
	Acetochlor	**Alachlor**	**Butachlor**	**Metolachlor**	**Propachlor**

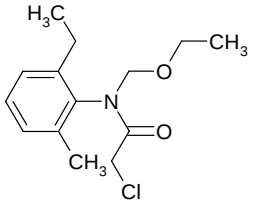
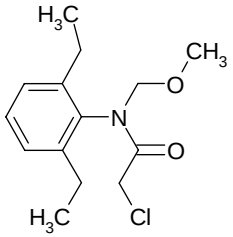
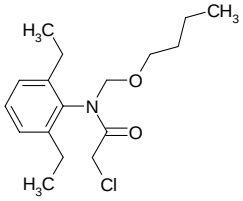
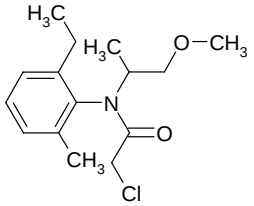
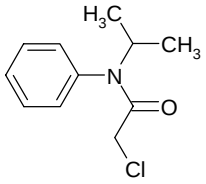
					
EndPoint	Nasal tumors: rats (+), mice (-)	Nasal tumors: rats (+), mice (-)	Nasal tumors: rats (+), mice (-)	Nasal tumors: rats (±), mice (-)	Nasal tumors: rats (-), mice (-)
Metabolism					
Oral Absorption	>70%	Extensive	Extensive	Extensive	>68%
Tissue Distribution	Extensive. Including binding to nasal epithelium in rats but not mice or other sp.	Extensive. Including binding to nasal epithelium in rats but not mice or other sp.	No Data	Extensive. Including binding to nasal epithelium in rats but not mice or other sp.	No Data
Biotransformation	Extensive, including *in vivo* precursors of quinone imine: sec-sulfoxide and sec-chloramide metabolites . *In vitro* p-OH-metabolite of sec-sulfoxide metabolite.	Extensive, including precursors of quinone imine: sec-methylsulfide, 4-amino-3,5-diethylphenol (as sulfate)	Extensive, including a precursor of quinone imine: 4-amino-3,5-diethylphenol (as sulfate)	Extensive. A possible quinone imine precursor: 2-methyl-6-ethylaniline (at less than 0.00055% of the dose.	Extensive, including 4-aminophenol (a quinoneimine precursor) and its glucuronide.
Active Metabolite	Derivatives diagnostic of dialkyl-benzoquinoneimine (DBZQ) identified in urine of dosed rats by Jeffries et al. (1998)	Derivatives diagnostic of DBZQ identified in urine of dosed rats by Jeffries etal. (1998)	Derivatives diagnostic of DBZQ identified in urine of dosed rats by Jeffries et al. (1998)	Derivatives diagnostic of DBZQ identified in urine of dosed rats by Jeffries et al. (1998)	No Data

Table 4. Summary of data supporting the MOA for nasal tumors in rats.

MOA Step	**Data for Acetochlor and Analogs**				
	Acetochlor	**Alachlor**	**Butachlor**	**Metolachlor**	**Propachlor**
Binding					

Binding to Nasal Protein	Dosing with C^{14}acetochlor or its C^{14} sec-sulfide showed concentration of label in nasal turbinates. Analysis of adduct indicated origin from binding of a benzo-quinone imine to protein	Dosing with C^{14}alachlorr, its C^{14} sec-sulfide, or DEA[1] showed concentration of label in nasal turbinates. Analysis of adduct indicated origin from binding of a benzo-quinone imine to protein.	No Data.	Dosing with C^{14}metolachlor showed concentration of label in nasal turbinates.	No Data.
Redox Cycling	Limited data. GSH depleted in liver ($p<0.05$). Lipofucsin pigment seen in olfactory epithelium in rats	Limited data. GSH depleted in liver ($p<0.05$). Genomic data on oxidative damage.	GSH depletion in stomach tissue and other tissues.	No data	No data
Cytotoxicity	Histopathology: Olfactory epithelial respiratory metaplasia.	In vitro cytotoxicity found using nasal tissue explants. In vivo histopathological: respiratory metaplasia of olfactory epithelium	Histopathology: Olfactory epithelial respiratory metaplasia	No data.	No data.
Cell damage	Lipofucsin pigment seen in olfactory epihelium	Ggenomic data on oxidative damage.	No data	No data	No data
Cell Replacement/ Increased cell turnover	Significantly increased cell proliferation in the olfactory epithelium of rats but not mice.	Significantly increased cell proliferation in the olfactory epithelium of rats	Significantly increased cell proliferation in the olfactory epithelium of rats	No data	Nodata
Nasal Tumors	See Above	See Above	See Above	See Above	See Above

[1] DEA = Diethylaniline

b. Mutagenicity Results

Acetochlor was evaluated in a variety of genetic toxicology assays submitted to HED which were summarized along with published articles by Dearfield et al. (1999) and are discussed below (and summarized in Table 5) according to endpoint.

GENE MUTATIONS

Results from published assays and studies submitted to HED in support of FIFRA registration were generally negative for gene mutations in bacteria (*Salmonella typhimurium*). There was a reproducible increase in mutant colonies (<2-fold increase) of strain TA1538 in one study (MRID 41565121). The response was, however, confined only to this strain. It was not observed in strain TA98 (the more sensitive derivative of TA1538) and was not concentration-related (observed only at 1000 and 2500 µg/plate +S9).

Hill et al. (1997) reported that a metabolite of acetocholor, diethylquinoneimine was "weakly" positive without S9 activation in a pre-incubation assay with *S. typhimurium* TA100. This finding, however, should be viewed with caution because the increase (2.1-fold) was limited to a single concentration (30 µg/plate -S9) that also induced cytotoxicity. Similarly, the "weak positive effect" seen in a Chinese hamster ovary (CHO)/HPRT gene mutation assay (~4x increase at 125 µg/plate -S9, with 71% cell survival) (MRID 00131395) was not observed in an independent second assay with this same cell line (MRID 42713106). There was, however, a positive response in the mouse lymphoma L5178Y forward gene mutation assay (MRID 00131394). In contrast to the weak effect observed in one of two CHO assays, the response in mouse lymphoma cells was seen in the presence of S9 activation with concentration dependency (*i.e.,* >2-fold ↑at 30 µL/L with 45% survival to 4-fold ↑at 40 µL/L with 12 % survival). At the time this assay was performed, mouse lymphoma colony size distribution was not preformed. Hence, it can not be ascertained whether the increased mutant colonies resulted from mutagenic or clastogenic action.

In keeping with the Agency's conclusions regarding the structural and toxicological similarities between acetochlor and **alachlor**, relevant genetic toxicology studies on **alachlor** are also included in this review. Wetmore et al. (1999) conducted a series of gene mutation assays in *S. typhimurium* TA100 on **alachlor** using S9 homogenates from the liver, olfactory mucosal or the respiratory mucosal of Long-Evans rats. Results showed an ~2- to 3-fold increases in revertant colonies of TA100 at 15,000 or 8430 µg/plate, respectively with rat liver S9; no response with 1500-15,000 µg/plate plus rat olfactory mucosal S9 but a 2-fold increase at 1250 µg/plate with rat olfactory mucosal S9 in a repeat experiment; and no response in the presence of rat respiratory. These findings should be considered equivocal since the positive data in the presence of rat liver S9 was not dose-related, was accompanied by cytotoxicity (thinning of the background lawn of growth), and the concentrations eliciting a ≥2-fold response were excessive. Similarly, the response in the presence of olfactory S9 homogenates was not reproducible. Kier et al. (1996) also tested *alachor* in the Ames assay using rat olfactory S9 homogenates and found only negative results. Wetmore et al. also reported a 2.9-fold increase in the mutation frequency of L5178Y mouse lymphoma cells at 5.6 µg/mL + rat olfactory S9. At this concentration, total cell survival was reduced to 8.3%. From results for gene mutation assay with bacteria or mammalian cells, Wetmore et al. concluded that positive results were only obtained in mouse lymphoma and TA 100 over a narrow concentration range immediately below levels that were lethal. "Thus, there appears to be a close association of a narrow concentration range that induces gene and chromosomal

mutations and concentrations that induce toxicity." Such findings, without support, are not considered by the genetic toxicology community to be valid evidence of a positive response.

Overall, the results from gene mutation assays are conflicting, show only sporadic positive findings, and, therefore, do not provide a convincing picture of gene mutation.

CHROMOSOME ABERRATIONS

In vitro test systems

Acetochlor induced a clastogenic effect in human lymphocytes (in whole blood cultures) at concentrations of 50 and 100 µg/mL -S9 and at 100 µg/mL +S9 (MRID 41565122). Cytotoxicity [i.e, ≥50%↓ in the mitotic index, MI was seen at 100 µg/mL -S9 (60-65% ↓MI). Induced chromosome aberrations were predominantly breaks, fragments and minutes. Ashby et al. (1996) (MRID 44496215) reported on the above study and presented data from two other assays with acetochlor demonstrating that acetochlor of analytical grade was a confirmed clastogen in human lymphocytes in whole blood cultures or in isolated human lymphocytes. Types of induced chromosome aberrations were breaks and fragments/minutes. The study authors stated that acetochlor appeared to be "more toxic to the isolated cells" than lymphocytes in treated-blood cultures and that the greater toxicity was probably due to "the absence of glutathione (GSH) in the isolated lymphocytes as compared to its presence in the whole blood cultures". Ashby et al. presented additional data showing that two other chloroacetyl non-carcinogens that were negative in the Ames assay (2-chloroacetophenone and 4-chloroacetylacetanilide) were also clastogens in human lymphocytes as was the N-butyl analogue of acetochlor. However, the des-chloro analogue was not clastogenic, which lead the authors to conclude that, "These results establish the chloro substituent in acetochlor as the clastogenic entity and indicate the protective cellular effect afforded by the free thiol (SH) group of glutathione".

In vivo test systems

There was no evidence of a clastogenic or aneugenic response in micronucleus assays in the bone marrow of CD-1 or C57 mice (MRID 00164941 or 41565123, respectively), and in a Sprague-Dawley rat bone marrow cytogenetic assay (MRID 00131392) up to toxicologically overt doses or cytotoxic levels causing reductions in the ratio of polychromatic to normochromatic erythrocytes (PCE:NCE) in mice or up to the limit dose using the oral route of exposure in rats. Similarly, acetochlor was not clastogenic in a series of dominant lethal mutation assays in rats (MRID 44069502 or 44093703) or mice (MRID 44093701) using either a dietary exposure or oral gavage up to toxicologically overt doses or the limit dose. Findings from one of the rat dominant lethal assays (MRID 44093703) showed significantly ($p < 0.025$) reduced enzyme levels for glutathione (GSH) in the testes, caput and cauda of the epididymis and the vas deferens of males 12 hours after treatment with 2000 mg/kg.

The overall results from chromosome aberration assays indicate that acetochlor is a confirmed clastogen in cultured mammalian cell test systems and the N-butyl analogue of acetochlor, but not the des-chloro analogue, was also clastogenic *in vitro*, as well as two chloroacetyl non-carcinogens. However, acetochlor's clastogenic activity in cultured mammalian cells results in breaks, fragments and minutes, which are generally associated with cytotoxicity because the types of induced aberrations are asymmetric and, therefore, not consistent with cell survival (Galloway 2000). Furthermore, the induction of chromosome aberrations is not expressed *in vivo* in either somatic or germinal cells of rats or mice.

OTHER MUTAGENIC MECHANISMS

In vitro test systems

Hill et al. (1997) reported suggestive evidence of sister chromatid exchange (SCE) induction in human lymphocytes at 10 µM acetochlor while 0.3 and 0.1 µM of a dialkylquinoneimine metabolite of acetochlor, (ethylmethylquinoneimine) induced a significant increase in SCE which was only 1.2-1.3-fold over background suggesting a weak response. Acetochlor was negative for unscheduled DNA synthesis (UDS) in primary rat hepatocytes up to cytotoxic concentrations (≥10.6 µg/mL) (MRID 00131393).

In vivo test systems

A weak positive UDS response was revealed in hepatocytes harvested from Sprague-Dawley rats treated for 12 hours with 2000 mg/kg acetochlor. Ashby et al. (1996) pointed out, however, that the weak UDS response was accompanied by major hepatic pathology [(e.g. necrosis, 60-fold increases in alanine transaminase and aspartate transaminase and 70% depletion of glutathione (MRID 44496215)]. A negative comet assay was reported in which DNA was harvested from the nasal, olfactory or respiratory tissue of male Sprague-Dawley rats administered dietary preparations of 1750 ppm (~88 mg/kg/day) acetochlor for either 7days or 18 weeks (MRID 44069503). Finally, a time- and dose-related significant ($p<0.05$-0.01) increase in cell proliferation (S-phase induction) was noted in the nasal turbinates of the olfactory region but not the respiratory region of male Sprague-Dawley rats receiving dietary dosages of 1750 or 5000 ppm acetochlor (~88 or 250 mg/kg/day, respectively) for 60, 90 or 160 days (MRID 44496207). This finding is of great importance since the increase in cell proliferation, which was time and dose-dependent, is most likely due to cytotoxicity to the nasal turbinates. Additionally, the sustained increase in cell proliferation is proposed a key event in the induction of nasal olfactory tumors in rats. It should be noted that the doses used in the comet assay or the cell proliferation assays caused nasal olfactory tumors in the Sprague-Dawley rat while the dose used in the UDS assay (2000 mg/kg) was in excess of the both the tumorigenic doses and the MTD (1000 ppm, ~50 mg/kg/day for 104 weeks).

CONCLUSIONS

Results for gene mutation assays are conflicting and provide no clear evidence of a positive effect in either bacteria or mammalian cell test systems. Similarly, the evidence from *in vitro* and *in vivo* UDS assays, *in vitro* SCE studies, and an *in vivo* comet test provide no convincing pattern of genotoxic activity. Although there is suggestive or weak evidence of *in vitro* SCE induction (only 1.3-fold higher than control for acetochlor), the impact of increased SCE induction is not well understood and this phenomena has not been linked to a cancer risk (Albertini et al., 2000). Thus, the results from these various assays are mixed and without confirmation. By contrast, results from chromosome aberration assays indicate that acetochlor is a confirmed clastogen in cultured human lymphocytes. There is also the possibility that the increased mutant colony counts observed in the positive mouse lymphoma assay resulted from a clastogenic rather than mutagenic response since this test system can detect chromosome breakage. Nevertheless, clastogenicity is confined to *in vitro* mammalian cell test systems and the types of induced aberrations suggest cytotoxicity. Based on data from three bone marrow assays in either mice or rats and three dominant lethal mutation studies also in rats or mice, acetochlor-induced clastogenicity is not expressed in either somatic or germinal cells of whole animals. This finding is consistent with a

similar profile of *in vitro* but not *in vivo* clastogenicity for the chloroacetanilides. Like acetochlor, **alachlor** has also been widely tested in a variety of assays either submitted to the Agency or published in the open literature. In agreement with the acetochlor data, alachlor is generally negative in gene mutation assays, clastogenic in CHO cells and human lymphocytes *in vitro* but negative in whole animal studies.

Ashby et al. (1996) claim that the clastogenicity of acetochlor results from a preferential reaction with GSH as opposed to the nitrogen or oxygen atoms of DNA, and "when these DNA-protective cellular nucleophiles are depleted (with increasing doses of acetochlor), a reaction will occur with chromatin sulphydryl groups leading to clastogenicity". Binding to macromolecules is characteristic of this chemical class as indicated by the preferential binding of acetochlor to sulfhydryl groups such as GSH and proteins. This would also appear to explain the weak UDS response in hepatocytes harvested from Sprague-Dawley rats treated for 12 hours with 2000 mg/kg acetochlor since non-protein sulfhydryl levels (consisting of >95% GSH in the liver) were reduced by 67%. The response was accompanied by liver necrosis which "reached such levels in some of the rats that panlobular destruction was observed." However, these findings conflict with the comet assay results showing that acetochlor did not produce DNA damage in Sprague-Dawley rat nasal olfactory tissue after dietary exposure to a tumorigenic dietary level (1750 ppm) for 18 weeks. The implications from these data have great impact since nasal olfactory tumors induced by acetochlor are considered by the Agency to be the neoplasia of toxicological importance. At this time, however, only a very weak case can be made for mutagenicity as the primary driver in the development of nasal olfactory tumors.

Mutagenicity studies with acetochlor are summarized below in Table 5.

Table 5. Summary of mutagenicity studies with acetochlor

MUTAGENICITY STUDIES WITH ACETOCHLOR		
GENE MUTATIONS		
870.5100 Bacterial Gene Mutation Assay *Salmonella typhimurium*	00050930 (1978) Acceptable/guideline 0.001-1 µl/plate - /+ S9	**Negative** up to the highest dose tested (1 µl/plate - /+ S9); higher concentrations (≥10 µl/plate - /+ S9) were cytotoxic
870.5100 Bacterial Gene Mutation Assay *Salmonella typhimurium*	41565121 (1989) Acceptable/guideline 1.6-5000 µg/plate - /+ S9	**Equivocal positive** in TA 1538 at 2500 and 1000 µg/plate + S9; reproducible at 1000 µg/plate but <2-fold, not dose-related and not seen in TA98
870.5100 Bacterial Gene Mutation Assay *Salmonella typhimurium* TA1538 only	44863202 (1989) Acceptable/nonguideline 100-5000 µg/plate - /+ S9 (Arochlor 1254 or Pheno-barbital /β-naphthoflavone induced rat livers)	**Negative** in TA1538 using 3 different Batches (89.8-99.6%) in two separate tests

870.5300 *In vitro* mammalian cell gene mutations Chinese Hamster Ovary (CHO) cells	00131395 (1983) Acceptable/guideline 25-150 µg/mL - S9 25-125 µg/mL + 10% S9	**Positive** ≥2-fold in mutation frequency (MF) at 125 or 150 µg/mL - S9 & 125 µg/mL + S9 accompanied by cytotoxicity (61% or 93%↓in cell survival - /+S9)
870.5300 *In vitro* mammalian cell gene mutations CHO cells	42713106 (1989) Acceptable/guideline 50-200 µg/mL -S9 50-300 µg/mL + 1, 2, 5 or 10% S9	**Negative** up to cytotoxic levels (≥200 µg/mL -/+ 10% S9)
870.5300 *In vitro* mammalian cell gene mutations Mouse lymphoma L5178Y cells	00131394 (1982) Acceptable/guideline 20-400 µL/mL -S9 5-250 µL/mL +S9	**Positive** 30-50 µL/mL +S9 2.2-5.2 fold increase accompanied by cytotoxicity (<10% survival at ≥50 µL/mL +S9)

MUTAGENICITY STUDIES WITH ACETOCHLOR		
CHROMOSOME ABERRATIONS		
870.5375 Cytogenetics *In vitro* mammalian cell chromosomal aberration assay human lymphocytes	41565122 (1989) Acceptable/guideline 0, 10, 50 100 µg/mL -/+ S9	**Positive** at 50 and 100 µg/mL -S9 and 100 µg/mL +S9 accompanied by marked reduction in mitotic indices at 100 µg/mL (≥59%↓). Types of aberrations: breaks, fragments and minutes
870.5375 Cytogenetics *In vitro* mammalian cell chromosomal aberration assay human lymphocytes (whole blood vs separated blood)	44863204 (1998) Acceptable/guideline 0, 10, 75 150 µg/mL -/+ S9 0, 100 µg/mL - S9 (whole blood) 0, 75 µg/mL - S9 (separated blood)	Whole Blood: **Positive** at 75 and 150 µg/mL -S9 and 150 µg/mL +S9 accompanied by slight reduction in mitotic indices at 150 µg/mL (31% -S9; 13 % +S9↓). Types of aberrations: breaks, fragments and minutes
		Whole Blood: **Positive** 9-fold↑ in aberrations at 100 µg/mL Separated Blood: **Positive** 26-fold↑ in aberrations at 75 µg/mL
SOMATIC CELLS		
870.5385 Mammalian Bone Marrow Chromosomal Aberration Test Rat	00131392 (1983) Acceptable/guideline 0, 40, 150, 500 mg/kg IP injection	**Negative** up to overt toxicity (significant ↓body weight gain)
870.5395 Mammalian Erythrocyte Micronucleus Test CD-1 Mice	00164941 (1986) Acceptable/guideline 0, 200, 660, 2000 mg/kg oral gavage	**Negative** up to overt toxicity (mortality) & cytotoxicity (significant ↓ PCE:NCE ratio at 2000 mg/kg, both sexes combined)
870.5395 Mammalian Erythrocyte Micronucleus Test CD-1 Mice	41565123 (1989) Acceptable/guideline 0, 898 or 1436 mg/kg ♂ 0, 1075 or 1719 mg/kg♀	**Negative** up to a cytotoxic dose (significant ↓ PCE:NCE ratio) seen at both doses in ♂♀
GERMINAL CELLS		

870.5450 Cytogenetics Dominant Lethal Rat	44069502 (1996) Unacceptable/guideline 0, 200, 1000, 1500 ppm for 10 weeks	**Negative** for dominant lethal mutations but dosage was insufficient
870.5450 Cytogenetics Dominant Lethal Mouse	44093701(1996) Unacceptable/guideline 0, 200, 1000, 3500 ppm for 8 weeks	**Negative** for dominant lethal mutations but dosage was insufficient
870.5450 Cytogenetics Dominant Lethal Rat	41963309/44093703 (1991/1996) Acceptable/guideline 0, 200, 1000, 2000 mg/kg oral gavage	**Negative**; earlier report of positive results now considered to be due to reproductive (infertility) toxicity
OTHER MUTAGENIC MECHANISMS		
Other Genotoxicity *In vitro* sister chromatid exchange assay Human Lymphocytes	Hill et al. (1997) 10 µM (2.7 µg/mL)	**Weak evidence of positive** response (1.5-fold ↑) in one of two donor cells
870.5550 Other Genotoxicity *In vitro* UDS in Primary Rat Hepatocytes	00131393 (1983) Acceptable/guideline 0.032-320 µg/well	**Negative** up to cytotoxic concentrations (≥10.6 µg/well)
Other Genotoxicity *In vivo* UDS in Primary Rat Hepatocytes	41565124 (1989) 0, 500, 1000, 2000 mg/kg oral gavage	**Weak positive response** accompanied by major hepatic pathology (necrosis, 70% ↓GSH, 60-fold increase in aspartate transaminase
Other Genotoxicity *In vivo* Comet Assay in Rat Olfactory and respiratory cells	44863208 (1999) 1750 ppm (175 mg/kg/day) 7 days	**Negative** at a tumorigenic dose *in vivo*

c. Mode of Action Data

1. Nasal tumor observations

As summarized in Table 6, three rat chronic studies reported statistically significant incidences of nasal tumors in rats at acetochlor dietary levels of ≥1000 ppm. Additional data indicate that these tumors are confined to the olfactory epithelium region. No nasal tumors were seen in two acceptable mouse carcinogenicity studies.

Table 6. Incidence of nasal tumors in rat chronic studies.

	Study (MRID)	Dose Level (ppm)							
		Males				Females			
#1	**PR-80-006 (00131088, 40484801)**	**0**	**500**	**1500**	**5000**	**0**	**500**	**1500**	**5000**
	papillary adenoma	0/69	1/70	6/69*****	18/69******	0/69	0/68	2/70	1/69
	pap. adenocarcinom.	0/69	0/70	0/69	2/69	0/69	0/69	0/70	0/69
	Combined	ND	ND	ND	ND	ND	ND	ND	ND
#2	**ML-83-200 (40077601)**	**0**	**40**	**200**	**1000**	**0**	**40**	**200**	**1000**
	papillary adenoma	1/58**	0/54	0/58	12/59******	0/69**	0/69	0/67	19/68******
#3	**88/SUC017/0348 (41592004)**	**0**	**18**	**175**	**1750**	**0**	**18**	**175**	**1750**
	papillary adenoma	0/69**	0/59	0/59	35/70******	0/69**	0/57	0/58	36/63******
	carcinoma.	0/69	0/59	0/59	2/70	0/69	0/57	0/58	1/63
	Combined	0/69**	0/59	0/59	37/70******	0/69**	0/57	0/58	37/63******
#4	Sulfoxide Metabolite 1 year[a](Special study)	**0**	**300**	-	-	**0**	**300**	-	-
	polypoid adenoma	0	7/32 **	-	-	-	-	-	-

* = p≤0.05; ** = p≤0.01. ; [a] Sex unspecified in this special study. Incidence at 26 weeks .

To determine if there was a similarity in morphology, origin, and location of proliferative lesions , the Registrant conducted a review (See MRID 44496214) of hematoxylin/eosin slides of nasal tissue of rats treated with acetochlor, alachlor or butachlor in previously conducted long-term oral studies. In the case of acetochlor, slides from study #3 (88/SUC017/0348, MRID 41592004) of Table 1 were used. In the case of alachlor, slides from an alachlor special rat chronic study (EHL 93049, MRID 43590001); and in the case of butachlor, slides from a butachlor rat chronic study (Biodynamics 79-2388, MRID 42244901) were used.

It was found that:

- All three of the chloracetanilides induced morphologically similar lesions confined **almost entirely to the olfactory epithelium lining** in specific regions of the posterior nasal passages.

- These lesions and tumors were found within all six ethmoturbinates, but were almost entirely absent from the dorsal septum and dorsal medial regions of the ethmoturbinates with the exception of foci of basal cell hyperplasia in female rats (16/69) exposed to acetochlor, of which 9/16 were mapped to those regions.

- Most of the benign tumors exhibited ciliation of the olfactory epithelial cells and were associated with **respiratory metaplasia of adjacent olfactory mucosa.** Many lesions were close to the olfactory-respiratory epithelial junctions (See pp. 14-16 in MRID 44496214).

- For all three compounds, these changes were apparently identical in nature and location and differed only in frequency of occurrence (comparison of frequency compromised by differences in animal sacrifice times, strains used and number of animals evaluated for each compound).

It was also concluded in this study that the similar morphology suggests that the same processes may be responsible for these preneoplastic and neoplastic changes for all three acetanilides.

These results are supported by observations with the rats from study #1 (MRIDs 00131088 and 40484801) in Table 6. In study #1 (conducted at 0, 500, 1500, & 5000 ppm) no nasal tumors were initially found, in spite of the higher doses used, because sections had been taken from the anterior part of the nasal cavity. When new histological sections were taken so as to include the posterior region of the nasal cavity, the tumors reported in Table 6 for study #1 were seen. The posterior region of the nasal cavity in rats is essentially olfactory epithelium, in contrast to respiratory epithelium, which is found in the more anterior part of the nasal cavity, Young (1981).

2. Metabolism

i. Absorption.

Bile-duct cannulated rats were administered C^{14}-alachlor excreted over 80% of a radioactive dose in the bile, in contrast to similarly dosed non-cannulated rats, that excreted 70% of the dose in urine. These values are consistent with high absorption by the oral route followed by extensive enterohepatic circulation of the metabolites.

ii. Tissue distribution

Sprague-Dawley rats were administered ^{14}C-Acetochlor in the diet at levels of 1750 or 5000 ppm. The animals were sacrificed after 14 days on the diet for examination by whole body autoradiography (WBA) and microautoradiography. WBA revealed significant localization of

radioactivity in the nasal turbinates. Micro radioautography in high-dose rats showed intense localization in Bowman's glands, a lower degree in the olfactory surface, and no evidence of localization in the respiratory epithelium. In low-dose animals only slight to moderate localization was seen in the Bowman's glands (MRID 44496210).

Male Sprague-Dawley rats received 5 consecutive daily doses of the ^{14}C-secondary sulfide metabolite of Acetochlor by gavage. Rats were sacrificed at 1 or 5 days after the last dose for examination by WBA. Examination of radioautographs from animals sacrificed one day after dosing show high levels of radioactivity in the intestinal contents & liver, nasal turbinates, and lining of the tongue. At 5 days after dosing in addition to residual radioactivity in the stomach and intestinal contents, there was clear localization in the nasal turbinates, radioactivity in surrounding areas was greatly diminished. Micro radioautography showed that the label was concentrated in the Bowman glands of the nasal turbinates (MRID 44496212).

iii. Biotransformation

As summarized in Figure 2, acetochlor in rats undergoes extensive biotransformation involving enterohepatic recirculation (MRIDs 41565125, 41565126, 41565127, 41592006, 41592007) leading to the precursors of the quinone-imine (e.g. the sulfoxide metabolite, Figure 2). Following conjugation with GSH or glucuronic acid in the liver, the conjugates are excreted in the bile. The GSH conjugate undergoes partial degradation in the gut and is reabsorbed resulting in the appearance of the sulfoxide metabolite (U11, Figure2) and its precursors in the blood.

The metabolites identified in Figure 2 amounted to about 77% of the dose. Other possible metabolites such as 2-ethyl-6-methylaniline (EMA) that would lead to a quinone imine were not discussed.

In more recent work (MRID 44530002), it was shown in vitro that microsomal fractions from rat liver and olfactory epithelium can metabolize the S-methyl sulfide metabolite of acetochlor to EMA (Figure 3). Thus, the Registrant summarized (MRID 46081801, and Green et al., 2000) the formation of precursors of the quinone imine by way of two paths (A and B) in the rat. Path A proposed amidase hydrolysis of the secondary methyl sulfide metabolite to 2-ethyl-6-methylaniline (EMA), hydroxylation of EMA to pOH-EMA and the subsequent formation of the quinone-imine. This pathway has been well characterized for **acetochlor**. Path B proposes oxidation of the secondary methyl sulfoxide metabolite to the sulphoxide (Acetochlor sulfoxide), hydroxylation of acetochlor sulfoxide to p-hydroxy sulfoxide leading to the formation of the sulfoxide quinone imine. Path B is an alternative pathway, which proposed a methyl sulfoxide as the major intermediate in the formation of DABQI as opposed to the aniline metabolite, formed by **alachlor**. In support of Path B, Green et al. found that acetochlor sulfoxide was the major metabolite in the plasma of rats fed 1750 ppm acetochlor in the diet for 6 months (~700 dpm sulfoxide, 20 minutes) or in rats 17 hours after being given a single oral dose of 200 mg/kg acetochlor (~190 dpm.sulfoxide, 20 minutes). In contrast, very little acetochlor sulfoxide (~75 dpm sulfoxide, 20 minutes) was detected in CD-1 mice administered single a oral dose of 200 mg/kg acetochlor.

However, results from the earlier comparative *in vitro* metabolism study in the rat and mouse (MRID 44530002) indicate that this is not a significant route of metabolism of acetochlor in the mouse because high levels of acetochlor sulfoxide were found in rat plasma as opposed to mouse plasma which contained very little acetochlor sulfoxide. Unlike the rat, therefore, acetochlor sulfoxide would not be considered a significant circulating metabolite for the mouse.

Overall, the data suggest that Path B is a plausible metabolic pathway for metabolism of acetochlor to the reactive quinone imine in the rat and that acetochlor sulfoxide is the major circulating metabolite available *in vivo* to the rat nasal tissues.

It should be pointed out, however, that while Path A is well established for **alachlor**, the Path B pathway has not been evaluated for this chloroacetanilide. Furthermore, the "accepted" Path A pathway has not been ruled out for acetochlor. Regardless of the pathway, however, the data presented by the Registrant do suggest that the secondary methyl sulphide substrate may not be available to the mouse because the major route of acetochlor metabolism in the mouse is through glucuronide conjugation. This is supported by data showing that acetochlor sulfoxide is not found in mouse plasma, and is, therefore, not a circulating metabolite. Nevertheless, there are concerns regarding these conclusion based on an independent analysis of radiochromatograms of the rat and mouse urine samples because not all of the rat or mouse urinary metabolites have been taken into account.

Either way, either quinone imine will then bind to tissue proteins and other nucleophiles such GSH.

Based on work by Green (1998) (MRID 44496203) conjugation with GSH plus path B seems the major source of quinone imine in the rat. When the mouse was studied (MRID 44496203) it was found that the major *in vivo* metabolic route was glucuronidation plus and excretion of the chloramide. Glutathione conjugation, enterohepatic circulation and formation of quinone imine precursors was not a major route. The author considered this interspecies difference to be consistent with the absence of nasal tumors in the mouse.

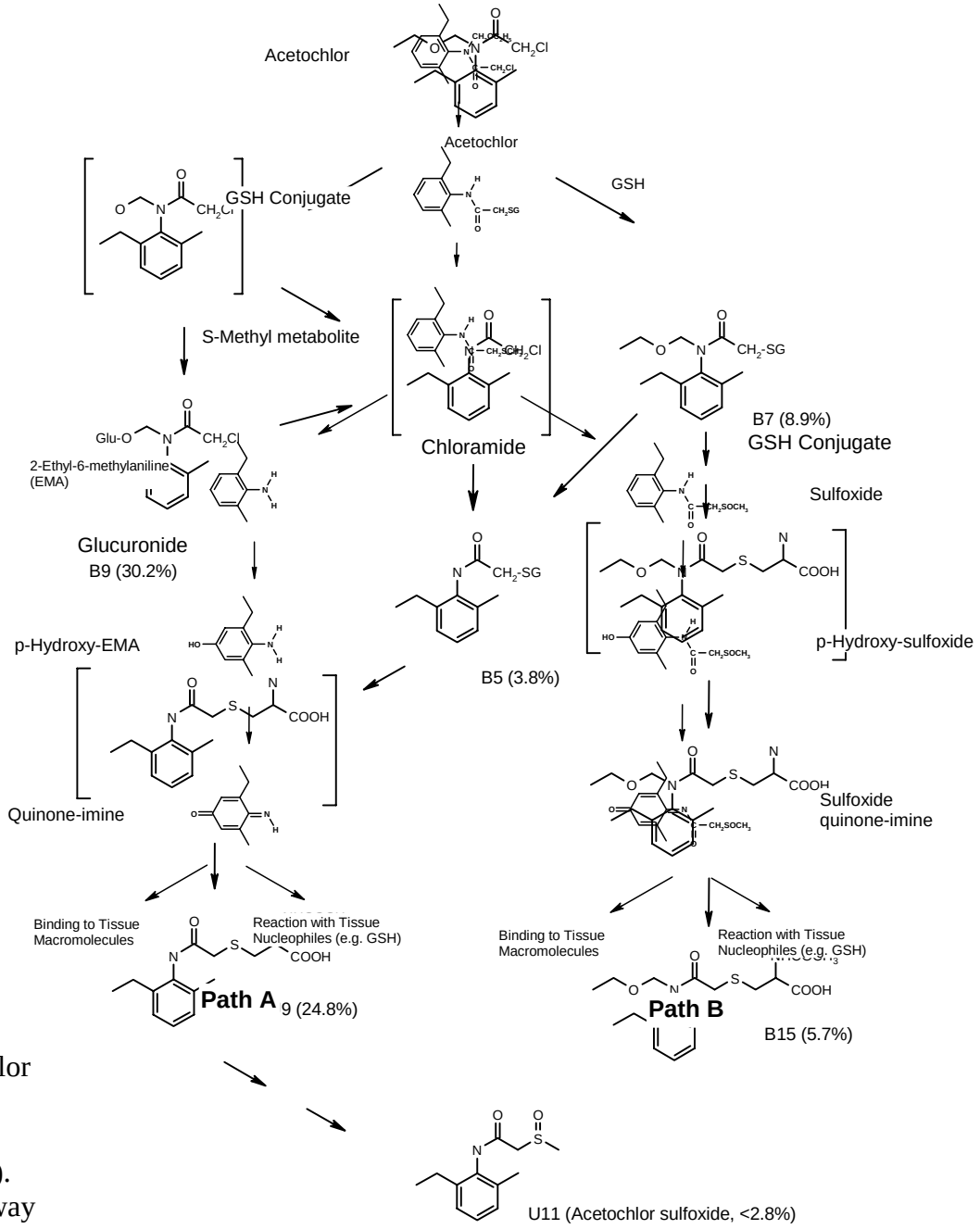


Figure 2. Biotransformation of acetochlor in SD rats (MRID 41565127). This pathway covers biotransformation from parent acetochlor up to formation of U11, the sulfoxide metabolite of acetochlor, one of the possible precursors of the quinone imine. (Percentages of metabolites, as percent of dose, are in parentheses).

Figure 3. Biotransformation of the S-methyl metabolite of acetochlor, leading to either methylethylquinone imine (MEQI) or methylethylquinone imine sulfoxide (Adapted from MRID 46081801).

3. Binding

i. Introduction.

The registrant has submitted a series of studies analyzing different aspects of binding of acetochlor and its metabolites to nasal tissue proteins.

In an initial study (MRID 44496210) the authors found a dose-related increase in adduct formation in SD rats administered ^{14}C-acetochlor in the feed at 1710 and 5170 ppm for 14 days. Nasal tissue proteins were extracted, subjected to acid hydrolysis and the released products were analyzed. As shown in Figure 4, two products were seen: EMIC-cysteine and EMA. One product, EMIC-cysteine, was formed from a quinone imine. EMIC-cysteine is formed regardless of whether the adduct comes from binding of EMA-quinone imine or sulfoxide metabolite-quinone imine. The second product (bottom reaction in Figure 4) EMA is formed if the adduct originated from direct binding of acetochlor or a metabolite still retaining the chlorine atom. Binding was confirmed by autoradiography. The level of binding was dose-related (Table 7). Notice that the level of product originating quinone imine binding (EMIQ-Cysteine) is smaller than that originating from binding from direct chlorine displacement.

TABLE 7 : Concentration of EMIQ-cysteine and EMA in nasal protein hydrolysate of rats fed with ^{14}C-acetochlor for 14 days[a]

Group (ppm in diet)	EMIQ-Cysteine (pmole/mg Protein)	EMA (pmole/mg Protein)
1710	119 ± 14.7	440 ± 273
5170	206 ± 64	1060 ± 445

[a]Data from Table 1, p. 32, MRID 44496210

Another pair of studies confirmed formation of nasal tissue adducts in rats dosed with two acetochlor metabolites which are quinone-imine precursors (see Figure 3 for structures): acetochlor sulfoxide metabolite (MRID 46009402) and acetochlor secondary sulfide metabolite (MRID 44496212). By dosing separately with [phenyl-^{14}C]-acetochlor and [carbonyl-^{14}C]-acetochlor, it was confirmed that the sulfoxide moiety was retained in the formed adduct.

Binding to nasal tissues of the mouse and rhesus monkey after ^{14}C-acetochlor administration was investigated in another pair of studies. In the case of the mouse (MRID 44496211) no EMIQ-cysteine was found after adduct analysis, only EMA was found, bound at dose related levels Table 8). EMA originated from binding of parent acetochlor or one of its metabolites still containing chlorine. In the case of the rhesus monkey (MRID 44496213) neither EMIQ-cysteine or EMA were found.

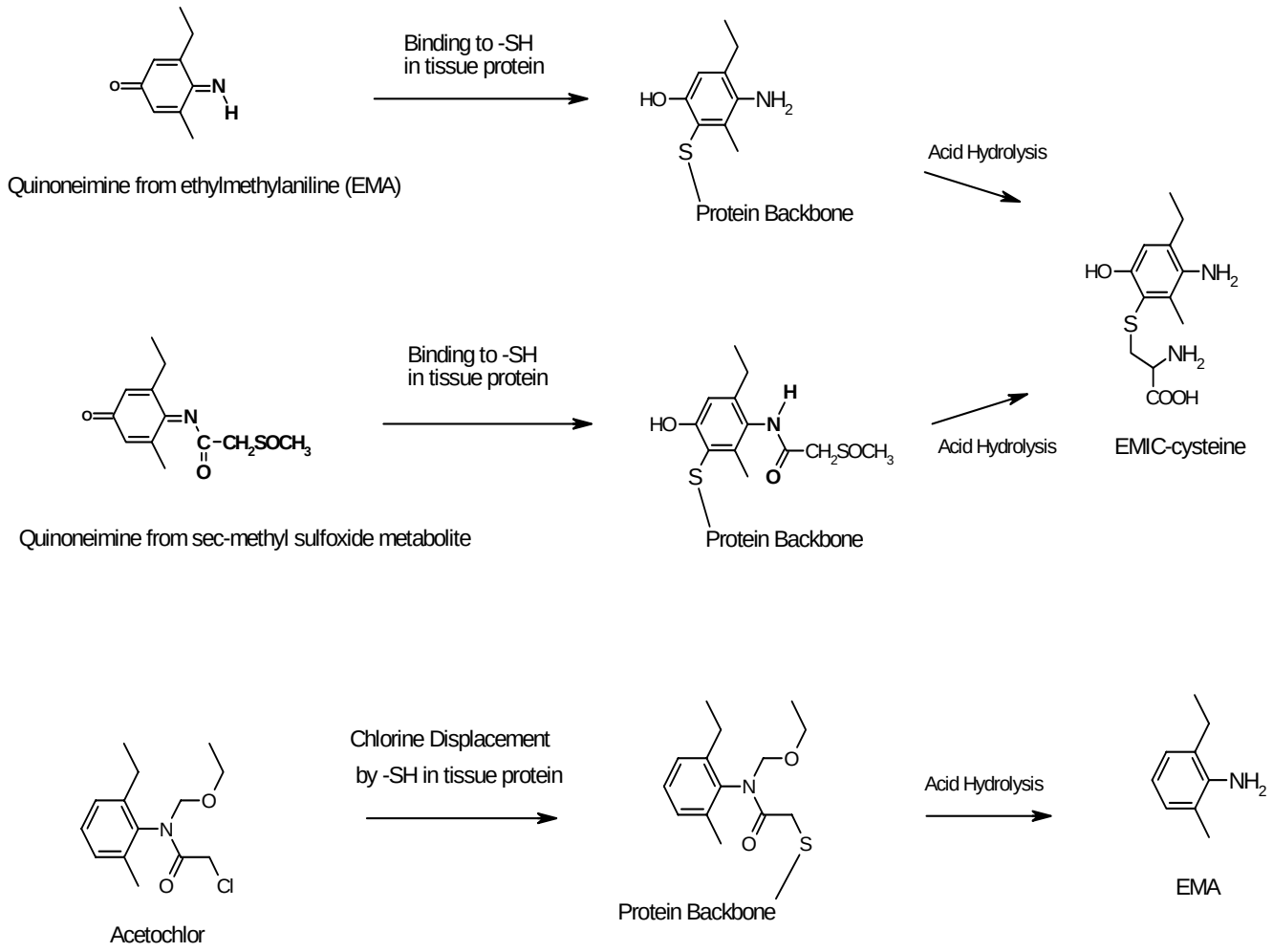


Figure 4. Identification of bound moieties in adducts to nasal epithelium proteins [adapted from MRID 44496212].

Microradioautography indicates that radioactivity is highest in the Bowman gland part of the olfactory epithelium for acetochlor (MRID 44496210) and acetochlor sulfoxide metabolite (MRID 46009402). Furthermore, HPLC chromatographic analysis of nasal protein hydrolysates after dosing with C^{14}-phenyl acetochlor show the label largely confined to hydrolysates from the olfactory epithelium (MRID 46009402).

TABLE 8: Concentration of EMIQ-cysteine and EMA adducts in nasal protein hydrolysate of mice fed ^{14}C-acetochlor for 14 days[a]

Group (ppm in feed)	EMIQ-cysteine (pmole/mg protein)	EMA (pmole/mg protein)
1800	<LOD[b]	128 ± 26.7
4750	<LOD	197 ± 54.4

[a]Data from Table 1, p. 26, MRID 44496211,
[b]Limit of detection

ii. Adduct formation in rats (Executive Summaries)

a) In a protein binding study (MRID 44496210), as explained by the investigators (p. 13 of the Study Report), acetochlor (95.2% a.i.) was administered to male Sprague Dawley rats in the diet at concentrations of 1710 ppm and 5170 ppm for 14 days to determine and characterize the nasal localization of ^{14}C-acetochlor. The results in this study address the potential binding of the putative metabolic adduct of acetochlor, 3-ethyl, 5-methylbenzoquinone-4-imine (EMIQ) to rat nasal proteins. The binding of acetochlor adducts to rat nasal turbinates was determined by an acid hydrolysis technique followed by HPLC analysis. HPLC analysis of the protein hydrolysate from both groups of animals showed a significant and dose-dependent formation of the 3-ethyl, 5-methyl-benzoquinoneimine-cysteine (EMIQ-cysteine) adduct *in vivo*. The average level of the EMIQ-cysteine adduct in rat nasal turbinates from rats fed 1710 and 5170 ppm was 119 pmole/mg protein and 206 pmole/mg protein, respectively. In addition to EMIQ binding, direct binding of acetochlor to nasal tissues was identified by the investigators.

The results of the whole body autoradiography showed significant localization of radioactivity in nasal turbinates at both dose levels. Microautoradiography studies showed intense localization of radioactivity within the Bowman's glands in high dose rats. There was a lower degree of localization of radioactivity in the olfactory surface epithelium and no evidence of localization within the respiratory epithelium. In low-dose rats, only slight to moderate localization of radioactivity was found in the Bowman's glands.

This protein-binding characterization study is **Unacceptable/Nonguideline**. This study may be upgraded to Acceptable/Nonguideline if the following data/information are submitted and are deemed to be satisfactory by the Agency: 1) the investigators did not present the rationale in support of the structure of the synthesized EMIQ-cysteine marker (p. 25 of the Study Report). The investigators should submit such rationale; 2) the investigators discussed the binding of EMIQ

to nasal proteins in the context of a mechanism of action for acetochlor. In addition to EMIQ binding, the investigators identified binding of acetochlor to rat nasal proteins after "non-enzymatic direct chlorine displacement of acetochlor by the sulfhydryl group of cysteine in rat nasal proteins" (p. 27 of the Study Report). However, the significance of these adducts is not included in the context of discussion of mechanism of action. The investigators should discuss this since this interaction with proteins may also underlie or contribute to the mechanism(s) of carcinogenicity, particularly since according to Table 1 (p. 32 of the Study Report) this form of binding to nasal protein is more extensive in terms of pmole/mg protein (as assessed by EMA release) than is EMIQ binding; 3) a clear statement describing the methodology employed in quantifying the concentration (pmole/mg protein) of EMIQ-cysteine and EMA in nasal protein hydrolysates, as reported, for example, in Table 2 of the Results section in this review; 4) citations to specific reference materials identified as needed in various sections of this review.

b) In a protein binding study (MRID 44496212), as explained by the investigators (p. 14 of the Study Report), acetochlor secondary sulfide (>99% radiochemical purity) was administered to male Sprague Dawley rats to determine and characterize the nasal localization of ^{14}C-acetochlor secondary sulfide. Four males were used in group M1, 2 males in group M2, 3 males in group M3, and 2 males were used in group M4. Groups M1, M2 and M3 were given five consecutive daily doses of approximately 7 mg/kg body weight and M4 group was given a single oral dose. In groups M1 and M2, half the animals were sacrificed one day after the final dose, and the other half were sacrificed five days after the final dose. In groups M3 and M4, all animals were sacrificed one day after the final dose.

The binding of acetochlor secondary sulfide to rat nasal turbinates was determined by an acid hydrolysis technique followed by HPLC analysis. HPLC analysis of the protein hydrolysate from treated animals showed the formation of a cysteine conjugate derived from 3-ethyl, 5-methyl-benzoquinoneimine (EMIQ-cysteine). The average level of this EMIQ-cysteine adduct was 19.3 pmole/mg protein following oral administration of acetochlor secondary sulfide for 5 days at approximately 7 mg/kg/day. The results of the whole body autoradiography showed significant localization of radioactivity in the nasal turbinates. Microautoradiography studies showed intense localization of radioactivity in the Bowman’s glands of treated animals.

In summary, the study supported the hypothesis that rat nasal tissue is capable of metabolizing acetochlor secondary sulfide to EMIQ [p. 14 of the Study Report]. It is noteworthy that Green et al. (2000) indicate that the nasal metabolism of acetochlor proceeds through a secondary sulfide, with subsequent branching through either EMA or sulfoxide pathways, both of which result in protein adducts via a reactive intermediate quinone-imine. The final hydrolysis step in the analytical procedure to assay for protein binding yields the same final quinone imine protein metabolite in both pathways, i.e. the EMIQ and other quinone imine pathway analytical end products are the same.

Principally, through the use of the acetochlor secondary sulfide, which precludes the direct binding to nasal tissue proteins via chlorine displacement that occurs with acetochlor in addition to the EMIQ, or other quinone-imine pathway (per Green et al.), this study nonetheless demonstrated the presence of a quinone-imine protein adduct in rat nasal tissue following administration of

acetochlor secondary sulfide. This study thus serves as further support for a quinone-imine protein binding mechanistic explanation for acetochlor induced nasal toxicity.

This study on secondary sulfide binding is **Unacceptable/Nonguideline**. This study may be upgraded to Acceptable/Nonguideline if the following data are submitted and are deemed to be satisfactory by the Agency: 1) a reference, preferably the best reference, wherein the rationale may be found for the hypothesis that the formation of EMIQ is critical to the induction of nasal tumors by acetochlor; 2) a reference to the chronic/carcinogenicity study upon which dose selection was based; 3) if possible, some indication of the comparative magnitude of localization of radioactivity in nasal tissue, versus those of liver, kidney, lining of tongue, for example; 4) any information that may be available on the nasal tissue cytotoxicity and carcinogenicity of acetochlor secondary sulfide; 5) any available information on the question of acetochlor metabolic conversion to EMIC in the liver.

c) In a special nonguideline mechanistic study (MRID 46009402), localization of protein adducts formed in the nasal cavity of rats by binding of [^{14}C-labeled] acetochlor sulfoxide or its metabolites was assessed in male Crl:CD(SD)BR rats by the following three techniques: (1) *In vivo* protein binding - comparison by HPLC of the total radioactivity bound to nasal cavity proteins in hydrolysates prepared from the olfactory mucosa vs. the respiratory mucosa of 4 rats given a single gavage dose of [^{14}C phenyl-labeled] acetochlor sulfoxide (10 mg/kg bw in 4 mL/kg bw PEG 600; 100 MBq/kg bw); (2) *In vitro* protein binding - comparison using SDS-PAGE, western blot and phosphor imaging of radioactivity bound to proteins following incubation of microsomal fractions derived from rat nasal cavity olfactory tissues with either [^{14}C phenyl-labeled]acetochlor sulfoxide or [^{14}C carbonyl-labeled] acetochlor sulfoxide (0.4 mM; 407-458 Kbq per incubation mixture) and (3) Histoautoradiographic localization of protein binding - localization of protein-bound radioactivity in sections of the olfactory and respiratory regions of the nasal cavities of rats exposed to a single gavage dose of either [^{14}C phenyl-labeled] acetochlor sulfoxide or [^{14}C carbonyl-labeled] acetochlor sulfoxide (10 mg/kg bw in 4 mL/kg bw PEG 600; 100 Mbq/kg bw) and sacrificed at 8 or 24 hr postdosing (1 rat/dose group/time point). Washed and unwashed slides were processed for autoradiography to compare levels of total and tightly bound radioactivity.

HPLC profiles of fractions eluted from the nasal olfactory tissue protein hydrolysates of rats treated with [^{14}C phenyl-labeled] acetochlor sulfoxide showed significantly higher levels of bound radioactivity than fractions from the nasal respiratory tissue protein hydrolysates. In addition to the largest peak, which appeared to elute as a doublet at 7.5 minutes, at least 3 other major peaks were identified along with numerous minor peaks (none were further characterized). No major peaks were isolated from the respiratory tissue fractions. [^{14}C]-labeled protein adducts formed by incubation *in vitro* of rat nasal olfactory mucosa microsomes with [^{14}C-carbonyl]- or [^{14}C-phenyl]-labeled acetochlor sulfoxide showed similar patterns when compared by SDS-PAGE/western blot/phosphor imaging, indicating that the sulfoxide moiety was retained in much of the bound radioactivity, although quantitative or qualitative comparisons of these proteins were not performed. Histoautoradiography of the olfactory and respiratory regions of the rat nasal cavity at 8 and 24 hrs postdosing showed the highest level of bound radioactivity occurring over Bowman's glands in the olfactory mucosa (as determined by visual inspection, not quantitative grain count). The radioactivity in Bowman's glands was determined to be tightly bound by comparing washed

(bound radioactivity) vs. unwashed (total radioactivity) slides. No tightly bound radioactivity was reported in the slides of the respiratory region of the nasal cavity, although histoautoradiograms of these sections were not presented in the study report. Although the highest grain count at 24 hr was observed over Bowman's glands, reported binding over the olfactory epithelial mucosal surface was less clearly visible due to background labeling.

This special nonguideline metabolism study in the rat is classified as **Unacceptable/non-guideline (upgradable)**. It was not conducted to satisfy guideline requirements for reregistration of acetochlors, but to provide supplemental data addressing the mechanism of nasal carcinogenicity of acetochlor in the rat. Although the study appeared to be well-conducted, reporting of the histoautoradiographic findings was incomplete (photos of the autoradiographs were not provided for some of the sections evaluated; see "Study Deficiencies", in Discussion section). The study may be upgraded to acceptable/nonguideline with submission of this confirmatory data.

iii) Binding in the mouse and monkey

a) In a protein binding study (MRID 44496211), as explained by the investigators (p. 13 of the Study Report), acetochlor (95.2%) was administered in the diet of female CD-1 mice at concentrations of 1800 and 4750 ppm to determine and characterize the nasal protein binding of ^{14}C-acetochlor. Acetochlor binding to mouse nasal turbinates was determined by acid hydrolysis followed by HPLC analysis. HPLC profiles of the protein hydrolysate from both treatment groups showed no significant formation of the 3-ethyl, 5-methyl-benzoquinoneimine-cysteine (EMIQ-cysteine) adduct *in vivo*. For both treatment groups, significant amounts of radioactive components were consistent with the 2-ethyl-6-methylaniline (EMA) standard. The concentration of EMIQ-derived cysteine adducts was below the limit of detection. The only detectable protein adduct formed between acetochlor and mouse nasal protein likely resulted from the chlorine displacement of acetochlor by the sulfhydryls of mouse nasal proteins. For whatever reason, on exposure to acetochlor via the diet, mouse (unlike rat) nasal mucosa does not yield the EMIQ-protein adduct, which may support the hypothesis that mouse nasal tissue lacks the capacity to metabolize acetochlor to the putative reactive metabolite, EMIQ. To the extent the formation of EMIQ *in vivo,* and its subsequent binding to nasal tissue protein, is critical as believed for the induction of nasal tumors by acetochlor, failure to detect EMIQ-cysteine adducts in the mouse after dietary exposure supports the hypothesis that the carcinogenic mechanism for acetochlor is not operative in the mouse, as contrasted with the rat. (p. 13 of the Study Report)

This study on the characterization of acetochlor protein binding in the mouse is **Unacceptable/Nonguideline**. This study may be upgraded to Acceptable/Nonguideline if the following data/information are submitted and are deemed to be satisfactory by the Agency: 1) the characterization of the structure of the EMIQ-cysteine marker as requested for the rat study (MRID 44496210), 2) a reference, preferably the best reference, wherein the rationale for the hypothesis that the formation of EMIQ is critical to the induction of nasal tumors by acetochlor, 3) comment on the question as to whether the EMA pathway of direct protein binding by chlorine displacement of acetochlor that occurred in this study would be associated with nasal cytotoxicity in the mouse.

b) In a 14-day oral toxicity study (MRID 44496213), as explained by the investigators (pp. 9, 13 of the Study Report), acetochlor (95.2%) was administered to 3 male Rhesus monkeys by gavage to determine and characterize the nasal localization of ^{14}C-acetochlor. The monkeys were administered ^{14}C-acetochlor at a dose level of 126 mg/kg body weight for 14 days. The results in this study address the potential binding of the putative metabolite of acetochlor, 3-ethyl, 5-methylbenzoquinone-4-imine (EMIQ), to monkey nasal tissue proteins. The binding of acetochlor to monkey nasal turbinates was determined by an acid hydrolysis technique followed by HPLC analysis. HPLC analysis of the protein hydrolysate showed no significant formation of the 3-ethyl, 5-methyl-benzoquinoneimine-cysteine (EMIQ-cysteine) adduct *in vivo*. The lack of detection of EMIQ-cysteine adducts in the monkey after oral dosing of acetochlor, according to the investigators, supports the hypothesis that the carcinogenic mechanism for acetochlor is species specific and among species tested appears to be restricted to the rat.

This Non-guideline oral toxicity study on nasal cell adduct formation is **Acceptable/Nonguideline** and contributes toward satisfying the intent of the study.

4. Cytotoxicity

There is data supporting cytotoxicity of acetochlor to the rat nasal olfactory epithelium. This data consists of observations of respiratory metaplasia of the olfactory mucosa and lipofucsin granules in the olfactory mucosa of rats treated with acetochlor. Additionally, supportive data from the analog alachlor.

a. Cytotoxicity data for acetochlor.

i. Respiratory metaplasia of the olfactory mucosa:

In a nonguideline study evaluating the distribution of rat nasal tissue proliferative lesions (MRID 44496214), hyperplasia, adenoma and adenocarcinoma of the nasal passages of rats exposed orally to alachlor, acetochlor or butachlor were mapped to determine site(s) of origin. Slides of nasal cavity tissue sections from the high dose male and female test groups of the 2-year dietary guideline studies on acetochlor (1750 ppm; 69 male and 70 female Sprague-Dawley rats) and butachlor (3000 ppm; 12 male and 13 female Long Evans rats) and from a one year oral non-guideline gastric initiation-promotion study (126 mg/kg; 10 male and 10 female Sprague-Dawley rats) that were previously determined to have preneoplastic lesions were reexamined by an experienced veterinary pathologist.

All three of these chloracetanilides induced morphologically similar lesions confined almost entirely to the olfactory epithelium lining in specific regions of the posterior nasal passages. Most of the benign tumors exhibited ciliation of the olfactory epithelial cells and **were associated with respiratory metaplasia of adjacent olfactory mucosa**. Many lesions were close to the olfactory-respiratory epithelial junctions. For all three compounds, these changes were apparently identical in nature and location and differed only in frequency of occurrence (comparison of frequency compromised by differences in animal sacrifice times, strains used and number of animals evaluated for each compound).

The appearance of respiratory metaplasia, described above was discussed by the two pathologists present at the meeting (J. Pletcher and D. Wolf) and they agreed that such metaplasia constituted a manifestation of cytotoxicity of acetochlor to the olfactory epithelium. Death (and loss) of the original olfactory epithelium cells results in their being replaced by the respiratory epithelium. cells, originating from differentiating stem cells.

ii. Lipofucsin pigment in the olfactory epithelium

In a 2-generation reproduction toxicity study (MRID 45357503), acetochlor was administered continuously in the diet to CD (SD) IGS BR (Sprague-Dawley) rats (26/sex/dose) at nominal dose levels of 0, 200, 600, or 1750 ppm (equivalent to 0, 21.2, 65.6, and 196.4 mg/kg/day in F1 males and 0, 22.4, 70.9, and 215.9 mg/kg/day in F1 females). F0 animals were given test article diet formulations for 10 weeks prior to mating to produce the F1 litters. On postnatal day (PND) 29, F1 animals (26/sex/dose) were selected to become the F1 parents of the F2 generation and were given the same concentration test formulation as their dams. F1 animals were given test

formulations for 10 weeks prior to mating to produce the F2 litters. Histopathological evaluation (Table 9) revealed treatment-related incidences of benign proliferative lesions (focal epithelial hyperplasia and polypoid adenomata) in the epithelial lining of the ethmoid region of the nasal cavity in F0 and F1 adult animals receiving 1750 ppm acetochlor and in F1 animals at the 600 ppm level. Minimally increased brown pigment (lipofuscin) was observed in the olfactory mucosa, mainly in the lamina propria and occasionally in the basal epithelium in most animals receiving 600 and 1750 ppm in both F0 and F1 generations and also in F1 females at the 200 ppm dose level.

Table 9: Selected histopathology findings in the nasal cavity [a]

Finding and severity	Sex	Dietary Concentration (ppm)							
		F0				F1			
		Control	200	600	1750	Control	200	600	1750
Nasal Cavity Examined No Abnormalities Detected	M	265	2612	263	250	2614	264	262	260
Nasal Cavity Examined No Abnormalities Detected	F	2510	254	250	250	2615	267	260	220
Nasal cavity - Polypoid adenoma (Benign)	M	0	0	0	4	0	0	3	8 [c]
	F	0	0	0	6 [b]	0	0	1	17 [d]
Nasal cavity - Hyperplasia of the olfactory epithelium (Minimal to slight)	M	0	0	0	3	0	0	0	7
	F	0	0	0	7	0	0	4	14
Nasal cavity - Hyperplasia of the respiratory epithelium (Minimal)	M	0	0	0	2	0	0	0	1
	F	0	0	0	2	0	0	0	0
Nasal cavity - Increased lipofuscin of the olfactory mucosa (Minimal to slight)	M	0	0	21	25	0	0	15	26
	F	0	11	25	25	0	9	25	22
Nasal cavity - Hyperplasia, squamous epithelium (Minimal)	M	0	0	0	0	0	0	0	0
	F	0	0	0	1	0	0	0	0

a Data extracted from Table 9 of DER for MRID 45357503.
b Includes 4 animals with single and 2 with multiple lesions.
c Includes 5 animals with single and 3 with multiple lesions.
d Includes 7 animals with single and 9 with multiple lesions.
e Minimal to moderate severity for F1 findings.

Lipofucsins are yellow-brown to reddish-brown pigments that occur widely throughout the body and are thought to be produced by an oxidation process of lipids and lipoproteins. The oxidation process occurs slowly and progressively and therefore, the pigments exhibit variable staining reactions (Bancroft and Stevens, 1996)

b. Cytotoxicity data for alachlor.

i. Alachlor *In vitro* data.

The registrant used a modification of the method of Trela and Bogdanffy (1991) to assess *in vitro* cytotoxicity. Olfactory and respiratory epithelial explants from the nasal cavity and were placed in tissue culture plates and incubated in Williams E media containing alachlor or its metabolites [2,6 diethylaniline (DEA), secondary sulfide or secondary amide]. Cytotoxicity was determined in terms of acid phosphatase released into the medium.

- Alachlor produced a statistically significant (vs controls) release of acid phosphatase with olfactory epithelium but not with respiratory epithelium.
- DEA produced a statistically significant release of acid phosphatase with olfactory and respiratory epithelium
- Secondary sulfide or amide did not produce statistically significant releases of acid phosphatase.

ii. Alachlor *In vivo* data.

Genter et al. (2002b) studied the progression of alachlor-induced olfactory tumors in rats. Male long-Evans rats were administered alachlor in the diet at levels of 0 or 126 mg/kg/day. The rats were sacrificed at 3, 4, or 5 months on the diet. Sections were taken through the ethmoid turbinates (Young's levels 3 & 4 or Mery's levels 22 & 30) and stained with H&E or Wright-Giemsa for light microscopic evaluation. Histological changes were seen in the olfactory mucosa after 3 months. "These changes consisted of respiratory **metaplasia** (i.e. replacement of olfactory mucosa by respiratory epithelium), increased cellularity and epithelial disorganization, with no evidence of cytotoxicity." Alachlor-induced neoplasms were first detected after 5 months of exposure. One of the 5 rats treated for 5 months had two neoplasms.

The pathologists present at the meeting (J. Pletcher and D. Wolf) noted at the meeting that even though Genter et al. (2002b) indicate "no evidence of cytotoxicity" in their study, the presence of "respiratory metaplasia" is indicative of prior cytotoxicity to the original olfactory epithelium.

As summarized below, studies of shorter duration than 3 months fail to show histopathological evidence of cytotoxicity.

In a cell proliferation study for alachlor (MRID 42852102) the authors found a dose-related increase in cell proliferation in the nasal turbinate epithelium, but did not find evidence of cytotoxicity in spite of looking for it.

- In an initial experiment (EHL 87112), female LE rats were administered via the diet at 0, 1, 126, or 252 mg alachlor/kg/day for 10, 30 or 60 days. The rats were administered ^{3}H-thymidine (ip) for evaluation of cell proliferation in nasal turbinates. Tissue sections were processed for microautoradiography and stained with nuclear Fast Red. Although, dose-related and statistical significant increases in cell proliferation were seen [e.g 0, 1.46, 4.10** , and 8.58** labeled cells/field at 60 days], no signs of cytotoxicity were seen at any dose level.

- In another experiment (EHL 90059), groups of Female LE rats were administered via the diet 0, 0.5, 2.5, 15, 42, or 126 mg alachlor/kg/day for 60 days and evaluated with ^{3}H-thymidine (ip) for cell proliferation in nasal turbinates. Two other groups of rats were treated with 0 and 126 mg alachlor/kg/day for 60 days and then placed on basal diet for another 60 days. Separately from processing for microautoradiography, nasal tissue from 3 rats/dose/time point was embedded, sectioned, and stained with H&E, nuclear Fast Red or thionin for histological examination of cytotoxicity. Although, dose-related and statistical significant increases in cell proliferation were seen in the olfactory epithelium, no signs of cytotoxicity were seen at any dose level in any section examined.

In connection to study EHL 90059, the authors noted that "the respiratory and olfactory portions of the nasal epithelium as well as the respiratory/olfactory junction were **specifically examined** for evidence of cytotoxicity by histological procedures. In no animal were cytological changes detected by the techniques employed. The nasal tumors induced by alachlor in the chronic rat feeding studies were generally very small (microscopic in size and only one or two occurred in the entire nasal mucosa. Therefore the likelihood of finding a cell with significant cytotoxicity or preneoplastic changes in this short term study is very small."

Wetmore et al. (1999) evaluated histopathology and cell proliferation nasal olfactory epithelium of rats treated ip with alachlor. Male Long-Evans rats were administered alachlor (ip, 0 or 126 mg/kg/day) for 1, 4, and 28 days (5 days per week). The animals were sacrificed 24 hours after the last dose. Two hours prior to sacrifice the rats received BrdU (ip) for evaluation of cell proliferation. There was no evidence of cytotoxicity (histological, H&E stain) or increased cell proliferation (immunohistochemical evaluation of S-phase cells) following examination of level III sections of nasal ecto- or endoturbinates.

iii. Genomics data for alachlor (oxidative damage)

In addition to the *in vitro* and *in vivo* cytotoxicity data presented above for alachlor, there is information that alachlor affects the redox status of the cell, leading to oxidative stress and which may result in DNA damage. Oxidative damage to DNA may lead to cytotoxicity followed by regenerative cellular proliferation (Clayson et al. 1994).

Based on a genomic analysis of **alachlor**-induced tumors in the olfactory mucosa of Long-Evans rats exposed to 126 mg/kg/day and sampled at various intervals from 1 day to 18 months, Genter et al. (2002b) proposed the following steps in the **alachlor**-mediated carcinogenesis model:

a. **Initial progression from histologically normal olfactory mucosa to foci of abnormal mucosa**

This step, which is regulated by genes in the acute phase of exposure, is accompanied by "upregulation" ($\geq$2-fold increase) of genes consistent with a mutagenic response possibly as a result of oxidative damage to DNA **(↑GADD 45, apurinic/apyramidinic endonuclease).** While the exact role of GADD (growth arrest and DNA-damage inducible) gene products is not known, this gene group is upregulated in response to stress

to allow cells time to repair macromolecular damage or to lead cells into apoptosis so that a genetic defect is not propagated. Types of environmental stress that induce GADD genes include UV irradiation, alkylating agents and glucose starvation (Takahashi et al. 2001; Jackman et al. 1994). Stokes et al. (2002) also demonstrated that GADD 45 gene induction occurs in response to reactive oxygen species (ROS) and quinones and is abolished in the presence of the antioxidant, ascorbic acid. It is of note that quinones, which are operationally non-genotoxic (Clayson et al., 1994), are highly redox active molecules which can redox cycle with their semiquinone radicals, leading to formation of ROS, including superoxide, hydrogen peroxide, and ultimately the hydroxyl radical. Production of ROS can cause severe oxidative stress within cells through the formation of oxidized cellular macromolecules, including lipids, proteins and DNA (Bolton et al. 2000). Supporting the hypothesis of oxidative stress, Genter et al.(2002a), also observed upregulation of other genes associated with oxidative stress, [***i.e.*, heme oxygenase** (Otterbein et al. 2000), **glutathione synthase and metallothionein** (Andrews 2000)].

b. **Progression from histologically altered olfactory mucosa to the development of adenomas**

This step was accompanied by expression of genes indicating inhibition of apoptosis **[Bid3(AI102299)]** and enhancement of cell proliferation **(zyxin)**. It is of note that Sarafian and Bredesen (1994) state that ROS can serve as common mediators of apoptosis.

c. **Progression to a malignant adenocarcinoma phenotype**

This phase was indicated by induction of genes related to the **wnt signaling pathway**, which are generally upregulated late in the carcinogenesis process.

d. **Transformation to adenocarcinomas**

In the late stages of tumor progression, the activation of **nuclear ß-catenin genes,** which is critical for tumor formation in other organs and is associated with mutations in the **wnt pathway**.

Several other studies support a role for oxidative stress in **Alachlor**-induced toxicity. Burman et al. (2003) show that dietary exposure of Long-Evans rats to 126 mg/kg/day for 1 day caused an ~20% depletion of the olfactory mucosa antioxidant, GHS followed by a significantly ($p<0.001$) increased expression of genes associated with increased GHS production after 2 and 4 days of treatment. A return to control values was seen by 10 days of treatment. A pattern somewhat similar to GSH was observed for ascorbate in the olfactory tissue of 126-mg/kg/day male rats (*i.e.,* initially, a significant decrease 1 day post-treatment, followed by significant increases 2 and 4 days after dosing). In contrast to the GSH data, there was a reduction in ascorbate at 10 days. We noted, however, that the response with either antioxidant was not dose related. From these results, the investigators concluded that, "Despite the fact that GSH levels recovered, acute antioxidant perturbations may have been sufficient to trigger other steps in the carcinogenic process. Therefore, acute depletion of GSH and ascorbate may trigger more sustained events involved in both the initiation and promotion of the carcinogenic process."

There is also evidence of the ability of **alachlor** to induce oxidative stress in other tissues. Bagchi et al.(1995) evaluated the potential of **alachlor** to induce oxidative stress and oxidative tissue damage, as measured by production of lipid peroxidation and DNA-single strand breaks (SSB), in the liver and brain of Sprague-Dawley rats administered two equal oral doses (at 0 and 21 hours) of 300 mg/kg. As noted by Clayson et al. (1994), SSB are considered by to be a good indicator of oxygen damage to DNA. Results from the study of Bagchi et al. (1995) show that **alachlor** induced moderate lipid peroxidation in liver and brain tissues and SSB in brain but not liver DNA in samples harvested 24 hours after exposure to the first dose. The same authors also conducted *in vitro* studies of chemiluminescence on liver and brain homogenates, and found that 1nmol/mL **alachlor** induced 3-fold increases in chemiluminescence in both tissues further suggesting that **alachlor** induced ROS. Finally, the results from *in vitro* studies with cultured PC-12 neuroactive cells exposed to 100 nM **alachlor** illustrate the sequence of early events postulated for this MOA (generation of ROS ➜DNA damage ➜tissue damage) with a 2-fold increase in DNA-SSB and a 3-fold increase in LDH leakage. Although olfactory nasal tissue was not examined in this series of assays, the ability of **alachlor** to generate ROS with subsequent DNA damage and tissue damage both *in vivo* and *in vitro* has been established. Finally, Bagchi et al. cite the work of Akubue and Stohs (1991) showing that the oral administration of 800 mg/kg **alachlor** to rats caused the increased urinary excretion of the "oxidative lipid metabolites, malondialdehyde, formaldehyde, acetaldehyde and acetone".

Based on the above considerations, the postulated MOA (generation of ROS ➜DNA damage ➜tissue damage ➜ cell proliferation➜olfactory nasal tumors) in rats is plausible and coherent. An additional factor favoring this MOA is the evidence of weak and sporadic mutagenic effects, generally seen only at concentration near or at cytotoxic concentrations. Nevertheless, these conclusions are based solely on data for **alachlor**. Similarly, the characterization of the hypothesized early events for this MOA draws heavily on the cDNA microarray findings of Genter et al. (2002a) for **alachlor** which have not as yet been independently confirmed. Additionally, there are no data available on acetochlor to fully test the plausibility of oxidative stress as an early and critical event leading to frank tumor formation in rat nasal olfactory tissue. Since oxidative damage to DNA induced by ROS is considered a mechanism related to carcinogenesis especially by operationally non-mutagenic carcinogens (Clayson et al. 1994), it may be prudent to test acetochlor for ROS formation. A possible analysis could be the production of increased 8-hydroxy-2'-deoxyguanosine (8-OHdG), one of the known markers of cellular oxidative stress during carcinogenesis (Kasai et al. 1997).

5. Cell Damage

The observation of respiratory metaplasia of the olfactory epithelium in rats treated with acetochlor, alachlor or butachlor (MRID 44496214) is indicative of death of olfactory cells and replacement of these cells by respiratory epithelial cells, differentiating from stem cells. The presence of lipofucsin granules in olfactory epithelium of rats treated with acetochlor in the diet for 3 months at 200 -1750 ppm (MRID 45357503) is a reflection of oxidative damage to cell membranes. This data are supported by observations of respiratory metaplasia produced by the

analog alachlor in rats treated with alachlor in the diet at 126 mg/kg/day for 3 months in a study by Genter et al. (2002b).

6. Cell Replacement/Increased cell turnover

The following data indicate that Acetochlor significantly increased cell proliferation in the olfactory region of the nasal turbinates in rats but not in mice. Acetochlor, however, significantly increased the rate of cell proliferation in mouse liver.

In a nasal cell proliferation study (MRID 44496207), acetochlor (95.2%a.i.) was administered to male Sprague-Dawley rats in the diet at concentrations of 0, 1750, and 5000 ppm. The two higher levels of acetochlor were carcinogenic to rats in a chronic study (see Table 6). Cell proliferation was measured after 60, 90 and 160 days of treatment in nasal turbinate respiratory and olfactory epithelium by measuring the DNA incorporation of ^{3}H-thymidine. ^{3}H-thymidine was administered IP for three consecutive days prior to sacrifice. In a separate study, nasal cell proliferation was also measured ~160 days with bromodeoxyuridine incorporation in rats receiving 0, 200, 1750 and 5000 ppm acetochlor in the diet.

Acetochlor (Table 10) significantly increased cell proliferation in the olfactory region of the nasal turbinates in rats administered 5000 ppm acetochlor in the diet for 60 days. Cell proliferation was also significantly increased at 90 and 160 days in the 1750 ppm and 5000 ppm treatment groups. There were no significant increases in cell proliferation in the respiratory region at any of the time points or doses tested.

In a non-guideline nasal olfactory and respiratory epithelial cell proliferation study (MRID 44496209), acetochlor (95.2% ai, lot/batch # T940059, MUS-9308-5458-T) was administered to 26 male CD-1 mice/dose in the diet at concentrations of 0, 1000, or 5000 ppm (equivalent to 0, 166.6, or 887.9 mg/kg bw/day) for 60 and 90 days. None of the mice died during the study. Acetochlor had no effect on nasal cell proliferation in mice administered acetochlor in the diet at 1000 or 5000 ppm for 60 days

These cell proliferation studies on acetochlor are supported by previous work done with the analog alachlor. As summarized in Table 11, alachlor produced dose-related increases in nasal cell proliferation in rats, with statistical significance after 60 days at the higher doses and after 30 days at the highest dose. Table 12 shows dose-related increases in nasal cell proliferation reaching statistical significance at the highest dose of alachlor (126 mg/kg/day) after 60 days on the diet. Table 12 shows that the cell proliferation effect of alachlor is limited to the olfactory epithelium and is reversible in rats returned to the basal diet after 60 days of treatment.

Table 13 (from Tables 6 and 10) compares doses for nasal adenoma formation in rats with doses for cell proliferation.

Table 10. Nasal cell proliferation in male SD rats treated for 60, 90, or 160 days with **acetochlor** (MRID 44496207).

Treatment (ppm)	No. of labeled cells/0.2 mm of basement membrane							
	Respiratory epithelium				Olfactory epithelium			
	^{3}H-Thymidine label			BrdU label	^{3}H-Thymidine label			BrdU label
	60-day	90-day	160-day	160-day	60-day	90-day	160-day	160-day
0	2.80 ± 2.37	1.37 ± 0.63	0.52 ± 0.23	2.43 ± 0.54	4.23 ± 2.37	4.75 ± 1.03	3.48 ± 0.55	7.17 ± 1.92
250	-	-	-	2.23 ± 0.34	-	-	-	6.25 ± 1.73
1750	1.37 ± 1.01	1.28 ± 0.59	0.79 ± 0.82	2.53 ± 1.12	3.81 ± 1.42	6.37 ± 1.06**	5.24 ± 0.92**	9.78 ± 1.80**
5000	2.07 ± 1.71	1.14 ± 0.38	0.86 ± 0.29	2.61 ± 0.73	6.66 ± 2.08*	7.90 ± 1.07**	7.06 ± 1.39**	11.46 ± 1.85**

Table 11. Cell proliferation at the respiratory-olfactory junction in the nasal turbinates of rats fed **alachlor** (MRID 42852102).

Dose (mg/kg/day)	Mean No. labeled cells/field ± SEM at various days on the diet			
	1 day	10 days	30 days	60 days
0	0.74 ± 0.2	0.30 ± 0.17	2.60 ± 0.08	0.76 ± 0.27
1	0.64 ± 0.17	3.48 ± 0.60*	2.02 ± 0.25	1.46 ± 0.28
126	0.82 ± 0.30	4.54 ± 1.15**	3.44 ± 1.03	4.10 ± 0.92**
252	0.34 ± 0.14	6.06 ± 0.94**	6.80 ± 0.48**	8.58 ± 0.59**

* = $p<0.05$, ** = $p<0.01$

Table 12. Effect of 60 day **alachlor** diet or 60 day **alachlor diet** followed by a 60 day recovery diet on cell proliferation in the respiratory and olfactory epithelia of the nasal turbinates of L-E female rats (MRID 42852102).

Dose (mg/kg/day)	Mean No. labeled cells/field ± SEM at various periods on the diet			
	Respiratory region		Olfactory region	
	60 days	60 days + 60 days recovery[1]	60 days	60 days + 60 days recovery
0	1.0 ± 0.2	0.8 ± 0.4	1.0 ± 0.3	0.8 ± 0.2
0.5	1.8 ± 0.6	-	0.6 ± 0.2	-
2.5	0.5 ± 0.1	-	1.4 ± 0.3	-
15	1.5 ± 0.7	-	1.2 ± 0.4	-
42	1.7 ± 0.5	-	2.9 ± 0.9*	-
126	0.7 ± 0.2	0.4 ± 0.0	3.2 ± 1.1*	1.0 ± 0.2

* = $p<0.05$, ** = $p<0.01$

[1] The animals were maintained in the alachlor diet for 60 days and then placed in alachlor-free diet for another 60 days.

III. DATA EVALUATION FOR MODE OF ACTION

In its evaluation of the MOA for the subject chemical, the CARC applied the 'IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis', developed by the International Programme on Chemical Safety, Geneva, Switzerland (Sonich-Mullin et al., 2001) The results of such an evaluation are summarized below.

1. Introduction

Previous pages have summarized acetochlor data submitted in support of a non-genotoxic MOA for the induction of tumors of the nasal olfactory epithelium in rats.

Although acetochlor also produces tumors at other sites in rats such as thyroid follicular cells and liver, this document covers only the MOA for nasal tumors in rats. The MOA for thyroid follicular tumors is discussed in the Cancer Assessment Document for Acetochlor (Fourth Evaluation) for the meeting dated April 21 and 22, 2004 (Part 1).

As summarized in Table 6, the endpoint of nasal tumors is clearly attained at the higher doses in three separate rat chronic rat studies, with the unexplained exception of females in Study #1. Although the Registrant's Study Report tables do not state explicitly that the nasal tumors originate from the nasal olfactory epithelium, there is evidence that the tumors originate in the olfactory portion of the nasal epithelium of the rats.

- To determine if there was a similarity in morphology, origin, and location of proliferative lesions (MRID 44496212), the Registrant conducted a review of hematoxylin/eosin slides of nasal tissue of rats treated with acetochlor, alachlor or butachlor in previously conducted long-term oral studies. In the case of acetochlor, slides from study #3 (88/SUC017/0348, MRID 41592004) of Table 6 were used. Among other findings , it was determined that all three of the chloracetanilides induced morphologically similar lesions confined **almost entirely to the olfactory epithelium lining** in specific regions of the posterior nasal passages.

- In rat chronic study #1 in Table 6 (conducted at 0, 500, 1500, & 5000 ppm) (MRIDs 00131088 and 40484801) no nasal tumors were initially found because sections had been taken from the anterior part of the nasal cavity. When new histological sections were taken so as to include the posterior region of the nasal cavity, the tumors reported in Table 6 for study #1 were seen. The posterior region of the nasal cavity in rats is essentially olfactory epithelium, in contrast to respiratory epithelium, which is found in the more anterior part of the nasal cavity (Young,1981).

Nasal tumors were also seen in rats treated with the sulfoxide metabolite of acetochlor. No nasal tumors were seen in acetochlor-treated mice in two acceptable cancer studies.

2. Postulated mode of action (theory of the case)

The postulated MOA for the induction of nasal tumors by acetochlor in rats involves the following steps:

i) Acetochlor conjugates with glutathione (GSH) and is excreted in the bile.
ii) The conjugate is biotransformed to a series of sulfur-containing products. Enterohepatic circulation of these products creates a pool of metabolites that are delivered to the nose.
iii) Biotransformation to tissue-reactive and toxic metabolites. Metabolism by nasal enzymes, results in formation of a benzoquinoneimine, an electrophile and redox-active molecule.
iv) Binding to cellular proteins plus possible generation of oxidative stress .
v) Cytotoxicity
vi) Regenerative cell proliferation.
vii) Sustained cytotoxicity and cell proliferation that results in neoplasia.

These steps are summarized in Figure 5 (same as Figure 1).

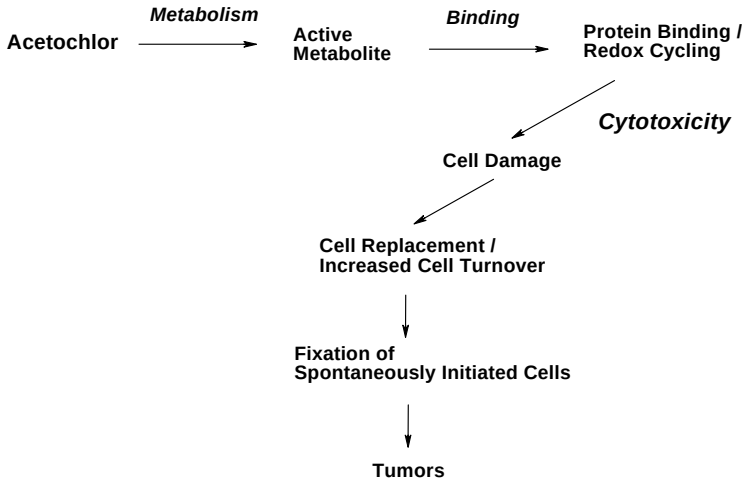


Figure 5. Postulated MOA of acetochlor in the induction of nasal tumors in rats

3. Initial events

As discussed in the metabolism section, there is strong evidence that the compound is well absorbed (high urinary excretion and other facts), is conjugated with GSH (major metabolites can be traced to GSH conjugation, e.g. mercapurates, sulfoxide etc.), undergoes enterohepatic recirculation (80% biliary excretion & over 70% urinary excretion), appears in plasma (e.g. the sulfoxide metabolite is found in rat plasma). Additional information (autoradiography) indicates that acetochlor and/or its metabolites distribute to the nasal turbinates, in particular to the olfactory epithelium (site of the nasal tumors) with no label at the respiratory epithelium. Autoradiographic evidence indicates that although, initially, radioactivity from acetochlor and its sulfide metabolite distributes widely, there is still significant residual radioactivity in the nasal turbinates and low background radioactivity in surrounding tissues, several days after a single oral dose. The mouse and other species do not show this pattern of nasal epithelium labeling.

4. Key events

The following three events are considered key for formation of nasal tumors by the proposed MOA:

QUINONE IMINE- FORMATION (PROTEIN BINDING) ➜ CYTOTOXICITY ➜ CELL PROLIFERATION

There is ample evidence that Acetochlor is metabolized to precursors of the quinone-imine.:

-After dosing with acetochlor, analysis of protein adducts obtained from nasal olfactory tissues reveals that hydrolysis of these adducts releases EMIC-cysteine (Figure 4). This product is consistent with a nucleophilic attack by an SH group in a protein on a quinone-imine [formed from EMA (path A) or sec-methyl sulfoxide metabolite (path B)].

- Administration *in vivo* of sec-methyl sulfide or sulfoxide metabolite of acetochlor (Putative precursors of nasal protein adducts) produces adducts that release EMIC-cysteine.

- Incubation of rat nasal tissue microsomal preparations will produce precursors of quinoneimines given appropriate substrates (e.g. sec sulfide, MEA)

- Administration of the sulfoxide metabolite of acetochlor (the postulated quinone imine precursor) to rats (see Figure 10b) produces statistically significant incidences of nasal polypoid adenomas after 26 weeks of treatment. Similar incidences were seen after 52 weeks.

Although it is likely that in the rat the quinone-imine is formed from the sulfoxide metabolite, the possibility that EMA might also be a precursor cannot be ruled out. Figure 2 indicates that more than 20% of the metabolites of acetochlor are not identified. Furthermore, the analysis of adducts

cannot distinguish whether the adduct originated from the sulfide or EMA. This issue is important since *in vitro* work of Coleman et al. (2000) indicates that human liver microsomes are as effective as the rat in forming EMA from acetochlor.

There is support for the idea that the quinone imine has to be generated locally (within the cell)

Consideration of high reactivity of the quinone imine coupled to the localized distribution of the label indicates that the active species must be formed at the site. If the active species were not locally activated at or within the cell, one would observe a very spread out pattern of labeling. This contention is supported by *in vivo* studies with N-acetyl-p-benzoquinone imine (NAPQI, the putative active species in the production of liver toxicity in mice dosed with acetaminophen). BALB/c mice were administered pure NAPQI by the ip route. At necropsy, liver cells were normal, with no apparent necrosis, and SGPT levels were normal. However, the blood was extensively coagulated, indicating that little or nothing reaches the liver, most of the material is used up in the intervening tissue fluids (Dahlin and Nelson (1982)).

There is support that the quinone imine is formed in the rat nasal epithelium in a dose related manner, but not in the mouse.

As summarized in Table 7, EMIQ-cysteine (indicator of quinone imine binding, or in general of its presence) is formed in a dose related manner. These levels of binding were seen in rats administered the test diet for 14 days at tumorigenic doses. There are no acetochlor data, however, to determine if there is a NOAEL for this binding. In experiments conducted in mice no EMIQ-cysteine was found, but EMA (indicative of direct Chlorine substitution) was found. The absence of quinone imine binding in the mouse (Table 8) is consistent with the negative autoradiography studies with the mouse and the negative nasal tumor results seen in the mouse chronic studies.

There is support for Cytotoxicity.

Re-reading of the slides (See MRID 44496214) for the 1988 acetochlor rat chronic study (MRID 41592004) and for butachlor (MRID 42244901) and alachlor (MRID 43590001) studies indicated that most of the benign tumors were associated with respiratory metaplasia of adjacent olfactory epithelium. This effect implies disappearance (death) of olfactory epithelium and replacement with respiratory epithelium. Furthermore, in a 2-generation reproductive toxicity study (MRID 45357503), F_0 and F_1 adult rats showed lipofucsin granules in the olfactory epithelium. These results are consistent with an oxidative process affecting cellular lipids and lipoproteins.

There is also additional supportive evidence for cytotoxicity in studies reported in the literature.

In a study by Genter et al. (2002b) with rats dosed with acetochlor at 126 mg/kg/day, it was noted that "Histological changes were seen in the olfactory mucosa after 3 months of dietary alachlor exposure. These changes consisted of respiratory metaplasia (i.e. replacement of olfactory mucosa by respiratory epithelium), increased cellularity and epithelial disorganization, **with no evidence of cytotoxicity**." Although these authors note "**no evidence of cytotoxicity",** their remarks suggest that there is actually evidence of cytotoxicity if:

- One interprets the phrase "epithelial disorganization" as a sign of cytotoxicity. In fact the expression "disorganization of the epithelial cell layer" was used as one of the characterizing features of the lesions seen in nasal mucosa of rats dosed with phenacetin in a study by Bogdanffy et al.(1989).

- One notes that there was "respiratory metaplasia", which requires disappearance of the olfactory epithelium cells to be replaced by respiratory epithelium cells.

Additionally, Wetmore et al. (1999) observed from their study of Long-Evans male rats dosed intraperitoneally with 126 mg/kg/day **alachlor** (prepared in dimethyl sulfoxide) for 1, 4 or 28 days that the lack of a cytotoxic and regenerative cell proliferation response in the nasal mucosa at an **alachlor** dose equivalent to a tumorigenic dose distinguishes **alachlor** from other nasal carcinogens. While Wetmore et al. considered the finding to be unusual, they state, "this observation is not unprecedented for chemical carcinogens in general". The authors further indicated that a recent survey of the carcinogenic compounds identified by the National Toxicology Program (NTP) revealed that approximately 44% do not cause cytotoxicity or enhance cell proliferation in the target tissue.

This is perhaps not unexpected for certain nasal carcinogens since D. Wolf (2004, private communication) indicates that equating cytotoxicity with the presence of dead cells in the epithelium lining of the nasal cavity is almost impossible because of the architecture of the lumenal structure. The entire nasal cavity is lined with a single layer of pseudostratified epithelium. The nasal cavity appears to be a couple of cell layers thick, however, all cells within the structure are attached to the basement membrane. When cells die, they pull away from the basement membrane and slough off. Since they are in a lumenal structure, they fall off into the air. Hence, one does not always see cells undergoing necrosis or apoptosis in tissue sections. It is, therefore, assumed that cell proliferation parameters such as BrdU labeling indices only increase when lost cells are being replaced. In general, increased labeling without significant hyperplastic expansion is considered secondary to loss of cells through necrosis or apoptosis. This is seen in the urinary bladder and kidney as well as the nose. With chronic treatment, the only indication of cytotoxicity is the increased proliferation rates. Data presented earlier from the nasal cell proliferation study with dietary administrations of 1750 or 5000 ppm acetochlor to Sprague-Dawley rats (MRID 44496207) support Wolf's position. Since the increase in cell proliferation, which was time and dose-dependent, is most likely due to cytotoxicity to the nasal turbinates, the sustained increase in cell proliferation is a key event in the induction of nasal olfactory tumors in rats.

Finally, if one accepts that a quinone imine has been formed inside of the nasal epithelium and the very high reactivity of the quinone imines [as electrophiles and oxidants, See Monks and Jones, 2002), then cytotoxicity is to be expected. Several studies found in the open literature suggest that DABQI may induce damage to DNA through oxidative stress. This is a reasonable effect to pursue since oxidative damage to DNA may lead to cytotoxicity followed by regenerative proliferation (Clayson et al., 1994).

There is support for nasal olfactory epithelium cell proliferation.

As summarized in Table 10, and graphically in Figures 6 and 7 acetochlor induces nasal olfactory cell proliferation. Figure 6 (using H^3- thymidine label) shows a statistically significant increase in cell proliferation vs controls at 1750 and 5000 ppm after 90 or 160 days of treatment. The statistically significant increase in cell proliferation vs controls is evident only at 5000 ppm after 60 days of treatment, with a NOAEL of 1750 ppm for this time period of treatment. Figure 7 (using BrdU label) shows a statistically significant increase in cell proliferation vs controls at 1750 and 5000 ppm after 160 days of treatment, with a NOAEL at 250 ppm. Taken together, Figures 6 and 7 point to a time relation for the NOAEL for cell proliferation: As the time of treatment increases, the NOAEL decreases (See Figure 8).

Although cell proliferation NOAEL values are defined for cell proliferation at two dose levels, there is no direct cell proliferation data to assure that the lowest of them (250 ppm) will remain a NOAEL for longer treatment periods than 160 days.

Examination of Table 10 indicates that the response is limited to the olfactory epithelium, consistent with the binding data. Table 13 lists nasal tumors and cell proliferation to facilitate comparisons.

Table 13. Values for nasal adenoma formation[a] and nasal olfactory epithelium cell proliferation at various dietary ppm levels of acetochlor. (This table was compiled from Tables 6 and 10 as an aid in determining a POD.) Data are for male rats only.

Effect	**No. adenomas/total examined** or **No. labeled cells/0.2 mm of basement membrane**										
	0	18	40	175	200	250	500	1000	1500	1750	5000
NasalTumors:: (3 studies) :											
PR-80-006	0/69	-	-	-	-	-	1/70	-	6/69**	-	18/69**
ML-83-200	1/58		0/54		0/58	-	-	12/59**	-	-	-
CTL/C/2191	0/69	0/59	-	0/59	-	-	-	-	-	35/70**	-
Cell Prolif. 60Days	4.2±2.4	-	-	-	-	-	-	-	-	3.81±1.4	6.66±2.1*
Cell Prolif. 90D	4.75±1	-	-	-	-	-	-	-	-	6.37±1.1**	7.9±1.1**
Cell Prolif. 160D (^{3}H-Thymidine	3.48±0.6	-	-	-	-	-	-	-	-	5.24±0.9**	7.06±1.4**
Cell Prolif. 160D (BrdU)	7.17±1.9	-	-	-	-	6.25±1.7	-	-	-	9.78±1.8**	11.46±1.9**

[a] The tumor data above refer to Chronic rat studies in Table 6.

[B] " - " means not tested ; $^{*}= p \leq 0.05$; $^{**} p \leq 0.01$

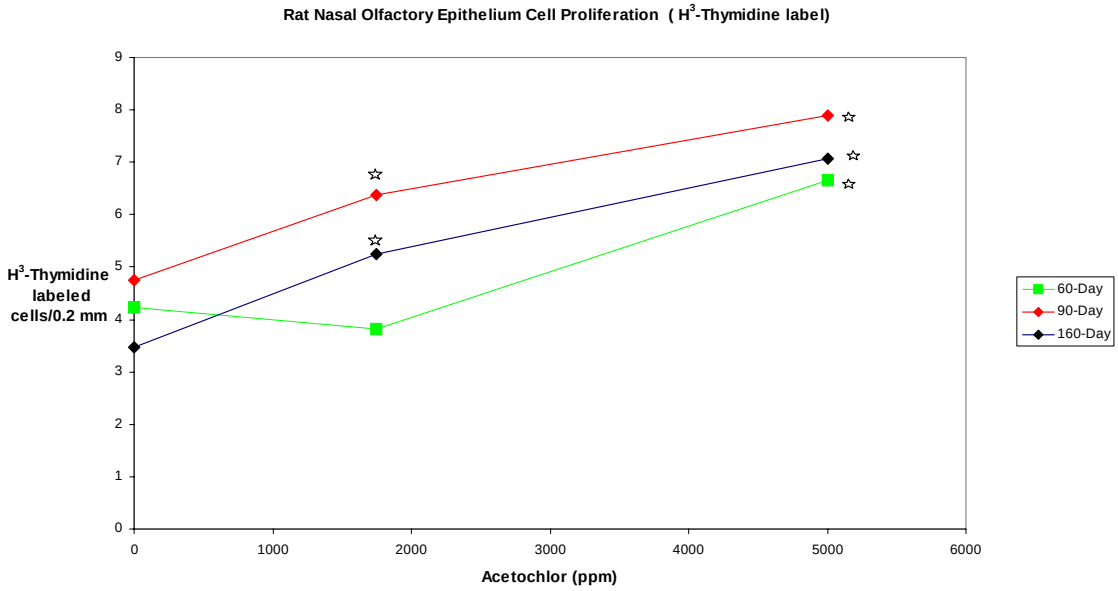


Figure
6. Cell proliferation dose response (H^3-Thymidine label) to various feed levels for 60, 90 or 160 days. Data from Table 5. * = p≤ 0.05

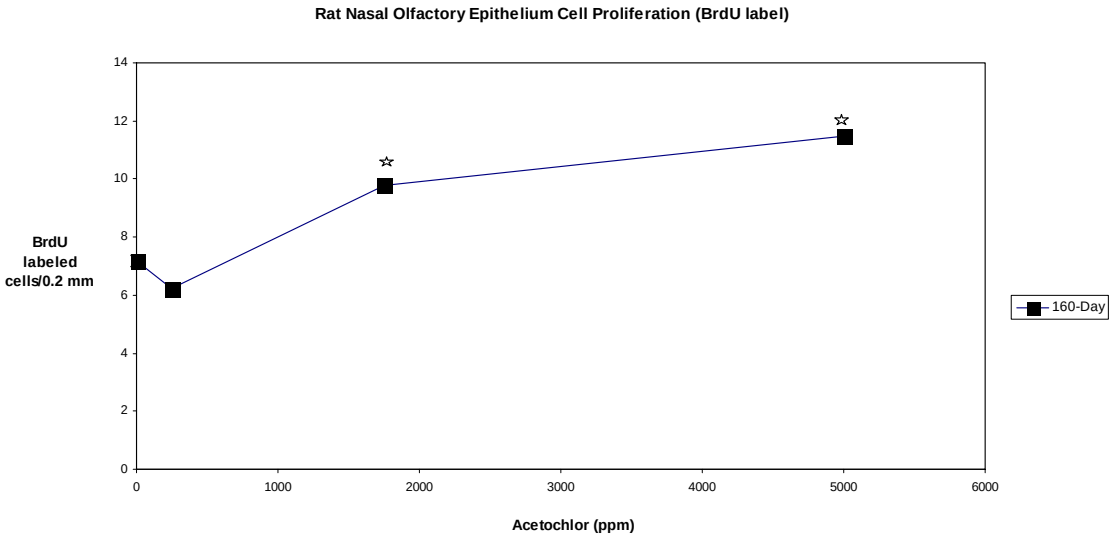


Figure 7. Cell proliferation dose response (BrdU label) to various feed levels for 160 days. Data from Table 5. * = p≤ 0.05

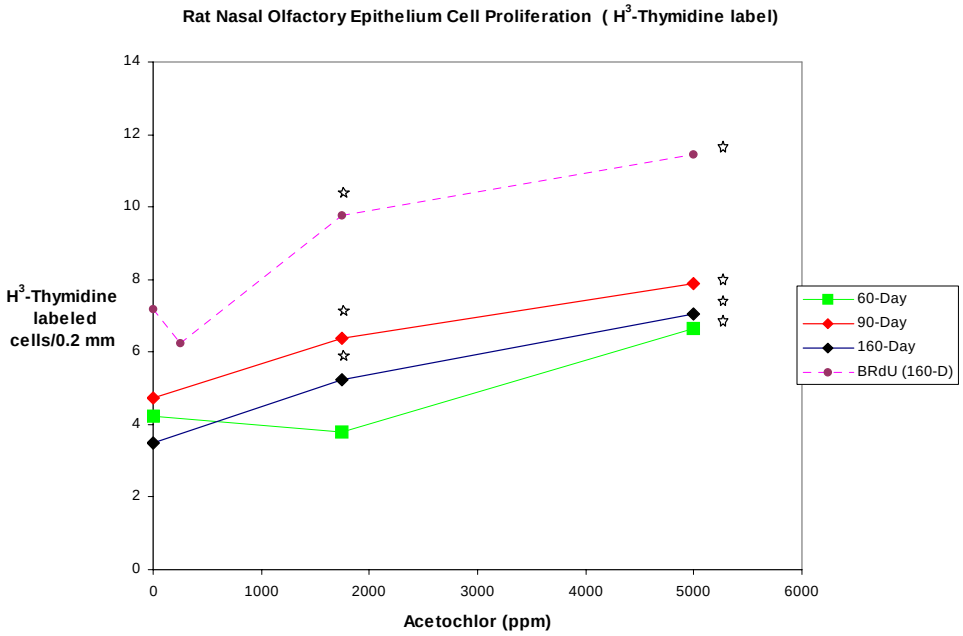


Figure 8. Same Figure 6, with the results of Figure 7, superimposed on it.
* = p≤ 0.05

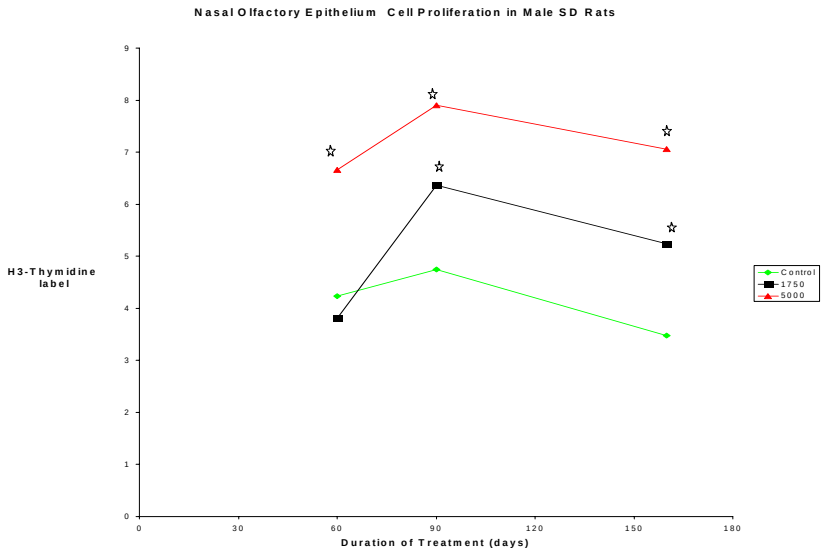


Figure 9. Effect of duration of treatment on cell proliferation. Values in Figure 6 were re-plotted using the duration of treatment in the x-axis. There is one curve for each dose level.

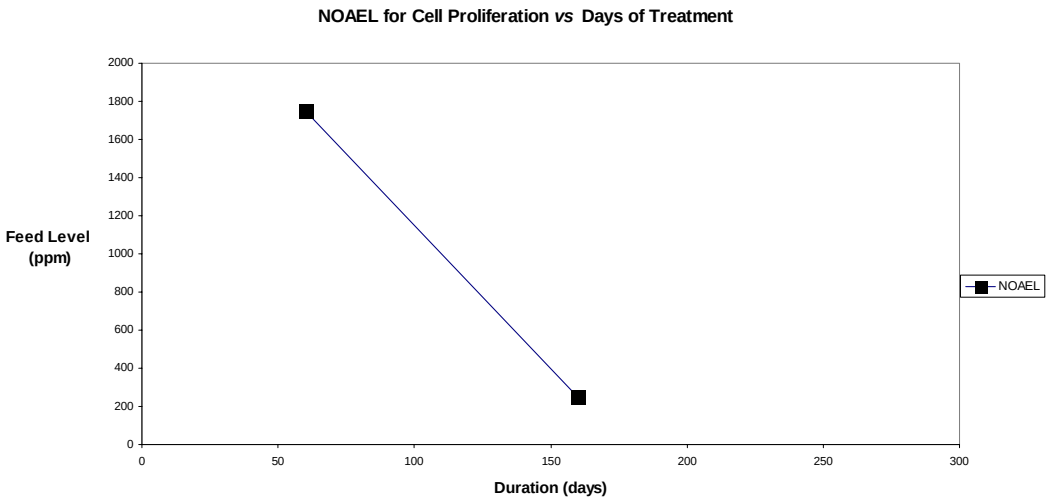


Figure 10a. NOAEL for cell proliferation vs days of treatment

5. Dose-response relationship

Several observations support a dose response relationship for the key events and the tumor endpoint.

a. Autoradiographic evidence indicates that when there is distribution to the nasal tissues and binding to the nasal turbinates (case of the rat), there are tumors. When there is no binding (presumably little or no dose of quinone imine, i.e. 0 dose), there are no tumors, such as in the mouse.

b. Chemical identification of bound residues indicates that the tumors are seen in species where the quinone imine is bound to proteins of nasal olfactory epithelium. When there is quinone imine binding, like in the rat, there are tumors. When there are undetectable levels of binding (i.e. no or undetectable levels of quinone imine) as in the mouse, there are no tumors.

c. Examination of Figures 6 and 7 reveals a dose-related increase in cell proliferation in the nasal olfactory epithelium of rats at doses coinciding with tumorigenic doses of acetochlor. In contrast, acetochlor does not produce cell proliferation in mice.

d. Examination of Table 14 indicates a dose-related increase in lipofucsin in the olfactory mucosa in both sexes. Lipofucsin formation is considered to be an indication of oxidative damage, which increases as a function of dose.

e. Using data from the analog alachlor (MRID 45852102), Table 8, it was shown that administration of alachlor in the diet for 60 days at tumorigenic doses resulted in significantly increased cell proliferation in the olfactory epithelium. Upon removal of the test material, cell proliferation had reverted to control levels after 60 days in basal diet.

Table 14: Histopathology findings in the nasal cavity [a]

Finding and severity	Sex	Dietary Concentration (ppm)							
		F0				F1			
		Control	200	600	1750	Control	200	600	1750
Nasal Cavity Examined	M	26	26	26	25	26	26	26	26
No Abnormalities Detected		5	12	3	0	14	4	2	0
Nasal Cavity Examined	F	25	25	25	25	26	26	26	22
No Abnormalities Detected		10	4	0	0	15	7	0	0
Nasal cavity - Polypoid adenoma (Benign)	M	0	0	0	4	0	0	3	8 [c]
	F	0	0	0	6 [b]	0	0	1	17 [d]
Nasal cavity - Hyperplasia of the olfactory epithelium (Minimal to slight)	M	0	0	0	3	0	0	0	7
	F	0	0	0	7	0	0	4	14
Nasal cavity - Hyperplasia of the respiratory epithelium (Minimal)	M	0	0	0	2	0	0	0	1
	F	0	0	0	2	0	0	0	0
Nasal cavity - Increased lipofuscin of the olfactory mucosa (Minimal to slight)	M	0	0	21	25	0	0	15	26
	F	0	11	25	25	0	9	25	22
Nasal cavity - Chronic inflammation, nasolacrimal duct (Minimal to slight [e])	M	12	13	8	8	10	17	14	11
	F	14	18	9	9	10	15	20	12
Nasal cavity - Rhinitis (Minimal to slight)	M	12	4	4	12	4	8	7	2
	F	5	2	3	7	3	1	0	0
Nasal cavity - Hyperplasia, squamous epithelium (Minimal)	M	0	0	0	0	0	0	0	0
	F	0	0	0	1	0	0	0	0
Brain - Astrocytoma	M	--	--	--	--	--	--	--	--
	F	--	--	--	--	--	--	--	1

a Data extracted from Tables 63 and 64 of the test report, **MRID 45357503,** (pages 223, 227, and 231).
b Includes 4 animals with single and 2 with multiple lesions.
c Includes 5 animals with single and 3 with multiple lesions.
d Includes 7 animals with single and 9 with multiple lesions.
e Minimal to moderate severity for F1 findings.

6. Temporal association

The database to assess the criterion of temporality is not complete. However, there are data to infer a sequence in time.

- The events associated with tissue distribution, binding, and genomic events are very early events. Radioautography of acetochlor-dosed rats shows that distribution and binding to nasal turbinates takes place after a single dose. If the binding is interpreted as due to the formation of a quinone imine, then one may say that the key event of quinone imine formation is taking place starting with the initial dose. In experiments with the analog alachlor, binding was seen to increase with time. It was found that nasal protein adducts (DEIQ-cysteine) increased with time in rats dosed for up to 2 weeks with C^{14}-labeled alachlor.

- Acetochlor produced significantly increased cell proliferation in cells of the nasal olfactory epithelium after 60 days (approximately 8 weeks) of treatment. There are no data to assess the earliest time of the proliferative response for acetochlor. However, Genter et al. (2000) reported no increase in cell proliferation after 1 month of dosing in rats treated with alachlor at a tumorigenic dose. Cell proliferation in the olfactory mucosa was seen by these authors at 6 months of alachlor exposure, at which time there were already nasal masses in 7 of 15 animals.

Although the time-frame for progression of acetochlor-induced olfactory mucosal tumors has not been studied, that of alachlor has been studied. Genter et al. (2002b) studied the progression of alachlor-induced olfactory mucosal tumors in rats. These authors did not observe histological changes after one month of treatment. At 3 months they reported respiratory metaplasia (i.e. replacement of olfactory mucosa by respiratory epithelium), increased cellularity, and epithelial disorganization. Alachlor-induced olfactory mucosal neoplasms were detected after 5 months of exposure.

The time-frame for tumor-progression for acetochlor-induced olfactory mucosal tumors is not known; however, short term data suggest that the time frame might not be too different from that of alachlor. In the case of acetochlor, polypoid adenomas of the olfactory epithelium have been observed in F0 and F1 animals in a rat multigeneration reproduction study with acetochlor. Additionally, preliminary data from a 1-year rat feeding study using the sulfoxide metabolite of acetochlor show a statistically significant increase in nasal polypoid adenomas in rats treated for 26 weeks (about 6.5 months).

Thus, one may conclude that there appears to be a time line for tumor formation with acetochlor that is consistent with the available data: early production and manifestation of the quinone imine and its effects, cell proliferation (60 days or less) that is reversible, and then tumor formation.

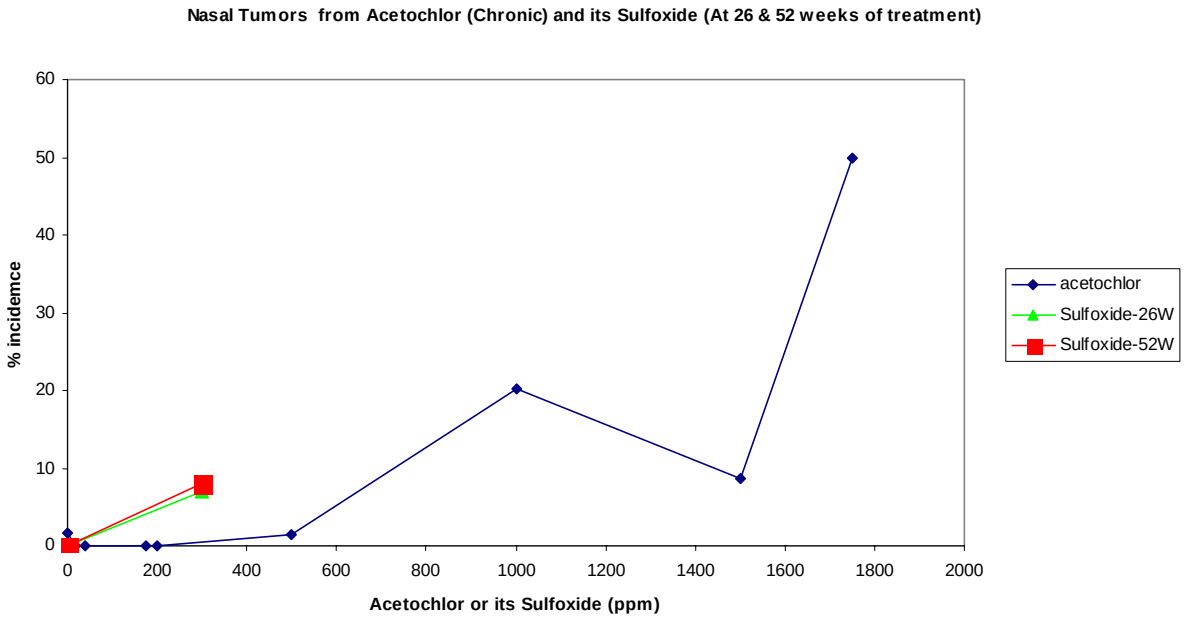
7. Strength, consistency and specificity of association of ultimate toxic effect with key events

a. There is consistency of formation of active species, tissue binding, and tumor formation site for acetochlor. The binding effect, like the tumors, is limited to the nasal olfactory epithelium.

b. The mouse, which does not bind the quinone imine to nasal olfactory epithelium, does not show nasal tumors in well conducted studies. In fact, there is binding of a mouse metabolite to nasal olfactory epithelium, but it is not the quinone imine.

c. Not only feeding of the parent compound produces nasal epithelium tumors, but also feeding of the sulfoxide metabolite of acetochlor, a proximate precursor of the quinone imine [Table 6 and Figure 10b].

d. Data for acetochlor are supported by data from the analog alachlor. Rats treated with alachlor for 1 month did not have detectable neoplasms when examined after a 5-month holding period in basal diet. No detectable olfactory mucosal lesions were observed in any of the "stop study" rats (Genter et al. 2002b).



Figur
e 10b. Dose dependance of nasal adenoma formation in rats with increasing level of acetochlor or its sulfoxide metabolite in the feed. Acetochlor data from Table 2 and sulfoxide metabolite from Registrant's White Paper (MRID 46081801)

8. Biological plausibility and coherence

The data discussed above indicate that this mode of action is coherent and biologically plausible. As reviewed by the FIFRA Sap in 1997 (USEPA 1997, 2001), this mode of action is shared by the chloroacetanilides (alachlor and butachlor).

9. Assessment of postulated mode of action

There is considerable evidence in support of the non-genotoxic MOA discussed in this document. The evidence for the key events has been detailed above. This evidence is supported by the following:

a. The absence of demonstrated positive mutagenic effect of the chemical.

b. Acetochlor administration results in dose related increases in the binding of the putative active quinone imine metabolite in the target tissue.

c. Acetochlor administration results in dose related increases in cell proliferation in cells of the target tissue.

d. Absence of nasal epithelial tumors in mice correlated with their inability to form adducts of the quinone imine at the target site. This evidence was confirmed autoradiographically.

e. Rats administered the sulfoxide metabolite of acetochlor (a proximate precursor of the toxic metabolite, the quinone imine) show nasal olfactory mucosa adenomas after 26 weeks of treatment (MRID 46081801). It is noted these results on the sulfoxide metabolite were submitted to the Agency as a brief preliminary report, part of an ARP's position paper (MRID 46081801). The full report, presently in draft form, has not yet been submitted to the Agency to undergo a full review.

The data on acetochlor are supported by the entire database for the analog alachlor, in particular:

> i. Reversibility of cell proliferation in rats treated with alachlor for 60 days at a tumorigenic dose, after placement in basal diet for 60 days (MRID 42852102)
>
> ii. Rats treated with the analog alachlor for 1 month at a tumorigenic dose (126 mg/kg/day) did not have detectable neoplasms when examined after a 5-month holding period in basal diet. No detectable olfactory mucosal lesions were observed in any of the "stop study" rats (Genter et al. 2002b).

The weight of the evidence in support for the mode of action evaluated in this document is high. The evidence would have been strengthened if corroborative experiments, such as prevention or reversal of a precursor event (e.g. cell proliferation) by appropriate administration of a chemical

(e.g. N-acetylcysteine) known to interfere with a key step (e.g. formation of quinone imine), had been available. Although dimethylaniline (DMA) and diethylaniline (DEA) [analogs of ethylmethyl aniline (EMA)] have been found to form *in vivo* DNA adducts in rat nasal mucosa, concerns about a genotoxic mechanism for acetochlor are mitigated by several factors. These include absence of formation of DNA adducts in nasal mucosa in parallel experiments in rats using alachlor and the reversibility of cell proliferation of olfactory epithelium observed with the analog alachlor

10. Uncertainties, inconsistencies and data gaps

a. Two key observations in support of the MOA (reversibility of nasal mucosal cell proliferation and the "stop study") utilized the analog alachlor. Although it is plausible that the same results will apply with acetochlor, there is no similar data to confirm this.

b. Although there were direct data available to support "cytotoxicity" for acetochlor (respiratory metaplasia and lipofucsin pigment), additional information was inferred from data for the analog alachlor.

c. Although the Registrant measured glutathione (GSH) levels in nasal mucosal epithelium for alachlor and acetochlor, no changes in levels were observed. GSH decrease is a well recognized effect of quinone imine generation in tissues. Although no GSH decreases were seen, the structure of the nasal epithelial tissue protein adducts confirms that a quinone imine was formed. Decreases in GSH, however, were seen in the liver of rats gavaged with acetochlor (MRID 44863205). Recent work by Burman et al. (2003) with the analog alachlor seems to indicate that alachlor may induce oxidative stress in the nasal mucosal olfactory epithelium, which is consistent with the presence of an electrophilic metabolite.

d. Although the structure of the nasal protein adducts supports the formation of a quinone imine in nasal olfactory epithelium, it would have been optimal if direct confirmation of the quinone imine as a key step were available. It would have been very valuable if administration of an agent known to counteract the effect of the quinone imine (e.g. N-acetylcysteine) or an inhibitor of its formation could have been seen to prevent or reverse the key steps.

e. Dimethylaniline (DMA) and diethylaniline (DEA) [analogs of ethylmethyl aniline (EMA), an acetochlor metabolite] have been found to form *in vivo* DNA adducts in rat nasal mucosa. Concerns about a genotoxic mechanism for acetochlor nasal tumorigenesis are mitigated, however, by several factors. These factors include absence of formation of DNA adducts in nasal mucosa in parallel experiments in rats administered alachlor (an analog of acetochlor) and the reversibility of cell proliferation of olfactory epithelium (a key event) observed with the analog alachlor.

DMA has been found to be a nasal carcinogen in feeding studies with CR CD rats (NTP 1990, Technical Report TR-278). Recent work (Duan et al., 2004) indicates that DMA may form DNA adducts in F-344 rats. Nasal mucosal DNA extracted from rats administered DMA by gavage at

310 mg/kg/day for 7 days showed clear spots by postlabeling. In a parallel series of studies by the same authors (Duan et al., 2004), DEA, the putative precursor for the nasal tumor-causing quinone imine for alachlor was also found to show evidence of adduct formation in nasal mucosal DNA. Nasal mucosal DNA extracted from rats administered DEA by gavage at 382 mg/kg/day for 7 days showed clear spots by postlabeling. Autoradiography of rats administered ^{14}C-DEA indicate binding of the compound to nasal turbinates.

On the other hand, as summarized below, although alachlor can form *in vitro* adducts with liver DNA or with deoxy-nucleosides and nucleotides, it appears not to be able to form *in vivo* DNA adducts with rat nasal mucosal DNA.

Brown et al. (1995) showed that alachlor and major metabolites, 2-chloro-N-(2,6-diethylphenyl)acetamide (CDEPA) as well as DEA, bind to mouse liver DNA and hemoglobin protein. Based on the *in vitro* reaction of alachlor and CDEPA with selected nucleosides and nucleotides, (thymidine 3'-monophosphate), Nesnow et al. (1995) demonstrated that both alachlor and CDEPA formed N-1 adducts with 2'-deoxyguanosine and N-3 adducts with thymidine as a result of chlorine displacement. Alachlor also formed N-7 adduct with 2'deoxyguanosine. In contrast to the *in vitro* work, recently Duan et al.(2004) administered alachlor to F344 rats to achieve doses of 126 mg/kg/day for 90 days. Analysis of extracted nasal mucosal DNA by postlabeling did not find evidence of DNA adduct formation, which is consistent with a non-genotoxic mode of action.

IV. CONCLUSIONS FOR MOA

The data supporting the mechanism of action for nasal olfactory epithelium tumors in rats by acetochlor have been evaluated by the CARC. It is concluded that the non-genotoxic MOA for nasal olfactory epithelium tumors in rats, discussed in this document, is supported by the data.

This evidence is supported by:

a. The absence of demonstrated positive mutagenic effect of the chemical.

b. Acetochlor administration results in dose related increases in the binding of the quinone imine metabolite to protein in the target tissue; this metabolite is considered to be the putative active species.

c. There is respiratory metaplasia of the nasal olfactory epithelium, an indication of cytotoxicity to the original olfactory tissue and its being replaced by respiratory epithelium, which originates from undifferentiated cells in the epithelium.

d. Lipofucsin granules are observed to increase in a dose related manner in the nasal olfactory epithelium of rats that show nasal olfactory tumors at the high dose. Lipofucsin granules are

associated with oxidative damage to lipids and lipoproteins, which is consistent with the redox alterations known to be produced by quinones and quinone imines.

e. Acetochlor administration results in dose related increases in cell proliferation in cells of the target tissue.

f. Absence of nasal epithelial tumors in mice correlated with their inability to form adducts of the quinone imine at the target site. This evidence of no quinone imine binding was confirmed autoradiographically.

g. Rats administered the sulfoxide metabolite of acetochlor (a proximate precursor of the toxic metabolite, the quinone imine) show nasal olfactory mucosa adenomas after 26 weeks of treatment (MRID 46081801).

The data on the non-genotoxic MOA for acetochlor are supported by the entire database for the analog alachlor, in particular:

i. Reversibility of cell proliferation in rats treated with alachlor for 60 days at a tumorigenic dose, after placement in basal diet for 60 days (MRID 42852102).

ii. Rats treated with the analog alachlor for 1 month at a tumorigenic dose (126 mg/kg/day) did not have detectable neoplasms when examined after a 5-month holding period in basal diet. No detectable olfactory mucosal lesions were observed in any of the "stop study" rats (Genter et al. 2002b).

The weight of the evidence in support for the mode of action evaluated in this document is high. The evidence would have been strengthened if corroborative experiments, such as prevention or reversal of a precursor event (e.g. cell proliferation) by appropriate administration of a chemical (e.g. N-acetylcysteine) known to interfere with a key step (e.g. formation of quinone imine), had been available. Although dimethylaniline (DMA) and diethylaniline (DEA) [analogs of ethylmethyl aniline (EMA)] have been found to form *in vivo* DNA adducts in rat nasal mucosa, concerns about a genotoxic mechanism for acetochlor are mitigated by several factors. These include absence of formation of DNA adducts in nasal mucosa in parallel experiments in rats using the analog alachlor and the reversibility of cell proliferation of olfactory epithelium observed with alachlor.

V. RELEVANCE TO HUMAN CANCER RISK ASSESSMENT

1. Data Presentation

This section covers the issue of relevance of the MOA for rat nasal turbinate tumors to human cancer risk assessment.

A. Introduction

In 1997, the SAP evaluated the weight-of-the-evidence for the biochemical transformation of **alachlor** to a reactive metabolite as the MOA for the induction of nasal olfactory tumors in the rat and also examined the relevance of this MOA for a human cancer risk assessment (USEPA 1996). Autoradiography data were presented showing localization of the alachlor metabolite only in the nasal tissues of the two rat strains and not in the mouse, hamster or squirrel monkey. These data were supported by *in vitro* metabolism results demonstrating a 30-fold increase in metabolism of **alachlor** in the rat compared to the mouse and a "several thousand-fold lower metabolism in the human". Since the rat responds with nasal tumors and the mouse does not, it was reasoned that the difference in formation of the reactive product, which is retained by nasal tissue, was the "critical mechanism". It was further reasoned that because there was a 30-fold lower *in vitro* metabolism of **alachlor** by the mouse and no nasal tumors, by analogy, the "several thousand-fold lower metabolism in the human" would presumably not result in nasal tumor in humans. However, the SAP stated that the findings were only "suggestive of interspecies differences ... Thus, because bioactivation is thought to play a key role in the mechanism of nasal tumor formation, evidence that bioactivation in humans occurs at significantly lower rates should be compelling". Based on the registrants claim that a rate-limiting step in the metabolism of alachlor in the mouse was not the only factor for lack of a tumorigenic response in the mouse, the SAP concluded that "the limitation in the metabolism in the mouse may not be the real reason for the lack of a tumorigenic response in the mouse. If this is accurate, the argument that limitation in the metabolism of alachlor in the human precludes alachlor being considered as a human carcinogen can not be supported."

The following paragraphs analyze the significance of recently reviewed data on **acetochlor** and evaluate the relevance to human cancer of the postulated MOA for rats.

B. Metabolic differences between rats and other species

The ARP has presented several studies in support of qualitative and quantitative differences in the disposition of acetochlor between rats and other species.

i. Qualitative Differences in Metabolism in Rats and Mice

Several pieces of information supporting the position that marked differences in the metabolism of acetochlor exist between rats and mice include:

The hypothesized MOA for nasal carcinogenicity of alachlor and acetochlor is the bioactivation of these pesticides through several steps ultimately leading to the formation of the corresponding carcinogenic product, dialkylbenzoquinone imine (DABQI). Ashby et al. (1996) (MRID 44496215) and Green (1998) (MRID 44496203) and Green et al. (2000) showed that primary glucuronidation and oxidation of the ethoxymethyl side chain on acetochlor takes place in the rat liver followed by biliary excretion and enterohepatic recirculation causing the removal of the glucuronide and formaldehyde to yield the chloroacetylamide. The subsequent metabolism of the chloroacetylamide involves conjugation of the chloroacetyl group with GHS followed by a sequence of transformations leading to various sulfur-containing metabolites including secondary methyl sulfide. By contrast, the mouse forms a series of glucuronides of the ethoxymethyl side chain through glucuronidation and oxidation in the liver which are then excreted in the urine (Figure 11). Based on these findings, the Registrant concluded that the major route of metabolism of acetochlor in the mouse was glucuronide conjugation.

In another study, whole body autoradiography from four Sprague-Dawley rats administered oral gavage doses of radiolabelled acetochlor sulfoxide metabolite (MRID 44496202) demonstrated that 8 hours after exposure, the highest concentration of radioactivity was found in the gastrointestinal tract and the nasal cavities. Five days postdosing, significant radioactivity was still found in the nasal passages. These findings are in agreement with the earlier whole body autoradiography of animals exposed to **alachlor** which indicated that the distribution to nasal turbinates is strain and species-specific to the rat and not observed in mice, hamsters or squirrel monkeys dosed with **alachlor** (MRID 43706001).

The absence of nasal cancer in mice administered acetochlor in the diet is attributed by the ARP (MRID 46081801) to differences in hepatic metabolism leading to a greatly reduced capacity to form DABQI. This decreased ability to form DABQI is manifested in mice, in addition to absence of tumors, as an absence of precursor events such as nasal tissue binding (MRID 44496211) and olfactory epithelium cell proliferation (MRID 44496209). It is not fully known if the qualitative differences between rats and mice exist between humans and rats.

ii. Quantitative differences in metabolism between rats, mice, monkeys and humans.

a. EMA hydroxylation (First reaction of Path A in Figure 3)

The ARP has studied *in vitro* quantitative differences in the rate of three reactions depicted in Figure 12 between rats, mice, and squirrel monkeys (MRID 44530002). The first reaction is the conjugation of acetochlor with GSH, the second reaction is the cleavage of secondary sulfide to

EMA, and the third one is the p-hydroxylation of EMA to p-hydroxy-EMA (a precursor of the quinone imine). These three reactions are part of Path A, in Figure 3, one of the possible paths, probably a minor one for rats, leading to quinone imine. These reactions were studied using cytosolic and microsomal fractions from liver and nasal epithelium of the tested species. Olfactory and respiratory epithelium were separated for rats and mice, but not for the squirrel monkey.

Comparison of the initial reaction rates of CD rat vs. squirrel monkey tissue fractions indicated that the rates of all three reactions (glutathione conjugation of acetochlor, hydrolysis of the secondary sulfide metabolite of acetochlor to form EMA, and hydroxylation of EMA to pOH-EMA) in liver and nasal tissue fractions were higher in rats than monkeys. Reported reaction rates in the liver fractions ranged from 2.9-fold higher in rats (hydroxylation of EMA) to 10.3- and 10.9-fold for GSH conjugation of acetochlor and hydrolysis of the secondary sulfide of acetochlor to form EMA, respectively. All three reaction rates were significantly higher in the nasal olfactory tissue of the rat (26.2-fold, GSH conjugation of acetochlor, 86-fold, hydrolysis of secondary sulfide metabolite of acetochlor and 23.7-fold, hydroxylation of EMA), suggesting that the rate of formation of precursors to reactive metabolites (imino quinones) that are implicated in nasal tumor formation in rats may be greatest in the rat and much lower in monkeys or other primates (MRID 44530002).

Review of the data, however, indicated that **there is uncertainty** in these rat-to-monkey ratios of activities because olfactory and respiratory epithelium were not separately analyzed for the monkey and the respiratory epithelium in the monkey may be acting as an enzymatically inert diluent for this *in vitro* assay. To evaluate the impact of this uncertainty, the ARP provided calculations (MRID 46081803) of the estimated rates (V_i) for p-hydroxylation of EMA assuming (1) a "conservative" estimate of monkey nasal tissue samples containing 10% olfactory tissue and (2) a "worst-case" estimate of samples containing 1% olfactory tissue. Based on these estimations, the presence of 10% olfactory tissue would give a primate $V_{olfactory}$ for p-hydroxylation of EMA that is 8.7-fold lower (not 23.7-fold lower, as reported initially) than that of rat olfactory tissue and 2.6-fold lower than rat respiratory tissues. If the samples contained only 1% olfactory tissue, the study author calculated that the primate $V_{olfactory}$ would be approximately 7.2-fold less than the rat olfactory tissue and 2.1-fold less than the respiratory tissues.

These results are summarized in Table 15. Examination of this table indicates that although-hydroxylation of EMA in the monkey is slower than in the rat, the rate is not so slow as to negate totally the possibility of oxidation of EMA in the monkey's olfactory epithelium, if EMA is available.

Table 15. Rat-to-monkey ratios of rate of p-hydroxylation of EMA to p-hydroxy-EMA using microsomal suspensions of rat olfactory epithelium and mixed monkey olfactory/respiratory

epithelium (in unknown proportion). [From MRIDs 44530002 and 46081803]

Experimental ratio with no correction	Corrected ratio assuming 10% contamination with respiratory epithelium	Corrected ratio assuming 1% contamination with respiratory epithelium
23.7	8.7	7.2

b. Hydroxylation of Acetochlor sulfoxide metabolite (First reaction in Path B, Figure 3)

The ARP has studied *in vitro* the hydroxylation of the acetochlor sulfoxide metabolite in the rat mouse, monkey, and human liver and nasal tissues (MRID 46009402 and 46081802). Hydroxylation of the acetochlor sulfoxide metabolite is the first reaction in Path B, Figure 3]. Acetochlor sulfoxide is the major circulating acetochlor metabolite in plasma, which requires hydroxylation in the formation of DABQI (the quinone imine).

In this study, the rate of hydroxylation of radiolabeled acetochlor sulfoxide metabolite to p-hydroxy-acetochlor sulfoxide was compared using microsomal fractions derived from Sprague-Dawley rat and CD-1 mouse separated nasal olfactory and respiratory tissues and from squirrel monkeys and 33 human morphologically normal nasal tissue surgical explants (olfactory and respiratory tissues from primates and humans were combined and not separated). Reported results indicated that the highest rates of hydroxylation of acetochlor sulfoxide were observed in the olfactory tissue of the rat and the mouse with comparable activities in these species (6 to 7-fold higher than the activity in rat respiratory tissue, respectively). There was no detectable activity in the primate or human samples.

The investigators concluded, therefore, that acetochlor-induced nasal tumors in the rat are not relevant to humans because the quinone-imine metabolite derived from acetochlor sulfoxide and believed to be responsible for olfactory tumorigenesis would not be produced at sufficient levels in the human. Review of the study by the Agency indicated, however, that there is uncertainty in these data because olfactory and respiratory epithelium were not separately analyzed and the respiratory epithelium in the human and primate may be acting as an enzymatically inert diluent for this in vitro assay.

Following Agency review of the data, because of **concerns raised regarding the possible dilution effect of inactive respiratory epithelium**, the Registrant provided estimations of the activity that would be present if the sample contained only 10% or 1% olfactory tissue (MRID 46081803). Based on these estimates, hypothetical rates of hydroxylation of acetochlor sulfoxide in human or primate olfactory tissue would yield rates that were 132-fold or 88-fold lower than the activity in the rat olfactory tissue. These values for primates, although much smaller than those for rats, are still consisitent with a finite probability of bioactivation of acetochlor metabolites in the nasal mucosa. These ratios of activities are summarized in Table 16.

Table 16. Rat-to-monkey ratios of rate of p-hydroxylation of acetochlor sulfoxide metabolite using microsomal suspensions of rat olfactory epithelium and monkey or human unseparated olfactory/respiratory epithelium (in unknown proportion). (From MRIDs 44530002 and 46081803).

Experimental ratio with no correction	Corrected ratio assuming 10% contamination with respiratory epithelium	Corrected ratio assuming 1% contamination with respiratory epithelium
Extremely high (no activity for primates)	132	88

As part of this investigation, assays were performed with purified human cytochrome p-450 CYP2A6 enzyme (coumarin hydroxylase) (MRID 46081802). Results demonstrated that CYP2A6 did not cause hydroxlyation of acetochlor sulfoxide but rat olfactory microsomes hydroxylated both coumarin and acetochlor sulfoxide. These data suggest that the enzyme that hydroxylates acetochlor sulfoxide is not coumarin hydroxylase but may be related to the CYP2A subfamily. A likely candidate would be the cytochrome p-450 CYP2A3 enzyme, the predominant olfactory cytochrome found in rats but not humans (Fernandez-Salguero and Gonzalez, 1995). Genter et al. (2002a) proposed involvement of CYP2A3 in the final bioactivation of **alachlor** metabolites to the reactive quinone imine; however, the genomic analysis of **alachlor**-treated rat olfactory mucosa revealed that CYP2A3 was downregulated after 2 or 4 days or 1 month of **alachlor** treatment.

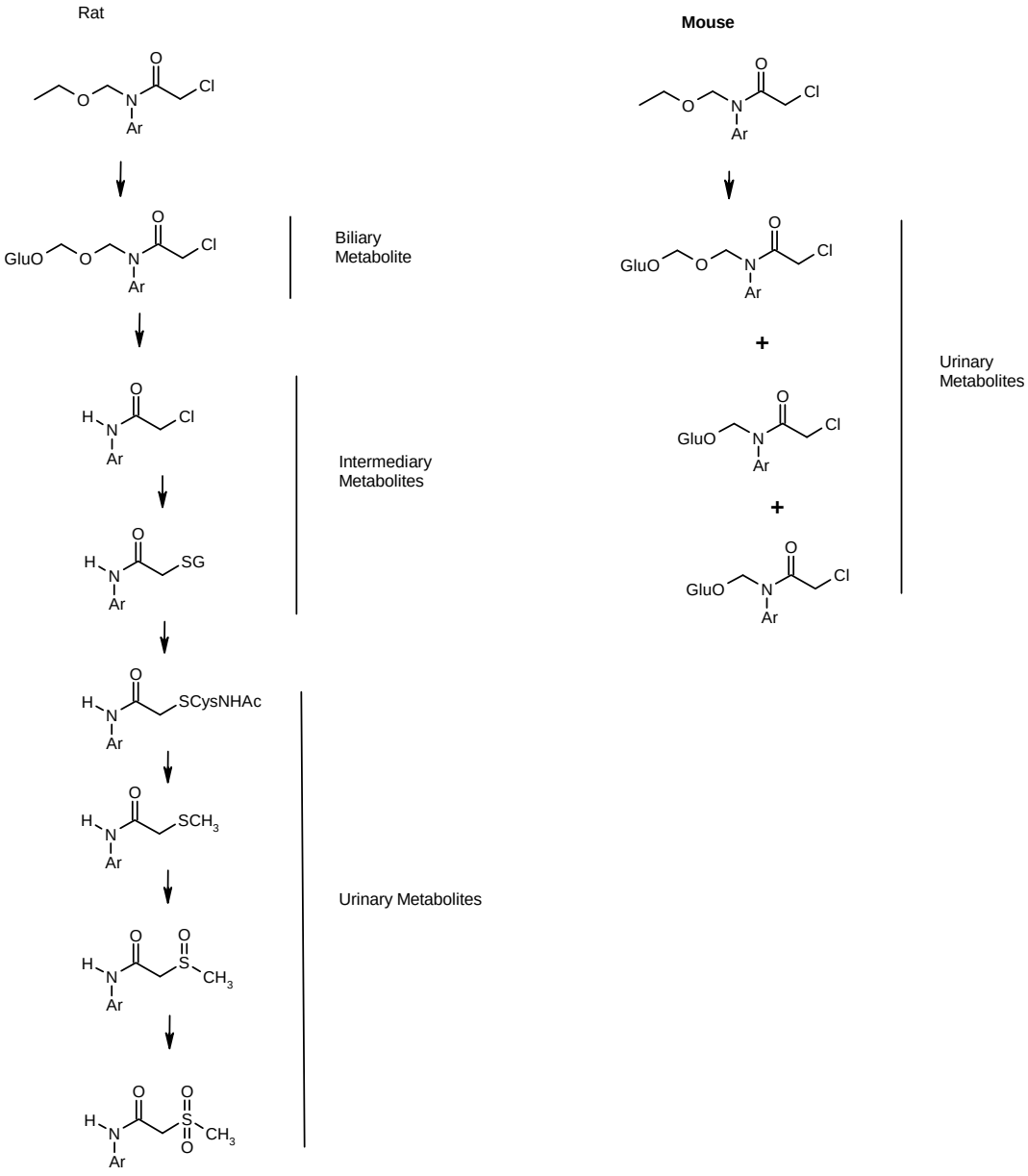
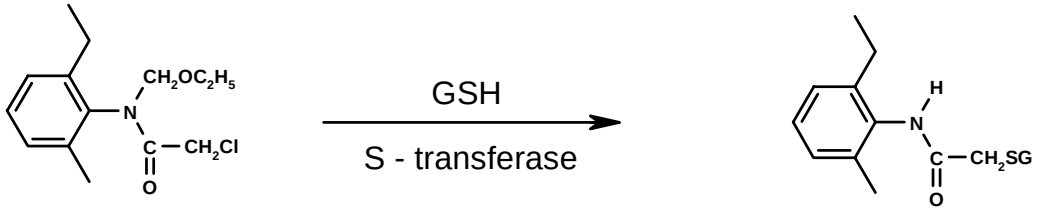
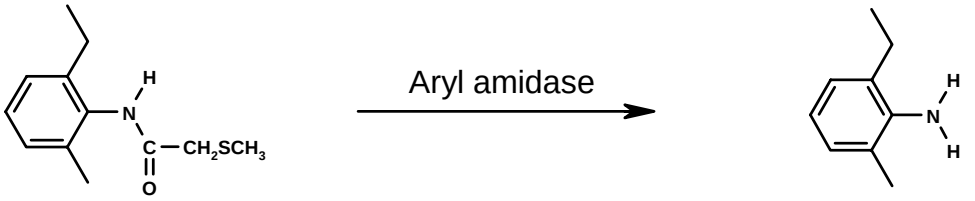


Figure 11. Biotransformation of acetochlor in the rat and mouse. (From MRID 44496203).

1) Acetochlor glutathione (GSH) conjugation



2) Secondary sulfide to 2-ethyl-6-methylaniline (EMA)



3) EMA to p-OH-EMA

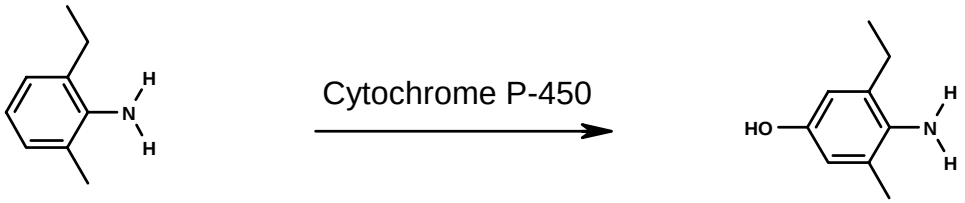


Figure 12. Metabolic reactions measured in rat, mouse, and primate liver and nasal tissues. (From MRID 44530002)

c. *In vitro* metabolism of acetochlor by rat and human liver

In contrast to the Registrant-sponsored studies indicating that acetochlor sulfoxide is not converted by humans to the quinone imine, an independent study by Coleman et al. (2000) demonstrated that human liver samples metabolized acetochlor to 2-chloro-N-(2-methyl-6-ethyl phenyl) acetamide (CMEPA, Figure 13), which is a step before transformation of acetochlor to the secondary methyl sulfide metabolite with subsequent bioactivation to the aniline substrate, 2-methyl-6-ethylaniline (EMA). Data presented further showed that commercially prepared liver microsomes from human males metabolized EMA at a 2-fold higher rate than liver microsomes from Long Evans rats (0.069 nmol/min/mg in human liver microsomes versus 0.035 nmol/min/mg in rat liver microsomes). Although human olfactory microsomes were not tested in this series of experiments, the potential for human liver microsomal fraction to produce the carcinogenic precursor EMA product is plausible. One may speculate that EMA will be carried in the blood to the nasal tissues and then could be further activated following Path A to produce the quinone imine. The related compound 2,6-dimethylaniline is a rat nasal carcinogen (NTP 1990, Technical Report TR-278) and 2,6-diethylaniline is the putative precursor to the alachlor rat nasal carcinogen.

Furthermore, Coleman et al. (2000) found that the cytochrome p-450 enzymes responsible for the human metabolism of acetochlor are CYP3A4 and CYP2B6.

2. Conclusion

On the basis of the *in vitro* metabolism data with microsomes derived from human livers showing the p-450 metabolism of acetocholor via the Scheme A pathway to the carcinogenic product, EMA, combined with the finding that the human microsomal fractions are about 2X more active than the rat in producing EMA (Figure 13), it is concluded that the Registrant argument that there is no relevance to humans can not be sustained.

This conclusion is supported by:

- The realization that production of a metabolite (EMA) with the capacity of undergoing transformation to a quinone imine is possible for humans (Coleman et al., 2000**).**

- *In vitro* studies of p-hydroxylation of EMA using olfactory epithelium enzymes indicate that rat-to-monkey ratios of activities (MRIDs 44530002 and 46081802) are not as large as 23.7 but could be as small as 7 or 8.

- In *in vitro* studies, the ratio of rat to monkey for p-hydroxylation of the sulfoxide metabolite of acetochlor may be not astronomically large, as initially postulated, but as small as 88 (MRID 46081802).

- Although nasal tissue was not included in the Coleman et al. (2000) study , the data indicate that human liver has the potential to produce EMA (Figure 13), a plausibly carcinogenic metabolite of acetochlor, which would then be available to all organs via the circulatory system.

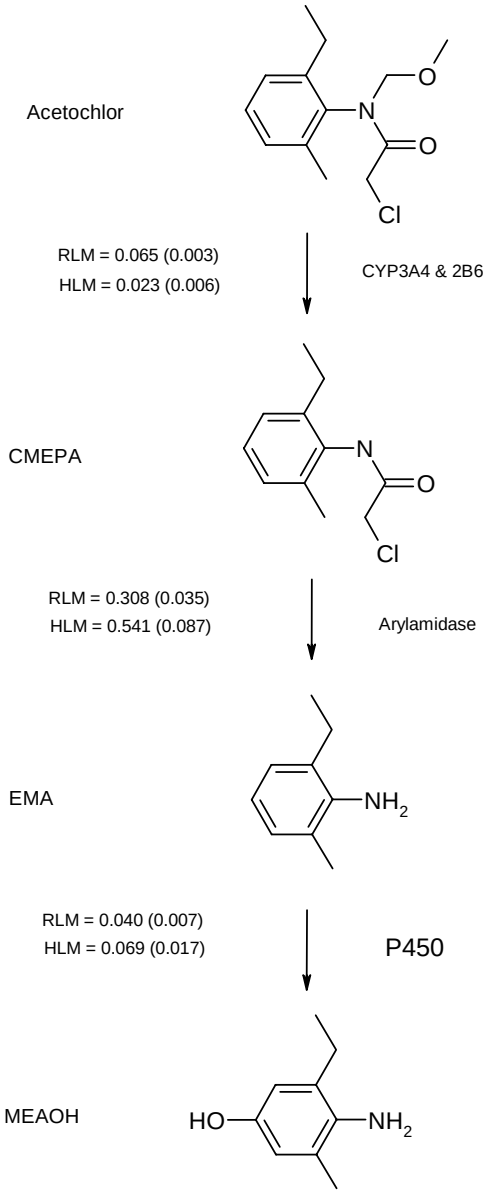


Figure 13. Comparison of the metabolism of acetochlor, CMEPA, and methyl ethyl aniline (EMA) between rat and human liver microsomes. HLM = Human liver microsomes, RLM = rat liver microsomes. Rates are nmol/min/mg ± (SEM). Adapted from Coleman et al. 2000).

VII. BIBLIOGRAPHY

SUBMITTED STUDIES

MRID No.	CITATION
00050930	Ross, W.D.; Kulik, F.A. (1978) Salmonella Mutagenicity Assay of CP-55097, DA-78-186: Report No. MRC-DA-838. Final rept. (Un- published study received Dec 12, 1980 under 524-EX-56; submitted by Monsanto Co., Washington, D.C.; CDL:099811-C)
00131088	Ahmed FE and Seely, JC (1983) Acetochlor: Chronic Feeding Toxicity and Oncogenicity Study in the Rat. Pharmacopathics Research Laboratories, Inc., Laurel, MD. Study No. PR-80-006. May 20, 1983. Unpublished report.
00131089	Ahmed., FE, Tegeris, A.S. and Seely, JC (1983) MON-097: 24-Month Oncogenicity Study in the Mouse. Pharmacopathics Research Laboratories, Inc., Laurel, MD. Report No. PR-80-007. May 4, 1983. Unpublished report.
00131392	Farrow, M.; Cortina, T. (1983) In vivo Bone Marrow Chromosome Study in Rats: Acetochlor (Mon 097): HL-83-006. Final rept. (Unpublished study received Sep 22, 1983 under 524-348; prepared by Hazleton Laboratories America, Inc., submitted by Monsanto Co., Washington, DC; CDL:071970-A)
00131393	Naismith, R.; Matthews, R. (1983) To Perform the Rat Hepatocyte Primary Culture/DNA Repair Test According to Standard Operating Procedure PH-311 on: MON 097: Study No. PH 311-MO-001-82. (Un- published study received Sep 22, 1983 under 524-348; prepared by Pharmakon Research International, Inc., submitted by Monsanto Co., Washington, DC; CDL:071970-B)
00131394	Mitchell, A., Rudd, C., Coleman, R (1982). An evaluation of Mutagenic Potential of Mon 097 Employing the L5178Y TK $^{+/-}$ Mouse Lymphoma Assay. SRI project No. LSC-2572: Monsanto Study No. SR- 81-150. Final report: prepared by SRI International, submitted by Monsanto Co. Washington DC Unpublished.
00131395	Farrow, M.. and Cortina, T.(1983). In Vivo Bone Marrow Chromosome Study in Rats: Acetochlor (Mon 097): HL-83-006. Final Report: prepared by Hazleton Laboratories America, Inc., Vienna , VA, submitted by Monsanto Co. Washington DC Unpublished.
00164941	Cortina, T. (1985) In vivo Micronucleus Assay in Mice: Mon 097(Acetochlor): Final Report: HL-84-405. Unpublished study pre- pared by Hazleton

Biotechnologies Corp. 56 p.

40077601 Naylor, M.W. and Ribelin, W.E. (1986) Chronic Feeding Study of MON 097 in Albino Rats. Study No. ML-83-200, Report No. MSL-6119; 93-190, Laboratory Project ID EHL-83107. September 25, 1986. Unpublished report.

40484801 Ribelin, W.E. (1987) Histopathology Findings in Noses of Rats Administered MON 097 in a Lifetime Feeding Study. Tegeris Laboratories, Laurel, MD and Monsanto Environmental Health Laboratory, St. Louis, MO. Laboratory Project No. ML-86-44/EHL 86027. November 4, 1987. Unpublished report (supplement to original study report).

41565119 Amyes, S.J. (1989) SC-5676: 78-Week Feeding Study in CD-1 Mice. Life Science Research Ltd., Suffolk, England. Study No. 87/SUC0012/0702. June 9, 1989. Unpublished report.

41565121 Callander, R., Priestly, K. (1989). Acetochlor: An Evaluation in the Salmonella Mutation Assay: Lab Project No. CTL/P2391: SV2370 prepared by ICI Central Toxicology Laboratory. Unpublished.

41565122 Howard, C. (1989). Acetochlor: An Evaluation in the in vitro Cytogenetic Assay in Human Lymphocytes. Lab Project No. CTL/P2617: SV0336 prepared by ICI Central Toxicology Laboratory. Unpublished.

41565124 Trueman, R. (1989) Acetochlor: Assessment for the Induction of Un- schedules DNA Synthesis in Rat Hepatocytes in vivo: Lab Project No: CTL/P/2604: SR0357. Unpublished study prepared by ICI Central Toxicology Laboratory. 49 p.

41565125 Hawkins, D.; Kirkpatrick, D.; Dean, G. (1987) The Biokinetics of [carbon 14|-Acetochlor after Oral Administration to Rats at a Nominal Level of 10MG/KG: Lab Project Number: HRC/STR 18/88502. Unpublished study prepared by Huntingdon Research Centre Ltd. 48p.

41565126 Hawkins, D.; Kirkpatrick, D.; Dean, G. (1989) The Metabolism of [carbon 14|-Acetochlor in the Rat after Oral Administration: Lab Project Number: HRC/STR 18/89603. Unpublished study prepared by Huntingdon Research Centre Ltd. 33 p.

41565127 Jones, B. (1990) Acetochlor: Biotransformation Study in the Rat: Lab Project Number: CTL/P/2809: UR0286. Unpublished study prepared by ICI Central Toxicology Laboratory. 49 p.

41592004 Virgo, D.M. and Broadmeadow, A. (1988) SC-5676: Combined Oncogenicity and Toxicity Study in Dietary Administration to CD Rats for 104 Weeks. Life Science Research Ltd., Suffolk, England. Study No. 88/SUC017/0348. March 18, 1988. Unpublished report.

41592006 Brooker, A.; Stubbs, A.; John, D. (1989) Acetochlor: Teratogenicity Study in the Rabbit: Lab Project Number: RB0432: ISN 205/89432. Unpublished study prepared by Huntingdon Research Centre Ltd. 102 p.

41592007 Hawkins, D.; Kirkpatrick, D.; Dean, G. (1987) The Biokinetics of [carbon 14|-Acetochlor after Oral Administration to Rats at a Nominal Level of 200 MG/KG: Lab Project No: HRC/STR 18/89184. Unpublished study prepared by Huntingdon Research Centre Ltd. 46 p.

41963309 Hodge, M. (1991) Acetochlor: Dominant Lethal Study in the Rat: Lab Project Number: CTL/P/3189: RR0527. Unpublished study prepared by ICI Central Toxicology Lab. 296 p.

42244901 Daly, I; Bolte, H. (1983). A Two Year Chronic Feeding Study of Butachlor in Rats. Bio/Dynamics, Inc. Lab. No. 79-2388. Sponsor No. BD-80-124. Submitted by Monsanto Co. Unpublished.

42713106 Lie, A, Myers, C. (1989). CHO/HGPRT Gene Mutation Assay with Mon 097: Lab Project No. ML-88-314: EHL 88183 prepared by Monsanto Co. Environmental Health Laboratory. Unpublished.

42852102 Brewster, D.; Hotz, K. (1991) A Study to the Effect of Alachlor on Cell Proliferation in Specific Tissues of the Rat and Mouse: Lab Project Number: HL 87112,90059,90114: R.D. 1156: 90114. Unpublished study prepared by Monsanto Co. 95 p.

43590001 Branch, D.; Thake, D. (1995) Gastric Tumor Promotion Study of Alachlor in Long-Evans Rats: Lab Project Number: ML-93-137: EHL 93049: RD 1295. Unpublished study prepared by Monsanto Co. 463 p. Author: Branch, D.

43706001 Hall, L. (1991) A Study of the Distribution and Localization of Dimethylaniline in Rats and Mice Using Whole-Body Autoradiography: Lab Project Number: MSL-11227: ML-89-129: EHL-89082. Unpublished study prepared by Monsanto Environmental Health Lab. 47 p.

44069502. Milburn, G.M. (1996). Acetochlor: Dominant Lethal Study in the Rat by Dietary

Administration, conducted at Zeneca's Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire (UK), Report No. CTL /P/4780 (Study No. RR0688), Final Report dated 16 May, 1996. Unpublished..

44069503 Ashby, J.; Kier, L.; Wilson, A.; et al. (1996) Evaluation of the Potential Carcinogenicity and Genetic Toxicity to Humans of the Herbicide Acetochlor: Lab Project Number: BJK01. Unpublished study prepared by Zeneca Central Toxicology Lab; Monsanto Chemical Co.; and Zeneca Agrochemicals. 107 p.

44093701 Milburn, G.M. (1996). Acetochlor: Dominant Lethal Study in the Mouse by Dietary Administration, conducted at Zeneca's Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire (UK), Report No. CTL /P/4781 (Study No. RM0693), Final Report dated 16 May, 1996. Unpublished.

44093703. Ashby, J., Tinwell, H., Lefevre, P.A., Williams, J., Kier, L. and Clapp, M.J.L. (1996). Evaluation of the Mutagenicity of Acetochlor to Male Rat Germ Cells, conducted at Zeneca Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire (UK), CTL Laboratory Project No. BJK03 Unpublished.

44496201 Clapp, M.; Heydens, W.; Ashby, J. et al. (1998) Acetochlor: Assessment of Oncogenic Potential in Rodents and Lack of Relevance to Humans: Lab Project Number: CTL/P/5667. Unpublished study prepared by Central Toxicology Laboratory.39 p.

44496202 Green, T. (1998) The Comparative Metabolism of Acetochlor in Rats and Mice. Central Toxicology Laboratory, Alderly Park, Macclesfield, Cheshire, UK. Report No. CTL/R/1318. January 12, 1998. Unpublished.

44496203 Green, T. (1998) The Comparative Metabolism of Acetochlor in Rats and Mice: Lab Project Number: CTL/R/1318. Unpublished study prepared by Central Toxicology Laboratory. 24 p.

44496207 Hotz, K.; Wilson, A. (1996) A Study of the Effects of Acetochlor on Rat Nasal Cell Proliferation: Lab Project Number: EHL 94096: 94096: ML-94-227. Unpublished study prepared by Ceregen, A Unit of Monsanto Co. and AniLytics, Inc. 160 p.

44496209 Hotz, K.J. and Wilson, A.G.E. (1998) A Study on the Effects of Acetochlor on Mouse Nasal Cell Proliferation. Ceregen (EHL), St. Louis, MO. Report No. MSL-14914. December 12, 1996. Unpublished report.

44496210 Lau, H.; Kraus, L.; Thake, D. et al. (1998) Characterization of Acetochlor Binding to Rat Nasal Tissues: Lab Project Number: EHL 95060: ML-95-171: 95060.

Unpublished study prepared by Environmental Health Laboratory. 66 p.

44496211 Lau, H.; Wilson, A. (1998) Characterization of Acetochlor Binding to Mouse Nasal Tissue: Lab Project Number: EHL 96063: ML-96-109: 96063. Unpublished study prepared by Environmental Health Laboratory. 48 p.

44496212 Lau, H.; Kraus, L.; Hotz, K. et al. (1998) A Study of the Protein Binding and Cellular Localization of Acetochlor Secondary Sulfide in Rats: Lab Project Number: EHL 96223: ML-97-10: 96223. Unpublished study prepared by Environmental Health Laboratory. 55 p.

44496213 Lau, H.; Wilson, A. (1998) Characterization of Acetochlor Binding to Rhesus Monkey Nasal Tissue: Lab Project Number: EHL 97167: ML-97-194: 97167. Unpublished study prepared by Coulston Foundation and Environmental Health Laboratory. 128 p.

44496214 Morgan, K. (1998) Tumor Mapping in Nasal Tissue from Long-Evans and Sprague-Dawley Rats Previously Treated with Various Chloracetanilides: Lab Project Number: CIIT 95044. Unpublished study prepared by Chemical Industry Institute of Toxicology. 45 p.

44496215 Ashby J, Kier L, Wilson AG, Green T, Lefevre PA, Tinwell H, Willis GA, Heydens WF, Clapp MJ. (1996). Evaluation of the potential carcinogenicity and genetic toxicity to humans of the herbicide acetochlor. Hum Exp Toxicol. 1996 Sep;15(9):702-35.

44530002 Green, T. (1998) The in vitro Metabolism of Acetochlor in Rat, Mouse, and Primate Liver, and Nasal Tissues: Lab Project Number: CTL/R/1319. Unpublished study prepared by Zeneca Central Toxicology Lab. 15 p.

44863202 Callander, R. (1998) Acetochlor: An Evaluation in the Salmonella/Microsome Assay: Lab Project Number: CTL/R/1379: YV3422: YV3423. Unpublished study prepared by Central Toxicology Labs. 24 p.

44863204 Fox, V. (1998) Acetochlor and Related Materials: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes: Lab Project Number: CTL/R/1386: SV0442: SV0655. Unpublished study prepared by Central Toxicology Labs. 36 p.

44863205 Citation Reference: Ashby, J.; Lefevre, P. (1993) Acute Toxicity of Acetochlor to the Liver: Pathological and Biochemical Studies (in Rats): Lab Project Number: CT/R/1177. Unpublished study prepared by Central Toxicology Labs. 19 p.

44863208 Ashby, J., Clapp, M.J.L. Tinwell, H Lefevre, P.A. (1999). Use of the Comet Assay tp Assess Genetic Toxicity in the Nasal Olfactory Cells of rats Exposed to Acetochlor in the Diet. Zeneca's Central Toxicology Laboratory (CTL)United Kingdom, Study Nos. XR5013 and XR4981(Report No. CTL/R/1289). July 1995.

45357503 Milburn, G.M. (2001) Acetochlor: Multigeneration Reproduction Toxicity Study in Rats. Central Toxicology Laboratory, Alderley Park Macclesfield, Cheshire, UK. Study No. RR0818, Report No. CTL/RR0818, ARP Submission No. 852-544. February 16, 2001. Unpublished report.

45367401 Kronenberg, J. (2001) Summary of the Acetochlor Registration Partnership Position Regarding the Oncogenic Effects of Acetochlor in Rats and Mice. Acetochlor Registration Partnership. Report No. ARP/852/556. March 29, 2001. Unpublished report.

46009402 Green, T. (2001) (Carbon 14) Acetochlor Sulphoxide: Binding and Localisation of Radioactivity in Rat Nasal Tissues. Project Number: Y11132/001, 011889, CTL/011889/RES/REPT. Unpublished study prepared by Central Toxicology Lab. (Syngenta). 22 p.

46081801 Kronenberg, J.; Dybowski, J. (2003) Acetochlor: Justification for Reclassification of Carcinogenic Potential. Project Number: XX/2003/094, GHC/5673, 852/585. Unpublished study prepared by Acetochlor Registration Partnership. 65 p.

46081803 Dybowski, J.A. (2003) Response to US EPA Data Evaluation: *In Vitro* Metabolism of Acetochlor in Rat, Mouse and Primate Liver and Nasal Tissues. CTL/R/1319. Regulatory Laboratories-Indianapolis Lab, Dow Agrosciences LLC, Indianapolis, IN. Laboratory Study ID GHC 5698. September 24, 2003. MRID No. 46081803. Unpublished study (supplement to MRID 44530002).

OTHER PUBLICATIONS

Akubue, PI and Stohs, SJ (1991). Endrin-induced production of nitric oxide by rat peritoneal macrophages. Toxicol Lett 62:311-316.

Albertini, RJ, Anderson, D, Douglas, GR, Hagmar, L, Hemminiki, K. et al. (2000). IPCS guidelines for monitoring genotoxic effects of carcinogens in humans. Mutat Res 463(2): 111-172.

Andrews, GK (2000). Regulation of metallathionein gene expression by oxidative stress and metal ions. Biochem Pharm 59: 95-104.

Bagchi, D, Bagchi, M, Hassoun, EA, Stohs, SJ (1995). In vitro and in vivo generation of ROS,

DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology. 104: 129-140.

Bancroft, JD and Stevens. A. (1996). Theory and Practice of Histological Techniques. Fourth Edn. Churchill Livingstone. p. 255.

Bogdanffy MS, Mazaika TJ, Fasano WJ (1989) Early Cell Proliferative Cytotoxic Effects of Phenacetin on Rat Nasal Mucosa. Toxicol. Appl. Pharmacol. 98: 100-112 (1989).

Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ (2000) Role of Quinones in Toxicology. Chem. Res. Toxicol. 13(3): 135-160.

Brown, MA, Kimmel, EC, Casida, JE (1988). DNA adduct formation by alachlor metabolites. Life Sci 49:2087-2094.

Burman, DM, Shertzer, HG, Senft, AP, Dalton, T, Genter, MB (2003). Antioxidant perturbations in the olfactory mucosa of alachlor-treated rats. Biochem Pharm 66:1707-1715.

Coleman, S, Linderman, R, Hodgson, E and Rose, RL (2000). Comparative metabolism of chloroacetamide herbicides and selected metabolites in human and rat liver microsomes. Environ Health Perspectives 108:1151-1157.

Clayson DB, Mehta R, Iverson F (1994) International Commission for Protection Against Environmental Mutagens and Carcinogens. Oxidative DNA damage-the effects certain genotoxic and operationally non-genotoxic carcinogens. Mutat.Res. 317(1):25-42.

Dahlin DC, Nelson SD (1982) Synthesis, Decomposition Kinetics, and Preliminary Toxicological Studies of Pure N-Acetyl-p-benzoquinone Imine, a Proposed toxic metabolite of Acetaminophen. J. Medicinal Chem. 25(8):885-886.

Dearfield, KL, McCarroll, NE, Protzel, A, Stack, HF, Jackson, MA, Waters, MD. (1999). A survey of EPA/OPP and open literature on selected pesticide chemicals. II. Mutagenicity and carcinogenicity of selected chloroacetanilides and related compounds. Mutat Res 443 : 183-221.

Duan Jd, Genter M, Jeffrey AM, Williams GM (2004) Formation of DNA adducts in F344 Rat Nasal Tissue by 2,6-Dimethylaniline and 2,6-Diethylaniline, but not alachlor. [Abstract 1901], The Toxicologist, SOT Annual Meeting 2004. Supplement to Toxicological Sciences 78 (S-1) 391.

Fernandez-Salguero, P and Gonzalez, FJ (1995). The CYP2A gene subfamily: species differences, regulation, catalytic activities and role in chemical carcinogenesis. Pharmocgenetics 5:S123-S128.

Galloway, S.M. (2000). Cytotoxicity and chromosome aberrations in vitro: Experience in industry

and the case for an upper limit on toxicity in the aberration assay. Environ Mol Mutagen, 35:191-201.

Genter, MB, Burman, DM, Vijayakumar, S. Ebert, CL, Aronow, BJ (2002a). Genomic analysis of alachlor-induced oncogenesis in rat olfactory mucosa. Physiol Genomics 12:35-45.

Genter, MB, Burman DM, Dingeldein, MW, Clough, I, Bolon, B. (2000) Evolution of alachlor-induced nasal neoplasms in the Long-Evans rat. Toxicol Pathol 28:770-781

Genter, M.B., Burman, D.W. and Bolon, B. (2002b) Progression of Alachlor-Induced Olfactory Mucosal Tumours. *Int. J. Exp. Path* 83: 303-307.

Green, T, Lee, R, Moore, RB, Ashby, J, Willis, GA, Lund, VJ, Clapp, MJL. (2000). Acetochlor-induced rat nasal tumors: Further studies on the mode of action and relevance to humans. Reg Toxicol and Pharm 32: 127-133.

Hill, AB, Jefferies, PR, Quistad, GB, Casida, JE (1997). Dialkylquinoneimine metabolites of chloroacetanilide herbicides induce sister chromatid exchanges in cultured human lymphocytes. Mutat Res 395:159-171.

Jackman, J, Alamo, I Jr, Forance, AJ Jr (1994). Genotoxic stress confers preferential and coordinate messenger RNA stability on the five *gadd* genes. Cancer res 54:5656-5662.

Kier, LD, Heydens WF, Lau, H., Thake, DC, Asbury, K, Wilson,, AG (1996). Genotoxicity studies of alachlor. Fundam. Appl. Toxicol. 30: 231.

Monks, TJ, Jones DC (2002) The Metabolism and Toxicity of Quinones, Quinoneimines, Quinone Methides, and Quinone-Thioethers. Current drug Metabolism 3:425-438.

Nesnow, S, Agarwal, SC, Padgett, WT, Lambert, GR, Boone, P. Richards, A (1995). Synthesis and characterization of adducts of alachlor and 2-chloro-N-(2,6-diethylphenyl)acetamide with 2'deoxyguanosine, thymidine and their 3'-monophosphates. Chem Res Toxicol 8(2): 209-217.

NTP (1990) National Toxicology Program.. NIH/NIEHS. Toxicology and Carcinogenesis Studies of 2,6-Xylidine (2,6-Dimethylaniline) (CAS No. 87-62-7) in Charles River CD Rats (Feed Studies). Technical Report No. 278 [TR-278]. Report date: January 1990.

Otterbein, LE, Augustine, MKC (2000). Heme oxygenase: colors of defense against cellular stress. Am J Physiol Lung Cell Mol Physiol. 279: 1029-1037.

Sarafian, TA and Bredesen. DE (1994). Is apoptosis mediated by ROS? Free Rad Res 21:1-8.

Sonich-Mullin, C., Fielder R, Wiltse J, Baetcke K, et al. (2001) IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis. Reg. Toxicol. Pharmacol. 34: 146-152.Sonich-Mullin, C., Fielder R, Wiltse J, Baetcke K, et al. (2001) IPCS Conceptual Framework for Evaluating a Mode of Action for Chemical Carcinogenesis. Reg. Toxicol. Pharmacol. 34: 146-152.

Stokes, AH, Freeman, WM, Mitchell, SG, Burnette, TA, Hellman, GM, Vrana, KE. (2002). Induction of GADD 45 and GADD153 in Neuroblastoma Cells by Dopamine-Induced Toxicity. NeuroToxicol 23:675-684.

Takahashi, S, Saito, S, Ohtani, N, Sakai, T (2001). Involvement of the Oct-1 regulatory Element of the *gadd45* Promoter in the p53-independent Response to Ultraviolet Irradiation. Cancer Res 61:1187-1195.

Trela, BA, and Bogdanffy, MS (1991). Carboxylesterase-dependent cytotoxicity of dibasic esters (DBE) in rat nasal explants. [Tox. App. Pharmacol. 107:285-301.

USEPA (1996). Scientific Advisory Panel (1996). A Set of Scientific Issues Being Considered by the Agency to Discuss and Evaluate the Weight-of-the-Evidence for Alachlor With Particular Reference to its Carcinogenic Potential.

USEPA (1997) SAP REPORT, April 28, 1997. Report of the FIFRA Scientific Advisory Panel Meeting, March 19-20, 1997, held at the Crystal Gateway Marriott, 1700 Jefferson Davis Highway, Arlington, VA 22202.

USEPA (2001) *The Grouping of a Series of Chloroacetanilide Pesticides Based on a Common Mechanism of Toxicity*, Office of Pesticide Programs, USEPA (June 7, 2001).

Wetmore, BA, Mitchell, AD, Meyer, SA, Genter, MB (1999). Evidence for site-specific Bioactivation of Alachlor in the Olfactory Mucosa of the Long-Evans Rat. Toxicol Sci 49: 202-212.

Wolf, D (2004) Personal Communications: E-Mail message from Doug Wolf (EPA/ORD), to Alberto Protzel (Branch Senior Scientist, EPA/OPP/HED/TOX), February 25, 2004.

Young (1981) Histopathological examination of the rat nasal cavity. Fund. Appl. Toxicol. 1:309-312.

Part 3: Dissenting Views on April 21-22, 2004 CARC Assessment of Acetochlor (Brian Dementi, Ph.D., DABT., May 6, 2004 (Revised May 19, 2004))

Dissenting Views on April 21-22 CARC Assessment of Acetochlor
Brian Dementi, Ph.D., DABT May 6, 2004 (Revised May 19, 2004)

On April 21-22, HED's Carcinogen Assessment Review Committee (CARC) appraised the carcinogenicity of acetochlor. The CARC classified acetochlor under the Agency's 1999 draft Carcinogen Risk Assessment Guidelines as "*Likely To Be Carcinogenic To Humans*", *with the quantitative risk assessment.* As I understood, the most conservative (dose-wise) neoplastic response, whether it be lung tumors, histiocytic sarcoma or possible other neoplastic response, would be used to quantify the risk, though that particular end point was not actually settled at the meeting. This *"Likely"* classification was undoubtedly a weight-of-the-evidence decision, taking into consideration all of the evidence for carcinogenicity.

However, acetochlor is a member of a class of pesticidal agents, the chloroacetanilides, among which the much studied alachlor is another notable analogue. Much of the toxicology of this class of agents is shared among its constituent members. One of the cancer end points discussed rather intensely at the meeting was that of the nasal epithelium, as this response is considered to be a common effect of the class. One Mode of Action (MOA) may be responsible for the nasal tissue neoplastic response for the chloroacetanilides. While the committee did not conclude at the meeting which of the various neoplastic responses would serve as the basis for the quantitative risk assessment, it did not appear to be the nasal tumors. However, for various reasons discussed below and in the exhibits for these comments, the nasal tissue neoplastic response *cannot be ruled out* as the most sensitive, inasmuch as the finding was observed after a short while in the reproduction study, there is in my view inadequate testing for this end point at ½ MTD, the tumors are exceedingly rare and for this reason real effects may not be picked up at low doses in the small animal group sizes used in cancer bioassays, etc.

One of the tasks of this joint MTARC/CARC meeting was to consider the MOA of the nasal tissue neoplastic response, and had the two meetings been conducted independently as originally planned, the nasal tissue neoplastic response MOA would have been in a separate MTARC report. However, in the case at hand, the subject of the MOA would appear to be part of the one report for the joint meeting. It is uncertain to me as to how the joint report will be formatted, as I have not seen any draft of the report.

Accordingly, the rationale for the hypothesized Mode of Action (MOA) for the one tumor type, *nasal neoplasia,* in the case of acetochlor, as reviewed at the joint MTARC/CARC meeting, derives in part from earlier work on alachlor. Alachlor and acetochlor are largely inseparable, toxicologically, and both agents along with other members of the chloroacetanilides have previously been viewed by CARC and possibly SAP as sharing a common mechanism of toxicity for neoplasia of the nasal olfactory epithelium. This particular neoplasia has been considered to operate by a MOA that would obviate employment of the linear low dose extrapolation (Q1*) approach in the regulatory setting. In any case, the prior CARC and SAP assessments of alachlor set the stage upon which the testing for this effect in the case of acetochlor was played out. *Little*

concern has been devoted recently to the consideration of how findings now in the case of acetochlor impinge the understanding of the MOA of alachlor already in place.
To the extent that CARC's recent decision affirming for acetochlor the same MOA for nasal neoplasia as that already in place for alachlor, there is only added support, or further endorsement, for this particular MOA as a common mechanism of toxicity for the class. Herein lies a principal aspect of this dissenting opinion, namely that in the interest of public health protection, the evidence in support of the MOA lacks the certitude that should be required before relaxing the regulatory posture in favor of an MOE (margin of exposure) approach *were nasal neoplasia to drive the risk assessment*. The MOA is an interesting and appealing hypothesis, and may indeed be applicable in explaining the nasal tumor response, but this toxicologist has too many concerns over the adequacy of the proof.

Furthermore, even if the MOA for nasal neoplasia were established as true, there is inadequate information to identify a nasal tissue "key effect" end point to employ in conjunction with nasal tumor incidence to identify a reliable Point of Departure (POD) for use in calculating the Margin of Exposure (MOE), as explained in the Agency's draft Carcinogen Risk Assessment Guidelines. This is best explained in Exhibit III (items 1 and 2 therein), as cited below.

This is all a very complicated subject. References that I would have appended to the CARC report as documenting the substance of my divergent opinions with respect to CARC are identified as follows: I) "Acetochlor Mechanism of Nasal Tissue Carcinogenicity" (1/26/04)(Doc #18) B. Dementi; II) Email B. Dementi/Colleagues on acetochlor issues of concern (2/4/04) (Doc #19); III) Email B. Dementi/Colleagues additional acetochlor concerns (3/2/04) (Doc #20); IV) Email B. Dementi/A. Protzel, comments on his proposed CARC presentation (4/7/04)(Doc # none): V) Email B. Dementi/Colleagues concerning absence of evidence of nasal tissue cytotoxicity (1/30/04) (Doc # none); VI) Email B. Dementi/J. Kronenberg, comments on Clapp et al (MRID 44496201) (5/28/03) (Doc #33); VII) Comments on Monsanto's "Acetochlor: Justification for Reclassification of Carcinogenic Potential - Acetochlor Reregistration Partnership" (10/24/03) (Doc #32) B. Dementi; VIII) Email B. Dementi/Colleagues comments on acetochlor rat reproduction study (3/9/04) (Doc #21); IX) Oral presentation to CARC on April 22 on acetochlor (4/21/04)(Doc # none) B. Dementi; X) Email W. Hirzy/B. Dementi forwarding 4/18 Email of S. Makris concerning request for MTARC meeting postponement (4/20/04) (Doc # none); XI) Email J. Kronenberg/L. Chitlik on subjects of histopathology (4/27/04)(Doc # none).

Examples of specific topics of divergent interpretation between myself and CARC are summarized as follows:

1) A principal element of the chloroacetanilide MOA for nasal neoplasia is that of cytotoxicity of the nasal olfactory epithelium hypothesized to result from protein binding by a quinoneimine metabolite of the chloroacetanilide in question. As explained in the 1/30/04 Email (Exhibit V), we have had difficulty obtaining evidence of such cytotoxicity. The registrant has claimed in the background materials that cell death and cytotoxicity follow treatment with these agents.

Phenacetin has been cited as a model for this response. And in the case of phenacetin, Bogdanffy et al (1989) [Bogdanffy, M. S. et al "Early cell proliferative and cytotoxic effects of phenacetin on rat nasal mucosa", Tox. Appl. Pharm. 98, 100-112] claim to find this cytotoxicity: "One or two weeks of daily phenacetin treatment resulted in degenerative changes in the olfactory epithelium and necrosis of Bowman's glands." (p. 100) The registrant has acknowledged no such histopathologic findings in the case of alachlor or acetochlor. The rat chronic bioassays do not illustrate this kind of histopathology, yet the tumors are found. *It is this toxicologist's opinion that cytotoxicity induced by quinoneimine must be demonstrated as partial proof of the MOA. Otherwise, the MOA remains in a hypothetical state.* There is qualitative evidence of quinoneimine protein binding, but inadequate quantitative data at doses up to and including the MTD to show what level of quinoneimine-protein binding is necessary to elicit meaningful cytotoxicity.

Since confirming this absence of substantial evidence of cell death and cytotoxicity, which I believe was surprising to persons in HED at this time, a hasty assessment of the subject just before the CARC meeting has taken place amongst pathologists. I offer comment on this in Exhibit IV. Furthermore, this very recent discussion has continued right up to April 21. I expressed my concerns over this untimely debate, and called for postponement of the April 21 CARC meeting to allow time for me as toxicologist (and any others who may take the interest) to review the subject (Exhibit X). In essence, to the extent that I have been able to evaluate the subject, there is no evidence for cell death or cytotoxicity in the carcinogenicity bioassays on acetochlor, while certain Agency pathologists now claim various findings (e.g. metaplasia) as constituting this evidence for cytotoxicity, when in fact there is nothing linking metaplasia to quinoneimine levels in a cause and effect relationship. Metaplasia may simply be a correlate of the neoplastic process whatever the underlying etiologic event (MOA) inducing cancer may be. There is no qualitative or quantitative data establishing a relationship between quinoneimine-protein binding and metaplasia. The registrant has acknowledged no evidence for cytotoxicity or cell death in the case of acetochlor, and I cannot accept our pathologists' claim to the contrary, i.e. that some other evidence for cytotoxicity can be discovered in the acetochlor data base that even the registrant himself to this point in time seems to lack awareness. Whose MOA is this to defend?

Now, I must acknowledge being on uncertain ground to a certain degree in this area, not because I am unable to appreciate the science, but because this has come upon us too precipitously to evaluate it in its fullest. Mine is more a protest over the lack of time to evaluate the subject than to assert certain knowledge in this area. I am a person with excellent scientific credentials and experience. I have many questions that I am being precluded from pursuing in a proper manner as toxicologist reviewer.

I believe Dr. Mary Beth Genter and colleagues should be permitted comment on our pathologists' recent comments, inasmuch as she is recognized even by the registrant himself as an expert

publishing in this very area (Exhibit XI), and has advised me that she does not accept the hypothesized MOA for chloroacetanilide induced nasal neoplasia. Enough on this subject.

2) A reproduction study on acetochlor has recently been reviewed in HED. This study disclosed a nasal tissue neoplastic response. Neoplasia has heretofore not been discovered in reproduction studies, at least as persons around HED can recall. This study reveals not only the nasal neoplastic response, but tumors were seen in rats when examined at just a little over 4 months of age. This suggests a brief order of latency of tumor expression that has not been explored to the fullest extent. Offspring may well be more susceptible as this study suggests, but the subject of offspring susceptibility has not been explored further to any point of resolution. There apparently are histopathology slides taken at PND 29 that were not examined. This study likely should have been treated as 6(a)(2). In raising these subjects at the CARC, I was advised that children's issues become moot when the "Likely To Be Carcinogenic To Humans" classification is rendered. I am not certain I agree with this. Acetochlor may now be so classified, but what about alachlor which has not been similarly evaluated in a reproduction study. A deficiency in the assessment of alachlor now becomes evident.

3) One of the central aspects in the case of the nasal tissue MOA is that the neoplasms arise exclusively in the olfactory epithelium, as this is the exclusive nasal tissue cite of metabolic activity for conversion of acetochlor to quinoneimine under the hypothesized MOA. Yet, few of the bioassays were dutiful in making a pathologic distinction as to just where the tumors occur, and there is some evidence for the occurrence of these rare tumors in the respiratory epithelium. Although, the neoplastic response certainly appear predominantly in the olfactory epithelium. In any case, studies are deficient to reach reliable conclusions that the neoplastic response is confined to the olfactory epithelium. For example, in MRID 40484801 where nasal tissues were reexamined for the acetochlor rat carcinogenicity study PR-80-006 (Doc #1) (doses 0, 500, 1500 and 5000 ppm), new sections of the nasal epithelium were prepared such that the study claims three nasal histopathology sections were examined, one each from three functional portions of the nose (squamous, respiratory and olfactory). And, yet, in reporting tumor findings they were not identified as to the specific nasal regions in which the tumors were located. Only total tumor incidence was reported. Now by the very nature and reason for the study, the reader is to assume they were predominantly olfactory, but there is that uncertainty as to their location, and whether or not for certain the neoplastic response excluded the respiratory epithelium. I might add, this study revealed no histopathologic evidence for cell death or cytotoxicity. Unrelated inflammation was reported.

4) Claims have been made that acetochlor and other members of the chloroacetanilides are non-genotoxic, while contrary claims appear in various sources in the data base. This is discussed somewhat in Exhibit IX and in other exhibits. This toxicologist would require a panel of outside experts to assess the entire mutagenicity data base for the chloroacetanilides before accepting what appears to be the official CARC position of negative genotoxicity except at cytotoxic doses. The studies must rule out site specific genotoxicity in the olfactory epithelium, for example.

Furthermore, were genotoxicity negative for acetochlor, there are other reasons under the Agency's draft 1999 Carcinogen Risk Assessment Guidelines to stick with the linear low dose extrapolation as opposed to the MOE approach to regulation (Exhibit I, p. 25; Exhibit III, p. 3).

5) Too much reliance is placed upon the absence of a nasal tissue neoplastic response in the mouse to conclude the MOA is peculiar to the rat, and thus irrelevant to man. I believe the CARC accepted that this neoplastic response is relevant to man. The mouse may be a poor model for assessing nasal tissue neoplasia (Exhibit I, pp. 9, 18-19, 21-22, 26).

6) At least where the nasal tissue neoplastic response is concerned, the three acetochlor rat studies are deficient in affording a reliable assessment in the 500 ppm dose range (½ MTD) (Exhibits I, pp. 27, 29, 32; VII, p. 4) It is important to identify as best we are able, the lowest dose exhibiting the neoplastic response, as this dose should be no lower than doses eliciting cytotoxicity and increased cell proliferation (if these can be reliably determined) under the hypothesized MOA. There is evidence in the alachlor data base that the neoplastic response goes to lower doses than increased cell proliferation [Exhibit IX, p. 6 (note a correction on p. 6: a single tumor was seen at 2.5 as opposed to the claimed 0.5 mg/kg/day dose level)].

END

Exhibit I

ACETOCHLOR MECHANISM OF NASAL TISSUE CARCINOGENICITY
Brian Dementi, Ph.D. 1/26/04

I) Introduction

Acetochlor is a member of the class of chloroacetanilide herbicides. The members of this class, which also most notably includes alachlor, have been associated with a number of neoplastic responses that have been the subject of review by HED's CARC and the FIFRA SAP. Another assessment of the carcinogenicity of acetochlor by the CARC is contemplated, which will involve consideration of the entire carcinogenicity data base. The extent to which this assessment will be confined to acetochlor is uncertain, as the data base for acetochlor is very much allied with that of the other members of the class.

The neoplastic response being focused upon in this assessment of *mechanism* of carcinogenicity of acetochlor is that of the nasal epithelium. The principal reason for setting this response apart for an assessment of mechanism is considered to reside with the fact that these neoplastic effects appear to occur at doses that possibly extend below the level of the MTD, and which cannot be discounted as occurring only at such high doses as to be irrelevant to human risk assessment.

The historical aspects of the carcinogenicity issues are presented as follows from the Acetochlor Registration Partnership's (ARP) draft July 18, 2003 submission entitled *"Acetochlor: Justification for Reclassification of Carcinogenic Potential"* a so called "white paper" submitted by ARP on July 28, 2003. **This presentation by the registrant also sets forth the registrant's objectives, and was provided at HED's request as a way of better understanding the registrant's claims and objectives .** Other documents which enhance the understanding of the history and current understanding of carcinogenicity will be employed. All quoted statements from the July 18 ARP report appearing as follows are italicized as well.

"Acetochlor was conditionally registered by the USEPA in 1994 and is currently classified by EPA as a B2 carcinogen. However, this classification was last evaluated by EPA in 1992. A substantial amount of additional information has subsequently been generated by the Acetochlor Registration Partnership (ARP) that suggests that this cancer classification is no longer appropriate. This document is intended to briefly summarize the new information and to provide the ARP's justification for requesting reclassification and a revision of the risk assessment approach."

"Numerous expert histopathological reevaluations of tissues from the previously submitted rat and mouse chronic studies support the conclusion that the only toxicologically significant oncogenic responses were nasal olfactory and thyroid follicular tumors in rats." The anticipated

cancer assessment would be expected to take these various Pathology Working Group (PWG) reports on a number of neoplastic responses under review along with such mechanistic studies as do focus upon neoplastic responses of the nasal epithelium and thyroid. *"The oncogenic modes of action for the production of these tumors has now been demonstrated in a series of mechanistic studies similar to those previously conducted for alachlor, a close structural analog with a similar toxicological profile."* The registrant claims: *"The results of these studies confirm that the oncogenic modes of action of acetochlor and alachlor are the same, and that the nasal and thyroid tumors are a result of non-genotoxic, species-specific mechanisms to which the rat is particularly sensitive."*

"Additional genotoxicity studies have now demonstrated that acetochlor does not possess significant genotoxic activity in vivo. Acetochlor is clastogenic in vitro by virtue of the sulfur-reactivity of its chloroacetyl substituent. However, no evidence of clastogenicity was observed in vivo, where normal levels of glutathione protect against this effect. In particular, the lack of any DNA damage in rat nasal epithelium, even after 18 weeks of dosing at an oncogenic dose level, provides further evidence that the in vitro clastogenicity of acetochlor is unlikely to be a significant factor in rodent oncogenicity." To the extent that genotoxicity is not operative in the nasal and thyroid neoplastic responses, renders more reasonable another mechanistic explanation.

"The thyroid follicular tumors in rats treated with acetochlor are a result of induction of hepatic UDP-glucuronyl-transferase (UDPGT), which in turn causes an increased clearance of thyroid hormone and a compensatory release of thyroid stimulating hormone (TSH) from the pituitary. Prolonged stimulation of the thyroid by TSH produces thyroid follicular hyperplasia and ultimately neoplasia. This is a well-known threshold-mediated response to which rats are highly sensitive, and is generally considered to be of little to no relevance to humans." Further along, ARP sites supporting documentation as follows: *"EPA Carcinogenicity Peer Review of Alachlor (EPA, 1997) 'Both the SAP and the CPRC agreed that the Agency requirements for demonstrating a hormonal mode of action were met by the registrant and that the tumors were observed only at an excessive dose.'"* (p. 17 of ARP report) Furthermore, ARP claims that EPA has concluded that acetochlor, along with other members of the class can be grouped together: *"EPA Common Mechanism Document for Chloroacetanilides (EPA, 2001) 'Acetochlor, alachlor and butachlor may be grouped together based on a common end-point and a known mechanism of toxicity (UDPGT induction). Data for all three chloroacetanilides exist (positive UDPGT induction, increased TSH, alterations in T3/T4 production, increased thyroid weights) to confirm that the postulated mechanism of action is indeed responsible for the effect.'"* (p. 17 of ARP report). Inspection of EPA (2001) confirms, as located on p. 26 of the EPA report, the ARP quotation. ***Although it therefore appears the Agency has reached a conclusion on this matter, EPA (2001) is claimed on its cover page to be a "preliminary draft", and thus insofar as it remains in the preliminary phase it would appear its "conclusions" are somehow tentative, and perhaps subject to further Agency review.***

"The nasal tumors in rats administered acetochlor are believed (emphasis added) *to be a result of*

metabolism of acetochlor to dialkylbenzoquinoneimine (DABQ1) metabolites by rat nasal tissue. These DABQ1 metabolites are highly reactive molecules which can bind to key cellular proteins and produce cytotoxicity, cell proliferation and ultimately neoplasia. Quinoneimines can also lead to formation of reactive oxygen species (ROS) that produce oxidative stress and activate a number of cellular signaling pathways that can lead to cancer. There is also some evidence that quinoneimines may be genotoxic in vitro. However, the lack of DNA damage in rat olfactory tissue following administration of acetochlor at oncogenic dose levels strongly supports a non-genotoxic mode of action. In any case, production of DABQ1 metabolites following administration of acetochlor is highly species-specific, and would be unlikely to occur in humans, particularly under anticipated levels of exposure." Of course, these claims on the part of ARP constitute the very substance of that which must be considered carefully by the Agency in its assessment of newly submitted materials, particularly as these pertain to the mechanism of acetochlor carcinogenicity of the nasal epithelium. It is surprising that if DABQ1 is as reactive as claimed, that it is also so devoid of genotoxic effects.

The following two paragraphs from the ARP submission, appear to constitute the essence of that which ARP would have the Agency support in terms of the carcinogenicity classification of acetochlor.

"Since the new data demonstrate that the only toxicologically significant tumors observed in chronic rodent feeding studies conducted with acetochlor are produced via highly species-specific, non-genotoxic mechanisms to which the rat is particularly sensitive, and which would be highly unlikely to occur in humans, particularly at anticipated human exposure levels, the current classification of acetochlor as a 'B2' or 'Probable Human Carcinogen' is no longer appropriate. Instead, the oncogenic potential of acetochlor would be more accurately described as 'Not Likely to Be Carcinogenic to Humans', as defined by EPA in the 2003 Draft Final Guidelines for Carcinogenic Risk Assessment. However, a more technically accurate descriptor of the oncogenic potential of acetochlor would be 'Animal Carcinogen Unlikely to be Carcinogenic to Humans', a weight-of-the-evidence descriptor recently suggested to EPA by Crop Life America (June 2, 2003, Docket OAR-2003-0008) as an alternative to the multiple descriptor 'likely at high doses, but not likely at low doses' described in the draft guidelines and currently assigned to alachlor."

"The USEPA and its Scientific Advisory Panel (SAP) have previously concluded that the mechanisms by which alachlor and acetochlor produce rat nasal and thyroid tumors are the same." Unfortunately, ARP did not cite at this point the specific references to those USEPA and SAP reports that substantiate these claims. *"They have also concluded that the potential oncogenic risks to humans following exposure to alachlor should be assessed using a non-linear, margin-of-exposure (MOE) approach. Therefore, any potential oncogenic risk to acetochlor should also be evaluated using a MOE approach. Furthermore, this approach should take into account the large species differences demonstrated,"* (pp. 6-7)

ARP says: *"Acetochlor is a close structural analogue of alachlor (see figure 1)"* (p. 8) Figure 1 presents the comparative structures for alachlor and acetochlor, which serves to illustrate the remarkable chemical organic structural similarities between the two, with but one methyl group substituent located in differing positions between the two molecules. Molecular weights are the same for both molecules, and functional groups are the same in both. There is good a priori reason based upon consideration of structural similarities bordering on being identical, to suspect that the two would behave very similarity pharmacologically and toxicologically, and as pesticides. This certainly favors a common mechanism classification.

"The toxicology profiles of the two chemicals are quite similar and the EPA has concluded that both alachlor and acetochlor share a common mechanism of toxicity for rat nasal and thyroid tumors (EPA, 2001)." Now, as indicated previously, while EPA (2001) does make this claim, EPA (2001) is but a "preliminary draft", that evidently was never finalized. Given that circumstance, it would appear its assertions are perhaps tentative, requiring further consideration and/or affirmation by the Agency.

"However, thus, much of the data developed for alachlor are also relevant for acetochlor and will be included within this document." Given the structural similarities between alachlor and acetochlor, it would certainly appear entirely reasonable to employ alachlor findings in concert with acetochlor findings toward the mutual understanding of the toxicology of both substances.

"Alachlor was classified by the EPA as a B2 carcinogen in 1986. However, following submission of a substantial amount of new mechanistic information, and following review by the Scientific Advisory Panel (SAP, 1996), the Agency reclassified alachlor as 'likely to be a human carcinogen at high doses, but not likely at low doses' (EPA, 1997) Furthermore, both the EPA Carcinogenicity Peer Review Committee (CPRC) and the SAP concluded that a non-linear margin of exposure (MOE) approach should be used when assessing potential oncogenic risks of alachlor to humans (EPA, 1997)." (p. 8) Conclusions such as these as rendered by EPA and SAP concerning the carcinogenicity of alachlor certainly have value in rendering decisions concerning acetochlor, for to the extent the Agency has recognized mechanistic studies on alachlor in addressing carcinogenicity, the way is made easier in the consideration of like studies on acetochlor. **A very important question is one of the level of certitude there is regarding EPA's assessment of the carcinogenicity, particularly nasal epithelium carcinogenicity, of alachlor. What is the full justification for an MOE approach? There needs to be a summary statement embracing all rationale/arguments in defense of an MOE approach. For if the assessment of alachlor is not as settled as ARP appears to be claiming, the way may be more difficult for acetochlor, wherein, in effect, both agents remain under review.**

At this point in the ARP (2003) report, the registrant reviews the overall *"Evidence of Carcinogenicity in Animals"* (pp. 8-10). The registrant cites the five (three rat, two mouse) carcinogenicity studies on acetochlor that constitute the basic evidence for carcinogenicity. It is

not the purpose of this assessment which focuses principally upon the mechanism of nasal tissue carcinogenicity, to review the ARP's discussion and interpretation of the several other neoplastic findings observed in these bioassays. Suffice it to say, apart from the findings of nasal tissue carcinogenicity in the rat, there are some nine additional neoplastic end points under review, namely rat (thyroid, liver, femur and stomach) and mouse (kidney, liver, lung, histiocytic sarcoma and ovary). Concerning all of these various findings, the basic persuasion of the registrant that EPA's CARC will be considering is summarized in the ARP report as follows: *"The only potentially toxicologically significant oncogenic response in the chronic rat and mouse studies with acetochlor were increased incidences of rat nasal olfactory tumors and rat thyroid follicular tumors. The other tumors cited by the EPA were either not considered to be treatment-related and/or were observed only at dose levels that greatly exceeded the MTD and thus not considered relevant for human risk assessment."* (p. 10). Now, the several pages that follow in the ARP report are devoted to the presentation of the registrant's views in search of the Agency's endorsement of findings that for various reasons, none of these findings pose any concern for the public health for the chemical as used. Much of the support for the registrant's case resides with the results of several Pathology Working Group (PWG) reports that have been produced since the Agency last considered acetochlor. The EPA cancer committee will be expected to render decisions on all of these arguments in addressing the carcinogenicity of acetochlor.

II) ARP Background Supporting Documentary

In our "Introduction", the registrant's position was presented as obtained largely from the ARP submission: *"Acetochlor: Justification for Reclassification of Carcinogenic Potential"* presented in a July 18, 2003 draft form as submitted informally to the Agency at a July 28, 2003 ARP presentation to HED. This submission by the registrant was provided in response to a recent HED request for a contemporary statement or summary of the registrant's position. This report itself asserts that: *"This document is intended as a supplement to, not a replacement for, a previous ARP document assessing the oncogenic potential of acetochlor (Clapp et al., 1998, MRID 44496201)"* (p. 8)

Accordingly, both of the ARP submissions, concertedly, provide the most contemporary perspectives of the ARP on the carcinogenicity of acetochlor, and are thus appended to this review. Both have received at least some level of HED written comment explained as follows..

The Email of Dr. Brian Dementi to ARP's Dr. Jerol Kronenberg, dated May 28, 2003, offers comment and raises questions in reference to Clapp *et al.* (1998). Dr. Kronenberg responded to these comments via his October 20, 2003 Email to Dr. Dementi. Perhaps the lag between the May 28 Email of Dr. Dementi and the October 20 Email of Dr. Kronenberg is explained in Dr. Kronenberg's Email of October 8, 2003 to Dr. Dementi.

Dr. Dementi further prepared commentary (October 24, 2003) on the ARP's July 18 draft on *"Acetochlor: Justification for Reclassification of Carcinogenic Potential"*, which is also a matter

of the record. It is to be noted that the July 18 ARP draft report was followed by a final report dated September 5, received by HED staff October 10, well after Dr. Dementi's October 24 comments on the July 18 draft were essentially complete. A cursory comparison between the draft July 18 and final September 5 reports reveals good concordance, such that with possibly a few exceptions the October 24 comments are applicable, page by page, to both the July 18 and September 5 ARP versions. ***There is an inherent problem wherein attempts are made to accommodate a dynamic situation where information is in flux, where informal draft submissions of reports are being addressed, though not having first been through the Agency's tracking system and provided to reviewers accompanied by bean sheets, and attendant projected dead lines for review, proper sign off and turn around. And where the matter is complicated by very complex scientific issues and a data base that has been accumulating for some time now, perhaps back to 1992 in this case.***

In any case, all of the above documents have been retained and are available as supporting materials for this mechanisms background paper. Namely, Clapp *et al.* (1998); Dementi (May 28, 2003); Kronenberg (October 8 and October 20, 2003); ARP (July 18, 2003 and September 5, 2003); Dementi (October 24, 2003); November 17, 2003 memorandum of B. Dementi to J. Jones; plus several others. All of these documents (and their background citations) are very relevant to the consideration of the carcinogenicity of acetochlor, and alachlor, with which the nasal carcinogenicity mechanism of acetochlor is inextricably woven.

III) Review of Mechanisms Study Submissions

This review will now focus upon presentation of the findings of a series of seven (7) mechanistic studies on the biological effects of acetochlor, principally as these pertain to neoplasia of the nasal olfactory epithelium. Several of these studies are of the same type as those previously conducted and reviewed by the Agency on alachlor. The alachlor studies have served in the assessment of the carcinogenicity of alachlor, and the findings and acceptability of those studies performed on a chemical of such like structure certainly should facilitate understanding and interpretation of the studies on acetochlor. Thus, the conclusions rendered by the Agency in its consideration of the mechanism of nasal tissue carcinogenicity of alachlor, sets the stage for the interpretation of similar studies on acetochlor. ***The level of certitude as to the mechanism of action of alachlor is of the utmost importance as fundamental to an interpretation of the mechanism of action of acetochlor.***

The mechanistic studies on acetochlor submitted by the registrant are identified, and the results summarized as follows via incorporation of the "Purpose of Study" and "Executive Summary" sections precisely as they appear in the respective HED Data Evaluation Records. It should be noted that all of these mechanistic studies are Nonguideline, and thus have no set of Agency criteria against which to judge the conduct, nor study specific criteria to evaluate, in determining their usefulness and acceptability. Judgements as to their acceptability and usefulness are thus very

subjective. Similar studies previously conducted on alachlor establish a precedence, or set the stage upon which to judge the studies on acetochlor. Much of what the Agency has concluded in terms of proofs of mechanism of action of alachlor have their direct application to the interpretation and acceptability of the studies on acetochlor. In other words, any particular rationale found acceptable by the Agency for alachlor, to be consistent, should be found acceptable for acetochlor when the same studies on the two compounds show the same things. Thus it is very important that prior conclusions for alachlor be definitive. The assumption being made here is that Agency decisions on the alachlor mechanistic studies have been properly reviewed/evaluated, and have application in the interpretation and understanding of the acetochlor studies.

1) MRID 44496210: Lau, H.H.S., et al (1998) Characterization of acetochlor binding to rat nasal tissue binding. 1/24/98

"PURPOSE OF STUDY: 'To determine and characterize the nasal location of ^{14}C-acetochlor in Sprague-Dawley Rats following dietary administration of acetochlor.' (p. 6)

"EXECUTIVE SUMMARY: In a protein binding study (MRID 44496210), as explained by the investigators (p. 13 of the Study Report), acetochlor (95.2% a.i.) was administered to male Sprague Dawley rats in the diet at concentrations of 1710 ppm and 5170 ppm for 14 days to determine and characterize the nasal localization of ^{14}C-acetochlor. The results in this study address the potential binding of the putative metabolic adduct of acetochlor, 3-ethyl, 5-methylbenzoquinone-4-imine (EMIQ) to rat nasal proteins. The binding of acetochlor adducts to rat nasal turbinates was determined by an acid hydrolysis technique followed by HPLC analysis. HPLC analysis of the protein hydrolysate from both groups of animals showed a significant and dose-dependent formation of the 3-ethyl, 5-methyl-benzoquinoneimine-cysteine (EMIQ-cysteine) adduct in vivo. The average level of the EMIQ-cysteine adduct in rat nasal turbinates from rats fed 1710 and 5170 ppm was 119 pmole/mg protein and 206 pmole/mg protein, respectively. In addition to EMIQ binding, direct binding of acetochlor to nasal tissues was identified by the investigators." As a point of clarification, this non-EMIQ protein binding upon hydrolysis yielded EMA standard. A non-enzymatic direct displacement of acetochlor by the sulfhydryl group of cysteine in nasal proteins is viewed as one such pathway for this binding. For comparative purposes, the levels of EMA identified following the hydrolysis procedure were 440 and 1060 pmole/mg protein at the respective doses of 1710 and 5170 ppm. So, quantitatively, binding to nasal proteins was evidently more extensive via the latter pathway than via the EMIQ-cysteine pathway. The study also notes that when EMIQ is synthesized and allowed to react with bovine albumin, adducts in addition to EMIQ-cysteine are observed."

[Note: this study did not differentiate proportions of olfactory versus respiratory epithelia collected in the assessment of level of EMIQ binding, and therefore is difficult to compare with such assessments of binding in the mouse (MRID 44496211) where the distinction was also not made.] (note added)

"The results of the whole body autoradiography showed significant localization of radioactivity in nasal turbinates at both dose levels. Microautoradiography studies showed intense localization of radioactivity within the Bowman's glands in high dose rats. There was a lower degree of localization of radioactivity in the olfactory surface epithelium and no evidence of localization within the respiratory epithelium. In low-dose rats, only slight to moderate localization of radioactivity was found in the Bowman's glands. [Note: it is important to note here that it be certain that localization was not observed in the respiratory epithelium, for the finding is not consistent with the location of tumors in the case of alachlor carcinogenicity testing, where the tumors were evidently observed in the respiratory epithelium. See CARC report (EPA, 1997; p. 2). If it turns out that the CARC report mis-identified the tissue as respiratory where it should have been olfactory, then this parenthetical statement should be interpreted as affirming the effect as consistent with the olfactory site localization of carcinogenicity in the alachlor carcinogenicity bioassay.] (note added)

"This protein-binding characterization study is **Unacceptable/Nonguideline**. This study may be upgraded to Acceptable/Nonguideline if the following data/information are submitted and are deemed to be satisfactory by the Agency: 1) the investigators did not present the rationale in support of the structure of the synthesized EMIQ-cysteine marker (p. 25). The investigators should submit such rationale; 2) the investigators discussed the binding of EMIQ to nasal proteins in the context of a mechanism of action for acetochlor. In addition to EMIQ binding, the investigators identified binding of acetochlor to rat nasal proteins after "non-enzymatic direct chlorine displacement of acetochlor by the sulfhydryl group of cysteine in rat nasal proteins" (p. 27). However, the significance of these adducts is not included in the context of discussion of mechanism of action. The investigators should discuss this since this interaction with proteins may also underlie or contribute to the mechanism(s) of carcinogenicity, particularly since according to Table 1 (p. 32) this form of binding to nasal protein is more extensive in terms of pmole/mg protein (as assessed by EMA release) than is EMIQ binding; 3) a clear statement describing the methodology employed in quantifying the concentration (pmole/mg protein) of EMIQ-cysteine and EMA in nasal protein hydrolysates, as reported, for example, in Table 2 of the Results section in this review; 4) citations to specific reference materials identified as needed in various sections of this review." (pp. 2-3)

This study confirms that when acetochlor is administered to the rat, binding occurs to nasal protein in the form of a quinoneimine (EMIQ) adduct, in a manner analogous to a quinoneimine (DEIQ) adduct formed in the case of alachlor. However, as stated previously, this aspect of this study makes no clear distinction as to whether this effect was observed in respiratory and/or olfactory epithelia. It does appear that in the microautoradiography study component, binding was observed only in Bowman's Gland and the olfactory epithelium. There was also the finding of non-enzymatic direct chlorine displacement protein binding, as is true in the case of alachlor. One might pose the question as to why this form of binding would not be expected to have a role in cytotoxicity? Thus acetochlor emulates alachlor in the binding mechanisms with nasal tissues.

This helps establish at a fundamental molecular level, equivalent responses between the two compounds, not unexpected given their remarkable structural similarities.

Commentary:

In response to the request for additional information to upgrade this and others of the mechnistic studies, the registrant has responded informally: "Responses to Questions Raised in EPA Reviews (DERs) of Several Acetochlor Mechanistic Studies", July 25, 2003, herein after referred to as ARP (2003). The formal submission, the text of which is the same, was received by this reviewer October 30, 2003.

Concerning DER question 1 "Investigators did not present the rationale in support of the structure of the synthesized EMIQ-marker." ARP (2003) responds by saying "The N-acetylcysteine-EMIQ adduct was formed using methodology developed and validated for the structurally related cysteine adduct DEIQ formed from alachlor (Monsanto ML-94-161, MRID 43641604)." (p. 1) The authors summarize the testing procedure used for alachlor, and then attempt to persuade that: "The experience with formation and confirmation of the DEIQ-cysteine adduct, were considered sufficient for confirmation of the identity and characterization of the EMIQ-cysteine and its use as a standard for the in vivo studies." Given the remarkable structural similarity between alachlor and acetochlor, to the extent the Agency has previously accepted the rationale for alachlor, the ARP response is reasonably applicable to answer the question on acetochlor, though the substance of the "proof" resides with alachlor, and but by analogy it would seem to acetochlor.

Concerning DER question 2: the registrant was requested to discuss the significance of adducts formed by non-enzymatic direct chlorine displacement in induction of nasal tumors by acetochlor. ARP (2003) responds: "HPLC analysis of the protein adducts from rat and mouse nasal tissues shows the presence of 14C-EMA. This is *most likely* (emphasis added due to lack of certitude) the result of the non-enzymatic direct chlorine displacement of acetochlor by the sulfhydryl group of cysteine (on proteins). However, the EMA-protein adduct does not result in tumor formation." This is shown clearly in the mouse, where administration of acetochlor resulted in an EMA-protein adduct but no nasal tumors." ***(Though acetochlor is evidently not carcinogenic in the mouse, it doesn't necessary follow that if an EMA-protein is not associated with a neoplastic response in the mouse, that it would not in the rat or other species. The registrant's statement is tantamount to saying if the EMA-protein adduct is associated with carcinogenicity, it must be so in all species. Hence, the registrant's perspective does not discount a concern that non-enzymatic direct chlorine displacement could play a role in the nasal tumorigenic response in the rat.)***

The registrant then again analogizes to alachlor: "A similar situation was previously demonstrated for alachlor, where there was no evidence of the DEIQ adduct, whilst the DEA peak was predominant. As with acetochlor, alachlor does not produce nasal tumors in the mouse."

"These data collectively support the view that the non-specific binding resulting via direct chlorine displacement does not have a causative role in the observed rat nasal tumors with acetochlor" (p. 2) So as before, the acceptability of this aspect of the registrant's expressed views concerning the carcinogenicity of acetochlor reside with the Agency's prior position on alachlor, which is the more fundamental assessment. Acetochlor is simply behaving in like manner with alachlor, as one would expect from its great structural similarity to alachlor. This study discloses no contrasts with those on alachlor.

Concerning DER question 3: the registrant is requested to provide a clear statement describing the methodology employed in quantifying the concentration (pmole/mg protein) of EMIQ-cysteine and EMA in nasal protein hydrolysates. The registrant again responds by saying: "The methodology for quantifying the concentration of EMIQ-cysteine and EMA in nasal protein hydrolysates involved preparation of nasal tissue homogenates, precipitation of cellular proteins and acid hydrolysis of the nasal protein samples. The acid hydrolysis procedure was analogous to that used for the preparation of authentic EMA, EMA-phenol and EMIQ-cysteine authentic samples. Nasal protein hydrolysates were then analyzed by HPLC. The procedures used for nasal tissue analysis were similar to those used in previous studies with alachlor (Haydens, et al., 1999)." (p. 2) So here again, the approach taken was modeled after that for alachlor, and to the extent the Agency has accepted alachlor, it would not be unreasonable to accept similar rationale for acetochlor.

Concerning DER question 4: The registrant was requested to provide references in support of certain claims made in the study, principally in reference to alachlor. The registrant has responded with many references. So many references portend much background reading. The question is one of whether the Agency in its consideration of alachlor is already satisfied with the documentation that supports conclusions regarding the mechanism of action of alachlor? If so, mechanistic studies with acetochlor need only yield the same results to establish a likeness with alachlor.

2) MRID 44496211: Lau, H.H.S. and Wilson, A.G.E. (1998) Characterization of acetochlor binding to mouse nasal tissue. 1/8/98

"**PURPOSE OF STUDY:** To determine and characterize the binding of radioactivity to CD-1 mouse nasal tissue following dietary administration of ^{14}C-acetochlor" (p. 6 of Study Report)

"EXECUTIVE SUMMARY: In a protein binding study (MRID 44496211), as explained by the investigators (p. 13 of the Study Report), acetochlor (95.2%) was administered in the diet of female CD-1 mice at concentrations of 1800 and 4750 ppm to determine and characterize the nasal protein binding of ^{14}C-acetochlor. Acetochlor binding to mouse nasal turbinates was determined by acid hydrolysis followed by HPLC analysis. HPLC profiles of the protein hydrolysate from both treatment groups showed no significant formation of the 3-ethyl, 5-methyl-benzoquinoneimine-cysteine (EMIQ-cysteine) adduct in vivo. For both treatment groups, significant amounts of

radioactive components were consistent with the 2-ethyl-6-methylaniline (EMA) standard. The concentration of EMIQ-derived cysteine adducts was below the limit of detection. The only detectable protein adduct formed between acetochlor and mouse nasal protein likely resulted from the chlorine displacement of acetochlor by the sulfhydryls of mouse nasal proteins. For whatever reason, on exposure to acetochlor via the diet, mouse (unlike rat) nasal mucosa does not yield the EMIQ-protein adduct, which may support the hypothesis that mouse nasal tissue lacks the capacity to metabolize acetochlor to the putative reactive metabolite, EMIQ. To the extent the formation of EMIQ in vivo, and its subsequent binding to nasal tissue protein, is critical as believed for the induction of nasal tumors by acetochlor, failure to detect EMIQ-cysteine adducts in the mouse after dietary exposure supports the hypothesis that the carcinogenic mechanism for acetochlor is not operative in the mouse, as contrasted with the rat. (p. 13 of Study Report) **[Note: as noted at this point under this section of the rat study MRID 44496210 given above, in neither the rat or mouse mechanism studies was there a differentiation made between olfactory and respirator nasal tissue binding by EMIQ.] (note added)**

"This study on the characterization of acetochlor protein binding in the mouse is **Unacceptable/Nonguideline**. This study may be upgraded to Acceptable/Nonguideline if the following data/information are submitted and are deemed to be satisfactory by the Agency: 1) the characterization of the structure of the EMIQ-cysteine marker as requested for the rat study (MRID 44496210), 2) a reference, preferably the best reference, wherein the rationale for the hypothesis that the formation of EMIQ is critical to the induction of nasal tumors by acetochlor, 3) comment on the question as to whether the EMA pathway of direct protein binding by chlorine displacement of acetochlor that occurred in this study would be associated with nasal cytotoxicity in the mouse."(pp. 2-3)

Commentary

Following are ARP (2003) responses to issues raised in the DER.

Concerning DER question 1: The characterization of the structure of the EMIQ-cysteine marker was addressed in their response to MRID 44496210. See commentary concerning DER question 1 for MRID 44496210, above.

Concerning DER question 2: The registrant lists some five reports that support the hypothesis that the formation of EMIQ is critical to the induction of nasal tumors by acetochlor. These publications and reports should prove beneficial in presenting the basic rationale in support of the hypothesis. Again, much of the fundamental pioneering evidence for the hypothesis resides with work on alachlor. Among these, it would be desirable to identify the article that is most comprehensive in presenting the hypothesis and the evidence in support of it.

Concerning DER question 3: "ARP (2003) says: No evidence of cytotoxicity or cell proliferation

was observed in mice administered acetochlor at dietary concentrations of 1000 and 5000 ppm." "These dose levels were equivalent to those used in the chronic bioassay, and higher than those that produced cell proliferation in the rat. ***The finding of only the EMA adduct and lack of cell proliferation clearly demonstrate the lack of association of this adduct with cytotoxicity, cell proliferation and nasal tumor formation*** (emphasis added). A similar observation was found in the monkey with alachlor (i.e. no DEIQ adduct, no nasal localization and no cell proliferation)." "Collectively, these observations support the critical importance of the EMIQ-cysteine adduct to nasal cytotoxicity/proliferation and tumor formation and lack of involvement of binding by direct chlorine displacement." (p. 6) (Note, the finding of EMA adduct in the mouse would be expected to yield microautoradiographic evidence of localization of acetochlor in the nasal epithelium of the mouse, but was evidently not studied in the case of acetochlor. When queried about this, the registrant advised that the data were in MRID 42852103. In this MRID, *alachlor* autoradiography evidently indicated preferential localization of radiolabel in the nasal epithelium of rat as opposed to mouse. This MRID needs to be examined more closely.)

Cytotoxicity would appear to be an important precursor event in this important mechanism of carcinogenicity. It is therefore of the utmost importance to have a clear characterization of the cytotoxicity, and in what chronic bioassays or other assays in which it must be present in the rat on the variety of chloroacetanilides that presumably share in common this mechanism of carcinogenicity. Does the cytotoxicity extend to lower doses than those exhibiting the neoplastic effect? If so, cytotoxicity could be classed as a "key event" under the draft 2003 Carcinogen Risk Assessment Guidelines, and quite likely employed in concert with tumor data in the dose response assessment as these guidelines prescribe. Is there any evidence of nasal cytotoxicity observed in offspring in the acetochlor Reproduction Study, in which a nasal tissue neoplastic response was observed?

3) MRID 44496212: Lau, H.H.S., et al (1998) Characterization of Acetochlor Secondary Sulfide Tissue Binding - Rat. 2/7/98

"PURPOSE OF STUDY: The stated purpose of the study was "To determine and characterize the nasal localization of ^{14}C-acetochlor secondary sulfide in Sprague-Dawley Rats following oral administration." (p. 6). Also, the study has as its purpose to determine if a similar metabolic pathway exists in rat nasal tissue to metabolize acetochlor secondary sulfide to EMIQ. (p. 27)

"EXECUTIVE SUMMARY: In a protein binding study (MRID 44496212), as explained by the investigators (p. 14 of the Study Report), acetochlor secondary sulfide (>99% radiochemical purity) was administered to male Sprague Dawley rats to determine and characterize the nasal localization of ^{14}C-acetochlor secondary sulfide. Four males were used in group M1, 2 males in group M2, 3 males in group M3, and 2 males were used in group M4. Groups M1, M2 and M3 were given five consecutive daily doses of approximately 7 mg/kg body weight and M4 group was given a single oral dose. In groups M1 and M2, half the animals were sacrificed one day after the

final dose, and the other half were sacrificed five days after the final dose. In groups M3 and M4, all animals were sacrificed one day after the final dose.

"The binding of acetochlor secondary sulfide to rat nasal turbinates was determined by an acid hydrolysis technique followed by HPLC analysis. HPLC analysis of the protein hydrolysate from treated animals showed the formation of a cysteine conjugate derived from 3-ethyl, 5-methyl-benzoquinoneimine (EMIQ-cysteine). The average level of this EMIQ-cysteine adduct was 19.3 pmole/mg protein following oral administration of acetochlor secondary sulfide for 5 days at approximately 7 mg/kg/day. The results of the whole body autoradiography showed significant localization of radioactivity in the nasal turbinates. Microautoradiography studies showed intense localization of radioactivity in the Bowman's glands of treated animals.

"In summary, the study supported the hypothesis that rat nasal tissue is capable of metabolizing acetochlor secondary sulfide to EMIQ." [P. 14 of Study Report] It is noteworthy that Green et al (2000) [Green, T. et al. "Acetochlor-induced Rat Nasal Tumors: Further Studies on the Mode of Action and Relevance to Humans, *Reg. Toxicol. Pharmacol*., 32, 127-133, 2000], indicate that the nasal metabolism of acetochlor proceeds through a secondary sulfide, with subsequent branching through either EMA or sulfoxide pathways, both of which result in protein adducts via a reactive intermediate quinoneimine. The final hydrolysis step in the analytical procedure to assay for protein binding yields the same final quinoneimine protein metabolite in both pathways, i.e. the EMIQ and other quinoneimine pathway analytical end products are the same.

"Principally, through the use of the acetochlor secondary sulfide, which precludes the direct binding to nasal tissue proteins via chlorine displacement that occurs with acetochlor in addition to the EMIQ, or other quinone-imine pathway (per Green et al), this study nonetheless demonstrated the presence of a quinoneimine protein adduct in rat nasal tissue following administration of acetochlor secondary sulfide. This study thus serves as further support for a quinoneimine protein binding mechanistic explanation for acetochlor induced nasal toxicity.

"This study on secondary sulfide binding is **Unacceptable/Nonguideline**. This study may be upgraded to Acceptable/Nonguideline if the following data are submitted and are deemed to be satisfactory by the Agency: 1) a reference, preferably the best reference, wherein the rationale may be found for the hypothesis that the formation of EMIQ is critical to the induction of nasal tumors by acetochlor; 2) a reference to the chronic/carcinogenicity study upon which dose selection was based; 3) if possible, some indication of the comparative magnitude of localization of radioactivity in nasal tissue, versus those of liver, kidney, lining of tongue, for example; 4) any information that may be available on the nasal tissue cytotoxicity and carcinogenicity of acetochlor secondary sulfide; 5) any available information on the question of acetochlor metabolic conversion to EMIQ in the liver." (pp. 1-2)

Commentary

ARP (2003) has responded to this DER as follows

Concerning DER question 1: The registrant refers to his response to the same question posed in DER 44496211, wherein he lists some five reports that support the hypothesis that the formation of EMIQ is critical to the induction of nasal tumors by acetochlor. These publications and reports should prove beneficial in presenting the basic rationale in support of the hypothesis. Again, much of the fundamental pioneering evidence for the hypothesis resides with work on alachlor. Among these, it would be desirable to identify the article that is most comprehensive in presenting the hypothesis and the evidence in support of it.

Concerning DER question 2: The registrant advises that dose selection for this study was intended to be comparable to that use in a similar study with alachlor methyl sulfide, and were considered to be "representative of the maximum amounts of secondary sulfide that would be present at the oncogenic LOEL." (p. 6)

Concerning DER question 3: The registrant responded by saying that the highest level (presumably the most dense) localization of radioactivity among the various tissues named, occurs in nasal tissues. And that the comparison among tissue types can only be spoken of in qualitative terms. The registrant says that the protein binding studies provide more quantitative species comparisons.

However it should be noted that there is no quantitative comparison between the nasal and other tissues, while the quantitative comparison spoken of by the registrant is with respect to species differences in nasal tissue binding, specifically. So while there is localization of radioactivity in certain tissues in addition to the those of the olfactory epithelium, knowing little more about the nature of binding in those tissues, there is the potential concern for possible carcinogenic responses in these other tissues should the mechanism remain the same as that of the nasal tissues.

Concerning DER question 4: The registrant responds by saying that: "No additional studies have been conducted with acetochlor secondary sulfide." So studies with this agent thus far do not shed further light on the cytotoxicity claimed as a precursor event in the quinoneimine mechanism of carcinogenicity.

The registrant does suggest that the secondary sulfide may be oxidized to the secondary sulfoxide in nasal tissue, localize there and elicit the carcinogenic process. So as we understand, there may be two species of quinoneimine derive metabolically from acetochlor, that bind nasal tissue proteins.

Concerning DER question 5: The registrant responds as follows: "Two studies have been conducted evaluating the *in vitro* p-hydroxylation activity of acetochlor metabolites in the liver. These studies demonstrated that the formation of EMIQ in rat liver is much lower than that in rat olfactory tissue (MRID 44530002; 44530001). Similar data have been generated with alachlor." So, though EMIQ does form in rat liver, as might be expected in this highly metabolic tissue, we

understand that the concentration there, unlike in nasal tissues, is not sufficient to elicit a carcinogenic effect.

4) MRID 44496213: Lau, H.H.S. and Wilson, A.G.E. (1998) Characterization of acetochlor binding to Rhesus monkey nasal tissue. 2/8/98

PURPOSE OF STUDY: "To determine and characterize the binding of radioactivity to Rhesus monkey nasal turbinate proteins following oral administration of ^{14}C-acetochlor." (P. 6 of Study Report)

EXECUTIVE SUMMARY: In a 14-day oral toxicity study (MRID 44496213), as explained by the investigators (pp. 9, 13 of the Study Report), acetochlor (95.2%) was administered to 3 male Rhesus monkeys by gavage to determine and characterize the nasal localization of ^{14}C-acetochlor. The monkeys were administered ^{14}C-acetochlor at a dose level of 126 mg/kg body weight for 14 days. The results in this study address the potential binding of the putative metabolite of acetochlor, 3-ethyl, 5-methylbenzoquinone-4-imine (EMIQ), to monkey nasal tissue proteins. The binding of acetochlor to monkey nasal turbinates was determined by an acid hydrolysis technique followed by HPLC analysis. HPLC analysis of the protein hydrolysate showed no significant formation of the 3-ethyl, 5-methyl-benzoquinoneimine-cysteine (EMIQ-cysteine) adduct *in vivo*. The lack of detection of EMIQ-cysteine adducts in the monkey after oral dosing of acetochlor, according to the investigators, supports the hypothesis that the carcinogenic mechanism for acetochlor is species specific and among species tested appears to be restricted to the rat.

This Non-guideline oral toxicity study on nasal cell adduct formation is **Acceptable/Nonguideline** and contributes toward satisfying the intent of the study.

5) MRID 44496207: Hotz, H.J. and Wilson, A.G.E. (1996) A study of the effects of acetochlor on rat nasal cell proliferation. 3/4/96

PURPOSE OF STUDY: To assess the effects of subchronic dietary administration of acetochlor on rat nasal cell proliferation. (p. 6)

EXECUTIVE SUMMARY: In a nasal cell proliferation study (MRID # 44496207), acetochlor (95.2%a.i.) was administered to male Sprague-Dawley rats in the diet at concentrations of 0, 1750, and 5000 ppm. Cell proliferation was measured after 60, 90 and 160 days of treatment in nasal turbinate respiratory and olfactory epithelium by measuring the DNA incorporation of ^{3}H-thymidine. ^{3}H-thymidine was administered IP for three consecutive days prior to sacrifice. In a separate study, nasal cell proliferation was also measured ~160 days with bromodeoxyuridine incorporation in rats receiving 0, 200, 1750 and 5000 ppm acetochlor in the diet.

Acetochlor significantly increased cell proliferation in the olfactory region of the nasal turbinates in

rats administered 5000 ppm acetochlor in the diet for 60 days. Cell proliferation was also significantly increased at 90 and 160 days in the 1750 ppm and 5000 ppm treatment groups. There were no significant increases in cell proliferation in the respiratory region at any of the time points or doses tested.

Terminal body weights of animals administered acetochlor in the diet at 5000 ppm for 60, 90 and 160 days were significantly decreased compared to control animals. A significant reduction in body weight gain was observed at the high dose level throughout the study.

This study which investigated the effects of acetochlor on nasal cell proliferation in the rat is **Acceptable/Nonguideline**.

6) MRID 44496209: Hotz, K.J. and Wilson, A.G.E. (1996) A study of the effects of acetochlor on mouse nasal cell proliferation. 12/12/96

PURPOSE OF STUDY: To assess the effect of subchronic dietary acetochlor on nasal cell (olfactory and respiratory epithelia) proliferation in CD-1 mice as measured via bromodeoxyuridine nuclear incorporation.

EXECUTIVE SUMMARY: In a non-guideline nasal olfactory and respiratory epithelial cell proliferation study (MRID 44496209), acetochlor (95.2% ai, lot/batch # T940059, MUS-9308-5458-T) was administered to 26 male CD-1 mice/dose in the diet at concentrations of 0, 1000, or 5000 ppm (equivalent to 0, 166.6, or 887.9 mg/kg bw/day) for 60 and 90 days.

None of the mice died during the study. Acetochlor had no effect on nasal cell proliferation in mice administered acetochlor in the diet at 1000 or 5000 ppm for 60 and 90 days.

This special study on nasal olfactory and respiratory epithelial cell proliferation in the mouse is **Acceptable/Non-guideline** and satisfies the intent of the study.

7) MRID 44496208: Hotz, K.J. and Wilson, A.G.E. (1996) Effects of dietary exposure of acetochlor on thyroid toxicity in male Sprague Dawley rats: time course. 2/5/96

PURPOSE OF STUDY: not in DER

EXECUTIVE SUMMARY: In a subchronic toxicity study, MRID 44496208, acetochlor (purity 95.2%, Lot No. T940059, MUS-9308-5458-T) was administered to groups of 20 male Sprague Dawley rats in the diet at concentrations of 0, 1750, and 5000 ppm (equivalent to 0, 100.6, and 280.9 mg/kg/day) for 14, 28, or 56 days. The effects on thyroid function of an earlier study (MRID 44496207) were partially reported in MRID 44496208. In that study, acetochlor (purity

95.2%) was administered to groups of 15 male Sprague Dawley rats in the diet at concentrations of 0, 200, 1750, and 5000 ppm (equivalent to 0, 10.4, 91.9, and 270.3 mg/kg/day) for a period of 90 days and to 10 male Sprague Dawley rats/dose for a period of 160 days.

In MRID 44496208, body weights, body weight gain, and food consumption were reduced compared to the control group at 5000 ppm. Food consumption was also statistically reduced at 1750 ppm during one time point (1-8 days). Absolute and/or relative liver weights were statistically increased at 1750 and 5000 ppm at all time points studied. Absolute and/or relative thyroid weights were statistically increased at 1750 and 5000 ppm at all time points excepting 56, 90 and 160 day time points at 1750 ppm and 160 days at 5000 ppm. Serum TSH was statistically increased at 5000 ppm at 14 and 28 days. T_3 levels were statistically decreased and T_4 levels statistically increased compared to the control group at 5000 ppm at 14 days only. T_4 was also statistically increased at 1750 ppm at this time period. Hepatic T_4-UDPGT activity was statistically increased at dose levels of 1750 and 5000 ppm after 14, 28, and 56 days of treatment. At 90 days, activity was affected at 5000 ppm only.

This study which investigated the effects of acetochlor treatment on thyroid function and T_4 metabolism is **Acceptable/Nonguideline.**

IV) GENERAL DISCUSSION

Acetochlor is a member of the chloroacetanilide group of herbicides. Extensive background information is on record within the Agency concerning its review of the carcinogenicity of members of this class of chemicals. It is not the purpose of this paper to provide an extensive review of the carcinogenicity assessments for all of the members of the class. Although that record may be quite extensive and certainly relevant in assessing the carcinogenicity of acetochlor as a member of the class, this paper focuses upon the carcinogenicity of acetochlor, and most specifically, carcinogenicity of the nasal olfactory epithelium. There are a number of neoplastic responses that have been identified in the acetochlor data base in addition to that of the nasal tissues. Several of these have been the subject of pathology working group (PWG) assessments and the entire carcinogenicity data base, apparently last visited in 1992, is to be considered by HED's Cancer Assessment Review Committee (CARC) in the near future. This present paper provides input on the mechanism of carcinogenicity of the nasal epithelium.

SAP/SAB Meeting of October 30, 1996

Acetochlor is being considered as a member of the class of chloroacetanilides. The mechanistic studies presented here as submitted by the registrant are analogous to such studies previously considered by the Agency on alachlor. For example, alachlor issues were considered at a joint SAP/SAB meeting in October 30, 1996, and the following are relevant excerpts from the December 6, 1996 report of that meeting.

In its first question to this SAP panel, the Agency is recorded as saying: "The proposed mode of action for nasal turbinate tumor induction is based on evidence demonstrating biotransformation of alachlor to a reactive metabolite, ***with binding of this metabolite to cellular protein, eventual cell death, and subsequent neoplasia*** (emphasis added). While rats and humans are recognized to possess the same biotransformation pathways involved in production of this metabolite of alachlor, it is also recognized that the activity of these pathways is substantially greater in the rat compared to the human, and that rats also demonstrate unique localization of this metabolite in the nasal turbinate compared to other species. Therefore, is the proposed mechanism for rat nasal tumorigenesis relevant for human cancer risk assessment?" (p. 46)

Excerpts from the SAP response read: "It is not clear why adenocarcinomas occurred rather than the usual squamous cell carcinomas occurring in this region. ***This would indicate an SAP perspective that the tumors in question are of a rare variety*** (comment added). The nasal tumors are the endpoint most appropriate for a cancer risk assessment since they occurred at doses *below the MTD* (emphasis added). ***The appropriateness of using the MOE approach is dependent on convincing data that alachlor metabolites are non genotoxic in rat nose*** (emphasis added). Numerous genotoxicity studies on alachlor itself have been conducted which indicate it is non genotoxic. The specific mechanism proposed for tumor formation involves biotransformation, translocation, and subsequent metabolic activation in situ in rat nasal tissue to a reactive metabolite. The genotoxicity of precursors to this metabolite are weakly genotoxic in bacterial mutagenesis assays. ***The strength of the evidence for the formation of very low levels of DNA adducts after alachlor administration should be commented on specifically by the EPA*** (emphasis added). DNA adducts would provide indirect support for the genotoxicity of alachlor."

HED's CARC considered this SAP report at its February 5, 1997 meeting (report dated June 27, 1997), but the report did not exactly address the question, saying only that: ".....alachlor demonstrated nasal tissue DNA binding after 24 hours. Qualitatively, a low level binding to nasal DNA was found, but could not be quantitated. A much higher level of protein binding in both liver and nasal turbinate tissue was observed. ***This suggests that metabolite(s) of alachlor bind macromolecules such as protein and DNA, and while protein binding is preferential at doses not considered excessive, both may contribute to the etiology of nasal tumors*** (emphasis added)." (p. 11) So it is not altogether clear what, if any, the role of DNA binding has in the etiology of nasal tumor induction, but even if protein binding is the more extensive, lesser levels of DNA binding could be of a character more influential, mechanistically, in carcinogenicity. ***The potential role of DNA binding, which evidently occurs, would appear not to have been addressed by CARC.***

"*The strength of evidence for these data on human metabolism of alachlor by human nasal tissue should also be addressed* (emphasis added). Interspecies differences in bioactivation of alachlor appear to be critical as biotransformation is the key step in *initiating the cytotoxicity* (emphasis added) and tumor response. Autoradiography data demonstrating localization of alachlor

metabolites only in rat nasal tissue and not mouse or monkey is suggestive of interspecies differences in formation of a reactive product that is retained by nasal tissues, providing *indirect evidence* (emphasis added) for the role of metabolic activation in the carcinogenic process. Thus, because bioactivation is thought to play a key role in the mechanism, for nasal tumor formation, the *evidence that bioactivation in humans occurs at significantly lower rates should be compelling* (emphasis added). The presence of these nasal enzymes in humans is indicative of a qualitative rather than quantitative response, suggesting that the shape of the dose response curve is very different across species rather than the mechanism for production of nasal tumors being not relevant for humans. The analogy to phenacetin is also noteworthy. Phenacetin also produces nasal tumors in rats. However, in humans it is carcinogenic to the lower urinary tract (urothelium), but only at extremely high doses (kg, total ingestion). Thus, although alachlor cannot completely be excluded from having activity in humans, it is highly likely that if it occurs at all, it would only occur at doses far in excess of exposure levels. *Therefore, an MOE approach to human risk assessment of alachlor is appropriate* (emphasis added)." (p. 47) ***So here, SAP does affirm an MOE approach for alachlor.***

"Data presented by Monsanto showed that there was a 30-fold higher metabolism of alachlor in the rat compared to the mouse. Since the rat does respond with nasal cancer and the mouse does not, this difference in metabolism is thought to be the critical mechanism. This rationale is extended to the human, where several thousand-fold lower activity in the metabolism of alachlor was found. When asked if the intermediate metabolite just beyond the most rate limiting step in the mechanism causes nasal tumors in the mouse, the registrants responded by saying that limitation in metabolism is not the only factor for lack of tumorigenic response in the mouse. *This instructs that the absence, or low level, of quinoneimine in the mouse is not sufficient rationale to explain a lack of tumorigenic response in the mouse*(comment added). *Therefore, the limitation in the metabolism in the mouse may not be the real reason for the lack of tumorigenic response in the mouse. If this is accurate, then the argument that limitation in the metabolism of alachlor in the human precludes alachlor being considered as a human carcinogen can not be supported* (emphasis added)." (pp. 47-48) This is a most notable observation by the SAP that requires an explanation. ***It appears the fundamental rationale supporting the hypothesis that the human, unlike the rat, lacks susceptibility to alachlor induced nasal neoplasia is here questionable. What did the registrant mean in saying: ".....limitation in metabolism is not the only factor for lack of tumorigenic response in the mouse."? Why didn't SAP search out from the registrant the one or more other factors evidently involved? The central element in the hypothesis offered as explaining the unique vulnerability of the rat to this neoplastic response is the metabolic conversion of alachlor to a quinoneimine, the proximate carcinogen in the olfactory epithelium. Why didn't the SAP pursue a more complete explanation before seemingly giving credence to the hypothesis? In fact, in the face of the registrant's incomplete response to SAP's question, it would appear the SAP did not accept the metabolism hypothesis as sufficient to preclude human vulnerability.***

The Agency's second question to the SAP panel concerned the mode of action for thyroid tumors.

The question posed was: "The proposed mode of action for the thyroid tumor is said to be the result of induction of hepatic glucuronyl transferase with subsequent decrease in circulating T3 and T4, a subsequent increase in TSH, and eventual hyperplastic response of the thyroid. Does the panel agree that interpretation of these data support the proposed mechanism for thyroid tumor induction?" (p. 48)

The SAP responded by saying: "The panel agrees that the interpretation of the data support a hormonally induced mechanism for the formation of thyroid tumors. This mechanism may be relevant for humans. However, since the tumors occurred only at doses in excess of the MTD, their usefulness for risk assessment is questioned." (p. 48)

SAP Meeting of March 19-20, 1997

A number of topics pertaining to Common Mechanism of Toxicity were considered at this meeting as recorded in the April 28, 1997 report of the meeting. Insofar as the meeting focused upon the chloroacetanilide group of pesticides, the Agency asserted that: “a) For the nasal tumors, a well-developed understanding of the underlying mechanism is available for one member of the class and appears to be applicable to others. For these pesticide chemicals, precursors to the putative, critical metabolite quinone imine have been identified for each chemical. b) For the thyroid tumors, a hypothetical mechanism has been developed for one chemical, linking the response to concurrent changes in microsomal enzymes that metabolize thyroid hormone. Effects on the liver for other members of the group are consistent with an influence on microsomal enzymes, suggesting a common mechanism of toxicity.” On a third subject, that of liver tumor induction, the Agency claimed no specific knowledge of a mechanism of action.

In response to these Agency findings, the SAP affirmed the Agency’s case study as “....excellent, well-presented and very appropriate.” And said: “The Scientific Advisory Panel agrees with the Agency’s conclusion that there is sufficient evidence to support the proposed groupings for nasal tumors.” It would have been helpful to this toxicologist if the SAP report had more specifically endorsed the quinoneimine hypothesis for the induction of nasal tumors, since it is more apparent that the chloroacetanilides as a group induce nasal tumors, than that the generation of quinoneimines is an essential precursor event in the mechanism of carcinogenicity.

Further, SAP says: “Regarding the thyroid tumors, even though the case study illustrated a common mechanism could be used to group certain chemicals for the development of thyroid tumors, the panel recommended that this endpoint not be used in combining margins of exposure because the toxic effects were noted a doses above the Maximum Tolerated Dose (MTD)**.** While the full range of doses employed can be used to determine common mechanisms, endpoints occurring solely at doses above the MTD should not be used in risk assessment.” In response to this latter statement, this reviewer would pose the question, what if tumors are confined to doses above the MTD, but the tumor precursors extend to lower doses, i.e. below the MTD? Agency

draft cancer guidelines would assess these as "key events" to be considered along with tumors in the assessment of the neoplastic response.

So, the 1997 SAP agreed with the Agency's groupings of chloroacetanilides for nasal and thyroid tumors.

Where the carcinogenicity of the nasal epithelium is concerned, acetochlor does not stand alone among the chloroacetanilide class in eliciting this response at doses at, and possibly below, the MTD. In fact, nasal tissue neoplasia would appear to be a concern for the class (EPA 2001). [It is noteworthy to indicate at this point that even if tumors of the olfactory epithelium are not seen below a certain dose, one must take into consideration incidences of the possible "key events" at all doses that may precede or occur concurrently with the appearance of tumors in the assessment of the carcinogenic response, as required under the Agency's draft Carcinogen Risk Assessment Guidelines. Such "key events" might be interpreted to include in this case such findings as a certain level of cytotoxicity, EMIQ-protein binding, genetic damage, preneoplastic lesions, etc. Such events may extend to lower doses than those yielding tumors, but in studies where numbers of animals in a low dose group may be too few (50-60) to be sufficiently sensitive to identify a low though nonetheless meaningful tumor incidence, such "key events" may arguably satisfy. Hence, their incidences must be appreciated in an assessment of the neoplastic dose response.

Also, there has been inadequate focus upon the assessment of historical rarity of the acetochlor induced tumors of the olfactory epithelium. Even a slight increase in a rare tumor (1% or less spontaneous incidence) may be biologically significant and may be interpreted as adequate evidence of carcinogenicity [Office of Science and Technology Policy (OSTP) (1985) (Fed. Re. Notice 50, vol. 50, p. 10418)]. Furthermore, in the case of rare tumors found at high doses, there may be inadequate group sizes at lower dose levels to identify meaningfully increased incidences of tumor expression. So, in this reviewer's perspective, it is not so simple a matter as to whether tumors occur or not only at MTD. Doses at which the precursor events occur should be included in any assessment in search of a no-effect-level for neoplasia.

In referring to the rarity of tumors of the olfactory epithelium in the rat, this reviewer is of the opinion that inadequate attention has been given the subject as an essential aspect of the interpretation of carcinogenicity at this site. ***There should be a review, a task unto itself, on the subject of historical control incidence to employ as a background document in the carcinogenicity assessment.*** It would take considerable time to properly research this subject. An example illustrative of the concern here is that of the incidence of nasal adenomas and carcinomas in the F344 rat as reported in the National Toxicology Program (NTP) 1997 data base. Taking into consideration all control groups from studies done via the oral feeding, oral gavage and inhalational routes, the control incidences of adenomas is 0/2205 (males), 1/2193 (females); and for carcinomas 0/2205 (males), 0/2193 (females). Among over 4000 F344 rats, but one nasal

adenoma and no carcinomas were recorded. A couple other publications bearing on the subject include: a) Brown, H. R. et al (1991) "Proliferative and Neoplastic Lesions in the Rodent Nasal Cavity", *Tox. Path.*, 19, 358-372; b) Feron, V. J. (1990) "Upper Respiratory Tract Tumors in Cpb:WU (Wistar Random) Rats", Env. Heath Per., 85, 305-315.

a) Brown et al say that spontaneous nasal tumors in rodents are "very rare", and in rats most often are squamous cell tumors. Adenocarcinomas of the olfactory epithelium would appear to be so rare in control rats as to be unrecorded, yet this paper does speak of adenocarcinoma of the olfactory epithelium as being chemically inducible: "Adenocarcinoma arising from the olfactory region is a somewhat controversial subject. ***Tumors in the olfactory region are typically malignant, highly pleomorphic, and exhibit both neural and epithelial features*** (emphasis added)." "This growth pattern is difficult to distinguish from malignant tumors arising from Bowman's glands. Because of the difficulty in defining the tumor cell origin and distinguishing characteristics of tumors of the olfactory region, all tumors arising from the olfactory surface epithelium are considered to be olfactory neuroblastoma regardless of the tumor morphology. Tumors which definitely arise from Bowman's glands are classified as adenocarcinomas. Bowman's gland tumors are frequently seen with chemicals causing tumors in the olfactory region. Tumors appear to arise within a background of diffuse Bowman's gland hyperplasia, hypertrophy, atypia, and necrosis." (p. 367)

Brown et al also say that "…..rats are usually more susceptible to the induction of epithelial tumors of the nasal cavity than mice." (p. 358) Now this statement is in no way peculiar to chloroacetanilides for which the differences in susceptibility between rats and mice are attributable by the registrant to a quinoneimine mechanism. Rather, for whatever reasons, according to Brown et al, ***rats are simply more susceptible to the induction of nasal tumors***. It is not particularly surprising or unusual that one specie of animal is more or less susceptible than another to induction of organ specific tumors. It is well recognized, for example, that the CD-1 mouse is much *more* susceptible than the F344 rat to liver tumor induction by a number of xenobiotics. And thus the more fundamental question is one of whether the rat or mouse is adequate surrogate for man. The answer to this question does not necessarily appear to reside with a quinoneimine mechanism, if chemicals in general elicit effects preferentially in the rat, i.e. this would suggest operation of an unknown mechanism that could be relevant to man, irrespective of the presence of an in situ generated quinoneimine as in the case of chloroacetanilides. The protein binding of quinoneimine in the rat as opposed to the mouse, may simply be co-incidental, not to be embellished by the absence of nasal olfactory epithelial tumors in the mouse.

It is also noteworthy that Brown et al also say that "…..tumors of the olfactory epithelium are almost uniformly malignant and invasive, while nonsquamous tumors of the respiratory epithelium are typically less invasive." (p. 358)

Tumor incidence data employed in this publication were for the F344 rat and the Wistar Random

rat, yet the conclusions appear to embrace the rat as a species. In the case of acetochlor, the carcinogenicity bioassays under review were performed in Sprague Dawley rats. The registrant says in Clapp et al (1998)(MRID 44496201): "Chronic dietary administration of alachlor, acetochlor and butachlor at high doses induces proliferative lesions in the posterior nasal passages of rats, but not in mice." "The alachlor study was conducted in Long Evans rats, while acetochlor and butachlor were studied in Sprague Dawley rats. All three compounds induced similar proliferative lesions [in two strains of rat (comment added)], including ciliated papillary hyperplasia, adenomas and sometimes adenocarcinomas." (p. 16) So strain of rat may not be a big factor in susceptibility of this species, but again this too needs to be examined more closely.

b) Feron, et al indicate that based upon a survey of data collected from ten 24- to 30-month toxicity/carcinogenicity studies and one 12-month study: "The incidence of nasal tumors in untreated male *controls* (emphasis added) was 1.1% (7/661), the tumors invariably being squamous cell carcinomas. *There were no nasal tumors found in untreated female controls* (emphasis added). The type of *compound-induced* (emphasis added) tumor most frequently observed was adenocarcinoma (of the olfactory epithelium)....." (p. 305) Compounds that induced adenocarcinomas included vinyl chloride, trichlorobutene and acetaldehyde. The adenocarcinomas induced by these agents had "....no obvious differences in the gross and microscopic appearance....." (p. 307). There is no mention in this article of involvement with Bowman's gland.

These two publications and the NTP data are but examples of evidence that olfactory epithelial adenocarcinoma is an extremely rare tumor type in the rat, and thus increased incidence among dosed animals need not be statistically significant to justify concluding a chemical to be carcinogenic when the tumors occur. Also, since several agents of differing organic structure induce these tumors in rats but not mice, renders less unique the same contrasting response observed for chloroacetanilides. In other words, the finding of quinoneimine-protein binding in the rat, but not in the mouse does not prove that the carcinogenic effect seen in the rat but not the mouse was due to this difference, since studies with several other agents similarly illustrate a lack of response in the mouse. Protein binding by a quinoneimine of acetochlor is an interesting finding, but may be nothing more than a correlate of the nasal tissue neoplastic response in the rat. The mouse apparently is just less responsive, for reasons inexplicable as of this time. The fact that acetochlor (or alachlor) is converted to the quinoneimine in the rat, but not in the mouse, may indeed preferentially elicit a neoplastic response in the rat, or have nothing to do with the contrasting olfactory epithelial tumorigenic responses in rat versus mouse. ***Thus, even in the event it can be demonstrated that humans do not transform acetochlor into the quinoneimine, this would not establish a lack of human vulnerability, since it has not been shown that quinoneimines, per se, are carcinogenic.***

The *proposed mechanism* of action for this neoplastic response appears to be most well developed for a principal member of the class, alachlor. So it is very important in considering the mechanistic evidence of nasal carcinogenicity of acetochlor that the mechanism of action of alachlor be

understood. Alachlor and acetochlor are remarkably similar in terms of chemical structure and properties, and have shown remarkably similar biological effects. *So much of the "proof" of mechanism of acetochlor resides with the reliability of the understanding of the mechanism of action of alachlor*. Certain of the acetochlor mechanistic studies simply track studies on alachlor for which there has been extensive review and understanding, so that to the extent that acetochlor behaves the same way in mechanistic studies, it arguably could be adopted into a mechanism of action previously understood with other compounds, such as alachlor. The real fundamental work on mechanism of carcinogenicity of the class has previously been worked out via testing of alachlor and perhaps other chloroacetanilide compounds.

Acetochlor, even prior to these immediate mechanistic studies, has been included among the group as having the common mechanism of action [e.g. EPA (2001)]. The current studies may not only enable acetochlor to be more fully "adopted" as sharing the same mechanism of carcinogenicity as alachlor, but to the extent it behaves in like manner it strengthens the arguments for the class. The studies seem to support a common mechanism of action, and as to decisions on the mechanism of carcinogenicity of the nasal passages, it would appear that one mechanism, *whatever that might be*, applies to both.

V) Summary of Findings in Acetochlor Mechanistic Studies

The seven acetochlor mechanistic studies presented here might be summarized as showing the following.

When radio labeled acetochlor is administered to rats via the diet at dosage levels 1710 ppm and 5170 ppm for 14 days (where the MTD in chronic studies is perhaps a little greater than 1000 ppm), binding of acetochlor to nasal protein sulfhydryl groups occurs as evidenced by the finding of the putative metabolic adduct (EMIQ-cysteine) upon protein hydrolysis. The level of binding was dose related, though not linearly, as evidenced by the finding of 119 and 206 pmole/mg protein EMIQ-cysteine adduct at the 1710 and 5170 ppm dose levels, respectively. No claims are made as to what level this binding must reach in tissues such as those of the olfactory epithelium in chronic bioassays in order to elicit a neoplastic response. If levels such as 119 and 206 pmole/mg protein were reached after 14 days of treatment, this adduct might well accumulate to much higher levels in chronic testing. We're aware of no data that would indicate what levels of this binding might be needed to result in a neoplastic response. So the finding of 119 and 206 pmole/mg protein, may only serve to confirm that like alachlor, an analogous adduct for acetochlor does occur in a dose related manner (though not linearly in this case) in the nasal olfactory epithelium.

An analogous study with acetochlor in the mouse and monkey, like alachlor, did not yield evidence of formation of the EMIQ-protein adduct. This would suggest that to the extent that this protein binding is essential in the nasal tissue neoplastic response (as established with alachlor), the particular metabolic process occurs in the rat but not the mouse.

When tested in the rat, acetochlor secondary sulfide was found to yield results similar to those for acetochlor, namely EMIQ-protein binding in nasal tissue, indicating that acetochlor is metabolized to the secondary sulfide via an alternative metabolic pathway, the secondary sulfide itself is then metabolized to EMIQ. Furthermore, as with acetochlor, acetochlor secondary sulfide studies reveal localization of radio label in nasal turbinates, and more specifically in Bowman's gland as with acetochlor.

Also, whole body autoradiographic studies on radio labeled acetochlor reveal localization of radio label in the nasal turbinates of the rat, but not so in the mouse (in the case of alachlor). Furthermore, microautoradiographic studies in the rat reveal dose related radio label accumulation in Bowman's gland of nasal tissues, the specific site of origin of the nasal tissue tumors, a finding not observed in analogous mouse studies. Hence, one way to view such mechanistic studies is that they indicate that mouse nasal tissue (Bowman's gland in particular) does not accumulate an EMIQ-protein adduct, or for that matter, any other metabolite of acetochlor. Now if the explanation for the radiographic findings in the rat is due to acetochlor quinoneimine binding to proteins, as part of the neoplastic process, as the registrant appears to advocate, ***it does not necessarily follow that an accumulation of acetochlor quinoneimine-protein has anything to do with explaining carcinogenicity.*** It may be an independent phenomenon. Indeed, bioaccumulation of radiolabel may simply mean that the biosynthesis of acetochlor quinoneimine and its retention bound to protein serves as a sink for acetochlor, that means little else of any consequence. Hence, the autoradiographic studies are mute with respect to establishing a quinoneimine mechanism of carcinogenicity. They only show preferential localization or accumulation of radiolabel in certain tissues. If the accumulation of quinoneimine-protein were critical to carcinogenicity, the studies should be extended for longer intervals to see if such accumulation becomes significant at lower doses. It would be interesting to examine nasal tissues from a rat chronic bioassay to see how far these levels may rise as compared with the 119 and 205 pmole/mg protein at 1710 and 5170 ppm, respectively, as reported in the Lau et al (1998) (MRID 44496210). In reference to Lau et al (1998), one might ask why testing was not done at doses up to and including the MTD (1000 ppm) in order to make the study more relevant to the acceptable dose range for chronic testing? Quinoneimine protein binding, over a longer time interval, at doses below the MTD could disclose a linear dose-response for this sort of binding within the dose range of primary interest, and thus point to an extension of the neoplastic response to lower dose levels than those in which increased tumors were actually observed. Such quinoneimine-protein binding (at least at a critical level) arguably could satisfy as a "key event", as could cytotoxicity or increased cell proliferation, in the neoplastic response as spoken of in the draft (1999 and 2003) EPA Carcinogen Risk Assessment Guidelines. Furthermore, these events could fall under one of the "Factors Supporting a Linear Approach" in the Guidelines, namely ***"Mode of action analysis does not support direct DNA effects, but the dose-response relationship is expected to be linear....."*** (p. 3-3) Generation of quinoneimine and its subsequent binding to cellular proteins could well be a linear response over an extensive dose range. Until the level of such binding that is necessary to induce cytotoxicity and increased cell proliferation is identified, an MOE cannot be

identified, regardless of the fact that tumors seen at high doses may not be observed at a lower dose level where these key events occur. This is very important for the nasal tumors, which are exceedingly rare, and where animal group sizes in carcinogenicity bioassays may be too small and lacking in sensitivity at lower doses to identify a tumor incidence of real concern to addressing human risk.

Along these lines, the July 2, 1999 draft Guidelines read as follows: ***[Note: these Guidelines say "Draft - Do Not Cite or Quote", but if the CARC is evaluating chemicals by these Guidelines, persons must be able to speak to and from these Guidelines.]*** "Cancer is a disease that develops through many cell and tissue changes over time. Traditional dose-response assessment procedures using tumor incidence as the response have seldom taken into account the effects of key events within the whole biological process, even though these events are the determinants of the overall dose-response. This has been due to lack of empirical data and understanding about these events. As more data become available and our understanding about how agents cause cancer improves, they can be use in dose-response assessment along with the traditional procedures. These guidelines encourage use of these new data as they become available to improve dose-response assessment." (p. 3-1) We have precisely that situation in the case of the acetochlor, where the mechanism focuses upon a quinoneimine, which is claimed to initiate a neoplastic response in the olfactory epithelium via binding to nasal proteins, such that a subsequent sequence of events unfolds: cytotoxicity > cell proliferation > neoplasia. Now if indeed this is a correct characterization of the neoplastic sequence of events, then according to these Guidelines, such parameters in this process as quinoneimine-protein binding, cytotoxicity, cell proliferation, are all potential "key events", which the Guidelines instruct should be employed along with tumors to assess the neoplastic response. So, to the extent that our studies rise to the occasion of pursuing mechanistic explanations of carcinogenicity, as the Guidelines encourage be done, we also incur the obligation to use the mechanistic data to more fully characterize the neoplastic response, as these Guidelines indicate. This means not only showing that a certain mechanism exists, say at high dose levels, but to determine more reliably the full dose range over which the neoplastic response occurs, and this may well extend to dose levels below those at which tumors are observed. Continuing along, the Guidelines say: "In this discussion, 'response' data include measures of key events considered integral to the carcinogenic process, in addition to tumor incidence. These responses may include changes in DNA, chromosomes, or other key macromolecules; effects on growth signal transduction, including induction of hormonal changes; or physiological or toxic effects that affect cell proliferation. Key events are precursors to cancer pathology; they may include proliferative events diagnosed as precancerous, but not pathology that is judged to be cancer. Analysis of such responses may be done along with those of tumor incidence to enhance the tumor dose-response analysis. If dose-response analysis of non tumor key events is more informative about the carcinogenic process for an agent, it is used in lieu of, or in conjunction with tumor incidence analysis for the overall dose-response assessment." (pp. 3-1 to 3-2) This is interpreted by this toxicologist to mean that if the recognized key events extend to lower doses than those at which tumors occur, the key events may serve to identify lower dose

ranges for the over-all neoplastic response than so identified by the traditional uses of tumor incidence data only.

The comparative acetochlor protein binding mechanism studies in rat and mouse appear to establish that a quinoneimine-protein adduct does occur in the rat, but this does not establish that such binding is a precursor event to nasal carcinogenicity in the rat as opposed to mouse. This binding simply may be co-incidental to, and independent of, another molecular process undergirding a carcinogenic effect in the rat. The fact that carcinogenicity of the nasal passages was not observed in the mouse may not be unexpected, irrespective of the absence of quinoneimine protein binding in the mouse, as it is recognized that generally rats are more susceptible than mice to the induction of epithelial tumors of the nasal cavity. [Brown et al (1991), Tox. Path., 19, 358-372]

Accordingly, in consideration of the various arguments, the absence of an olfactory nasal epithelial carcinogenicity finding in the mouse, accompanied by the absence of quinoneimine protein binding in mouse, monkey and man cannot be used to establish this carcinogenic effect, any more than any other in the rat, as peculiar to that species and thus irrelevant to human risk assessment. ***Lacking is the clear evidence that acetochlor quinoneimine-protein binding in the rat is an essential, obligate, event to the carcinogenicity observed.*** This has simply not been shown. All of the evidence used in support of this argument (autoradiography, microautoradiography, evidence of acetochlor quinoneimine protein binding in the rat versus other species) cannot establish that the carcinogenic effect in the rat proceeds by a quinoneimine mechanism, as opposed to these two events occurring independently of one another. Acetochlor induced cell proliferation in the rat is consistent with a neoplastic response in the rat, but bears no inextricable relationship to quinoneimine protein binding, and increased cell proliferation should not be unexpected if other mechanisms of carcinogenicity were at work. The absence of testing at doses up to and including the MTD (1000 ppm) in the protein binding, autoradiographic, and cell proliferation studies, and the design of such studies so as not to test for protein binding at lower doses after longer time intervals, obviated obtaining any findings in this dose range from these studies that could have proved helpful in understanding the dose-response for carcinogenicity in the rat in the dose range of primary interest.

Nasal cell proliferation studies in the rat show increased olfactory (as opposed to respiratory) epithelial cell proliferation after 60, 90 and 160 days treatment with 5000 ppm, and after 90 and 160 (but not 60) days treatment with 1750 ppm dietary acetochlor as evidenced by incorporation of tritiated thymidine at both doses and all time points. Incorporation of bromodeoxyuridine at 160 days in rats receiving 200, 1750 and 5000 ppm acetochlor also confirmed increased nasal olfactory cell proliferation at the top two doses. These studies thus illustrate that acetochlor elicits nasal cell proliferation, specific to the olfactory epithelium, at dose levels well above the MTD (1000 ppm). Why did testing not include the MTD? It is quite possible that proliferation would not have been observed at the MTD, the dose level accepted as positive for nasal carcinogenicity. Furthermore,

why was there no olfactory cell proliferation testing at one-half the MTD (500 ppm)? It is noteworthy that the acetochlor carcinogenicity bioassays of record lacked proper testing at 500 ppm (one-half MTD) [See Dementi (October 24, 2003) (pp. 3-4, as cited below] A positive and dose-related finding at both 500 and 1000 ppm (and possibly below) would have suggested that, in contrast to the registrant's position on carcinogenicity, proliferative effects extend to doses below the MTD. Such effects could serve as "key events" in the assessment of carcinogenicity according to EPA's draft Carcinogen Assessment Guidelines. The finding of what might have been positive data showing increased cell proliferation at doses up to and including the MTD has been effectively precluded by simply not testing in that important dose range. Such data as that which might have proved useful in the assessment of carcinogenicity within the critical dose range is not available. This study unfortunately neglects to address cell proliferation, a central element to the registrant's hypothesis of progression (cytotoxicity > proliferation > neoplasia) within the very dose range said to be of interest in the neoplastic response. So while it appears to be true that acetochlor fosters cell proliferation of the olfactory epithelium *at doses well exceeding the MTD,* the study fails to establish whether this does or does not occur, and how differentially so, at important dose levels up to and including the MTD.

It is noteworthy that evidently a steady state increased cell proliferation was reached after 60 days at 5000 ppm, as the rate was essentially the same at days 60, 90 and 160 days at this dose level, and so it may have been a useful study to detect increased cell proliferation at yet lower doses such as those of the MTD and somewhat below that level. It could be very worthwhile to repeat the study at lower, more relevant, dose levels, and after a little longer period of dosing to detect possible increased proliferation at progressively lower doses. Recall that it took longer (90 days) for proliferation to be seen at 1750 ppm than it took (60 days) at 5000 ppm.

The finding of acetochlor-induced nasal olfactory epithelial cell proliferation is consistent, to be sure, with a carcinogenic response at that site, but it adds no support for any sort of specific effect as mediated via a proposed quinoneimine mechanism, or any other mechanism. Rather, it is consistent with the observed neoplastic response that is peculiar to this portion of the nasal epithelium. ***In other words, whatever entity induces the neoplastic response, that response might well be anticipated to be characterized by increased cell proliferation in conjunction with tumor induction.***

Hence, these mechanistic studies confirm for acetochlor, as in the case of alachlor, the formation of a quinoneimine in nasal tissues that binds to sulfhydryl groups of nasal proteins, that acetochlor localizes, probably in a protein bound form, in nasal olfactory epithelium, and that acetochlor induces increased nasal cell proliferation, all of which findings are consistent with the finding of the nasal cell carcinogenicity of acetochlor. Two mechanistic studies submitted here, one in mice and the other in monkeys, fail to show any acetochlor nasal cell binding. Such contrasts between the rat on the one hand, and the mouse and monkey on the other hand, tend to support the concept that nasal cell carcinogenicity is peculiar, at least to the rat as opposed to mouse and monkey, in the limited test comparisons had.

Although these limited mechanistic studies indicate an effect peculiar to the rat, they are not sufficient in themselves to say the effects are not of concern to man. Rather, they indicated that to the extent they confirm similar studies in alachlor, and to the extent that it is established by the Agency that alachlor is not of concern, an argument exists for adopting acetochlor. ***But this needs to be examined very closely, i.e. not only how well acetochlor parallels alachlor, but whether the mechanism of nasal tumor induction in the case of alachlor can be attributed at a quinoneimine-protein complex.***

In summary, alachlor and acetochlor have demonstrated the same, or certainly very similar responses in various studies. They both yield nasal olfactory tumors in the rat at the MTD and possibly below; the nasal neoplastic response is the same, and of a rare character; in both cases, the olfactory epithelial neoplastic response is said to proceed via the progressive sequence: cytotoxicity > cell proliferation > neoplastic response; both yield quinoneimine-protein adducts in the nasal olfactory epithelium, which adducts are considered to elicit cytotoxicity; as a consequence both compounds exhibit localization in nasal tissues in autoradiographic studies, where Bowman's gland is a focal region of that localization; neither compound elicits a nasal tissue neoplastic response in the mouse; neither evidences quinoneimine-protein binding in nasal tissues of the mouse; neither compound concentrates radiolabel in respiratory epithelial tissue in autoradiographic studies; etc.etc.

Because of these analogous responses for both compounds in rat and mouse, attended by the evidence that the monkey behaves more like the mouse in certain more limited studies, fuels the registrant's notion that the nasal tissue tumorigenic response in the rat, is indeed rat specific, and that nasal carcinogenicity illustrated in the rat is not relevant to man.

As explained previously, it is not entirely clear that this particular mechanism of olfactory neoplasia as hypothesized for *alachlor* has been accepted by SAP. The question of the mechanism of nasal tissue neoplasia must be settled for the class before it can be concluded that the bioassay findings in rats are not relevant to man. In addressing this concern of mechanism of nasal tissue neoplasia and relevance to man, one cannot consider only the mechanism studies

Concerns transcribed from the October 24, 2003 comments of Dr. B. Dementi on Monsanto's July 18, 2003 draft "white paper" (final report dated September 5, 2003; MRID 46081802)

1) The mechanism, or mode of action, for nasal neoplasia is said to proceed via: quinoneimine-protein binding > cytotoxicity > cell proliferation > neoplasia. This is all very vague and ill defined for that which must be an extremely complex molecular process. In the case of acetochlor, it is still not clear as to what is meant by "cytotoxicity". The registrant has not provided a characterization of cytotoxicity despite the fact that cytotoxicity is claimed repeatedly as fundamental to the neoplastic response. Gross and/or histopathologic evidence of cytotoxicity should be provided if the claim of cytotoxicity is to have evidentiary meaning in support of the hypothesis.

2) The evidence for nasal tissue carcinogenicity of acetochlor in the rat derives from the results of three carcinogenicity studies. The registrant maintains that the finding of nasal carcinogenicity is restricted to doses at and above the MTD (said to be 1000 ppm). This reviewer does not accept the registrant's conclusion that testing was adequate at doses below the MTD, having concluded that another study is needed at proper dose levels up to and including the MTD. ***The data as it stands is notably absent an acceptable study at a dose level equivalent to one half the MTD.*** For rationale in support of this claim, see Dementi,10/24/03 as cited above, pp 4-5). Furthermore, the assessment of the existing studies does not address the significance of the rarity and unusual character of the nasal tissue neoplastic response. So, in essence, the bioassays have not been properly conducted nor evaluated in reaching a conclusion that the findings are irrelevant to human risk assessment.

3) A reproduction study on acetochlor, now under review in HED, yielded findings of nasal tumors in both the F0 andF1 generations. This study must be carefully reviewed, and included among the three rat chronic bioassays in assessing this neoplastic response in the rat. The histopathologic characterization and rarity of the neoplastic responses in offspring versus that in adult animals is needed.

4) Once the question were settled as to just what the cancer bioassays reveal concerning carcinogenicity in the rat, the question for acetochlor then becomes one of whether the neoplastic effect in the rat is even relevant to man. The mechanism studies are said to show that the carcinogenic effect in the rat is peculiar to the rat, as opposed to the mouse, monkey or human. If true, once the rat bioassays are excluded as irrelevant for human risk assessment, there are no other animal model bioassays illustrating a nasal tissue neoplastic effect, and, hence, no evidence exists for a concern to humans.

Now the mechanism studies purport to support that the nasal tissue neoplastic response in the rat proceeds via a mechanism involving metabolic conversion of acetochlor to a reactive quinoneimine (the proximate carcinogen) in sufficient quantity to elicit the neoplastic response observed at the MTD. For background information on this mechanism of action for nasal tumors, see, for example, Li et al (1992) Short Communication: Metabolism of Alachlor by Rat and Monkey Liver and Nasal Turbinate Tissue. Drug Metabolism and Disposition, 20, 616-618. It should be noted, however, that this paper in making the speculation, does not provide any proof of a causative relationship between quinoneimines and carcinogenicity in the rat. Need to site the most authoritative paper in realm of proof.

The focus upon the MTD rests with the claim that this is the only dose at which a neoplastic effect is acknowledged to occur in the case of acetochlor, based upon the three chronic rat bioassays, the reproduction study not withstanding. In reality, the mechanism studies themselves would not rule out a neoplastic response in the rat at doses below the MTD. The mechanism study reviewed above, Lau et al (1998) (MRID 44496210), assessed acetochlor nasal protein binding at 1710 and

5170 ppm, both of which doses yielded protein binding, in the dose related manner indicated, but included no testing at lower doses which might have identified a NOAEL for such binding. Hence, the mechanism studies merely show that the quinoneimine protein adduct, *believed to be critical* to the rat neoplastic response, proceeds only in the rat. The mechanism studies are of no use in defining the LOAEL/NOAEL for protein binding or in characterizing tumorigenicity in the rat, since these are but short term studies, that provide no information as to the levels to which protein bound quinoneimines may rise in the rat during chronic testing. The mechanism studies are of value only in the qualitative sense and useful perhaps to assess comparative responses among species.

5) Concerning the mechanism of carcinogenicity, there is serious question over the possibility of a genetic mechanism in addition to the quinoneimine-protein binding hypothesis.

Burman et al (2003) [Burman, D.M., et al (2003) *Antioxidant pertubations in the olfactory mucosa of alachlor-treated rats*. Biochem. Pharmacol., 66, 1707-1715], a publication recently received in HED, and not formally reviewed makes the following relevant claims: a) The basic result of new research reported in this publication, which has to do with assessments of endogenous antioxidants depletion in response to alachlor administration may best be expressed in the authors' text: "Dietary exposure to alachlor depletes olfactory mucosa antioxidants, which may contribute to DNA damage and tissue-specific tumor formation." (p. 1707) Should the mechanism of tumor formation reside with DNA damage, then this would be at variance with the quinoneimine-protein binding hypothesis as the sole mechanism undergirding the explanation of the mechanism of nasal carcinogenicity. The question of the mutagenicity of the chloroacetanilide group must be carefully considered if claims of a nongenotoxic mechanism of carcinogenicity are to be defended. ***It is not sufficient to confine the mutagenicity assessment to those studies submitted by the registrant to satisfy mutagenicity Guideline testing requirements.***

Burman et al (2003) indicate that: "The complete mechanism of alachlor-induced nasal tumor formation in rats has not been elucidated, but we have evidence that metabolic enzymes present in the olfactory mucosa, but not in the liver, bioactivate alachlor to one or more mutagenic species (7). The sites of alachlor-induced tumor formation in the olfactory mucosa correspond with the distribution of cytochrome P450 2A3 [7], suggesting a role for this enzyme in the formation of a *mutagenic/carcinogenic* (emphasis added) metabolite. The basis for the apparent resistance of mice to the development of alachlor-induced olfactory mucosal tumors [1] is also unclear." (p. 1708)

According to the contributions of this publication, it appears the mechanism of chloroacetanilide induction of nasal tumors is not settled science. This very recent publication would appear to be of value in contributing to the understanding of the mechanism of nasal carcinogenicity, and requires full review and consideration by those who desire to understand the mechanism.

Dearfield et al (1999), as cited by the registrant in ARP (2003) in reference to chloroacetanilides,

mentions a: '.... consistent pattern of mutagenic activity, probably mediated via metabolites. This mutagenic activity is a mechanistically plausible factor in the development of tumors.....'. (p. 15). If certain mutagenicity studies justified this conclusion in Dearfield et al, *specifically* what subsequent studies undercut the conclusion and why? EPA's Dr. Kerry Dearfield's most recent views should be obtained at this time, given the importance of the genotoxicity interpretation.

Then one might say there is lack of certitude that the proposed mechabism can be limited to the rat. For protection of public health, the rationale needs to be more definitive

So, while the registrant's essential thesis is that acetochlor is metabolized in the nasal olfactory epithelium to a quinoneimine, which in turn binds proteins to yield a cytotoxic effect, such that a progression ensues: cytotoxicity > cell proliferation > neoplastic response, there is no concrete linkage between quinoneimine-protein binding and cytotoxicity. Cytotoxicity in the case of acetochlor has not even been identified, let alone characterized. Nor has it been shown, nor could it be shown with the existing data, that cytotoxicity arising from quinoneimine-protein binding is an essential event in fact leading to the nasal olfactory neoplastic response. The registrant's claim of the sequence of events: cytotoxicity > cell proliferation > neoplastic response is no more than an assertion, capitalizing upon the vague concept that tumorigenic responses in general may proceed by such steps, though lacking any evidence that such steps obtain in this very neoplastic response.

If the quinoneimine-protein ultimately leads to cytotoxicity, one would need evidence from a study of longer duration than 14 days, possibly a chronic study, showing that in the course of time a buildup or accumulation of this product occurs, reaching such levels (pmole/mg protein) as are critical to elicit a cytotoxic response. This cytotoxic response should be well identified if there is to be proof of a role of cytotoxicity in the neoplastic response. Furthermore, if the progression to neoplasia as claimed by the registrant is to be shown, in the further course of time both of these expressions (quinoneimine-protein binding and cytotoxicity) would enhance in tandem and somehow be linked to an onset of increased cell proliferation and so on to a neoplastic response. These things must be shown. It is not sufficient to conclude from a 14-day study, where 119 and 206 pmole/mg protein quinoneimine-protein were observed at the 1750 and 5000 ppm dose levels, respectively, that such binding ever increases in the course of time at these doses to the point of eliciting, an as yet uncharacterized, cytotoxicity; the initial parameter in the registrant's claim of progression to a neoplastic response. There needs to be evidence of both an accumulation of this quinoneimine-protein complex in the olfactory epithelium, and an attendant cytotoxicity in the course of time, during dosing over a sub-chronic or even chronic time frame. The binding that has been shown in the 14-day study is but qualitative evidence that such binding occurs, as it has not been shown that 119 or 206 pmole/mg protein bears any relationship to cytotoxicity. One is unable even to speculate as to what levels the quinoneimine-protein must rise before evidence of attendant cytotoxicity occurs. And until this is shown, one has no way of knowing whether the quinoneimine-protein binding is but an innocuous sink for an acetochlor metabolite in olfactory tissues, or whether it indeed plays an etiologic role in the neoplastic response.

Furthermore, such evidence must be had from longer term testing at dose levels up to and including the MTD. Doses of 1750 and 5000 ppm are recognized by the registrant as excessive who claims the MTD to be 1000 ppm. This sort of evidence where it can be shown not only in short term studies that quinoneimine-protein binding occurs, but rather in longer term studies that the concentration of this complex continues to accumulate to the point of cellular detriment (cytotoxicity) of a character that can be identified (but not yet so identified) and assessed is necessary. Along these lines, recall that increased olfactory epithelial cell proliferation (MRID 44496207) may have required 60 days to be observed at 5000 ppm and 90 days to be seen at 1750 ppm. How much time might it take to achieve increased proliferation (key element in the registrant's progressive scheme, which presumably follows onset of cytotoxicity) at say 200, 500 and 1000 ppm (MTD) in order to compare with an acceptable (MTD-wise) carcinogenicity study in the rat.

To the extent that the registrant desires to discount neoplastic responses at doses exceeding the MTD, he should not rely upon short term mechanistic studies at these same excessive dose levels. Now that the binding has been shown at those high doses, mechanistic studies must be conducted within the relevant dose range (up to and including the MTD) if a mechanistic argument is to be used convincingly to conclude that these "key events" on the course to neoplasia do not occur at doses below the MTD. The mechnistic studies as they stand are mute with respect to any consideration as to whether there is a NOEL for the mechanism of carcinogenicity. Though no quinoneimine-protein binding was observed at 200 ppm, this is not to conclude that in the course of time beyond 14 days in the chronic bioassay, the quinoneimine-protein levels could not have exceeded some threshold for cytotoxicity expression.

Furthermore, from this reviewer's perspective, it cannot be concluded at this time that the neoplastic response does not occur at doses below the MTD, because testing has been inadequate at doses below the MTD, nor can it be said these rare nasal tumors would not be of concern for human risk assessment even if they were seen only at the MTD and above in the chronic bioassays, given their rarity. We are unable with the available information to postulate alternative mechanisms of nasal olfactory carcinogenicity, since this is a complex subject. There is uncertainty in the data base as to the character of acetochlor effects on the mouse nasal epithelium, and uncertainty as to a possible mutagenicity role in the mechanism. The most reasonable and conservative approach, in consideration of public health protection, is to consider the neoplastic response in the rat as surrogate for man, and as useful in risk assessment to consider the rat as the basis for addressing human risk assessment. END

Exhibit II

Brian Dementi
02/04/04 02:53 PM

To: Alberto Protzel/DC/USEPA/US@EPA, Linnea Hansen/R9/USEPA/US@EPA, Larry Chitlik/DC/USEPA/US@EPA, Nancy McCarroll/DC/USEPA/US@EPA, Susan Makris/DC/USEPA/US@EPA
cc:
Subject: Acetochlor issues of concern

Colleagues,

Having wrestled extensively with the acetochlor and alachlor carcinogenicity issues, focusing upon the mechanism, or mode, of action of carcinogenicity of the nasal olfactory epithelium, I am concerned about interpretations and the work that evidently remains to be done. So I decided to assemble these in the form of a list of concerns.

This list is not complete, and it is likely that more will follow, but I wanted to express at least these at this time.

I would be pleased to have your comments as to the validity of these concerns.

Best Wishes,
Brian Dementi

ACETOCHLOR: LIST OF CONCERNS Brian Dementi, Ph.D. 2/4/04

1) Given that the various Guideline carcinogenicity studies in the rat reveal a carcinogenic effect on the nasal tissues for both alachlor and acetochlor, demonstrated in the latter case even in offspring in the reproduction study, it would appear this effect is real and shared as a common effect of alachlor and acetochlor, and likely so for the chloroacetanilid class. The fundamental questions are whether adequate evidence has been presented to confirm as reliable the registrant's mechanistic hypothesis that serves to discount these findings as relevant to humans, and would justify employment of an MOE approach as opposed to a linear low dose extrapolation method for purposes of regulation.

The cancer bioassays are Guideline studies, have been reviewed (i.e. have their own DERs), and reveal what they reveal. There have been PWG assessments. A concern is that essentially two bodies of information exist, namely, 1) the Guideline bioassays and how these have been interpreted in the usual way within HED, and 2) the other body, i.e. the non-guideline work which the registrant would use to prove: a) that the nasal tumor findings occur *only* in the rat (via a quinoneimine protein-binding effect confined to the olfactory epithelium that in turn results in cytotoxicity leading to neoplasia within that epithelium), and therefore since the neoplastic effect is peculiar to the rat, would preclude human relevance; b) that the mechanism of the neoplastic response is non-genotoxic in nature; and c) that the cytotoxicity eliciting effect has a NOEL such that the MOE approach for carcinogenicity can be taken as opposed to linear low dose extrapolation approach.

Has the Agency already accepted this claim for alachlor, or what can be conclusively said as to the Agency's assessment of alachlor?

2) All of the work being considered in regard to *mechanism* derives from non-guideline studies, literature publications and various other reviews submitted by the registrant, such as the Acetochlor Registration Partnership's (ARP) September 5, 2003 "white paper" . There are no study protocols established by the Agency for such work, which if followed in testing and review, would be instructive in deciding whether various data should be accepted as proving a *mechanism* or *mode* of action of interest to the Agency. *How much reliance is to be placed upon non-guideline studies, journal articles, and perspectives on the subject submitted by the registrant?*

The non-guideline acetochlor mechanistic studies submitted by the registrant have been reviewed. They provide that which the registrant wishes to provide, but who is to say whether these studies incorporate assessments that a naive independent peer committee would advocate? For example, doses employed in the studies designed to prove quinoneimine-protein binding in the olfactory epithelium of the rat, well exceeded the MTD (1000 ppm) as identified in the chronic bioassays. Should testing in the range up to and including the MTD have been included? The actual design of the study thus excluded testing at doses well below the MTD, where a positive response in the lower dose range would be meaningful as a "key event" in the interpretation of carcinogenicity.

Furthermore, the quinoneimine-protein binding studies were conducted for a dosing period of but 14 days duration. The rat acetochlor nasal tissue protein binding study (MRID 44496210) did illustrate the presence of quinoneimine in the form of quinoneimine-protein binding, and that it was dose related at the two dose levels tested, i.e. 119 and 205 pmole/mg protein @ at 1710 and 5170 ppm, respectively. Yet, in not conducting the study for longer periods precluded the possible identification of a continuous time and dose dependent increase in magnitude of quinoneimine-protein binding and its accumulation to such levels that might result in a cytotoxic effect. If the quinoneimine-protein binding were assessed in a suchronic or chronic study in the dose range up to and including the MTD (1000 ppm), the finding of greatly enhanced levels of quinoneimine-protein binding, well above those observed after 14 days of dosing, accompanied by cytotoxicity/cell death

would further support the registrant's hypothesized acetochlor induced mode of action, namely, "cytotoxicity > cell proliferation > neoplasia".

Concerning publications, there are many of these that have been referenced by the registrant and identified from other sources. While these have been read by Agency personnel, many of these are quite complicated, and have received no official written reviews. Who is to defend or refute their many claims, particularly when they express contradictory perspectives. *The proper review and interpretation of this great body of information is of concern.*

Similarly, documents submitted by the registrant, most notably the recently submitted ARP September 5, 2003 "white paper" have been examined and comments rendered, *but many questions remain.*

3) The registrant's thesis is that nasal neoplasia arises from metabolic/toxic events in the olfactory as opposed to the respiratory epithelium. What is the level of certainty that tumors actually found are confined to the olfactory epithelium as the source of origin? This requires a consideration of all of the cancer bioassays for both alachlor and acetochlor, as well as perhaps others of the chloroacetanilide class. Susan Makris, who has been reviewing the acetochlor reproduction study says tumors of both epithelia are reported.

4) What is to be concluded under FQPA concerning offspring susceptibility given the finding of very rare nasal tumors among offspring in the acetochlor reproduction study, where dosing is much abbreviated compared with that in chronic bioassays? It should be noted that the registrant has provided his interpretation of the nasal tumor findings in the acetochlor reproduction study (see the ARP September 5, 2003 "white paper", Appendix 2, "Supplement to Rat reproduction Study"). The "white paper" says the tumors are of the olfactory epithelium, while not mentioning respiratory epithelial tumors as noted at the end of the preceding paragraph.

Under the circumstance that treatment results in both respiratory and olfactory epithelial tumors, there should be independent assessments of the two responses, as the olfactory and respiratory tissues are metabolically and physiologically distinct.

Once HED's review of the reproduction study is complete, the reviewer of the reproduction study, or someone else, will need to address these questions, as well as the views of the registrant as expressed in Appendix 2. *Finalizing all that pertains to the reproduction study would require time, and there needs to be a schedule for the completion of this work.*

The DER for the reproduction study should comment on issues such as: a) the early onset of these tumors, and precursor events; b) precise comparative histopathology between the nasal tumors in this reproduction study and those tumors identified in the rat chronic bioassays; c) the role of hyperplasia in the assessment; d) the implications of brown pigment identified in the "olfactory mucosa" at the top two doses among males and across all doses in females; e) the precise location

of these various neoplastic findings with respect to olfactory versus respiratory epithelia.

5) Since there has been the finding of nasal tumors in offspring in the acetochlor reproduction study, how should this influence the prior assessment of alachlor, which evidently has not been similarly evaluated for offspring nasal pathology in a reproduction study?

6) An on-going one year cancer study in the rat with the acetochlor sulfoxide metabolite is claimed to be giving the same results (neoplasia) as acetochlor itself (see ARP September 5, 2003 "white paper", Appendix 3). The report for this carcinogenicity study is affirmed by the registrant to be in draft form, and will be submitted "in the near future". Some of the key data are presented in Appendix 3 (pp. 54-59) of this report, but little can be done with this study until it has been received and reviewed. *It is of concern that to the extent the study is important to the Agency's assessment, it is uncertain at this time just when a study report would be available, and an Agency review generated.*

7) In the ARP's September 5, 2003 "white paper" (p. 27), the registrant refers to a publication, Coleman et al (2000), which appears to be a subject of disagreement with the registrant. Under Appendix 4 (pp. 61-65) the registrant provides an evaluation of the Coleman paper. This is a complex paper dealing with subjects of chloroacetanilide metabolism and genotoxicity. It appears as though microsomal enzyme metabolism of the various chloroacetanilides does not necessarily follow the same pattern as would be desired if all members of the class are to be treated as behaving by a common mechanism of toxicity. This is critical to the hypothesis of their oneness of behavior (or common mechanism). *Coleman et al (2000) requires a full Agency review in order to respond properly to its contents and to the registrant's review and comments on the same.*

8) Tumors of the olfactory epithelium are exceedingly rare If such tumors are vanishingly rare or indeed non-existent in the rat model, then acetochlor induced tumors need not be statistically significant to be interpreted as positive. *There needs to be developed, documentation focusing upon the assessment of rarity of nasal tumors (olfactory and respiratory epithelia).*

9) The June 27, 1997 CARC report for alachlor speaks of the nasal tumors as respiratory epithelial tumors. This quite possibly is a mistake in the CARC report (see 1/11/04 Email of B. Dementi to W. Burnam), *and requires clarification by CARC.*

10) Monsanto [Heydens, W. F. (1996)] says: "DEIQ has been shown to bind to cellular protein, most <u>likely</u> *(emphasis added)* changes its structure/function *(not an unreasonable hypothesis, though it has not been shown for alachlor or acetochlor)*, thereby causing cell death." (p. 10) Cell death as a consequence of alachlor quinoneimine-protein binding in nasal tissues has not been shown. Similarly, there is no finding of cell death among the acetochlor mechanistic studies either. (see 1/30/04 Email of B. Dementi to "Colleagues", which in turn "forwarded" the 1/21/04 Email of J. Kronenberg to B. Dementi).

11) Again, the registrant's publication [Heydens, W. F. (1996) Monsanto] claims not only species

(rat) specific effects of alachlor, but strain specificity as well, saying that in autoradiographic studies: “Significant labeling of the nasal turbinates was apparent in the Long-Evans rat, but hardly detectible in the Sprague-Dawley or F344 rat.” (p. 6) Yet, in the case of acetochlor, the finding was had in the Sprague Dawley rat (MRID 44496210). This suggests an unexpected difference, or departure from commonality, between the chemically nearly identical alachlor and acetochlor.

In view of any lack of commonality among responses in differing test animals, the registrant goes on to say: “When the phylogenetically similar rat and mouse do not respond alike, or when different strains of the same specie do not respond alike, it is difficult to justify the extrapolation of these rat nasal tumors to humans.” (p. 6) On the other hand, this is perhaps the good reason to test in different species and strains, lest a positive finding that may be relevant to humans be missed. *This lack of consistency among animal models introduces uncertainty in the reliability and relevance of animal model testing to address human health effects.* Yet, in the absence of testing procedures more relevant to human experience, this remains the prescribed manner of testing to which we must be resigned.

12) The registrant claims as the “mode” or “mechanism” of nasal carcinogenicity, one proceeding as follows as a consequence of nasal (olfactory epithelium) quinoneimine-protein binding: “cytotoxicity > cell proliferation > neoplasia”, yet, as acknowledged by the registrant, there has been no showing of cytotoxicity as a result of acetochlor administration, and inadequate evidence that alachlor elicits nasal cytotoxicity (see 1/30/04 Email of B. Dementi to “Colleagues”). *What must the Agency conclude regarding the adequacy of the evidence to substantiate the registrant’s hypothesis that it would have the Agency accept?*

The registrant developed for consideration a complete statement summarizing all of the evidence for cytotoxicity. That evidence was very week for alachlor and non existent for acetochlor (see 1/21/04 Email of J. Kronenberg to B. Dementi).

However, further to the evidence for a cytotoxic response, a June 19, 1991 Monsanto report [Brewster and Hotz (1991) "A Study of the Effect on Cell Proliferation in Specific Tissues of the Rat and Mouse" (MRID 42852102)] claims, as studied in the Long Evans rat that ".....rats were administered alachlor at 0, 0.5, 2.5, 15, 42 and 126 mg/kg/day and cell proliferation was determined in nasal tissue at 10 and 60 days after initiation of treatment. Also in this study, nasal tissue was examined histologically for specific effects of alachlor." (p. 2). While the study did identify increased cell proliferation confined to the olfactory epithelium, *there was no evidence of cytotoxicity.*

However, as to the question alachlor induced cell proliferation, there is a contrasting finding in the literature [Wetmore et al (1999) "Evidence for Site-Specific Bioactivation of Alachlor in the Olfactory Mucosa of the Long-Evans Rat", Tox. Sc. 49, 202-212]. This publication finds that when alachlor is administered ip for up to 28 days at doses that are carcinogenic in chronic studies, olfactory regenerative cell proliferation, as assessed by BrdU labeling, was not increased. This

negative finding was obtained as assessed in both Level III of the olfactory mucosa, and when localized to Bowman's glands. (p. 205)

So these findings in Wetmore et al (1999) appear to be in contrast to those studies of record on alachlor, but also conflict with the more recent mechanism study on acetochlor [Hotz, K.J. and Wilson, A.G.E. (1996) "A study of the effects of acetochlor on rat nasal cell proliferation", Monsanto Company (MRID 44496207)] as conducted in the Sprague Dawley rat. Results in this recent submission by the registrant, where acetochlor was administered via the feed for 30 and 60 days, were positive for increased cell proliferation in the olfactory epithelium, but not in the respiratory epithelium, as assessed by labeling with both 3H-thymidine and BrdU. The report concluded that increased cell proliferation was confined to the olfactory epithelium. It should be noted that doses in this study which yielded the positive response (namely, 1750 and 5000 ppm), exceeded the estimated MTD (1000 ppm) in the carcinogenicity bioassays.

An independent investigator, Dr. Mary Beth Genter, manifestly maintains the absence of any nasal cytotoxicity in her bioassays of alachlor, while her research clearly confirms that alachlor induced the neoplastic response in the olfactory epithelium. Studies by Dr. Genter and her associates require their own level of review if the Agency is to employ the work in its assessment. What level of review and write-up of this work must be undertaken, or is it simply to be presented, as is, to CARC for consideration in its assessment? *If journal review work is to be done on the Genter publication (s), this remains to be done, and will likely be quite time consuming.*

It should also be noted that while the registrant cites nasal cytotoxicity by phenacetin as a model of alachlor induced cytotoxicity, Dr. Genter insists from work in her laboratory that though phenacetin does elicit the cytotoxicity, alachlor clearly does not elicit such cytotoxicity, even at very high doses. Hence, she refutes the argument that phenacetin behaves mechanistically the same as alachlor (see 1/8/04 Email of B. Dementi to A. Protzel and L. Hansen, conveying notes of a 1/7/04 conference call with M. Genter). In a subsequent phone conversation of B. Dementi with B. Genter, 1/28/04, she affirmed the remarkable contrast between the cytotoxicity of phenacetin and alachlor, saying the former elicits massive sloughing of the olfactory epithelium, with no comparable effect in the case of alachlor.

13) What was EPA's conclusion on alachlor mutagenicity? Reference is made again here to Wetmore et al (1999). In this publication, the authors acknowledge as negative for alachlor a wide variety of genotoxicity studies. Yet, as reported in this publication, when *in vitro* mutagenicity testing is made specific to the olfactory mucosa, by incorporating in the test medium an S9 fraction from the rat olfactory mucosa, a positive mutagenic response was found. Similar testing using an S9 fraction from the respiratory mucosa was negative. The authors say: "This study suggests that target tissue bioactivation of alachlor results in the formation of one or more mutagenic metabolite(s), which may be critical in alachlor-induced nasal tumorigenesis." (p. 202). Of added interest here, and by analogy, if acetochlor were tested in this way it could yield similar findings. *A definitive review of this Wetmore et al (1999 is indicated. The obvious concern that*

needs to be addressed is whether mutagenicity of these agents can be, or should be, discounted from consideration as the mode or mechanism of action for carcinogenicity. Appropriate comparative review on the question of genotoxicity appears essential, and could be quite time consuming.

14) The ARP September 5, 2003 "white paper" claims that both the non-human primates and humans *"apparently"* lack the enzymes in the nasal epithelium which are required to metabolize acetochlor to the DABQ1. (p. 26) *The evidence in support of this claim needs to be assembled.* The registrant could be sought to summarize the relevant data.

15) Autoradiographic studies do not differentiate between quinoneimine protein binding and other mechanisms of acetochlor binding in cells, so it cannot be said with certainty that the autoradiographic evidenced of bioaccumulation necessarily reflects only quinoneimine protein binding, or some other form of binding, or at least in what proportions. In other words, what exactly is to be concluded from autoradiographic evidence of radioactive compound localization in a tissue? *Commentary/review on this subject is needed.*

16) Registrant claims nasal tumors to be small and non-progressing to malignancy. In a Monsanto report [Brewster and Hotz (1991) (MRID 42852102)], the statement is made that: "The nasal tumors induced by alachlor in the chronic rat feeding studies were generally very small (microscopic) in size and only one or two occurred in the entire nasal mucosa." (p. 13) Similarly, Ashby et al. (1996) (p. 731) claim the olfactory tumors are *only visible microscopically*. Also, concerning the very important question regarding the character and time of onset of nasal tumors in the rat, the registrant (ARP) claims in the July 18, 2003 draft of the "white paper": under "3.4 ARP Conclusions Regarding Carcinogenic Potential to Rodents": "The nasal olfactory tumors produced by acetanilides are *unusual* (emphasis added) and *can be detected within 5-6 months of continuous exposure* (emphasis added)." (p. 12) This should be viewed as a focal statement in addressing carcinogenicity. It is to be noted that for reasons unknown, this above particular quotation as it appears under Section 3.4 (p. 12) in the July 18 draft, has been removed from Section 3.4 (p. 12) of the same title of the September 5 final document.

By contrast to these claims, Genter and colleagues have investigated the nasal carcinogenicity and genotoxicity of *alachlor*, and *various publications by these investigators need to be summarized.* For example, in Burman et al (2003) [Burman, D.M., et al (2003) "Antioxidant perturbations in the olfactory mucosa of alachlor-treated rats." Biochem. Pharmacol., 66, 1707-1715], the authors say: "We have shown that alachlor-induced olfactory mucosal tumors (polypoid adenomas and *adenocarcinomas*) (emphasis added) occur with a relatively short latency (i.e. following 5 months of exposure at 126 mg/kg per day in the diet) and *high multiplicity* (emphasis added), in that rats treated continuously at this level for 12-18 months often exhibited *10-20 tumors per nasal cavity [7-9]* (emphasis added)" (pp.1707-1708) These lesions are said to be life threatening, at least in the sense of causing air-way obstruction. *So the reliability of the registrant's claims versus those of Burman et al (2003) to the effect that alachlor induced nasal tumors are small, lacking*

multiplicity and non-progressing to malignancy requires consideration.

17) Mouse lung tumors were identified in the carcinogenicity bioassays for both alachlor and acetochlor. These two studies should be evaluated together, as they may be complementary in revealing a positive response that is less dismissible than when either study is interpreted in isolation. Did these two studies yield the same neoplastic finding? *This constitutes additional that would be required to evaluate the data base.*

18) In the case of acetochlor, there are three Guideline carcinogenicity studies of record conducted in the rat. Concern has been expressed that there is inadequate testing at doses below the MTD (October 24, 2003 Review/Commentary by B. Dementi on the ARP's draft July 18, 2003 "white paper"). In one of the bioassays (Acc No 071962071965) (dose levels 0, 500, 1500 and 5000 ppm), there was virtually no nasal tumor response in females at any dose level, up to 5000 ppm, an inexplicable absence of finding in consideration of the other two studies. This was essentially a negative study among females. In the other two studies, there were positive nasal tumor findings at the highest dose levels in each, 1000 ppm (MRID 400770601) and 1750 ppm (MRID 41592004), where the MTD was estimated at 1000 ppm. The next highest dose level in the two positive studies were 200 ppm and 175 ppm, respectively. In consideration of all three studies, there is in effect an absence of adequate testing in the range of ½ the MTD, or 500 ppm. Now while the first study did include a 500 ppm dose, the study appears aberrant in not yielding a nasal tumor effect at any dose across all doses, 500-5000 ppm. Therefore, the negative finding at 500 ppm should not be taken as reliable. This was the case for nasal tumor response in females. Among males a similar disparity of nasal tumor response existed, but not to the same degree of contrast. *In this reviewers opinion, the three studies should be carefully reviewed as to question of whether there has been adequate testing at doses up to and including the MTD*

19) This reviewer maintains that the PWG histopathology re-reads should be submitted directly to the Agency without any attendant risk assessment interpretation by the PWG. The PR Notice 94-5 under which re-reads are conducted simply provides for re-reading of slides, not risk assessment. This is important in securing the objectivity and integrity of the PWG process, since these assessments are conducted at the request and expense of the registrant. (See 11/17/03 Email of B. Dementi to J. Jones) Certainly, at least one of these PWG re-reads should be read again by truly independent pathologists.

20) “White paper” (p. 19) Very important statement: EPA (1998) says “.....entity responsible for alachlor cytotoxicity response is not known with certainty.” *To what extent has the Agency (EPA, 1998) already accepted this as evidence of cytotoxicity critical to the schematic (cytotoxicity > regenerative proliferation > neoplasia) for neoplasia ? This requires clarification.*

21) In the June 27, 1997 CARC report, we find that in the case of alachlor, nasal tumors were observed at 2.5 (single tumor), 15, 42, and 126 mg/kg/day in two Long Evans rat studies combined, one tested up to 126 and the other up to 15 mg/kg/day. No tumors were seen at 0.5

mg/kg/day. In regard to these findings, the report concludes: ".....the MOE for the nasal tumors should be determined with 0.5 mg/kg/day as the "point of departure" as no tumor response was seen at this dose level." CARC appears to be oblivious to the concern that though no tumors were seen at the 0.5 mg/kg/day, it may not be the true MOE simply because so few animals are in the test group, i.e. not enough animals to pick up on a very meaningful incidence of this rare tumor type that would be of concern to the human population. In cancer bioassays, higher doses are used in part because there are too few animals per dose group to be representative and protective of a huge human population exposed to very low doses. Given the findings in this study at the higher doses, there is no justification for concluding that an MOE has been identified at the lowest dose level where tumors no longer appear, especially rare tumors. So while an MOE approach might be justified, which itself is to be questioned, the lowest dose in this study where tumors no longer appear cannot be relied upon as the dose at which relevant tumorigenic expression has ended.

22) What is the status of EPA's draft Carcinogen Risk Assessment Guidelines under which the acetochlor assessment will be conducted? *It would be a very notable contribution to have comments on this subject of Guideline status*.

END

Exhibit III

Brian Dementi
03/02/04 02:18 PM

To: Alberto Protzel/DC/USEPA/US@EPA, Linnea Hansen/R9/USEPA/US@EPA, Nancy McCarroll/DC/USEPA/US@EPA, Larry Chitlik/DC/USEPA/US@EPA, Susan Makris/DC/USEPA/US@EPA
cc:
Subject: Acetochlor: additional concerns

Colleagues,

In my Email to you of February 4, I appended a list of some 22 concerns pertaining to the assessment of the acetochlor database. I indicated that more may follow. Well here are an additional six items, and this second document is a draft, as more may be added to it. But I wanted to get these out on the table now, as time in growing short.

Best Wishes,
Brian D.

ACETOCHLOR: ADDITIONAL CONCERNS Dr. B. Dementi March 2, 2004

1) The mechanism or mode of action of acetochlor induced carcinogenicity of the nasal olfactory epithelium postulated by the registrant might briefly be characterized as: quinoneimine-protein binding > cytotoxicity > cell proliferation > neoplasia. Cell death has been claimed as an aspect of the cytotoxicity.

The registrant, having postulated a mechanism or mode of action for acetochlor induced nasal neoplasia, is advocating a Margin of Exposure (MOE) analysis for nasal carcinogenicity. The Agency's 1999 draft Guidelines for Carcinogen Risk Assessment provide for such an approach in the assessment of carcinogenicity potential. According to those Guidelines: "A margin of exposure is defined as the point of departure divided by the environmental exposure of interest." "A margin of exposure analysis is applicable if data are sufficient to presume a non-linear dose-response function containing a significant change of slope. If, in a particular case, the evidence indicates a biological threshold, as in the case of carcinogenicity being secondary to another toxicity that has a threshold, an RfD or RfC like approach may be estimated and considered in cancer assessment." (p 3-10)

The Guidelines indicate: “For a margin of exposure analysis, the point of departure would ideally be the dose where the ***key events*** in tumor development would not occur in a heterogenous human population, thus representing an actual ‘no effect level.’ Therefore, it is recommended that margin of exposure analyses be ***based on precursor responses rather than tumor incidences***, since precursor events can often be detected with greater sensitivity (i.e. both earlier and at lower doses), providing further input to the decision regarding acceptability of the margin of exposure.” (emphases added) (p. 3-11) Similarly, EPA’s 2003 draft final Guidelines for Carcinogen Risk Assessment say: “The goal is to use precursor data to extend the observed range below what can be observed in tumor studies.” (p. 3-13)

In the analogous case with ***alachlor***, where the proposed mechanism, or mode of action, for olfactory neoplasia is the same as that for acetochlor, the calculation of the MOE for alachlor as performed by the registrant was as follows [see Monsanto (1996) *Executive Summary section on Human Exposure and Margins of Exposure,* from “Information on the Carcinogenicity of Alachlor for the FIFRA Scientific Advisory Panel October 1996"] (p. 10): Monsanto employed for the purpose of this calculation, EPA’s numerical value for the Anticipated Residue Contribution (ARC) for the overall U. S. population of 1.3 x 10(-5) mg/kg/day, and for the Points of Departure, the NOEL and LOEL values of 2.5 mg/kg/day and 15 mg/kg/day for neoplasia (tumor expression) obtained from the rat carcinogenicity bioassays. The registrant thereby obtained the following Margins of Exposure (MOE): as derived from the NOEL, MOE = 2.5 mg/kg/day/1.3 x 10 (-5) mg/kg/day = 192,000; and as derived from the LOEL, MOE = 15 mg/kg/day/1.3 x 10(-5) mg/kg/day = 1,153,000. ***So the MOE in terms of the NOEL for carcinogenicity is the first figure, 192,000.***

The criticism to be made of this calculation, and its interpretation in the protection of public health, is that it derives purely from the NOEL/LOEL for nasal tumor incidence data, and not from the “key events” incidence data peculiar to the proposed mechanism.

Further, the 1999 Guidelines treat that “.....lack of quantitative information on the key event may make it necessary to use tumor data instead of key event data. In this case, the analysis of the margin of exposure must contain an estimate of the dose-response curve for tumors plus have sufficient discussion of the difference (on the dose scale) between no effect levels and effect levels for key events and tumors. A larger margin of exposure may be needed to account for possible differences between the dose-response curves for the key events and for tumors, and to assure decision-makers that cancer risk for the heterogeneous population (including sensitive subgroups) is not appreciable.” (p. 3-12) Hence, the Guidelines in effect require that, in the assessment of cancer risk assessment, a departure from the use of the *conservative linear approach* in dose-response assessment to the *MOE approach* is conditioned upon adequate knowledge concerning the mode of action to at least permit an estimate of the NOEL/LOEL for the precursor event(s) if not providing such quantitative data for the same as to replace tumor data in the assessment.

Unfortunately, Monsanto's calculation of the alachlor MOE employed tumor incidence data to the exclusion of either "key events" data, or any rationale or estimate of just what the impact of such data, were it available, might have upon the all important MOE.

The registrant's approach employed the use of tumor data itself, and that alone, despite the insensitivity of the carcinogenicity bioassay to quantitate tumor incidence, particularly in the low dose group, for these very rare nasal olfactory epithelial tumors. The computation in essence violated the real intent of the Guidelines to permit MOE assessments in lieu of the linear approach.

In the case of ***acetochlor***, were this approach taken, there is not sufficient information to competently discuss "differences (on a dose scale) between no effect levels and effect levels for key events and for tumors". In this toxicologist's view, the lack of quantitative information on the key event severely compromises the defensibility, and hence the reliability, of the proposed mechanism, and serves to weaken public health protection when a departure from the tradition and long standing linear approach to risk assessment is taken in favor of an MOE approach based upon tumor incidence data alone. Cancer bioassays are simply not sensitive enough to conclude a NOEL for cancer, and particularly so for extremely rare tumors as are those of the olfactory epithelium.

In the particular case at hand with acetochlor, if the postulated mechanism, or mode of action, is correct, the "key event" would appear to be acetochlor quinoneimine-protein binding. So, to properly assess the MOE, arguably one would need to know what level, or concentration, of the quinoneimine-protein is necessary to trigger cytotoxicity of such magnitude as to lead to neoplasia. Thus the NOEL/LOEL dose levels for generation of this critical level of quinoneimine-protein would be needed to empoly as the Point of Departure for the MOE assessment. However, in the case of acetochlor, quantitative data are not available for this end point. No attempt has been made to obtain such quantitative data in the critical dose range up to and including the MTD. Arguably one could empoly the NOEL/LOEL for cytotoxicity as the "key event", but likewise, the mechanistic studies for acetochlor were not designed to obtain this end point for cytotoxicity. Furthermore, even if such data for cytotoxicity were available, cytotoxicity itself is somewhat removed from the proposed mechanism or mode of action, as a variety of events other than quinoneimine-protein binding could in principal elicit cytotoxicity. Thus the justification for the MOE as opposed to the linear approach based upon the proposed mechanism of action becomes less defensible. Further along in the proposed mechanistic scheme, one might employ regenerative cell proliferation as the "key event", but here again the mechanistic studies are not sufficiently rigorous to identify the point of departure for this effect as a precursor event to neoplasia. Furthermore, to empoly cell proliferation in the identification of the point of departure, must be viewed as yet further removed from the proposed singular event (quinoneimine-protein binding) of the proposed mechanism, since increased cell proliferation quite commonly precedes neoplasia, whatever the mechanism might be.

2) In the assessment of the mechanism or mode of action of acetochlor, the position being taken by

the registrant and possibly by the EPA is that the MOE approach is appropriated for carcinogen risk assessment for those agents shown to be non-genotoxic. However, according to the 1999 draft Carcinogen Risk Assessment Guidelines, another rationale exists in support of remaining with the linear approach, which may have been neglected. The Guidelines indicate under "Factors Supporting a Linear Approach" "Any of the following conclusions leads to selection of a linear dose-response assessment approach:" and the fourth such listing reads *"Mode of action analysis does not support direct DNA effects,* ***but the dose-response relationship is expected to be linear (e.g., certain receptor-mediated effects)****"* (p. 3-3) There is good reason to suggest, in the case of the acetochlor proposed mechanism or mode of action for nasal olfactory neoplasia, that the quinoneimine-protein binding is linear with dose. There appear to be no mechanistic or mode of action studies that provide evidence for a *change of slope*, as the Guidelines speak of, in the "key events" dose response data, that could be employed to identify a "point of departure" for such data. It is not known just what level of such binding is necessary to elicit a neoplastic response. Presumably, though, it must be the minimal level to elicit cytotoxicity. But a point of departure or threshold for this binding has not been identified, nor could it be, since the mechanistic studies for acetochlor unfortunately were not designed with that aim in mind. Cytotoxicity as an element in the neoplastic progression might also be viewed as a "key event", but cytotoxicity has not even been identified for acetochlor, and, hence, no data exists to identify a threshold for this endpoint in the progression. Nevertheless, the Agency has the obligation to consider the cited perspective in the Guidelines in support of remaining with the conservative linear risk assessment, even in the face of no evidence for genotoxicity. ***In substance, the linear approach should not be abandoned and the MOE approach resorted to simply because there is an inadequate finding of genotoxicity***.

We should not leave this point without adding that there also remains considerable uncertainty surrounding the question of acetochlor genotoxicity that must be addressed.

3) The following citation is introduced here by way of enhancement of concern #16 in Dr. Dementi's February 4, 2004 "Acetochlor: List of Concerns", see 2/4/04 Email of Dementi to Colleagues.

Chu et al (1981) "*Factors in the Evaluation of 200 National Cancer Institute Carcinogen Bioassays*." J. Tox. Env. Health, 8, 251-280. "Ideally, a distinction should be made between truly benign tumors, which never progress to malignancy, and tumors that are in a benign state according to histopathologic criteria at the time of diagnosis. Scientific judgements in this area are limited by inability to predict the biological behavior of a lesion on the basis of morphological criteria, *but it appears that there are few, in any, truly benign tumors in rodents* (emphasis added). If this were true, all chemicals that induce benign tumors would be termed carcinogens." (p. 257-258)

4) As in the case of the previous concern, the following citation is introduced here by way of

enhancement of concern #18 in Dr. Dementi's February 4, 2004 "Acetochlor: List of Concerns". This is intended to emphasize the importance of the presence of reliable testing at ½ MTD in carcinogenicity bioassays.

Haseman (1985) "*Issues in Carcinogenicity Testing: Dose Selection."* Fund. Appl. Toxicol., 5, 66-78. In reference to dose selection issues for some 50 National Toxicology Program (NTP) carcinogenicity bioassays, and in arguing for at least three dose levels in these studies, the author says: "These data also indicate that more than two-thirds of the carcinogenic effects detected in feeding studies would have been missed had the high dose been reduced from the estimated MTD to 1/2MTD." (p. 66) One would thus be concerned that if sensitivity drops remarkably from testing at the MTD to that at 1/2MTD, that detection may become quite insensitive at doses < 1/4MTD. It is important therefore that for optimum sensitivity and for the obtaining of reliable dose response data, in addition to testing at the MTD, there should also be reliable testing at 1/2MTD, rather than to employ a next dose below the MTD that drops well below 1/2MTD. Further along, Haseman says: "Second, the NTP has begun to incorporate routinely a third, lower-dosed group into its 2-year carcinogenicity studies (i.e., a typical NTP design employs doses of 0, 1/4MTD, 1/2MTD, and MTD)." (p. 71)

5) ***Where carcinogenicity bioassays are concerned, there is no justification for the out of hand discounting of any and all neoplastic findings simply because they are observed at doses exceeding the MTD.*** Furthermore, when such finding are discounted one must question whether the remaining dose groups in the study are adequate to define an acceptable study. Many of the neoplastic findings in the three rat and two mouse acetochlor bioassays have been discounted simply because the doses at which they were observed exceeded the MTD.

Haseman (1985) expresses concerns over discounting carcinogenicity findings simply on the grounds that MTD was exceeded. "Metabolic overload and secondary carcinogenesis are legitimate issues that should be considered in the design and interpretation of carcinogenicity studies. However, they should not be the justification (with no supporting evidence) for routinely 'explaining away' all tumor increases seen only at high dose levels. In order for these factors to influence the interpretation of carcinogenicity studies, a direct cause and effect relationship with the induction of cancer must be established. For example, it is insufficient merely to demonstrate that metabolic overload occurred; ***one must show explicitly how this overload produced carcinogenic effects.***" (p. 70)

6) Haseman (1985) also says: "When considering the relationship between *cytotoxicity* (emphasis added) and carcinogenicity, it must be noted that tissue damage does not always lead to increased tumor incidences." (p. 70) He provided examples of two agents in NTP studies that yielded "toxic liver lesions" at high incidences in dosed groups, but with no corresponding increase in liver tumors.

Exhibit IV

Brian Dementi
04/07/04 02:38 PM

To: Alberto Protzel/DC/USEPA/US@EPA
cc:
Subject: Comments on Proposed MTARC Presentation

Alberto,

Please find appended comments on the "Proposed Data Presentation to MTARC for the April 21 meeting.

Feel free to share this with the MTARC committee.

Best Wishes,
Brian D.

Alberto, 4/7/04

I have read the draft “Proposed Data Presentation To METARC”, for the April 21, 2004, which I received around March 30. I find it to be very excellent in many ways. The Introduction, list of data available, data on acetochlor and analogs, metabolic pathways and their diagrams, and proposed diagrammatic presentation of the proposed mechanism, and much more.

I have a number of concerns that pertain primarily to the evaluation and interpretation of the data said to support the MOA. I have already expressed much of this in my Emails of February 4 and March 2, 2004 to our staff that find no distinct representation in your presentation, though these documents along with my January 26, 2004 paper on mechanisms and other writings are among the attachments. So these documents expressing my views are there to be reviewed by the committees.

Some of my concerns with the proposal are enumerated as follows;

1) You indicate: “Nasal turbinate tumors have also been found in F1 pups in a rat 2-generation study.....” (p. 5). I believe it would be more correct to say the tumors were found in F0 and F1 offspring, in the young adult (about a little over 4 months age) stage but beyond the pup stage in development. However, nasal tissues were taken (preserved) from F1 and F2 pups at PND 29, but never examined histopathologically, an obvious deficiency in my view.

Also, both olfactory (primarily) *and* respiratory tumors were found. Such tumors of either epithelium are considered exceedingly rare. Respiratory tumors do not reconcile with the proposed mechanism, and is another issue where this study is concerned. In my view, the finding of these nasal tumors in offspring in a reproduction study is so important that the finding should have had the priority level usually accorded 6(a)(2) findings. I should note that as I understand, alachlor has not been similarly evaluated for nasal histopathology and carcinogenicity in a reproduction study. This finding in acetochlor poses questions about alachlor, and how it would perform in like testing. The evidence of offspring susceptibility requires some quantitation under FQPA on behalf of children's interests.

2) You indicate: “In 1996 the FIFRA SAP was presented and did not refute a postulated mode of action (MOA) for alachlor for nasal epithelium in rats similar to the one to be discussed in this document.” (p. 5) The level of endorsement of the MOA by SAP lacks the certitude this toxicologist would desire. It is clear the SAP recognized the commonly shared nasal tumorigenicity of these agents, but I would prefer to have seen a clear statement endorsing the quinoneimine hypothesis as undergirding the tumorigenicity. That SAP requested that CARC follow-up with certain clarification, it is not altogether clear that the MOA was endorsed, *or could have been endorsed*, given the nature of the questions and comments posed by the SAP. The December 6, 1996 SAP/SAB report on the October 30, 1995 alachlor meeting, and one should read their complete comments, says for example: "Therefore, the limitation in the metabolism in the mouse may not be the real reason for the lack of tumorigenic response in the mouse. If this is accurate, then the argument that limitation in the metabolism of alachlor in the human precludes alachlor being considered as a human carcinogen can not be supported." (p. 48) But if indeed the panel did endorse the MOA, would they have done so if they knew claims of cytotoxicity and cell death, repeatedly made by the registrant, were so questionable? The registrant now acknowledges there is no evidence for cell death of the nasal olfactory epithelium following alachlor administration, and in my view the evidence the registrant recently cited for the cytotoxicity of alachlor is not sufficiently convincing to support the hypothesis (see the 1/30/04 Email of Dr. Dementi with Dr. "Jerol Kronenberg's Email of 1/21/04 appended).

3) In Table 1 (p. 12), under tissue distribution you say for three of the compounds, there is no nasal epithelium binding in mice or other species. Does this statement include direct binding to proteins and possibly other macromolecules by the chlorine displacement mechanism that you and I have often discussed?

4) In Table 1 (p. 13), in the penultimate horizontal column, you say “Significantly increased cell proliferation in the olfactory epithelium of rats but not mice.” In the case of acetochlor, this is certainly true in the mechanism study we reviewed, but it should be note that this was at doses that exceeded the MTD (1000 ppm) (namely, 1750 and 5000 ppm, MRID 44496207). To my knowledge, we do not have on record evidence that acetochlor induces cell proliferation within the critical dose range of up to and including the MTD. *The study does not prove that increased cell proliferation actually occurs at the MTD tumor eliciting doses. I think the registrant should not*

have avoided doses such as those at MTD (1000 ppm) and ½ MTD (500 ppm), particularly since tumors are claimed to be seen as early as 5 months (4 months or less in the reproduction study), and it took 90-160 days (3 to 5 or more months) to see cell proliferation at doses exceeding the MTD. Based upon the weakness of the response at 1750 ppm, I seriously doubt that cell proliferation would be detected in-time before tumors if tested at the MTD (1000 ppm). If increased cell proliferation can only be detected at doses exceeding the MTD, how could increased cell proliferation serve as a stage in the hypothesized progression to neoplasia at 1000 ppm?

5) Concerning Table 2 (p. 22) on the carcinogenicity findings, I would indicate the following:

a) In support of the nasal tumors as being of the olfactory epithelium, you say the Registrant's tables do not state explicitly that the above tumors originate from the nasal olfactory epithelium, but go on to explain that when tissues were reexamined in posterior regions, tumors were found, though they had not been found in the previous assessment since it involved the anterior regions of the nose. You cite this as evidence the tumors were of olfactory epithelium. I would agree that it is more likely the tumors were for this reason olfactory, but it is the pathologist's responsibility to make the diagnoses. Olfactory epithelium is neural, and I understand tumors in this region have certain features that mark them as olfactory versus respiratory. And while it may be likely that tumors in the re-cut were olfactory, we do not know that for a fact, nor do we know what if any fraction of these may have been respiratory tumors. Part of the proof of the quinoneimine mechanism hypothesis is that nasal tumors be exclusively of the olfactory region, including Bowman's Gland. Therefore, if the hypothesis is to be defended, tumors must be diagnosed by the pathologist as olfactory. It is noteworthy that a 1992 pathology rat nasal tissue mapping study (MRID 44496214), Doc# 26, indicated that nasal lesions in the 1988 acetochlor study, high dose group 1750 ppm were essentially confined to the olfactory region. But it is not clear whether these lesions were of the respiratory epithelium that replaced olfactory epithelium at this high dose, or whether the tumors were neural. More needs to be said about this.

b) You indicate tumors of nasal passages were found in PR-80-006 on re-examination, but incidences among males were not as high as in the other two studies, especially when one compares incidences at the various doses, but what's more, the study by marked contrast with the other two studies and with males in the same study, females were virtually non-responsive. This still was essentially or comparatively a negative study for females across all doses.

6) On page 23, in reference to the autoradiography mechanism study, you note that there was significant localization in the olfactory region at 5000 ppm and only slight to moderate localization was seen at 1750 ppm. This is important, but I must express the concern that both doses, 1750 and 5000 ppm were in excess of the MTD (1000 ppm), and these autoradiographic effects may have resulted from metabolic overload that might not be observed at doses up to and including the MTD where tumors occur. It is most disturbing the registrant did not include doses in the range up to and including 1000 ppm, in order to prove an effect in the acceptable dose range, and to help in

the search for a key effects NOEL.

7) You say: "In support.....acetochlor sulfoxide was the major metabolite in the plasma of rats fed 1750 ppm acetochlor in the diet for 6 months." (p. 24) Again, 1750 ppm exceeded the MTD (1000 ppm), and may have resulted from metabolic overload. This effect would have much greater significance if observed at doses up to and including 1000 ppm, especially after 6 months, for this is the time period within which tumors occurred.

8) You say (p. 24): "Either way, either quinoneimine will then bind to tissue proteins and other nucleophiles such as GSH." *I would add, and DNA?*

9) The acetochlor quinoneimine-protein binding reported after 14 days at 1710 and 5170 ppm is a good qualitative indicator of the transformation of acetochlor to quinoneimine and of its binding to protein, but this does not establish any adverse effect. It could be an innocuous sink for the quinoneimine. Carcinogenicity may be proceeding via an entirely different route that bears no relationship to quinoneimine-protein binding except that the two events may occur contemporaneously. Beneficial toward substantiating the hypothesis would be information of a more quantitative nature linking a buildup of the quinoneimine-protein complex with a subsequent onset and enhancement of cytotoxicity, a claimed element in the progression to neoplasia.

Casarett & Doull's *Toxicology, The Basic Science of Poisons*, 4th Edition (1996), says under a section: "Models Derived from Mechanistic Assumptions": "The development of biologically based dose-response models for endpoints other than cancer are limited ; however, several approaches are being explored in developmental toxicity, utilizing cell cycle kinetics, enzyme activity, litter effects and cytotoxicity as critical endpoints (reference cited). Unfortunately, there is a lack of specific quantitative biological information for most toxicants and endpoints." (p. 83)

Also, as before, 1710 and 5170 ppm exceeded the claimed MTD (1000 ppm), and protein binding may have resulted from metabolic overload. The studies should have included dose groups up to and including 1000 ppm in search of a more relevant effect in support of the hypothesis, and in search of a NOEL for this effect as a possible "key event" to employ under EPA's draft cancer guidelines as a point of departure in an MOE calculation. *However, I fear a true and meaningful NOEL for this endpoint lies below the level of detection by the methodology which was employed.*

10) Concerning mechanism studies MRIDs 44496210 (rat), 44496211 (mouse), 44496212 (rat), 44496213 (monkey), although the studies provide qualitative evidence for quinoneimine-protein binding in the nasal epithelium of only the rat, the studies do not differentiate between olfactory and respiratory epithelia as having been individually examined. There is no certitude that all the studies reflect effects occurring within the olfactory epithelium, knowledge that is necessary to fairly compare effects across species. Unlike these studies, MRID 46009402 (rat), which tested acetochlor sulfoxide binding with the nasal epithelium, was clearly designed to distinguish between effects in the olfactory versus respiratory epithelia. (p. 30) Though this study is currently classified

as unacceptable, it appears to show a preferential (though not exclusive) effect for the olfactory epithelium. It is uncertain just how much weight should be assigned to this study in proving the hypothesized MOA. It is noteworthy that the study differentiated between findings in the two epithelia, and underscores the fact that this destination *was not made* in the aforementioned studies, where had it been made, a more reliable species comparison would have been obtained.

11) Concerning the topic of "Cytotoxicity" (p. 34), I would say the following. In his 1/21/04 Email correspondence with Dr. Dementi, pertaining to the "cytotoxicity" element of the hypothesized nasal tissue MOA, Dr. Joel Kronenberg acknowledges that the registrant has no evidence for cell death for either alachlor or acetochlor, nor having any evidence for cytotoxicity in the case of acetochlor. He cites some evidence for cytotoxicity of alachlor. This was a surprising revelation. This evidence requires further consideration as to whether such evidence should indeed qualify as cytotoxicity of a character to support the proposed mechanism. It would appear the needed rationale supporting cytotoxicity as a meaningful effect was not previously in place when the MOA for alachlor was considered. This acknowledgment of the lack of evidence for cell death and cytotoxicity has spawned just now a debate on the issue among pathologists. Unfortunately, this is a complex subject, and this toxicologist maintains that a thorough review of the subject is required that goes well beyond the quick responses that are reflected in the indicated correspondence given below.

12) As to the question of alachlor induced *in vivo* cytotoxicity (pp. 35-37), certain works of the registrant and those by Dr. Genter's group are compared. The registrant's studies (EHL 87112 and 90059) yielded evidence for cell proliferation, they were negative for cytotoxicity. By contrast, the two of the three Genter works that assessed cell proliferation were reportedly negative for this effect, and all three studies were negative for cytotoxicity. So there is quite a bit of evidence here that does not support cytotoxicity as an effect of alachlor. In the hypothesized MOA, central elements in the progression from quinoneimine-protein binding to neoplasia include the elements of cytotoxicity > cell proliferation. Since these studies do not confirm the alleged cytotoxicity, and two published works claim no increased cell proliferation, how can the MOA be reliably defended?

Now one of the published Genter works, the 2002 paper, indicates that there was respiratory metaplasia "(i.e. replacement of olfactory mucosa by respiratory epithelium)." (p. 36). Dr. Genter explained to this toxicologist (conversation of 3/31/04) that this metaplasia is actually a transformation of olfactory cells into respiratory cells, as opposed to the death of olfactory cells followed by a new growth of respiratory cell. So metaplasia would not yield histopathologic evidence of cell death. Since the published works failed to show increased cell proliferation, one must question whether metaplasia, heretofore not claimed as an element in the proposed MOA, might server as an element in the MOA.

13) Phenacetin, which induced nasal tumors, elicits nasal cytotoxicity, and which like acetochlor

would be expected to be metabolized to a quinoneimine, has been cited in the registrant's works as a model for the MOA in support of the chloroacetanilides. However, it has become apparent from the Genter work that phenacetin exerts an unequivocal cytotoxicity of the nasal epithelium, in marked contrast with that of alachlor. See my 1/8/04 Email to you and Linnea conveying the notes of the 1/7/04 conversation with Dr. Genter. So it would appear that while phenacetin and alachlor share in common the induction of nasal tumors, and both would form a quinoneimine, the MOA for the two is not shared nor could serve to explain the nasal neoplasia. Both likely induce nasal tumors by some other mechanism. This is my concern at least, as toxicologists.

14) Concerning the dialogue among pathologists (Drs. Wolfe, Pletcher, Genter and Bolon) on the subject that you have presented, I think the whole matter is shrouded in confusion that renders highly questionable at this time the hypothesized MOA for alachlor. For example, Dr. Wolf's initial comments of 2/25/04 (p. 38) and Dr. Genter's response (p. 39) to his comments are contrary statements, where she is saying dead cells would not disappear from view histopathologically. Her statement appears more credible to me. She has explained to us in the past that the sloughing of dead cells in the case of phenacetin is abundantly evident, and that such is not the case for alachlor. I might add, the veracity of the proposed MOA must reside with positive evidence of cytotoxicity, and not a cytotoxicity that one cannot see. A phantom cytotoxicity will not do, i.e. as providing proof. Dr. Wolf's stated support for the MOA does not flow from any logic presented in the earlier part of his text. In Dr. Wolf's follow up response of 3/15/04 (p. 39) to Dr. Genter's comments, he suggests the cell death would be seen at high doses. However, the fact of the matter is that at high doses, where neoplasia is abundant for both alachlor and acetochlor, cell death has not been observed, as freely acknowledged now by the registrant. If one must look at the proper time and a critical dose level to witness the cytotoxicity, this has not been even spoken of heretofore, let alone done for these chloroacetanilides. Again, a phantom cytotoxicity and cell death is now being invoked to explain the missing hard core evidence that everyone thought existed until now. For Dr. Wolf to then conclude that he and Dr. Genter are "...saying the same thing" does not stand the test of reason. Again, Dr. Genter has explained to us that in the case of phenacetin, the evidence for cytotoxicity and cell death is abundant, and that this is in remarkable contrast with alachlor, at doses in both cases which are neoplastic.

Upon entering the discussion, Dr. Pletcher (3/17/04) says: "Anyway, metaplasia (of whatever sort) is evidence of a noxious effect (toxicity) .....as is necrosis, of course.....so I think everyone should agree that we have a toxic effect manifested in the olfactory epithelium of the nasal cavity of the rats treated with Alachlor/Acetochlor, and that this effect must have at some stage involved necrosis or an increase in apoptosis (accelerated programed cell death, hyperplasia and eventually metaplasia." (p. 40) Metaplasia is a term not heretofore not invoked in the MOA sequence of events. Here again, a phantom cytotoxicity (cell death) is being proclaimed. Furthermore, if metaplasia is involved, it follows cytotoxicity (cell death) in the sequence of events, and thus when observed cannot be expected to serve as substitute for cytotoxicity (cell death) evidence that is missing. According to Dr. Pletcher, cytotoxicity (cell death) is a presumed event, if not witnessed, and it has not been witnessed for alachlor. This toxicologist must reiterate that in the case of

phenacetin, Dr. Genter claims to have witnessed in abundance cytotoxicity (cell death) at neoplastic doses that appear consonant with the hypothesized MOA. *The same is not observed with alachlor*. In the alachlor case at neoplastic doses there is no comparable cytotoxicity (cell death), but a transformation of olfactory cells into respiratory epithelial cells. In the case of alachlor, Dr. Genter says: "If cells had died and miraculously vanished, one would see a thinning of the epithelium. This is not the case, ...the olfactory mucosa of the treated rat .....is HYPERCELLULAR and thicker than that of the age-matched control rat!!!" (p. 39)

In this toxicologist's view, given the registrant's surprising recent acknowledgment of the absence of any evidence for cell death for either alachlor or acetochlor, no evidence for cytotoxicity for acetochlor, and questionable evidence of cytotoxicity for alachlor, coupled with this dialogue among pathologists, serves to question the veracity of the proposed MOA for the chloroacetanilides, the assertions of Drs. Wolf and Pletcher to the contrary not withstanding. Before any further endorsement of the MOA is rendered, a panel of experts outside the Agency should be convened for a more definitive opinion. This new debate comes too close to the date for the MTARC meeting for proper assessment of this issue.

15) Concerning cell damage comments (p. 41), it would be helpful to let your audience know what the implications for the mechanism might be if indeed alachlor & acetochlor had no impact upon GSA levels. The finding of quinoneimine-protein by the method employed is a qualitative indicator for the presence of a quinoneimine, but there is no basis for deciding what level of this binding is necessary to trigger a neoplastic response, if it did trigger such a response.

16) Concerning the presentation on "Cell replacement/increased cell turnover" (p. 41-44):

a) There is evidence for acetochlor induced cell proliferation specific to the olfactory epithelium of the rat. However, there is no speculation even as to what level of increased cell proliferation would be expected to result in a neoplastic response. So as with quinoneimine-protein binding, this is a qualitative indicator of an effect. Also effects were seen at doses that exceeded the MTD (1000 ppm), and may be a consequence of metabolic overload. It is unfortunate that the registrant neglected to test more fully in the dose range of up to and including 1000 ppm. There may be no significant effect in that range.

The comparable mouse study (MRID 44496209), did it test both respiratory and olfactory tissues independently? As I recall, the distinction was not made in the mouse.

b) Studies on nasal cell proliferation for alachlor are cited in support of those on acetochlor, Tables 7 & 8 (p. 44). These studies do show an effect specific to the olfactory epithelium, but the dose of 126 mg/kg/day may be in excess of the MTD (we need to know the MTD for alachlor), and the effect possibly due to metabolic overload. On the other hand, incredible as it may seem, there may be a positive effect at the lowest dose of a mere 1 mg/kg/day @ 10 and 60 days. If not true, what does this say as to the reliability of the methodology? There is no comparable mouse study cited

for alachlor. If the mouse study were done, we would need assurances it was negative for the olfactory epithelium.

Under topic III. Data Evaluation, please consider the following:

1) Concerning affirmation of neoplasia of the olfactory epithelium of the rat: "....the endpoint is clearly attained at the higher doses in three separate chronic rat studies." (p. 45) *Not so among females in PR-80-006, and the effect is less pronounced for males than would be expected based upon the other two studies.* At this time this anomaly is inexplicable. This is reviewed in Dr. Dementi's October 24, 2003 comments (Document #32)(item #13) on the "white paper" submitted by Dr. Kronenberg. As explained in those comments, there is inadequate testing in ½ MTD dose range for the composite of three studies.

"No nasal tumors were seen in mice in two acceptable studies." (p. 45). In what epithelium? There is a serious question as to whether the effects in the olfactory epithelium were ferreted out in the mouse studies.

2) Under the postulated MOA, item "v) Cytotoxicity" (p.45), it should be noted that in the registrant's background materials one often sees *cytotoxicity (cell death)*, while now we know the registrant disowns cell death for alachlor and acetochlor as having been seen.

3) Evidence by autoradiography of nasal localization (p. 46), but what is the real meaning of this? It may well be an innocuous sink for the material that happens to be coincidental with neoplasia and a probable host of other unknown biochemical events, each equally as likely to be responsible for the neoplastic effect if one desired to make the claim.

4) The section "4. Key Events" (p. 46) sets forth the key events, but the rationale in support of the reality of these key events as somehow explaining nasal carcinogenicity is not well documented here. Many assertions are made without adequate documentation. All that we have are qualitative indicators of certain metabolic events that are used to speculate a mechanistic link between these and a neoplastic outcome.

5) "There is support that the quinoneimine is formed in the rat nasal epithelium in a dose related manner, but not in the mouse" (p. 48) First, too much emphasis through out these considerations is focused upon the lack of an effect in the mouse. We are never certain whether this absence of effects in the mouse resides unambiguously with the olfactory epithelium in the mouse studies. Furthermore, if there is no operation whatsoever of the proposed MOA in the mouse, how much reliance is to be placed upon the absence of this effect in the mouse? The mouse model is recognized in the literature [see discussion on Brown et al, Document #18, p.21(157)] as a poor one for detecting nasal neoplasia for various xenobiotics, and the absence of such finding with acetochlor and alachlor is not necessarily surprising or particularly revealing. Little interpretative

reliance can be placed upon the mouse studies for this lesion.

6) "There is indirect support for Cytotoxicity" (p. 48) Since the registrant's acknowledgment of no finding for cytotoxicity and cell death for acetochlor, now we must begin to rely upon "indirect evidence" in support of this critical element of the MOA for acetochlor. As rendered above, the discussion now in progress among pathologists does not explain Dr. Genter's clear distinction between the effects of alachlor and phenacetin, such that both cannot satisfy the proposed MOA, nor could be cited as mutually supportive in this regard. This matter as stated needs to be addressed by an external unbiased peer review panel.

7) "Several studies found in the open literature (these should be cited here) ***suggest*** that DABQ1 ***may induce*** damage to DNA through oxidative stress." (p. 49) Terms such as "suggest" and "may induce" are not of the definitive character needed for public health protection. Do we have any studies on alachlor and acetochlor that test this hypothesis on a par with that which has been used to rule out a mutagenic effect?, or are we falling back on this ill characterized explanation lacking no other reliable explanation for what could be a DNA directed effect?

8) Concerning the discussion on acetochlor induced cell proliferation, it appears true from the evidence presented that: "As the time of treatment increases, the NOEL decreases." (p. 49) And ".....there is no direct cell proliferation data to assure that the lowest of them (250 ppm) will remain a NOAEL for longer treatment periods than 160 days." (p. 49) Also: "It is possible to conjecture that 250 ppm might be at or close to a NOAEL for cell proliferation for more extended periods of treatment, *because 500 ppm is a non-tumorigenic dose (Table 2) for nasal olfactory epithelium tumors in rats." (p. 50)* (emphasis added) Words such as "conjecture", "might be", "close to", reflect a lack of certitude that is needed for reliable risk assessment for public health protection. Also, according to the hypothesized MOA, increased cell proliferation precedes and spawns tumor development, while this data does not appear to preclude the advent of tumors as occurring in time *before* detectable expression of cell proliferation.

Furthermore, to argue that cell proliferation at 250 ppm is predictably a NOAEL since the tumor NOEL was 500 ppm, again suffers in that: a) 500 ppm is highly to be questioned as a NOAEL for neoplasia, as in study PR-80-006 where the dose was tested, females were virtually non responsive at any dose (up to 5000 ppm), and males were less responsive in this study than in the other two studies at equivalent dose levels. This toxicologist has no confidence that 500 ppm in the study in which it was tested revealed the magnitude of response that would have been seen at this same dose in the other two studies. And thus 500 ppm cannot be claimed as a NOAEL for neoplasia among females any more than could the 1500 and 5000 ppm dose levels in that study; b) 500 ppm may not be a true NOAEL for neoplasia, as carcinogenicity bioassays lack sensitivity to pick up on meaningful incidences, particularly of rare tumors. This is one reason for employing linear low dose extrapolation in cancer risk assessment; and c) The rationale cedes authority to the hypothesis that itself is the subject of scrutiny. The data should prove the hypothesis, and not that which is

hypothesized employed to conclude what the supporting data (which is missing) would likely be in support of the hypothesis.

Again, needed is cell proliferation data in the missing range of between 400-1000 ppm, and to compare time of onset of tumors and increased cell proliferation to assure their proper ordering in time, as prescribed in the MOA.

9) Concerning "Dose-response relationship", consider the following:

a) Autoradiographic evidence for acetochlor's binding to nasal tissues in the rat but not the mouse, while tumors occur in the rat but not in the mouse is but a correlation and not a proof of causation or of species specificity. As stated earlier, autoradiographic studies at doses up to and including the MTD (1000 ppm) should have been employed to test the critical dose range for such localization. Doses tested exceeded the MTD. The localization may reflect an innocuous sink for the compound that in effect removes it from harms way.

b) Furthermore, the absence of a neoplastic response in the mouse may simply reflect a fundamental lack of susceptibility of the mouse olfactory epithelium to tumor induction by xenobiotics.

c) The same argument applies in the case of quinoneimine-protein binding.

10) Concerning "Temporal Association"

a) "The database to assess the criterion of temporality is not complete. However, there are data to infer a sequence in time." (p. 55) Again, this toxicologist must question the reliability of inferential data.

b) For many reasons already stated in these comments and elsewhere by this toxicologist, disagreement must be acknowledged with respect to the following statement: "Thus one may conclude, there appears to be a time line for tumor formation ....." (p. 56)

11) Concerning "Strength, consistency and specificity of association of ultimate toxic effect with key events" (p. 56)

This section essentially reiterates the same assertions.

In Figure 10b, the acetochlor incidence data as plotted appears to be male data only for study PR-80-006 (500 and 1500 ppm), as the female response is virtually nil @ 500, 1500 and 5000 ppm. This study should not be compared with the other two on the same scale of comparison. Furthermore, the 500 ppm dose cannot be viewed as fairly supplying needed data in this dose range, especially for females. This is discussed more fully in Dr. Dementi's October 24, 2003

comments (Document #32, p. 4) on Dr. Kronenberg's September 9, 2003 "white paper".

12) Concerning “Biological plausibility and coherence” (p. 59)

This toxicologist agrees that the MOA is plausible, and that this class of agents clearly share in common the induction of exceedingly rare tumors of the olfactory epithelium. However, there are so many and diverse issues that have been raised, so much unknown and so many inconsistencies in the data as to render unreliable the implementation of this MOA to support an MOE approach for regulatory purposes. In the interest of public health protection, certainty regarding the hypothesized MOA must be at a higher level.

13) Concerning "Other modes of action" (pp. 59-62)

The review/comments in this section are very interesting, and could have their application. But how much reliance is to be based upon this information as actually addressing the issue at hand regarding mechanism of action in nasal neoplasia? To this toxicologist, your text is all very speculative, and affords nothing to hang your hat on. You have not shown this has anything to do with acetochlor. You say "Additionally, there are no data available on acetochlor to fully test the plausibility of oxidative stress as an early and critical event leading to frank tumor formation in rat nasal olfactory tissue." (pp. 61-62) You then well suggest studies that could be done.

14) Concerning "Assessment of postulated mode of action" (pp. 63-64)

I have no additional comments as these would be redundant, i.e. having been said previously. I must say however, I disagree that the confidence on this mode of action is as high as "moderate to high". (p. 64) *In my view, it is not high enough to embrace a MOE approach to regulation.*

15) Concerning "Uncertainties, inconsistencies and data gaps" (p. 64)

It would be redundant to express again my concerns. They are presented elsewhere.

16) Concerning "Relevance to Human Cancer Assessment" (pp. 66-end)

a) The SAP quotation in the first paragraph (p. 66) is very indicative of SAP's concern that absence of a mouse neoplastic response may be misleading insofar as such findings indicate a lack of human relevance. This is a very essential matter that was never addressed.

b) The section on metabolic differences between rats and other species is very interesting. Much of the reasoning and evidence would probably be very relevant if the quinoneimine is the carcinogen, but if not the discussion may be moot. Agreed that "It is not fully known if the qualitative differences between rats and mice exist between humans and rats" (p. 67)

You have done a good job. There is so much information that has accumulated on this subject, that it becomes well nigh impossible to address it in one episode of review.

Brian Dementi

Exhibit V

Brian Dementi
01/30/04 01:05 PM

To: Linnea Hansen/R9/USEPA/US@EPA, Alberto Protzel/DC/USEPA/US@EPA, Larry Chitlik/DC/USEPA/US@EPA, Nancy McCarroll/DC/USEPA/US@EPA, Susan Makris/DC/USEPA/US@EPA
cc:
Subject: RE: Dr. Kronenberg's Email, subjects of interest pertaining to acetochlor.

Colleagues,

In response to this Email from Dr. Kronenberg, I would like to offer the following comments.

Concerning the question of nasal olfactory epithelial cytotoxicity and cell death following alachlor and acetochlor administration to rats, the registrant has claimed in several documents that the neoplastic response in such tissues proceeds, mechanistically, or as a mode of action, as follows, : "cytotoxicity > cell proliferation > neoplasia", where cytotoxicity is said to result from quinoneimine-protein binding within the cell. In certain Monsanto documents, cell death is claimed as an aspect of the evidence of cytotoxicity [see for example, Heydens, W. F. (1996) "An Evaluation of the Oncogenic Potential of Alachlor", where the following relevant text appears: "DEIQ has been shown to bind to cellular protein and most likely changes its structure/function, thereby causing cell death(emphasis added)" (p. 10) I should note that the Heydens (1996) article was among those documents in an October 1, 1996 package assembled by Monsanto for presentation to the SAP at its October 1996 meeting to consider alachlor. This claim of cell death associated with alachlor administration finds its expression in other Monsanto documents. Thus I was seeking from Dr. Kronenberg any works on alachlor or acetochlor where there was a showing of cytotoxicity, and cell death in particular, that would justify Monsanto's claims.

Well, as it turns out, there are no research papers or publications in which cell death has been demonstrated as a result of alachlor treatment. So I believe the registrant overextended his claims concerning the

magnitude of toxicologic effects on the olfactory epithelium, and I believe Dr. Kronenberg is here acknowledging the same.

I should qualify one response from Dr. Kronenberg wherein he speaks to my question of whether cell death is critical in demonstrating the mode of action. I was not suggesting that it is critical, only questioning whether the claim of cell death can be substantiated as at least one of the many possible evidences of cytotoxicity, as cell death would certainly qualify as most compelling evidence of cytotoxicity.

So we understand at this point that the registrant does not have evidence to support a claim of cell death as resulting from alachlor administration, even at high doses. And the same is true for acetochlor. Per Dr. Kronenberg: "As discussed, we do not have any data demonstrating olfactory death from exposure to either acetochlor or alachlor."

So what other evidence is there for cytotoxicity? It is clear from Dr. Kronenberg's response and information we have on hand that there is no evidence to support a claim of acetochlor induced cytotoxicity, even at doses exceeding the MTD. Perhaps the necessary studies to demonstrate the effect have not been conducted with acetochlor, although a finding of this sort (e.g. ablation or sloughing of the olfactory epithelium) might have been confirmed, were that the case, in the carcinogenicity bioassays, particularly at those doses that exceeded the MTD.

In the case of alachlor, Dr. Kronenberg asserts there is adequate evidence in support of a claim of cytotoxicity, and proceeds in his correspondence to document that evidence. Please bear in mind as I comment on that evidence, the evidence is expected to represent the registrant's best and most substantial documentation known to him for alachlor induced cytotoxicity. The evidence is as follows:

1) Increased acid phosphatase leakage after in vitro exposure (MRID 43641602).

2) Increased expression ot two cellular stress genes (HSP70 and NMO) following 60 days of in vivo exposure at the oncogenic dose level of 126 mg/kg/day (MRID 43590002).

3) Dr. Kronenberg then cites a publication, Genter et al (2002). The full citation is not provided, but appears to be Genter et al (2002) "Genomic analysis of alachlor-induced oncogenesis in rat olfactory mucosa",

Physiol. Genomics 12, 35-45. His cite reads as follows; "'alterations in extracellular matrix components, induction of oxidative stress, upregulation of ebnerin, and .....wnt signaling pathway activation' when administered to rats at the oncogenic dose level of 126 mg/kg/day".

This appears to be the sum total of the evidence for alachlor induced cytotoxicity. I should note that 126 mg/kg/day is a very high dose, approaching if not at the MTD for alachlor. There would appear to be no testing for this kind of evidence of cytotoxicity (if indeed it is to be viewed as cytotoxicity) at lower doses that might be performed in search for a NOEL for this "key event(s)" needed to properly assess carcinogenicity dose response under EPA's draft Carcinogen Risk Assessment Guidelines.

The evidence for cytotoxicity is unimpressive. Regrettably, I know of no published guidelines that might help identify or characterize the kinds of evidence that would or would not support a claim of cytotoxicity. Thus, in my view, I find it difficult to accept this evidence as justifying a claim of cytotoxicity. Cells may well endure this level of insult without significant degeneration, or inducement to proliferate, but who knows for certain. The studies were not conducted at lower doses to answer the question of whether these types of perturbations occur that could lead to meaningful incidences of these rare nasal tumors at lower doses in a larger cohort of animals than those employed in the cancer bioassays.

Phenacetin has been claimed as inducing olfactory cytotoxicity and as supporting a claim for this effect in the case of alachlor. However, as a few of us will recall from our recent conference call with Dr Genter, she acknowledged from work in her laboratory the induction of cytotoxicity (specifically, sloughing of the olfactory epithelium) by phenacetin, but by contrast said there was no such effect with alachlor. Dr. Genter was very emphatic in owning a remarkable contrast existing between phenacetin and alachlor in this respect. Phenacetin induces cytotoxicity, alachlor does not. So as I see it, the use of work on phenacetin, which does indeed induce the nasal tumors, in support of the claimed mechanism for alachlor is inappropriate.

Dr. Kronenberg mentions the evidence for increased cell proliferation, but has this been studied adequately at lower doses? Cell proliferation in the proposed mode of action is viewed as following cytotoxicity, which itself has not been established. Cell proliferation need not be linked to or necessarily follow only cytotoxicity, but may result from a plethora of

other molecular events that are unknown, and being unknown cannot be assayed to determine the NOEL/LOELs for such possible "key events" in the neoplastic process. So, cytotoxicity cannot be used as an event ("key event") to assess dose response, or used as the basis for identifying a NOAEL, or dose level below which a carcinogenic effect would be exempted.

Dr. Kronenberg says that "No cell proliferation was noted in rat nasal respiratory tissue or in mice." Presumably he refers to both respiratory and olfactory tissue in mice. It is important that if this effect is peculiar to the olfactory tissues of rats that tumor induction be noted at that cite. However, in the rat reproduction study now under review on acetochlor, tumors in offspring are reported in both epithelia. That study as well as the carcinogenicity studies need to be carefully reviewed and characterized in this respect.

Dr. Kronenberg then says "Therefore, based on the overall weight of the evidence, the EPA and SAP agreed with the ARP that this was the likely common mechanism of action for alachlor and acetochlor induced nasal tumors." In my view, alachlor, acetochlor and perhaps others of the chloroacetanilide class share in common the induction of nasal olfactory tumors, an exceedingly rare neoplastic response. The sheer rarity of these lesions in control animals supports the reality of their induction by these chemicals, even at low incidences. There is little doubt these chemicals share in common the induction of these tumors. Can it be said for certain that EPA and SAP decisively concluded anything more in terms of what explains the induction of these tumors? Having read the EPA and SAP reports, I find a lack of clear affirmation of the mode or mechanism. But these documents need to be examined carefully for the conclusion on this issue. Furthermore, the acetochlor work might influence the views on alachlor, and in that sense the question of mechanism or mode of action for the class is unsettled.

The registrant appears to have revised his characterization of the mode or mechanism of action to say that: "We now recognize that there may be more than one possible molecular mechanism by which a DABQ1 metabolite produces cell proliferation and ultimately tumors." And ".....the rat nasal tumors result from formation of DABQ1 metabolites by rat nasal tissue. It is the species-specific formation of these DABQ1 metabolites, not cytotoxicity or cell death (emphasis added), that is considered the key precursor event to neoplasia." First, is this anything more than speculation? If this is true, there is no longer a parameter, such as cytotoxicity, to serve as a basis for identifying a NOAEL for the effects of the chemical. What

parameter might one use in this case to assess the propensity for the DABQ1 to have a toxicologic effect? Might there be a genotoxic component? Quinoneimine-protein binding in olfactory tissue, which might possibly be viewed as a "key event" in the neoplastic process as claimed, has not been assessed in any kind of long term study that might show dose-related and time-dependent increases in protein binding that could possibly be expected to rise to such levels as to result in cell proliferation, and, thence, neoplasia. In other words, there are no parameters other than tumor incidence itself upon which basis to identify a NOAEL for the neoplastic response. But the carcinogenicity bioassay may not incorporate enough animals, particularly in the low dose groups, to identify a real and meaningful increase of incidence in these very rare tumors. Cell proliferation might possibly be used, but in the case of acetochlor, cell proliferation has not been adequately evaluated at lower doses in long term studies, in oder to fathom or characterize any possible dose response that could lead to cancer.

On any case, these are some of my views, and I would be pleased to hear yours.

Best Wishes,
Brian Dementi

----- Forwarded by Brian Dementi/DC/USEPA/US on 01/28/04 10:44 AM -----
"KRONENBERG, JOEL M [AG/1000]" <joel.m.kronenberg@monsanto.com>
01/21/04 06:12 PM

To: Brian Dementi/DC/USEPA/US@EPA
cc: Alberto Protzel/DC/USEPA/US@EPA, Linnea Hansen/R9/USEPA/US@EPA, "'Juberg, Daland (DAS)'" <drjuberg@dow.com>
Subject: RE: Questions of interest pertaining to acetochlor.

Brian:

This note is an attempt to summarize our discussions of last week concerning the mode of action for nasal tumors produced by alachlor and acetochlor. The two main issues discussed were: (1) lack of evidence of cell death in the nasal tissues and (2) whether or not evidence of cell death is critical in demonstrating the mode of action. I believe your concern was that cytotoxicity leading to cell death was an essential

element of the proposed mode of action but was not sufficiently demonstrated for either alachlor or acetochlor.

As discussed, we do not have any data demonstrating olfactory cell death from exposure to either acetochlor or alachlor. However, there is sufficient evidence of olfactory cytotoxicity following exposure to alachlor. This evidence consists of increased acid phosphatase leakage after in vitro exposure (MRID 43641602) and, more importantly, increased expression of two cellular stress response genes (HSP70 and NMO) following 60 days of in vivo exposure at the oncogenic dose level of 126 mg/kg/day (MRID 43590002). No change in the activity of the stress genes was noted after 30 days of exposure. Further evidence of olfactory cytotoxicity is provided by the recent publication of Genter et al. (2002) which concluded that alachlor produced "alterations in extracellular matrix components, induction of oxidative stress, upregulation of ebnerin, and ... wnt signaling pathway activation" when administered to rats at the oncogenic dose level of 126 mg/kg/day.

As you mentioned in our conversation, the mechanism of action previously proposed for nasal tumors induced by alachlor and acetochlor was formation of DABQI -> nasal protein adducts -> cytotoxicity -> cell death -> cell proliferation -> neoplasia. Although no direct evidence for cell death was observed, a dose-related, reversible increase in cell proliferation was produced in the olfactory tissue of rats following repeated exposure to the oncogenic dose levels of 42 and 126 mg/kg/day of alachlor. No cell proliferation was noted in rat nasal respiratory tissue or in mice. Therefore, based on the overall weight of evidence, the EPA and SAP agreed with the ARP that this was the likely common mechanism of action for alachlor and acetochlor induced nasal tumors.

In the past few years, new information has become available that suggests that cell death may not necessarily be an essential precursor step in the progression to cell proliferation and neoplasia. There are now a number of possible mechanisms by which quinones such as those produced by alachlor, acetochlor and phenacetin (which also produces olfactory tumors) are believed to produce cytotoxicity and ultimately neoplasia (see Bolton et al., 2000 or Section 5.2.5 of our September 5th document). However, while the precise molecular mechanism(s) by which these quinones actually produce cytotoxicity, cell proliferation and neoplasia may not be known, this information isn't critical to the human risk assessment process or to the determination of a "common mechanism of toxicity" as described by FQPA and current EPA policy. As discussed in our September 5 document

(summarized in last paragraph in Section 5.2.5), the ARP has now slightly revised the proposed mode of action for the!
chloroacetanilides. We now recognize that there may be more than one possible molecular mechanism by which a DABQI metabolite produces cell proliferation and ultimately nasal tumors. It is possible that the DABQI-nasal protein adducts are just markers of exposure and that cell death may not be an obligatory step to cell proliferation. However, regardless of the precise molecular mechanism leading to cell proliferation and ultimately neoplasia, the overall "mode" of toxicity remains the same as previously proposed: the rat nasal tumors result from formation of DABQI metabolites by rat nasal tissue. It is the species-specific formation of these DABQI metabolites, not cytotoxicity or cell death, that is considered to be the key precursor event to neoplasia.

I hope this helps address your concerns. Please let me know if I can be of any further assistance.

Regards,

Joel

-----Original Message-----
From: Dementi.Brian@epamail.epa.gov
[mailto:Dementi.Brian@epamail.epa.gov]
Sent: Thursday, January 08, 2004 11:29 AM
To: KRONENBERG, JOEL M [AG/1000]
Cc: Protzel.Alberto@epamail.epa.gov; Hansen.Linnea@epamail.epa.gov
Subject: RE: Questions of interest pertaining to acetochlor.

Dear Dr. Kronenberg,

Thank you for the responses to my questions.

As a follow-up to question #4, I continue to have problems with understanding the precise nature of the cytotoxicity said to follow alachlor administration, where that cytotoxicity is hypothesized to be a component in the progressive stages to neoplasia of the nasal olfactory

epithelium.

I am very much interested in having the complete characterization of alachlor induced cytotoxicity in the registrant's own words, accompanied by supporting documentation. As I attempt to characterize this effect, I find myself uncertain as to whether I have covered all bases, and haunted by the possibility of having neglected to locate some one or more element of that expression of cytotoxicity. My question here is not so much focused upon what causes the cytotoxicity, but rather, how that effect is expressed, as for example in terms of molecular or cellular pathology.

Again, since cytotoxicity is such an important aspect to the neoplastic response as Monsanto claims, it would be particularly noteworthy to have the full characterization presented by the registrant himself, the one who knows it best in its fullness, and who could find no fault with that characterization. Also, this is something that, with confidence, I can then refer to others as the most authoritative characterization of that cytotoxicity which precedes cell proliferation in the hypothesized continuum to neoplasia.

In response to your question, as I understand committee meetings have not been scheduled.

I hope to hear from you soon.

Best Wishes,
Brian Dementi

"KRONENBERG, JOEL M [AG/1000]" <joel.m.kronenberg@monsanto.com>

To: Brian Dementi/DC/USEPA/US@EPA

cc: Linnea

Hansen/R9/USEPA/US@EPA, Alberto
12/19/03 02:07 PM
Protzel/DC/USEPA/US@EPA, Larry

Chitlik/DC/USEPA/US@EPA, Nancy

McCarroll/DC/USEPA/US@EPA, Susan

Makris/DC/USEPA/US@EPA
Subject: RE: Questions of interest pertaining to acetochlor.

Brian:

I am in the process of moving offices and many items are still in boxes. In addition, I am leaving for vacation in a few minutes and won't be back until January 5. Thus, rather than cause you any delay, I will try my best to provide quick answers now and can provide further details if needed in January. I have inserted my responses below your questions.

Hope this helps.

Have any of the committee meetings been scheduled yet?

Happy Holidays!

Joel

-----Original Message-----
From: Dementi.Brian@epamail.epa.gov
[mailto:Dementi.Brian@epamail.epa.gov]
Sent: Wednesday, December 17, 2003 1:30 PM

To: KRONENBERG, JOEL M [AG/1000]
Cc: Hansen.Linnea@epamail.epa.gov; Protzel.Alberto@epamail.epa.gov; Chitlik.Larry@epamail.epa.gov; McCarroll.Nancy@epamail.epa.gov; Makris.Susan@epamail.epa.gov
Subject: Questions of interest pertaining to acetochlor.

Dear Dr. Kronenberg,

To further assist in our on-going assessment of the carcinogenicity of acetochlor, it would be helpful if you could respond to the following questions.

1) Are the nasal olfactory tumors the same, histopathologically, as identified in the rat Guideline carcinogenicity studies on alachlor and acetochlor, and in the acetochlor reproduction study now under review?

** YES, the same tumors were noted in all of these studies

2) The structural and toxicological similarities between alachlor and acetochlor are remarkable and well recognized. I would be curious if you could provide any information you may have summarizing any striking, or particularly noteworthy, biological effects differences existing between the two?

** As you indicated, the tox profiles for alachlor and acetochlor are very similar. There are some minor differences but these could be caused, at least in part, by differences in strain of animal, laboratory, methodology, animal variability, etc. The 2 most prominent differences that come to mind are that alachlor caused ocular lesions, which were thought to be strain-specific (Long-Evans used for alachlor, Sprague-Dawley used for acetochlor), and stomach tumors in the chronic rat studies while acetochlor did not.

3) Please provide any summaries you might have of historical control incidences for rat (all strains) nasal (olfactory) tumors of the kind identified in the alachlor and acetochlor Guideline cancer bioassays and in the reproduction study now under review.

** Unfortunately, we do not have any summary of historical data for nasal tumors readily available. However, although these tumors are uncommon, single instances of such tumors have been observed in control animals in at least 2 studies, one of which was the 2nd Monsanto chronic rat study with acetochlor (ML-83-200).

4) Cytotoxicity is a fundamental element of the claimed progression to neoplasia in the rat for acetochlor, i.e. cytotoxicity > cell proliferation > neoplasia. As we have discussed in the past, I understood there was no available evidence for cytotoxicity of the nasal olfactory epithelium following treatment with tumor inducing doses of acetochlor. Is it fair to conclude this remains so for acetochlor, or might you add anything in this regard? Mainly though, would you please summarize for us the evidence for such comparable cytotoxicity as may follow alachlor treatment at doses inducing nasal tumors. Please make distinctions as to the dose levels at which cytotoxicity is observed. I would greatly appreciate receiving your own characterization of this cytotoxicity.

** We have no new data since our presentation on July 28, 2003, or submission of our summary document (Acetochlor: Justification for Reclassification of Carcinogenic Potential, dated September 5, 2003). Thus, you are correct in that we have not demonstrated cytotoxicity for acetochlor, only for alachlor. Cell proliferation was demonstrated for both alachlor and acetochlor but the response for alachlor was much stronger (it is also a more potent nasal carcinogen). The data for alachlor are briefly summarized in the RED (EPA, 1998) and in Sections 5.2.2.5 and 5.2.2.6 of our September 5 summary document. In addition, please keep in mind that the mode of action for the acetanilide-induced rat nasal tumors is not unique, as best evidenced by the fact that similar tumors are also produced by quinonimines formed from phenacetin. The various mechanisms by which quinones are believed to induce cytotoxicity were discussed during our July meeting and are briefly summarized in Section 5.2.5 of our September 5 document.

5) In reference to the protein binding studies in the rat (MRID 44496210), wherein the EMIQ-cysteine adducts in "nasal turbinates" were found to be 119 and 206 pmole/mg protein @ the respective dietary dose levels of 1710 and 5170 ppm, and in the mouse (MRID 44496211), what

would be the expected proportions of olfactory versus respiratory epithelium of the nasal tissues that were taken to assess the levels of protein binding?

** Unfortunately, I do not have any specific information available to answer this question. I would guess that both rats and mice have reasonably similar ratios of olfactory to respiratory tissue, but am not certain. I believe they both have MUCH higher amounts of olfactory tissue than either monkeys or humans. I will try to find a better answer in January.

Best Wishes,
Brian Dementi

Exhibit VI

Brian Dementi
05/28/03 10:39 AM

To: Joel.M.Kronenberg@Monsanto.com
cc: Linnea Hansen/R9/USEPA/US@EPA, Alberto Protzel/DC/USEPA/US@EPA
Subject: Comments on acetochlor MRID 44496201

Dear Dr. Kronenberg,

As promised in our conference call of May 20, please find here my comments directed to: "Acetochlor: Assessment of Oncogenic Potential in Rodents and Lack of Relevance to Humans", Clapp, MJL et al, January 28, 1998; Report No:CTL/P/5667; MRID 44496201

The following comments are rendered primarily to help secure ready access to fundamental sources of scientific information referred to in the text. This will facilitate evaluation of the data base, and render conclusions more defensible. In citing reference materials, it would be particularly helpful and most desirable to provide MRID numbers where known. Documents are most readily retrievable under MRID numbers.

In certain cases these comments call for further assessment.

There comments are not necessarily thorough, as this is a learning process for at least some of us, and the comments have been somewhat hastily prepared in order to expedite the review process. In any case, it is important that the record carry complete documentation for the benefit of various individuals and committees in performing their tasks, and for purposes of any future reference.

Furthermore, these comments are pertinent to your documentation as of early 1998, thus we assume that the white paper you are preparing will contain new information that you might have acquired since then, and will address the issues raised in our phone conversation of May 20.

1) P. 8: EPA's Proposed Guidelines for Carcinogen Risk Assessment (are now draft EPA, 2003)

2) P. 9: "This metabolite forms protein adducts, which lead to

cytotoxicity,....." Cite reference(s) illustrating cytotoxicity.

3) P. 9: "For example, the ability of rat nasal tissue to metabolize alachlor to DEA-phenol .....is approximately 22, 000-fold higher than that of humans." Citation

4) Pp. 9-10: "Results from other mechanistic work (citation).....provide strong evidence that this non-genotoxic mechanism is operative in the production of alachlor-induced tumors in rats."

5) P. 10: "It was concluded (by the Agency), that acetochlor could be grouped together with alachlor and butachlor for nasal tumor induction....."

6) P. 10: "The Acetochlor Registration Partnership (ARP) has generated additional studies which further support the Agency's conclusion (citation) that acetochlor produces tumors in rats by the same modes of action established previously for alachlor".

7) P. 10: "This document (full citation) reviews all of the data showing a common oncogenic mode of action for acetochlor and alachlor....., and outlines the basis for concluding that the oncogenic responses.....in rats should not be extrapolated to humans exposed to low levels of acetochlor."

8) P. 11: "The MTD for acute oral exposure in the rat when used in in vivo genotoxicity screens was established at 500-800 mg/kg based on lethality and hepatotoxicity." Citation

9) P. 11: Text indicates that the long term dietary MTD is 1000 ppm for both rats and mice (citation), while three out of five carcinogenicity studies were conducted at top doses exceeding the 1000 ppm dietary levels. Please offer comment on the question as to whether there is adequate carcinogenicity testing at doses up to and including an MTD.

10) P. 11: "Extensive studies.....have led to the (registrants'?) conclusion that acetochlor is not mutagenic to....."

11) P. 14: "Supporting this conclusion is the fact that acetochlor is not genotoxic to the olfactory nasal epithelium (citation), ..."

Note: Concerning the preceding pages on genotoxicity, further comment is reserved pending HED's mutagenicity assessment.

12) P. 14: "These tumors were predominantly benign....." It would be helpful to include a brief statement as to the relative prevalence of benign and malignant tumors, and to comment on the historical incidence (rarity) of these particular olfactory tumors in this strain of rat. Analogous comment was rendered (p. 15) in reference to lung tumors in the mouse: "These tumors are common in the mouse strain (CD-1) used....."

13) P. 15: The results of the PWG review.....indicate that the apparent increase (statistical significance ?) in incidence with treatment is of no biological significance (Hardisty, 1997c)." It is my understanding that under Pesticide Regulation (PR) Notice 94-5, a PWG involves the re-evaluation of pathology readings. As quoted from Notice 94-5: "From time to time the Office of Pesticide Programs receives requests for re-consideration of Peer Reviewed decisions based on re-evaluations of the pathology readings. These re-evaluations reflect voluntary activity on the part of the registrants, and are not the result of a requirement imposed by the Agency. The Agency is then asked to disregard the original readings and base its evaluation (emphasis added) on the most recent ones. As a result the Agency may have two (or at times even more) pathological diagnoses for the same study." This notice provides a mechanism for formally re-reading histopathology slides. It is not a provision for rendering risk assessment. Thus to say that the tumors are of no biological significance goes well beyond the Notice's provision for simply the re-diagnosing of slides. Rather, once histopathology readings have been rendered under this Notice, it becomes the duty of the Agency's CARC to render conclusions regarding such matters as biological relevance. Further, it is my understanding that pathologists who participate in a PWG, are highly expert in reading (interpreting, diagnosing) histopathology slides. Risk assessment, on the other hand, draws upon more diverse expertise, e.g. toxicology, clinical chemistry, biochemistry, statistics, pathology, exposure assessment, etc. and derives from a wide range of background materials usually not available at a PWG.

The PWG is expected to provide authoritative and impartial diagnoses of histopathology slides, and that is all which is provided for under the August 24, 1994 PR Notice 94-5. This is my understanding, at least.

These views similarly apply to the Hardisty 1997a and 1997b PWGs as

discussed on p. 16.

14) P. 18: "Studies (citations) with alachlor have provided support for the involvement....."

15) P. 18: "In contrast, a similar study (citation) with mice showed no evidence of localization." One might be inclined to conclude from your discussion that this absence of nasal tissue localization in mice was observed in Lau et al, 1997b, while that study, apparently MRID 44496211, did not employ whole body or microautoradiography studies.

16) P. 19: First paragraph under 4.1.3, suggest providing citations for the alachlor studies mentioned.

17) P. 19: "The formation of these adducts, under conditions which produced nasal tumors in the chronic bioassays, ..." Provide comment as to whether formation of these adducts would be expected to occur at doses below, possibly well below, those that produced nasal tumors in chronic bioassays. This is particularly important in that to the extent that such protein binding is to be interpreted as a "key event" in the neoplastic process, EPA's draft Proposed Carcinogen Risk Assessment Guidelines call for consideration of such "key events" along with tumor incidence in the assessment of dose response.

18) P. 19: "In vitro studies (citations) have revealed significant interspeciese differences in nasal turbinate metabolism of alachlor and its metabolites."

19) P. 20: "Similar studies (citations, presumably for example, Green 1997d, as on p. 36) with acetochlor....."

20) P. 20: "Acetochlor administration at 200 ppm for 160 days did not induce cell proliferation (at all, or just not statistically significantly so?), nor were tumors observed ....." It is noteworthy that a vast untested dosage range exists between 200 ppm and 1750 ppm.

21) P. 21: "It is widely recognized that prolonged disturbance of thyroid-pituitary homeostasis is associated with the development of thyroid follicular hyperplasia and neoplasia in experimental animals." It would be very helpful to provide at least one very excellent authoritative citation which presents these concepts, particularly as developed in the first two paragraphs under 4.2.1.

22) P. 21: "Studies (citations) with alachlor have shown that high dose exposure results....."

23) P. 21: "Both the SAP (citation) and the CPRC (EPA, 1997a) agreed....."

24) P. 22: Under 4.2.2, what, if any, effects were observed on these thyroid parameters at 200 ppm. If none, you might so indicate.

25) P. 22: "These results provide strong evidence that acetochlor.....as demonstrated previously for alachlor (citation)."

26) P. 23: "This metabolite forms protein adducts, which lead to cytotoxicity (how manifested?),prolonged cell proliferation, and ....." "Critical differences (citation) in enterohepatic circulation and tissue specific metabolism.....to humans."

27) P. 23: "Similar studies (citations) have been conducted with acetochlor which have demonstrated....."

28) P. 23: "These data provide evidence that a threshold dependent non-genotoxic mechanism is operative....." What is your best estimate as to magnitude of that threshold?

Best Wishes,
Brian Dementi, Ph.D., D.A.B.T.

Exhibit VII

October 24, 2003

Review/Commentary by Dr. Brian Dementi on the July 18, 2003 draft document: ***"Acetochlor: Justification for Reclassification of Carcinogenic Potential" - Acetochlor Reregistration Partnership.***

This July 18 draft document was informally provided attendees at the July 28 "ARP-EPA Meeting on Oncogenicity of Acetochlor" held at the Crystal City Marriott. The *final version* of the ARP paper, dated September 5, was received by this reviewer on October 8. *Hence, the comments presented here were developed in reference to the July 18 draft.* A scan of the September 5 final report reveals but little though some significant departure, as noted, with respect to the July 18 draft. One very notable and significant departure between the two versions is presented in the last paragraph of item 16 below.

This paper is said to be a "supplement to, and not a replacement for a previous ARP document assessing the oncogenic potential of acetochlor (Clapp et al., 1998, MRID 44496201." (pp. 7-8)

According to the registrant: "The primary purpose of this document is to *'briefly'* (emphasis added) outline the available data and summarize the rationale of the Acetochlor Registration Partnership (ARP) regarding its request for cancer reclassification and modification of the approach used for oncogenic risk assessment." (p. 7)

1) This paper has been particularly helpful to this reviewer in characterizing alachlor status, and in paving much of the way toward an understanding of the like behavior of acetochlor.

2) Since the two compounds, alachlor and acetochlor, are so similar, structurally, one methyl group transposed, same molecular weight - they are in effect isomers of a kind. It would not be surprising that chemically they behave very similarly. It would be instructive for the registrant to list or summarize the toxicologic and efficacious differences between the two. Has the registrant found any fundamental toxicologic differences between the two that are worthy of note in understanding comparative mechanisms of behavior?

3) (p. 6) What does it mean to say that acetochlor was "conditionally" registered by EPA?

4) What is EPA's conclusion on acetochlor mutagenicity?

5) (p. 6) It is still not clear as to what is meant by "cytotoxicity" in the case of nasal tissue response to acetochlor. Registrant has not provided a characterization of cytotoxicity despite the fact that cytotoxicity is claimed repeatedly as fundamental to the neoplastic response. Gross and/or

histopathologic evidence of cytotoxicity should be provided if the claim of cytotoxicity is to have evidentiary meaning in support of the hypothesis. To the extent that acetohlor may behave like alachlor, it would be worthwhile to provide a full assessment characterizing any such effects in alachlor. Some of this rationale is presented at section 5.2.2.5 "*In vitro* Cytotoxicity", but is that the extent of the evidence, and were there no other evidence, is it sufficient to support a claim of cytotoxicity in the neoplastic process, in search of an MOE approach to risk assessment?

Is the absence of such data for acetochlor due to the lack of comparable testing, or simply because acetochlor behaves differently? Nevertheless, for the claim to carry sway for acetochlor, on so important a subject, the evidence of cytotoxicity must be demonstrated for acetochlor. Ashby et al (1996) indicate that in the case of acetochlor, nasal tumors ".....were preceded by a dose- and time-related increase in the incidence of S-phase cells in the nasal olfactory cells ...." (p. 731) Would this phenomenon constitute some form of molecular "cytotoxicity" that would help satisfy the missing evidence for cytotoxicity critical to the proposed neoplastic progression for acetochlor? Should this be assayed for and employed as a "key event" as defined and employed to extend the carcinogenic dosage range under EPA's 1999 or 2003 draft Carcinogen Assessment Guidelines?

6) (p. 6) The progression: cytotoxicity > cell proliferation > neoplasia - is all very vague and ill defined, little more than a brush- stroke accounting of what must be an extremely complex molecular process.

7) (p. 7) Mentions 2003 EPA draft Carcinogen Guidelines - what is the status of these Guidelines?

8) (p. 7) Apparently alachlor is classified under 1999 Guidelines as "Likely to be carcinogenic at high doses but not likely at low doses". Is this in fact its current classification?

9) *The registrant is here requesting "cancer reclassification and modification of the approach used for oncogenic risk assessment."* (p. 17) The registrant evidently desires the same MOE approach for acetohlor (if carcinogenic) as EPA has granted alachlor.

10) (p. 8) Has EPA confirmed or concurred on proof of structure of acetochlor?

11) (p. 8) It would appear the registrant desires reclassification from B2 (1986 Guidelines) to "Likely at high doses only (1999 draft Guidelines) - and if the latter - use MOE approach - but really prefer "not likely in humans".

As a point of interest, is a quantitative risk assessment currently applied to acetochlor?

12) (p. 9) Says with regard to acetochlor, nasal tumors predominantly benign/non-life threatening/with little progression the second year. Note from my own observations - Genter, M.B. and colleagues have investigated the nasal carcinogenicity and genotoxicity of alachlor, and

various publications by these investigators need to be summarized. For example, in Burman et al (2003), as cited in comment 16 below, the authors say: "We have shown that alachlor-induced olfactory mucosal tumors (polypoid adenomas and adenocarcinomas) occur with a relatively short latency (i.e. following 5 months of exposure at 126 mg/kg per day in the diet) and high multiplicity, in that rats treated continuously at this level for 12-18 months often exhibited 10-20 tumors per nasal cavity [7-9]" (pp.1707-1708) These lesions are said to be life threatening, at least in the sense of causing air-way obstruction.

13) (p. 9) For all tumor types - is there adequate testing (acceptable Guideline) at and below the true MTD? Where the focus is upon nasal tumor assessment, in this reviewer's opinion, it is doubtful that the three two-year rat carcinogenicity studies on acetochlor provide adequate dose level testing at doses below the MTD. This reviewer's concern is that when discounting tumors at doses exceeding the MTD, that what remains of a study at lower doses constitutes a full and adequate study. For example, should the MTD be 1000 ppm, needed would be doses of say 200, 500 and 1000 ppm, i.e. a completely acceptable study, dose-wise. If the high dose in a particular study were found to be excessive such as to discount positive evidence of neoplasia on the grounds that dosing was excessive, there should be testing at the true MTD before accepting the study. In other words, there is a purpose for testing at proper dose levels that include an MTD and properly spaced lower levels. Studies should embody a proper number of dose groups at acceptable dose levels.

Dosage levels of acetochlor employed in three rat studies are tabulated. (p. 8) The registrant says: "MTD established at approximately 1000 ppm ..... in rats....." (p. 9), for which no supporting documentation is referenced. The registrant should provide supporting documentation for this important claim of MTD in the rat. Actually, the February 11, 1997 PWG report on hepatocellular neoplastic responses (MRID 44496205) concluded that: "Examination of mortality and body weight data from these three studies indicate that the MTD for acetochlor in Sprague Dawley rats is estimated to be greater than 1000 ppm and less than 1500 ppm in the diet when exposed for up to 104 weeks." (p. 26)

As the neoplastic response in rats concerns *nasal olfactory tumors*, the views expressed here follow those developed by the registrant in Ashby et al. (1996): "The only biologically significant tumors induced in rodents by acetochlor at MTD doses were of the nasal olfactory epithelium in both sexes of rat (Figure 10). *This response was confined to the MTD dose (Figure 7; 1000 ppm acetochlor), and above* (emphasis added). Five characteristics of these tumors were, first, that they were only visible microscopically and did not affect the health or longevity of the animals. Second, there was no significant development of the tumors during the second year of the bioassay. Third, no metastasis were recorded. Fourth, that they were preceded by a dose- and time-related increase in the incidence of S-phase cells in the nasal olfactory cells (Table 18). Finally, the tumors were not preceded by DNA damage to nasal olfactory or respiratory cells....." (p. 731)

This reviewer would like to offer comment on the question as to whether the chronic rat studies cited should be viewed as to their adequacy of addressing this neoplastic response at doses below the MTD. But first, be it noted that in Ashby et al, there is no mention of the rarity of the nasal tumors in their characterization of the response. By contrast, in discounting mouse lung tumors (p. 730), the authors advise that "....the incidence of lung (and liver) tumors in CD-1 mice is known to be variable, and was particularly low in the concurrent control groups. This is illustrated by the recent historical control lung tumor data shown for the laboratory that conducted one of the mouse bioassays....." "Further, the highest incidence of lung tumours in the acetochlor groups (11 of 60; 18.3% tumor bearing animals) falls within the control range for the laboratory." (p. 730) Indeed, the bulk of the authors' rationale for discounting mouse lung tumors in the acetochlor study, resided with the high historical incidence. Yet, in discussing nasal tumors, the authors' provide no mention of the historic incidence of these tumors. One might ask, are these ever observed in the control rats, and if so, with what incidence? Furthermore, there are no histopathologic descriptors for what may well be very unusual nasal tumors. There is an obligation on the part of the registrant to discuss rarity, historic incidence and histopathologic features of these unusual nasal tumors. It should be noted that rare tumors need not reach statistical significance in cancer bioassays to be considered treatment related and of concern in human risk assessment.

Further to the question of whether there has been adequate testing for nasal tissue tumors in the rat at doses below the MTD. The concern that testing may not be adequate is best exemplified in consideration of the female data. There are three chronic bioassays, the results of which in terms of nasal adenomas are consolidated in Figure 10 (p. 730) of Ashby et al (1996). In one of the bioassays (Acc No 071962071965) (dose levels 0, 500, 1500 and 5000 ppm) (Ashby et al. p. 706), there was virtually no nasal tumor response in females at any dose level, up to 5000 ppm. This was essentially a negative study among females. Yet, by contrast, in a second study (MRID 400770601) (dose levels 0, 40, 200 and 1000 ppm), there was a remarkable response at the top dose, 1000 ppm, the dose claimed in Ashby et al to be the MTD. The next lower dose in this study, 200 ppm, was negative. And in a third study (MRID 41592004) (doses 0, 18, 175 and 1750 ppm, there was, also in contrast to the first study, a remarkable response at the top dose, 1750 ppm, and similarly to the second study, the next lower dose, 175 ppm was negative. Given the positive findings in two studies at the top doses (1000 and 1750 ppm, respectively), in order to properly test at and below the MTD of 1000 ppm, there should be a dose level of testing somewhere in the general range of 400 to 600 ppm before concluding that this neoplastic response is limited to the MTD. Now, the first study did incorporate a dose level of 500 ppm, but this study, as explained at the outset, was in essence a negative study. Ideally, there should have been a dose level of 400-600 ppm *in the very same study which illustrated the positive response at the MTD (1000 ppm).* The negative finding at 500 ppm in the second study, which was negative as well even at 1500 and 5000 ppm, cannot serve in this regard. For reasons unknown, one study on a particular compound often fails to confirm another study. The positive study (in this case two studies) in the same rat strain (Sprague Dawley in this case) is being used to identify the positive response, as it should.

This reviewer's basic conclusion is that the two chronic studies illustrating the nasal tissue neoplastic response among *female* rats, do not incorporate adequately high (intermediate) doses below the MTD of 1000 ppm, to justify concluding the effect is limited to doses at and above the MTD. The *male* data tend to support the same conclusion, but not as solidly so. Male data tends to be the same as the female data in that the first chronic study yielded less remarkable findings at 1500 and 5000 ppm than one would expect from the effects seen at 1000 ppm and at 1750 ppm, respectively, in the second and third studies. Thus, in this reviewer's opinion, the 500 ppm group in the first study cannot serve as a surrogate to address needed intermediate dose testing below the MTD for males. Hence, for both sexes, females in particular, there is an intermediate dose testing void for a study wherein testing at the MTD (1000 ppm) was achieved.

Furthermore, that the nasal tissue neoplastic effect is a real effect, and should have been detected in the first chronic bioassay as discussed above, is further attested to by the finding of nasal tumors in the F0 and F1 rats of a new reproduction study (Appendix 2, p. 42), now under review in HED, and in a new one-year chronic bioassay on both acetochlor and its sulfoxide metabolite (Appendix 3, p. 54), yet to be submitted by the registrant.

The above views are in contrast to the registrant's dogma as expressed in Ashby et al (1996) concerning dose selection in the chronic bioassays, and the attendant interpretation of findings: "Analysis of this combined toxicological database is dominated by the problem of non-linear metabolism and non-linear toxicity dose responses, and our initial use of inappropriately high dose-levels. Thus, all of the adverse rodent toxicities encountered were observed only at elevated, and usually toxicologically inappropriate, dose-levels. These high doses were initially selected to ensure regulatory acceptance of the data, but were subsequently found to exceed regulatory requirements. At these elevated dose-levels, associated tissue changes were encountered that compromised interpretation and extrapolation of the toxicities observed to lower dose-levels - in particular , to those levels of exposure likely to be encountered by humans." (p. 703)

Now, while it may be true that in certain studies, doses chosen were excessive, i.e. exceeded the MTD, the registrant's remedy has not been to repeat the studies, or at least one rat study, at well chosen high and properly spaced lower dose levels, but rather to discount findings observed at high doses on the grounds of MTD exceedance, treating such findings as irrelevant, while accepting what remains of these studies in the lower dose range as constituting adequate testing. Once the MTD has been characterized, as it should be prior to conducting definitive chronic testing, the longer term study should employ the MTD and at least two, properly spaced lower dose levels, this as prescribed in EPA's testing Guidelines. When the MTD is hugely exceeded as it was in two of the three cancer bioassays discussed above, and findings are discounted for that reason, what remains are but partial studies that do not satisfy Guideline testing requirements. So to accept the registrant's argument that the neoplastic findings at high doses should be discounted, and leave it there, is tantamount to affirming the study as inadequate, un-interpretable, and in need of being repeated at proper dose levels if reliable conclusions are to be drawn. So, in this reviewer's opinion, it is woefully inadequate to discount findings for the reason of MTD dosing exceedance,

while yet accepting in the face of that reasoning, that which amounts to an unacceptable study. It becomes, more or less, a game of picking and choosing one's findings as best suits one's desires.

At this point in time, and in the interest of public health protection, preferred would be to require new properly conducted combined chronic toxicity/carcinogenicity bioassay.

14) (p. 10) Under the topic "New Pathology Information", the registrant summarizes the results of various PWG assessments. This reviewer is of the opinion that under Pesticide Regulation PR Notice 94-5, a means is provided for the re-reading of histopathology slides by a panel of expert pathologists. There is no provision in this PR Notice for conducting study risk assessments. This is the Agency's responsibility. Mouse lung tumor is a prime example here of the PWG exercising liberties that go beyond reading (re-reading) slides: "Mouse lung: The PWG concluded that the lack of dose-response, absence of preneoplastic lesions, lack of tumor multiplicity, and similarity to historical control data indicates that the apparent slightly increased incidence of lung tumors was not related to treatment (MRID #44496206)." (p. 10). The PWG should have submitted a report tabulating their agreed to histopathology diagnoses, and walked away from the matter. To speak to such issues as dose-response, absence of preneoplastic lesions, lack of tumor multiplicity and similarity to historical control data in arguing that the lung tumors were not related to treatment, in essence preempts the responsibility of the Agency to interpret the data, and does the registrant's bidding. This reviewer maintains that the PWG histopathology re-reads should be submitted directly to the Agency without interpretation by the PWG. This is important in securing the objectivity of the PWG process since these assessments are conducted at the request and expense of the registrant. This needs to be properly addressed for all of the acetochlor PWG submissions. Furthermore, this reviewer believes all lesions should be examined by yet other entirely independent pathologists who submit their re-reads directly to the EPA, and who are thus not also employed in the apparent conflict of interests of reading histopathology and assessing human risk in concert with the registrant, where the latter process resides outside and beyond the provisions of the PR Notice.

15) (p. 11) Concern about the bottom line on neoplasia observed in a Reproduction Study. This reviewer needs to examine the Reproduction Study and the DER (which has not yet been completed) and evaluate it with respect to the claims rendered in this "white paper". There should be in particular close examinations of comparative dosing with the chronic/carcinogenicity studies, time of nasal tumor onset in offspring (latency), comparative histopathology of nasal tumors and tumor incidence. ***This Reproduction Study should assume its place among the chronic studies in the assessment of nasal neoplasia, particularly as it concerns offspring.***

Do we often find reproduction studies confirming neoplastic findings observed in the chronic toxicity/carcinogenicity studies?

16) (p. 12) Concerning the very important question regarding the character and time of onset of nasal tumors in the rat, under "3.4 ARP Conclusions Regarding Carcinogenic Potential to

Rodents", the ARP says: "The nasal olfactory tumors produced by acetanilides are *unusual* (emphasis added) and *can be detected within 5-6 months of continuous exposure* (emphasis added)." (p. 12) This should be viewed as a focal statement in addressing carcinogenicity. If "unusual" is consonant with rare, which it likely is, there is no compelling need for incidences of such tumors to achieve statistical significance in order to be considered treatment related. Given that these tumors are acknowledged to be "unusual", the record should include a full histopathologic characterization of the tumors, and a reliable assessment of historic incidence. This should involve obtaining the National Toxicology Program's (NTP) historic incidence of the tumor. Also, to the extent these tumors may be of early onset (particularly with respect to time of onset in control animals, if indeed they are ever seen in controls), there is an attendant concern for enhanced carcinogenicity potential.

It is to be noted that for reasons unknown, this above particular quotation as it appears under Section 3.4 (p. 12) in the July 18 draft, has been removed from Section 3.4 (p. 12) of the same title of the September 5 final document.

This is an appropriate section of these comments to introduce text from a very recent publication [Burman, D.M., et al (2003) *Antioxidant pertubations in the olfactory mucosa of alachlor-treated rats*. Biochem. Pharmacol., 66, 1707-1715] This publication has just been received in HED, and has not been reviewed. However, many other journal articles in-house pertaining to this subject, and cited in the registrant's text have not received any formal review either, and yet are being referenced in the deliberations. Burman et al (co-authored by M.B. Genter) makes the following relevant claims: a) The basic result of new research reported in this publication, which has to do with assessments of endogenous antioxidants depletion in response to alachlor administration may best be expressed in the authors' text: "Dietary exposure to alachlor depletes olfactory mucosa antioxidants, which may contribute to DNA damage and tissue-specific tumor formation." (p. 1707) Should the mechanism of tumor formation reside with DNA damage, then this would be at variance with the quinoneimine hypothesis as the sole mechanism undergirding the explanation of the mechanism of nasal carcinogenicity.

b) In speaking of their past work, the authors' say: "We have shown that alachlor-induced olfactory mucosal tumors (polypoid adenomas and adenocarcinomas) occur with a relatively short latency (i.e. following 5 months of exposure at 126 mg/kg per day in the diet) and high multiplicity, in that rats treated continuously at this level for 12-18 months often exhibited 10-20 tumors per nasal cavity (7-8)." (pp. 1707-1708) This claim concerning latency affirms the above quotation from the July 18 draft "white paper" concerning chloroacetanilides, which has been removed from the final September 5 version. And the claim of progression as expressed in terms of multiplicity after 12-18 months of *alachlor* treatment is at variance with the registrant's claim of lack of progression of the nasal tumor expression during the second year of treatment with *acetochlor,* as cited in item 12 above.

The text of Burman et al continues: "The complete mechanism of alachlor-induced nasal tumor

formation in rats has not been elucidated, but we have evidence that metabolic enzymes present in the olfactory mucosa, but not in the liver, bioactivate alachlor to one or more mutagenic species (7). The sites of alachlor-induced tumor formation in the olfactory mucosa correspond with the distribution of cytochrome P450 2A3 [7], suggesting a role for this enzyme in the formation of a *mutagenic/carcinogenic* (emphasis added) metabolite. The basis for the apparent resistance of mice to the development of alachlor-induced olfactory mucosal tumors [1] is also unclear." (p. 1708)

According to the contributions of this publication, it appears the mechanism of chloroacetanilide induction of nasal tumors is not settled science. This very recent publication would appear to be of value in contributing to the understanding of the mechanism of nasal carcinogenicity, and requires full review and consideration by those who desire to understand the mechanism.

17) (p. 12) If existing cancer studies lack a proper dosage range (meaning an MTD plus at least two properly spaced lower dose groups) - then the positive findings at doses exceeding the MTD should serve as a driving force for more definitive carcinogenicity testing.

18) (p. 12) Claims "No evidence of increased carcinogenic potential in offspring" - how much data is there to support this claim? The registrant should summarize all findings that support a claim that there is no offspring susceptibility. This is essential under requirements of FQPA.

19) (p. 8) For reference purposes, registrant provides a consolidated table of dosage levels employed in the five carcinogenicity studies on acetochlor.

20) (pp. 13-15) This reviewer must rely upon HED for genotoxicity assessment. This ARP paper notes that a Dearfield et al assessment expressed muta concern which ARP discounts: "Dearfield et al. (1999) [refers to acetanilides in general, but apparently did not include all of the most recent studies]: '.... consistent pattern of mutagenic activity, probably mediated via metabolites. This mutagenic activity is a mechanistically plausible factor in the development of tumors.....'." (p. 15). By apparent contrast, further along the registrant says: "Genotoxicity not a significant factor in formation of rodent tumors" (p. 15) If certain mutagenicity studies justified this conclusion in Dearfield et al, *specifically* what subsequent studies undercut the conclusion and why? EPA's Dr. Kerry Dearfield's most recent views should be obtained at this time, given the importance of the genotoxicity interpretation.

21) (p. 15) "..... the EPA concluded that the oncogenic modes of action for nasal and thyroid tumors produced by alachlor and acetochlor are the same (EPA, 2001)." Upon reading EPA (2001), this would appear to be the case. See for example section VII, p. 27. However, it should be noted that EPA (2001), entitled "The Grouping of a Series of Chloroacetanilide Pesticides Based on a Common Mechanism of Toxicity", June 7, 2001, includes a notice on the cover page indicating: "This document is a preliminary draft and has not yet been released by the Agency." Was there ever a final report? From EPA's perspective, what is the status of finality of views

expressed in this June 7 "preliminary draft"? Are the conclusions rendered somehow tentative? The registrant says further along under the heading of "Oncogenic Potential of Acetochlor": "The EPA has *tentatively* (emphasis added) concluded that acetochlor, alachlor and butachlor share a common mechanism of toxicity for formation of nasal tumors (EPA, 2001)." (p. 45) See also p. 17.

22) (p. 16) Speaks of a well recognized mechanism for thyroid tumors concurred in by EPA (1998) - into which mode acetochlor fits.

23) (p. 17) In reference to thyroid tumors, registrant says for alachlor both SAP and CARC concluded that Agency requirements for this hormonal mode for thyroid tumors met by this agent, and that the tumors were observed only at an excessive dose. This is an excellent affirmation of the Agency's position on thyroid tumors. Furthermore, the registrant cites EPA (2001) as saying: "'Acetochlor, alachlor and butachlor may be grouped together based on a common end-point and a known mechanism of toxicity (UDPGT induction). Data for all three chloroacetanilides exist (positive UDPGT) induction, increased TSH, alterations in T3/T4 production, increased thyroid weights) to confirm that the postulated mechanism of action is indeed responsible for the effect.'" Confirmed as found on p. 26 of EPA (2001). This affords excellent affirmation of EPA's prior conclusion with regard to thyroid neoplasia. Yet, EPA (2001) remains a "preliminary draft" as explained above.

24) (p. 17) Note again that registrant indicates EPA says *tentatively* for nasal tumors - alachlor, butachlor and acetochlor share a common mechanism. Seems to be saying this is recognized by (EPA?), as proceeding via a quinoneimine. Heydens et al (1999) is cited as a principal review article on this mechanism. It is noteworthy that a good structural depiction of the metabolism of acetochlor via EMA and sulphoxide pathways, both leading to quinoneimines is presented in Fig. 5.2.1, p. 25.

The paper also speaks in vague terms of progression: cytotoxicity > regenerative proliferation > neoplasia. To the extent that cytotoxicity plays a key role in this critical sequence of events, there should be some gross and/or histopathologic evidence for cytotoxicity in the critical studies. If this evidence is not present, what is the justification for such a claim? Furthermore, if there is no evidence of cytotoxicity, there may be some other "key event" leading to neoplasia which may occur at lower doses, and should be considered in an assessment of the neoplastic response under the draft 2003 Carcinogen Assessment Guidelines. **This reviewer must reiterate that the registrant explain the nature and evidence of "cytotoxicity".**

25) (p. 18) It is very significant in support of a specific tumorigenic effect in the rat that the autoradiography studies revealed localization in the rat as contrasted with mouse, hamster and monkey.

26) (p. 19) No DABQ1 adducts in mice or monkey - but yes in rat. The registrant claims the

phenomenon is species specific, yet only a few species have been tested. It would be more appropriate to say at this time the effect is observed in the rat, but not the mouse or monkey if this is extent of what the data reveals.

27) Question for staff: do EPA (CARC) and SAP agree that the alachlor nasal neoplastic response proceeds via the immunoquinone mechanism, and that only - in other words, for alachlor, is it fair to conclude as settled science that the EPA has already concurred with the registrant's hypothesis? If this is true, it is considerably easier to accept acetochlor as acting via the same mechanism if it proves a likeness to alachlor in many ways in testing which is somewhat less pioneering (me-too type testing) than what has been done with acetochlor. Yet, there still remains the concern that compounds that are very close in structure, may have markedly different biochemical and pharmacologic properties.

28) (p. 19) Presents a significant perspective that several parameters support the DABQ1 mechanism in rat as opposed to other species, i.e. species specificity.

29) (p. 19) Very important statement: EPA (1998) says "entity responsible for alachlor cytotoxicity response is not known with certainty." Is this uncertainty in reference to an immunoquinone mechanism? This question follows the registrant's apparent claiming of evidence of *in vitro* cytotoxicity to the nasal epithelium. This needs to be reviewed closely. Would such evidence satisfy as proof of cytotoxicity though no such evidence is seen in the usual Guideline *in vivo* studies? To what extent has the Agency (EPA, 1998) already accepted this as evidence of cytotoxicity critical to the schematic (cytotoxicity > regenerative proliferation > neoplasia) for neoplasia ?

30) (p. 20) Will need to examine MRID 42852102 concerning cell proliferation. Study said to show increased cell proliferation in rat olfactory epithelium as opposed to the respiratory epithelium. No such proliferation was observed in the mouse. Effect in rats was observed at 42 and 126 mg/kg/day. Was 42 mg/kg/day the lowest dose tested? This study yields a very important finding in support of specificity for the olfactory epithelium, and that it occurs in the rat but not mouse. Which rodent model is considered the preferred surrogate for man?

31) (p. 20) Need to confirm this attribution to the World Health Organization (WHO): WHO (2001) concluded that in the case of alachlor, nasal tumors "are induced by a mechanism not relevant to humans." WHO (2001) citation not rendered in "References"

32) (p. 21 and elsewhere) Does EPA accept, for alachlor at least and already possibly for acetochlor the claimed routes of metabolism - especially in nasal tissue?

33) Major Question: how much of this so-called 'white paper' do we accept at face value, w/o reviewing the numerous cited references? How much of this does EPA already agree with, such as to narrow down what background information needs to be confirmed?

34) Question for registrant: In all of your studies when both alachlor and acetochlor were studied, did you find any case (es) wherein the properties and/or the conclusions were different between the two compounds?

35) (p. 22) Autoradiography for acetochlor - locates in nose of rat but not mouse. Is this true for alachlor as well? Importantly, M.B. Genter in ongoing reach on the nasal tissue carcinogenicity of alachlor, has advised by personal communication (M. Genter/B.Dementi 10/20/03) that when tested in mice (AJ mouse) after one year of dosing at 260 mg/kg/day (acknowledged to be a high dose) by the feed, mice developed an extensive nasal tissue response so described as "eosinophilic globules or inclusions" located within nasal cells, which cells thus appear greatly enlarged. Though no tumors were found. She does not consider this phenomenon as "cytotoxicity", which may be debatable, though it does represent a notable effect on the nasal epithelium in mice of this particular strain. This work is preliminary, as rendered by telephone and is yet to be published. Regardless of whether this effect is to be considered evidence of cytotoxicity in the mouse, it would constitute evidence of the compounds presence in the nasal epithelium. Genter hypothesizes the effect may be a consequence of cellular antioxidant depletion in the nasal epithelium. However, it is uncertain whether acetochlor would elicit the same effect, and thus the findings are significant insofar as alachlor and acetochlor act by the same mechanism. At the very least, these findings in the mouse would need to be explained if there is to be confidence in the claim that acetochlor does not bind or otherwise localize in mouse nasal epithelium of the mouse, a distinction essential to the registrant's hypothesis that the effect is species specific to the rat.

36) (p. 22) Formation of DABQ1 protein adducts from acetochlor, like alachlor, occurs in the nose of the rat but not mouse (by positive analytical chemical structural analysis?)

37) (p. 22) 5.2.3.4 explains the remarkably different potential for primates versus rat to metabolize acetochlor, which is remarkably similar to alachlor in this comparison.

38) (pp. 22-23) Olfactory (but not respiratory) cell proliferation increased at 1750 and 5000 ppm in rat, which agrees with neoplasia at the high dose.

39) Important - need to take each respective claim of evidence that neoplasia is peculiar to rat/olfactory/low dose (i.e. at/below MTD) - and say whether or not the point (or claim) can be accepted to the Agency's satisfaction. For example: a) does the Agency accept there is positive evidence for neoplasia not only at HDT? b) does the Agency accept evidence that rat nose is peculiarly responsive, considering radiography, cell proliferation, metabolism, etc. ? *This will take time.*

40) (pp. 22- 23) Concerning cell proliferation, the report speaks of testing at 0, 200, 1750 and 5000 ppm in the rat. Given that 1750 and 5000 ppm both exceeded the estimated MTD (1000 ppm) in chronic testing, there seems to be no testing within the important 200 to 1000 ppm range

in this study. Would the Agency accept the registrant's interpretation in the absence of testing in the 200 to 1000 ppm dose range?

41) (p. 23) Since 1999, newer findings focus more on the importance of the methyl sulfoxide metabolite rather than from the aniline metabolite in neoplasia. Since this is newer, it may receive closer scrutiny. Evidently this was not done with alachlor, so there is not that precedent. (This may be an example of how convinced we were that the aniline metabolite was the way - only to find we're wrong, and there was yet another explanation. Might there be yet others unknown to us at this time?

42) (p. 23) The sulfoxide metabolite locates in Bowman's gland, which is the site of tumors.

43) (p. 24) Middle paragraph - need reference for the claim that CYP2A6 human form does not metabolize acetochlor sulfoxide.

44) (p. 24) An on-going one year cancer study in the rat with the acetochlor sulfoxide metabolite is giving the same results (neoplasia) as acetochlor. The report for this study is said to be in a draft form, and will be submitted "in the near future". Some of the key data are presented in Appendix 3 (pp. 54-59) of this report. Beyond noting the fact that the data suggest that acetochlor sulfoxide and acetochlor, at dosage levels anticipated to yield equivalent plasma levels of the sulfoxide, both yield polypoid adenoma and hyperplasia of the nasal tissues. The effect appears more remarkable with acetochlor itself (see p. 58). Until this study has been received and reviewed no further comment will be offered here.

45) (pp. 24-25) Under "Conclusions for Acetochlor Mode of Action", the ARP provided a very good concluding statement to the effect that the quinoneimine mechanism for nasal neoplasia may proceed via both an aniline and/or a sulfoxide quinoneimine metabolite of the common precursor acetochlor metabolite, namely an S-methyl sulfide metabolite. The particular figure (Figure 5.2.1, p. 25) in question depicting the alternate metabolic routes, would be useful for presentation to HED's mechanism and cancer committees. The registrant says: "In addition, regardless of relative contributions from the methyl sulfoxide and aniline pathways, the putative mode of action for the formation of rat nasal tumors by either the sulfoxide DABQ1 or DEA DABQ1 metabolite is the same." (p. 26)

46) The registrant has done a much better job of documenting his claims than in Clapp et al (1998). How thoroughly must the Agency now examine their many references? How much has already been accepted by the Agency?

47) (p. 26) Registrant claims: "Species differences in the overall metabolism of acetochlor to either (aniline or sulfoxide) metabolite indicate that the rate of conversion is significantly higher in the rat than in the mouse and several orders of magnitude greater than in primates and humans." This claim needs to be buttressed with adequate documentation and presentation of the

comparative rate data in the differing species as claimed. Again, the registrant speaks of cellular damage (analogous to cytotoxicity) as a principal event in the "....cascade of events.....that ultimately leads to the formation of olfactory tumors." (p. 26) This evidence for cytotoxicity remains to be presented.

48) (p. 26) Both the non-human primates and humans *"apparently"* lack the enzymes in the nasal epithelium which are required to metabolize acetochlor to the DABQ1. "Apparently" does not rise to the level of certitude that is necessary in support of the hypothesis. What evidence is there to support the claim?

49) (p. 26) “.....the mode of action for induction of nasal olfactory tumors is specific to the rat and is not relevant to human health risk assessment." This is a key element in the registrant’s hypothesis, and must be correct if acetochlor’s neoplastic effects in rat nasal tissue are to be dismissed as irrelevant to human risk assessment. Is the Agency prepared to accept this claim for acetochlor? Has the Agency already accepted the claim for alachlor?

50) (p. 26) "The generation of a reactive DABQ1 following exposure to chloroacetanilides is the currently accepted (*by whom?*) mode of action responsible for the subsequent nasal tumor formation in rats (EPA 2001)." Is this a true EPA conclusion? As explained elsewhere, EPA (2001) is a preliminary report, and there appears to be no final. Thus its conclusions might be regarded as but tentative.

51) (p. 27) “The mechanism by which quinones induce cytotoxicity is complex with many plausible pathways." This reviewer's concern is whether the Agency has correctly identified *the one* true mechanism, if there be but one, that underlies the problem.

52) (p. 27) The registrant speaks of a Coleman et al (2000) paper, which appears to be a subject of disagreement with the registrant. Under Appendix 4 (pp. 61-65) the registrant provides an evaluation of the Coleman paper, a copy of which publication is appended to Appendix 4. This is a complex paper dealing with subjects of chloroacetanilide metabolism and genotoxicity. This publication requires a full Agency review in order to respond properly to its contents and the registrant's review and comments on the same. It appears as though microsomal enzyme metabolism of the various chloroacetanilides *does not necessarily follow the same pattern* as would be desired if all members of the class are to be treated as behaving by a common mechanism of toxicity. *This is critical to the hypothesis of their oneness of behavior.* This reviewer has posed the question elsewhere in these comments as to whether there might be evidence that acetochlor and alachlor may not act in the same way (see comments 2, 32, 55), even though the two compounds are so remarkably similar, structurally.

The Coleman paper says among other very important things: "However, human liver microsomes metabolize acetochlor to CMEPA at a similar rate to that of rat liver microsomes, and subsequent metabolic rates of CMEPA and MEA with human liver microsomes *exceed those of rat liver*

microsomes (emphasis added), *suggesting that acetochlor has the greatest potential to be genotoxic to humans."* (p. 1157) For this and possibly many other reasons, Coleman et al requires a critical review.

53) Look more closely at pp. 27-28.

54) (p. 28) Mentions DABQ1 induced cytotoxicity and tumors. What does this mean? Look at this more closely.

55) Make a list of all identified *differences* between acetochlor and alachlor.

56) (p. 30) "These studies have demonstrated that the oncogenic modes of action by which alachlor and acetochlor produce rat nasal and thyroid tumors are the same (EPA 2001)." How thoroughly is EPA already committed to accept this mechanism as one for both agents?

57) (p. 30) "..... the ARP believes that the oncogenic weight-of-the-evidence descriptor 'Not Likely to Be Carcinogenic to Humans', as described by EPA in the 2003 Draft Final Guidelines for Carcinogenic Risk Assessment (EPA, 2003), would be more appropriate than the current B2 or 'probable human carcinogen' classification." So this appears to be the principal focus and objective of the registrant, i.e. to change the classification for acetochlor, and probably for alachlor and other members of the chloroacetanilide group as well.

58) (p. 30) Obtain structures of t-ESA and t-OXA metabolites of acetochlor. These have been detected in surface and ground water, and are generated via microbial action on acetochlor. "Based on the available studies, the EPA has previously concluded that alachlor t-ESA is less toxic than parent alachlor and unlikely to be carcinogenic, and thus should not be included in an oncogenic risk assessment for alachlor (EPA, 1998a). Similar studies have been conducted on the acetochlor metabolites, apparently resulting in the same conclusion. *However, there are no carcinogenicity studies on the two degradates, and in this reviewer's opinion, and in the interest of public health protection, the question of the agents' carcinogenicity can not be reliably appraised by structure-activity analysis.*

59) (p. 31) Under 9.2 "Linear vs. Non-Linear Approach" (p. 31), the ARP claims the following: "Because of the compelling evidence of species-specificity of the oncogenic modes of action for alachlor, both the EPA and SAP have previously concluded that the potential oncogenic risks to humans for alachlor should be assessed using a non-linear, margin-of-exposure (MOE) approach (SAP, 1996; EPA, 1997). The SAP stated: '....although alachlor cannot completely be excluded from having activity in humans, it is highly likely that if it occurs at all, it would only occur at doses far in excess of exposure levels. Therefore, an MOE approach to human risk assessment of alachlor is appropriate.'"

"The species differences between rats, mice, and humans in production of DABQ1 metabolites, the

key event for nasal oncogenicity, appear to be even greater for acetochlor than previously demonstrated for alachlor." (p. 31) This latter statement regarding the comparative potentials of alachlor and acetochlor, should be supported by reference to documentation.

This is a very important statement affirming prior EPA and SAP conclusions, which if valid certainly support prior EPA and SAP sanctions that would support the registrant's desired regulatory posture for acetochlor. (This reviewer came into middle of these deliberations and must acknowledge the influence of decisions already rendered on these chemicals, indeed this class of chemicals, and thus what has already been concurred in or agreed to by EPA/SAP.)

60) On pp. 41-53, there is presented under Appendix 2 a "Supplement to the Rat Reproduction Study." This study is currently under review in the Agency, and thus there is no final Agency DER from which this reviewer may speak. However, since nasal tumors were observed in *offspring* of the F0 and F1 generations at the time of sacrifice after but 19-22 weeks of acetochlor administration, the findings in this study are particularly relevant to the current consideration of nasal tissue mechanism of neoplasia, to the overall cancer assessment, and to the question of offspring susceptibility under the requirements of FQPA.

Dosing (0, 200, 600 and 1750 ppm) in both generations occurred up until the time of sacrifice 19-22 weeks of age. Benign proliferative lesions (hyperplasia and adenoma) of the nasal turbinate epithelium were observed in both sexes in both the F0 and F1 generations. The neoplastic response thus appears to be of early onset. Unfortunately, there is no inherent capability in the protocol for this study to determine the time of onset of the hyperplasia/adenoma that was observed for the first time at the normal sacrifice time. In any case, the lesions as assayed appeared very early. It should be noted that given the importance of tumor latency in assessing carcinogenicity and possible offspring susceptibility (important under FQPA), results of this study indicate that a determination of comparative offspring versus adult latency be undertaken.

"Polypoid adenomas and hyperplasia of the olfactory epithelium were noted in F0 and F1 animals at 1750 ppm and in F1 animals at 600 ppm...." "No lesions were observed at 200 ppm, which was the overall NOAEL for the study." It would be necessary to affirm the presence/absence of hyperplasia in all dose groups. It is also to be considered that the tumors were observed at the much lower dose level of 600 ppm in the F1 as opposed to 1750 ppm in the F0 generation, suggesting an across generation transfer of susceptibility that requires comment. Given the incidence of these lesions after so short a time period, one of necessity would be curious to know what the outcome would have been in terms of incidence and severity had these animal's exposures continued for longer periods. What is the historical control incidence of this lesion among offspring in reproduction studies? Indeed have such lesions ever been seen before among offspring in a reproduction study?

The position being taken by the registrant on this study as presented in the "white paper", might be quickly summarized as follows: 1) Although neoplastic lesions of the nasal passages among

offspring were observed at lower doses (as expressed in ppm) in the reproduction study than in the chronic bioassays among adult animals, this disparity can be attributed to greater food intake among offspring than adults, which in effect equalizes the doses at which tumors were seen as expressed in mg/kg/day in the two types of studies. 2) Although the neoplastic response may seem to have had an earlier onset (decreased latency) in the reproduction study, the registrant argues that this is an artifact of sacrifice time, such that in effect one cannot be certain when the neoplastic response would have been first observable in either type of study. The data don't exist that would more rigorously compare time of onset, or latency, in the two studies. 3) In terms of tumor incidence, the registrant argues these were in effect the same in the reproduction and chronic bioassays. This despite the fact that the chronic studies extend for longer periods of time, which would have afforded a longer period of challenge by the test material, and would be expected to afford greater opportunity for both tumor expression and progression.

In this reviewer's opinion, much of the rationale employed by the registrant to discount concerns over possible evidence in the reproduction study of enhanced offspring susceptibility, namely the apparent response at lower doses in offspring, apparent decreased latency among offspring, and increased potential for there to be enhance tumor incidence as a result of exposure during early life exposure of offspring is to be questioned. These views should be critically evaluated once the review of the reproduction has been finalized. Until the review of that study is finalized, it would be preemptive for this reviewer to address these subjects. The review of the reproduction study should be viewed as an essential task prior to the Agency's assessment of carcinogenicity of acetochlor. In other words, insofar as the reproduction study yields what appears to be for such studies a rare expression of tumorigenicity of a rare tumor type in the nasal passages of offspring after short periods of dosing, mandate that this study be carefully interpreted (under FQPA), pared against the registrant's "Supplement to Rat Reproduction Study" as presented in Appendix 2 (pp. 42-53) of this "white paper" and included along with the chronic bioassays in the assessment of the nasal tissue neoplastic response.

END

Exhibit VIII

Brian Dementi
03/09/04 10:53 AM

To: Alberto Protzel/DC/USEPA/US@EPA, Larry Chitlik/DC/USEPA/US@EPA, Linnea Hansen/R9/USEPA/US@EPA, Nancy McCarroll/DC/USEPA/US@EPA, Susan Makris/DC/USEPA/US@EPA
cc:
Subject: Comments on acetochlor reproduction DER

Colleagues,

Please find appended comments I have developed on the recenrly reviewed acetochlor reproduction study.

Best Wishes,
Brian dementi

Colleagues, March 5, 2004

Today I read for the first time the Data Evaluation Record (DER) for the February 16, 2001 acetochlor reproduction study (MRID 45357503), which I received via Email on 3/4/04. The hard copy I printed out is, of course, not itself a signed copy.

I read (and in places, at least scanned) the DER with the principal interest of understanding the the character and assessment of nasal tissue responses observed in the F0 and F1 generations, as my current interest for which time grows short, is upon the mechanism or mode of carcinogenicity of acetochlor, to be taken under advisement of CARC at the end of this month.

The DER is excellent. I have the following perspectives to offer:

a) As I understand, it is very unusual, if not unheard of, for carcinogenicity to be a finding in a reproduction study. Am I mistaken in this belief? If this is an unusual outcome for a reproduction study, does not that observation itself merit mention in the DER? Now I realize that these studies are not usually tailored to identify neoplasia, and that this study may have been unusual in its focusing upon the nasal tissues as a suspect site. So mention in the DER of this rare finding (rare in the senses of being both observed in a reproduction study and in being intrinsically rare neoplasms) could be **caveated** as resulting from an atypical microscopic examination of the nasal tissues, driven by nasal neoplasia observed in the chronic bioassays.

b) Was this data submitted, or ever considered, as 6(a)(2)? A comment on this would be appropriate given the unusual nature and significance of the neoplastic response, and the fact that the study may be indicating enhanced offspring susceptibility for neoplasia..

c) The nasal olfactory tissue neoplasia now identified in the chronic carcinogenicity bioassays for acetochlor (and alachlor) appears to have a short latency period. However, it may not be possible to say whether the carcinogenicity studies reveal decreased latency of induction of these neoplastic effects simply because they are so rare, i.e. there appears to be no control data to which time of onset is to be compared. Yet, it may be apparent that the induction period for the nasal tissue neoplastic response is less in this reproduction study even than in the carcinogenicity studies. Since latency is such a critical aspect in defining carcinogenicity, the subject should be addressed.

d) From another perspective regarding tumor latency, there appear to be no time-to-tumor studies for the carcinogenicity bioassays, and certainly not for this reproduction study. Neoplasia was seen at sacrifice, which according to this DER would have been at a point as early as somewhat in excess of 4 months in age. Specifically, the DER reads: "Histopathological evaluation revealed treatment-related incidences of benign proliferative lesions (focal epithelial hyperplasia and polypoid adenomata) in the epithelial lining of the ethmoid region of the nasal cavity in F0 and F1 adult animals receiving 1750 ppm acetochlor and in F1 animals at the 600 ppm level. Although no clear evidence of malignant change was apparent, the animals were just over 4 months of age and had been exposed to acetochlor for approximately 18 weeks (F0) or 25 weeks (F1) when these lesions were observed." (p. 1) So the neoplasia is observed at the earliest point examined, and there is no definitive information on time of onset in either the carcinogenicity or reproduction studies, but the subject is central to the question of defining carcinogenicity and to assessment of offspring susceptibility.

Now in the reproduction study, nasal tissues (among a certain few other tissues) were taken and preserved from pups at day 29 post partum. Presumably this was true for both F1 and F2 pups, which the DER should affirm in the paragraph on "Offspring", p. 7. But according to the DER, citing the study report, none were examined histologically. (p. 7). The nasal tissues were taken from pups for a purpose, but were not evaluated along those lines. *Should this be viewed as a study deficiency, particularly with reference to examining F2 pups, if one were concerned about both latency and offspring susceptibility? This toxicologist views the microscopic examination of these tissues as essential.*

e) The DER says: "Minimally increased brown pigment (lipofuscin) was observed in the olfactory mucosa, mainly in the lamina propria and occasionally in the basal epithelium in most animals receiving 600 and 1750 ppm in both F0 and F1 generations and also in F1 females at the 200 ppm dose level; however , this finding was not considered to be of toxicological significance." (p. 1) The question is, for what reason the findings are not considered as of toxicological significance? The DER indicates on p. 28 that though dose-related, the reasons may be that the findings were minimal to slight in severity, were not observed in the carcinogenicity bioassays, and are not clearly

associated with the purported mechanism of action for tumor induction in the nasal epithelium. However, the question of the mechanism of nasal tissue neoplasia is not settled.

Brian Dementi, Ph.D., DABT

Exhibit IX

ORAL PRESENTATION, Brian Dementi, Ph.D., DABT April 21, 2004

General Comments

This oral statement is but an overview of information presented in my written assessments. *I must refer to my written documents as constituting my actual scientific presentation, as I believe recorded there is a very substantial contribution to this project that simply cannot be adequately conveyed in a brief oral presentation*. All that I would have planned to say here is set forth and documented in much greater detail in the written documents, principal among these being my January 26, 2004 "Acetochlor Mechanism of Nasal Tissue Carcinogenicity" paper (Doc #18) and follow-up expressions of my concerns (Doc #s 19 & 20). A very noteworthy Email correspondence is that of January 30, 2004 on evidence for nasal cytotoxicity (cell death) of the olfactory epithelium. However, there is much more of substantial importance in the other materials I have contributed to the package.

As I embrace the data base for the Mode of Action (MOA), I have found this to be a very complicated and challenging subject. I find that with the exception of the carcinogenicity bioassays and the reproduction study, that essentially all studies under review are non-guideline studies (including the mechanism studies, journal publications and various reviews submitted by the registrant). The mechanism studies were designed to illustrate that which the registrant desired to illustrate, but do not test for added evidence that I would have sought had I designed the studies. Nor do they test for what an Agency protocol might have sought in designing Guideline studies. So one of the major problems is in deciding how much reliance is to be placed on these materials, and when they disagree, which carries greater weight.

In any case, to the best of my ability I am unable to confirm that the MOA has a credible scientific basis. It is an interesting hypothesis, and I agree that the MOA is plausible, and that this class of agents clearly share in common the induction of exceedingly rare tumors of the olfactory epithelium. However, there are so many and diverse issues that have been raised, so much unknown and so many inconsistencies in the data as to render unreliable the implementation of this MOA in support of an MOE approach as opposed to the linear low dose extrapolation method for cancer risk assessment. In the interest of public health protection, certitude regarding the hypothesized MOA must be at a higher level.

The registrant (Monsanto) claims that the MOA for acetochlor is the same as that of alachlor and other members of the chloroacetanilide class, and comes now with new mechanistic evidence for acetochlor that would justify its regulation along side the other members of the class, most notably as seen with alachlor. The mechanism studies in question were designed to emulate those previously designed and worked out for alachlor. These acetochlor studies, all non-guideline, and their respective DERs are in the package for today's meeting. Briefly, these studies assessed quinoneimine-protein binding in nasal tissues of rat and mouse, autoradiography for nasal tissue location, and nasal cell proliferation. Basically these studies on acetochlor affirm earlier work on alachlor, that would appear to justify a conclusion that acetochlor behaves like alachlor, mechanistically. As I see it, both agents induce nasal tumors, and probably share a common mechanism in inducing nasal tumors. As I see it, to the extent that the MOA for alachlor is settled science, acetochlor could possibly be adopted as a "me too" compound. If this were the case, my work would have been made easy as the secondary reviewer of seven mechanistic studies on acetochlor.

Early on in the review process this was recognized by me as a complex subject, and I was not certain as to what the registrant's best case was for the assessment of carcinogenicity. In order to attempt to understand just exactly what the registrant's perspectives and desires were, I suggested to our work group that we ask the registrant, in the person of Dr. Jerol Kronenberg, to provide the Agency with an up to date assessment of the data base, and what the registrant was seeking from the Agency. Dr. Kronenberg gladly responded and provided last September 5 a document entitled "Acetochlor: Justification for Reclassification of Carcinogenic Potential", the so-called "white paper", Document #31 in your package. Since it appeared to be my assignment, I developed comments on this submission, dated October 24, Document #32 in your package. I should add that Dr. Kronenberg advised that this "white paper" should be viewed as a supplement to an earlier work by Clapp et al (1998) (Document #25) My comments on Clapp et al, dated May 28, 2003 appear as Document #33. These documents raised many issues.

1) The proposed MOA is interesting and possibly true, but I cannot accept that the evidence in support of it is sufficiently rigorous to justify its adoption over that of the linear low dose extrapolation approach.

2) I do not accept there to be adequate proof that the tumors are caused by a quinoneimine metabolite of the parent compounds. Again I say, it may be true, but in the interest of public health protection, evidence to adopt the hypothesis as real must be more compelling.

3) When SAP considered this matter in 1996 and 1997, I am convinced the SAP accepted that these compounds share a common mechanism in eliciting nasal tumors in the rat, but I would like to have witnessed a stronger affirmation from the SAP to the effect that it endorsed the quinoneimine MOA. Reading the SAP reports one might conclude that the panel did accept the quinoneimine hypothesis, but I would have appreciated an actual statement to the effect, such as "we concur on the validity of the quinoneimine hypothesis for the MOA". I say this because they asked certain

questions that in the absence of appropriate answers it is difficult to see how they could have accepted the hypothesis. For example, the December 6, 1996 SAP report for the October 30 meeting says: “Therefore, the limitation in the metabolism in the mouse may not be the real reason for the lack of tumorigenic response in the mouse. If this is accurate, then the argument that limitation in the metabolism of alachlor in the human precludes alachlor being considered as a human carcinogen can not be supported." (pp. 47-48) This is a most notable observation by the SAP that requires an explanation. It appears the fundamental rationale supporting the hypothesis that the human, unlike the rat, lacks susceptibility to alachlor induced nasal neoplasia is here questionable. What did the registrant mean in saying: “.....limitation in metabolism is not the only factor for lack of tumorigenic response in the mouse.”? Why didn’t SAP search out from the registrant the one or more other factors that could explain why the mouse is non-responsive? It may be the mouse is simply a poor model for xenobiotic induction of nasal tumors by a mechanism other than quinoneimine that is effective in the rat.

4) When these SAPs considered the materials, the MOA was depicted as quinoneimine > cytotoxicity (cell death) > cell proliferation > neoplasia. This is a rather generic set of steps often cited as explaining neoplasia. The acetochlor mechanism study (Doc #9) that assayed quinoneimine-protein binding in the rat, as reported after 14 days at 1710 and 5170 ppm (119 and 205 pmole/mg protein, respectively), is a good qualitative indicator of the transformation of acetochlor to quinoneimine and of its binding to protein, but this does not establish any adverse effect. Quinoneimine-protein binding could be an innocuous sink for the quinoneimine. Carcinogenicity may be proceeding via an entirely different route that bears no relationship to quinoneimine-protein binding except that the two events may occur contemporaneously. Beneficial toward substantiating the hypothesis would have been information of a more quantitative nature linking a buildup of the quinoneimine-protein complex at doses up to and including the MTD (e.g. 400-1000 ppm) with a subsequent onset and enhancement of cytotoxicity, a claimed element in the progression to neoplasia.. To my knowledge such needed data does exist.

Concerning mechanism studies Doc #9 (rat), Doc #10 (mouse), Doc #11 (rat) and Doc #12 (monkey), although the studies provide qualitative evidence for quinoneimine-protein binding in the nasal epithelium of only the rat among these three, the studies do not differentiate between olfactory and respiratory epithelia as having been individually examined in any of them. There is no certitude that all the studies reflect effects occurring within the olfactory epithelium, knowledge that is necessary to fairly compare effects across species, and to validate (help prove) the MOA which proclaims the effect as peculiar to the olfactory epithelium. In my view, this is a significant data gap.

Cytotoxicity

5) In his presentation, Dr. Protzel has set forth the proposed metabolic pathways for the conversion of acetochlor to a quinoneimine, where the latter is said to be a very reactive material, and appears to be viewed as the proximate carcinogen under the hypothesized MOA. The MOA for nasal tissue

neoplasia in the rat involves the binding of the quinoneimine with sulfhydryl groups on tissue proteins, which is considered adverse to cells that leads to a claimed cytotoxicity (cell death), and so on to neoplasia, depicted diagrammatically as follows: quinoneimine-protein binding > cytotoxicity (cell death) > cell proliferation > neoplasia. Under this hypothesis, nasal neoplasia does not occur in the mouse because the mouse does not metabolize acetochlor to the quinoneimine. It is the binding of the quinoneimine to protein that is hypothesized as the initiating toxic insult to the cell. Little more has been said or shown in the supporting materials as to what circumstances of dosing are minimally necessary to bring the process to fruition in the expression of neoplasia. It would certainly seem to be a necessary condition under this hypothesized MOA that quinoneimine-protein binding be sufficiently manifest as to bring about a recognizable state of cytotoxicity (cell death) in the nasal epithelium. But available data do not address just what that level of sufficiency might be. And herein lies one of the most recent questions, which is whether cytotoxicity (cell death) has ever been positively identified, and is anything more than an effect presumed to be real under the hypothesized MOA. I am not certain that quinoneimines, per se, have ever been shown to elicit cytotoxicity, or to be carcinogenic.

One of the key steps in this sequence is cytotoxicity (cell death). As I embraced the huge volume of background materials for the first time, I accepted that there must be substantial evidence for this cytotoxicity and cell death so liberally claimed in the registrant's materials. But as I examined the materials more closely, I was having difficulty finding that evidence for cytotoxicity and cell death, and also in consultation with Dr. Mary Beth Genter, University of Cincinnati researcher, I found she was claiming no cytotoxicity or cell death in her research on alachlor. This inspired my request that the registrant, again in the person of Dr. Joel Kronenberg, to provide the evidence for these effects. After my repeated attempts to obtain a clear statement on the subject, he responded on 1/21/04 (see Dr. Dementi's Email of 1/30/04) that there was *no evidence for nasal tissue cell death for either alachlor or acetochlor*, and no evidence for acetochlor induced cytotoxicity, while he did cite some evidence for cytotoxicity in the case of alachlor. That evidence in my opinion does not rise to the level that people would have anticipated had been present all along and what would be acceptable. I think others of our staff who have had longer experience with this chemical than I were surprised by this revelation. So, as far as I am concerned, the evidence for cytotoxicity is inadequate to support the hypothesized MOA. More needs to be done to prove the existence of nasal cell cytotoxicity at tumor inducing doses and below, i.e. in the range up to and including 1000 ppm. Later we will see that nasal tumors extend to lower dose levels than does cell proliferation as assayed.

Cytotoxicity at doses approximating 1000 ppm (a clearly positive dose for neoplasia) must be demonstrable and not presumed for this MOA to hold water. One would expect to witness this obligate cytotoxicity at doses even below the lowest dose at which tumors are observed.

This evidence of cytotoxicity cited by Dr. Kronenberg now requires further consideration as to whether it constitutes reliable proof of this step in the MOA. *It would appear a definitive supporting cytotoxicity as a meaningful effect was not previously in place when the MOA for*

alachlor was considered, otherwise it would need not be debated so at this point. This acknowledgment of the lack of evidence for cell death and cytotoxicity has spawned just now a debate on the issue among pathologists. Unfortunately, this is a complex subject, and this toxicologist maintains that a thorough review of the subject is required that goes well beyond the quick responses that are reflected in the indicated correspondence. This is why I called for postponement of this meeting, namely to allow me the time as toxicologist to evaluate the materials, but that request was just recently denied.

6) The registrant has cited the nasal neoplasia of phenacetin as providing primary evidence in support of the MOA, where phenacetin clearly induces cytotoxicity (cell death). Phenacetin has evidently been a model compound cited in support of the MOA for nasal cell tumor induction. However, Dr. Genter has explained to us in no uncertain terms that in her laboratory, while phenacetin does indeed elicit profuse cytotoxicity (cell death), alachlor does not in the induction of nasal tumors. There is a marked contrast in Dr. Genter's view as to the mechanism of induction of nasal tumors by alachlor and phenacetin in this respect. So it would appear that while phenacetin and alachlor share in common the induction of nasal tumors, and both would form a quinoneimine, the hypothesized MOA may not be reliable in explaining the carcinogenicity shared by the two agents. Both may well induce nasal tumors by some other mechanism. This is my concern at least, as toxicologists.

This takes quite a swipe at what the registrant has used to support the MOA for alachlor, based upon the effects of phenacetin. I believe that when SAP took up the alachlor issue, the panel may not have been aware of this distinction between alachlor and phenacetin, while phenacetin was being cited as supportive. I postulate that it would have been more difficult for SAP were they fully aware of the absence of evidence of cell death, weak evidence for cytotoxicity, and the marked contrast between alachlor and phenacetin as discussed. So if SAP did support the MOA, I would question but whether that would be the case *now* in the face of this evidence.

7) Concerning the dialogue among pathologists (Drs. Wolfe, Pletcher, Genter and Bolon) being considered on the subject, I think the whole matter is shrouded in confusion that renders highly questionable at this time reliable defense of the hypothesized MOA for alachlor. More time is needed for a balanced assessment, and for me to appraise the subject.

8) There is no evidence in the three acetochlor rat carcinogenicity bioassays, as represented in the histopathology sections of the respective study DERs, recording histopathologic evidence for cytotoxicity or cell death that should be anticipated under the proposed MOA at doses up to and including those that illustrated profound nasal tissue neoplastic responses for these exceedingly rare tumors. Hyperplasia is seen.

Cell Proliferation

9) Another element in the MOA is that of "cell proliferation". This is a complicated subject. The

mechanism study (Doc #13) submitted by the registrant for acetochlor did show increased cell proliferation, but in my view this was only observed at high doses (1750 and 5000 ppm) that exceeded the MTD (1000 ppm), and may have been a consequence of metabolic overload. I think the registrant should not have avoided testing at the 1000 ppm and below dose levels. I seriously question that increased cell proliferation would have been observed at lower doses, particularly of a character to explain the remarkable neoplastic response observed at 1000 ppm. In any case, this needs to be shown for acetochlor. I consider this a deficiency.

In the case of alachlor, a June 19, 1991 Monsanto report [Brewster and Hotz (1991) "A Study of the Effect on Cell Proliferation in Specific Tissues of the Rat and Mouse" (MRID 42852102)] claims, as studied in the Long Evans rat that ".....rats were administered alachlor at 0, 0.5, 2.5, 15, 42 and 126 mg/kg/day and cell proliferation was determined in nasal tissue at 10 and 60 days after initiation of treatment. Also in this study, nasal tissue was examined histologically for specific effects of alachlor." (p. 2). While the study did identify increased cell proliferation confined to the olfactory epithelium at 42 and 126 mg/kg/day after 60 days, there was no evidence of cytotoxicity of the olfactory epithelium. So this study focused the assessment upon the olfactory epithelium, and observed increased cell proliferation**,** but did not confirm cytotoxicity in terms of cytologic changes as assessed by histologic procedures. So if cytotoxicity is expected under the MOA to precede increased cell proliferation in such way as to foster increased cell proliferation, this study which assayed these effects in the olfactory epithelium found only the increased cell proliferation. This study found no effect on cell proliferation in the olfactory epithelium of the mouse.

At doses of acetochlor that elicit tumors (1000 ppm), it is not known whether increased cell proliferation would occur, since it was not tested. And if it did occur, it is not clear the effect would have been seen prior to tumors. Further study would be necessary to address these issues, which constitutes another deficiency. The data on cell proliferation is not adequate to fully support the role of increased cell proliferation as a stage in the proposed MOA for neoplasia of acetochlor, but appears to be so for alachlor. Again, needed is cell proliferation data in the missing range of between 400-1000 ppm, and to compare time of onset of tumors and increased cell proliferation. I should note that whatever the proximate carcinogen may be, increased cell proliferation as an aspect of the neoplastic response is non-instructive as to what that agent might be. In other words, whatever the proximate carcinogen, or mechanism of carcinogenicity, increased cell proliferation and increased tumors move in tandem and constitute the neoplastic response. Together they say no more than tumors alone would say in explaining mechanism or mode of action.

Also, while increased cell proliferation and an increased incidence of cancer may both be identified in a given study when both are tested, it is not necessarily to be presumed that an assay for cell proliferation is necessarily more sensitive than the assay for carcinogenicity. In the case of alachlor induced cell proliferation in the study cited above (dose levels: 0, 0.5, 2.5, 15, 42 and 126 mg/kg/day, the NOEL was 15 mg/kg/day. Yet, in the alachlor cancer bioassay, conducted at the same doses for the full length of the study, nasal neoplastic response was considered real at doses possibly all the way to 0.5 mg/kg/day NOEL. In other words, one of these very rare nasal tumors

was observed at 0.5 mg/kg/day. One cannot therefore presume the use of increased cell proliferation as more sensitive than the neoplastic response in attempting to identify a point of departure (POD) for risk assessment purposes. Tumors occurred at doses well below the NOEL for cell proliferation. This means either the MOA is inaccurate or cell proliferation assays lack sensitivity.

Cell proliferation should be examined in a reproduction study to help address possible earlier onset of the neoplastic effect in pups and offspring.

Autoradiograph

10) In reference to the autoradiography mechanism study in the rat, there was significant localization in the olfactory region at 5000 ppm and only slight to moderate localization was seen at 1750 ppm. However, both doses, 1750 and 5000 ppm, were in excess of the MTD (1000 ppm) in the chronic studies, and these autoradiographic effects may have resulted from metabolic overload that might not be observed at doses up to and including the MTD where tumors occur. It is most disturbing the registrant did not include doses in the range up to and including 1000 ppm, in order to prove an effect in the acceptable dose range, and to help in the search for a key effects NOEL. Another deficiency.

11) The 1996 SAP said "Autoradiography data demonstrating localization of alachlor metabolites only in rat nasal tissue and not mouse or monkey is suggestive of interspecies differences in formation of a reactive product that is retained by nasal tissues, providing *indirect evidence* (emphasis added) for the role of metabolic activation in the carcinogenic process." The committee should be reminded that autoradiographic localization is but "indirect evidence" of a role in neoplasia. As I have said elsewhere, the localization may represent an innocuous sink of the radio labeled material.

Furthermore, autoradiographic studies do not differentiate between quinoneimine protein binding and other mechanisms of acetochlor binding in cells, so it cannot be said with certainty that the autoradiographic evidenced of bio-accumulation necessarily reflects only quinoneimine protein binding, or some other form of binding, or at least in what proportions. This constitutes a weakness in the evidence.

What exactly is to be concluded from autoradiographic evidence of radioactive compound localization in a tissue? The SAP indicated this is but indirect evidence. The fact that one or more metabolites of the parent localized in a tissue, might be misleading that it necessarily is responsible for any particular adverse effect in that tissue. It should not be used in unqualified manner in support of the proposed MOA simply because it and neoplasia were both positive in rat and negative in the mouse. There may be another mechanism of carcinogenicity to which the rat is simply more susceptible than the mouse, i.e. for which the mouse just happens to be a poor model for detecting the neoplastic potential. In my view the registrant and CARC have placed far too

much reliance upon the autoradiography findings. This response should be more seriously evaluated as to its precise contribution in proving the MOA. In my view, it may be a very misleading enticement intending to downplay the real implications of the positive evidence for carcinogenicity. The evidence for carcinogenicity is without contention as to its meaning, and speaks far more definitively than this short term autoradiographic testing to downplay its significance for the public health. Cancer is a lion, radio labeling here a kitten.

12) Given what has been said, two elements of the MOA are in serious question interpretively, namely cytotoxicity (cell death) and cell proliferation.

Mutagenicity Concerns

13) Concerning the question of mutagenicity, the suggestion that alachlor and/or acetochlor is(are) mutagenic finds its expression in the data base. For example: Burman et al (2003) indicate that: "The complete mechanism of alachlor-induced nasal tumor formation in rats has not been elucidated, but we have evidence that metabolic enzymes present in the olfactory mucosa, but not in the liver, bio-activate alachlor to one or more mutagenic species (7). The sites of alachlor-induced tumor formation in the olfactory mucosa correspond with the distribution of cytochrome P450 2A3 [7], suggesting a role for this enzyme in the formation of a *mutagenic/carcinogenic* (emphasis added) metabolite. *The basis for the apparent resistance of mice to the development of alachlor-induced olfactory mucosal tumors [1] is also unclear* (emphasis added)" (p. 1708)

Another example is a concern expressed in the June 27, 1997 CARC report for its February 5, 1997 meeting: ".....alachlor demonstrated nasal tissue DNA binding after 24 hours. Qualitatively, a low level binding to nasal DNA was found, but could not be quantitated. A much higher level of protein binding in both liver and nasal turbinate tissue was observed. This suggests that metabolite(s) of alachlor bind macromolecules such as protein and DNA, and while protein binding is preferential *at doses not considered excessive, both may contribute to the etiology of nasal tumors* (emphasis added)." (p. 11) So it is not altogether clear what, if any, the role of DNA binding has in the etiology of nasal tumor induction, but even if protein binding is the more extensive, lesser levels of DNA binding could be of a character more influential, mechanistically, in carcinogenicity.

Yet another example, the January 27, 1992 "Third Peer Review for Acetochlor" (Doc C3 in the package) says: *"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 <u>in vivo</u> result, and the primary analogue, alachlor, is also positive in this assay. The overall mutagenicity concern would support a concern for carcinogenicity." [pp. 20 (617)-21(618)]*

There are other cases in this data base where mutagenic concerns are raised. I do not have time to assemble these. They are cited at various places in my written presentation. Though I have not personally evaluated any of studies assessing mutagenic activity, mutagenic concern has been often

enough expressed in the overall data base to call for an evaluation of these claims by an independent group of specialists in the field.

Point of Departure (POD)

14) In the assessment of the mechanism or mode of action of acetochlor, the position being taken by the registrant and possibly by the EPA is that the MOE approach is appropriate for carcinogen risk assessment for those agents shown to be non-genotoxic. However, according to the 1999 draft Carcinogen Risk Assessment Guidelines, another rationale exists in support of remaining with the linear approach, which may have been neglected. The Guidelines indicate under "Factors Supporting a Linear Approach" "Any of the following conclusions leads to selection of a linear dose-response assessment approach:" and the fourth such listing reads *"Mode of action analysis does not support direct DNA effects, but the dose-response relationship is expected to be linear (e.g., certain receptor-mediated effects)"* (p. 3-3) There is good reason to suggest, in the case of the acetochlor proposed mechanism or mode of action for nasal olfactory neoplasia, that the quinoneimine-protein binding is linear with dose. There appear to be no mechanistic or mode of action studies that provide evidence for a *change of slope*, as the Guidelines speak of, in the "key events" dose response data, that could be employed to identify a "point of departure" for such data. It is not known just what level of such binding is necessary to elicit a neoplastic response. Presumably, though, it must be the minimal level to elicit cytotoxicity. But a point of departure or threshold for this binding has not been identified, nor could it be, since the mechanistic studies for acetochlor unfortunately were not designed with that aim in mind. Cytotoxicity as an element in the neoplastic progression might also be viewed as a "key event", but cytotoxicity has not even been identified for acetochlor, and, hence, no data exists to identify a threshold for this endpoint in the progression. Nevertheless, the Agency has the obligation to consider the cited perspective in the Guidelines in support of remaining with the conservative linear risk assessment, even in the face of no evidence for genotoxicity. *In substance, the linear approach should not be abandoned and the MOE approach resorted to simply because there is an inadequate finding of genotoxicity.*

Even if the hypothesized MOA is correct, the mechanism studies do not provide the kind of data needed to identify a POD for key events for use in calculating a MOE. The calculation of the MOE that was provided by the registrant in his 1996 package (Doc #24) submitted to the SAP completely ignored key events in the calculation of the MOE based on the NOEL for tumors for the POD, clearly in contrast to what the guidelines advocate. My comments on this are developed under item 1 (Doc #20).

Comments on MOA

15) The MOA clearly claims a nasal tissue neoplastic response that is peculiar to that of the nasal olfactory epithelium of the rat, as contrasted with that of the mouse. In my view, too much emphasis through out these considerations is focused upon the lack of an effect in the mouse as supportive of the hypothesized MOA. First, we are never certain whether this absence of effects in

the mouse resides unambiguously with actual test results as obtained on the olfactory epithelium itself in the mouse studies. Second, even if there is no operation whatsoever of the proposed MOA in the mouse, how much reliance is to be placed upon the absence of this effect in the mouse? The mouse model is recognized in the literature as a poor one for detecting nasal neoplasia for various xenobiotics [see discussion on Brown et al, Doc #18, p. 21(157)], and the absence of such finding with acetochlor and alachlor is therefore not necessarily surprising or particularly revealing. Little interpretative reliance can be placed upon the mouse studies for this lesion.

Furthermore, it is not entirely clear that the neoplastic response is confined to the olfactory epithelium. Respiratory epithelial tumors are another ball game according to the hypothesized MOA. Tumors in the reproduction study were predominately olfactory, but respiratory tumor were observed. The pathology for the various Guideline cancer studies does not appear to differentiate the tumors as to location, except possibly in study PR-08-006, which was re-cut, but even in that case, as I understand, tumors of the olfactory epithelium were not identified as such by the pathologist. Rather, these tumors have been presumed, by other interpreters, to be of the olfactory epithelium since the sections taken were from posterior regions of the nasal epithelium, and that alone. In other words, it does not appear the pathologist characterized these tumors as olfactory tumors.

16) I believe that Dr. Kronenberg's acknowledgement of the absence of any evidence for cell death and minimal or questionable evidence for the cytotoxicity of alachlor alone has been a sourtse of surprise to many on the staff. And in the face of this, were it not a surprise to CARC or SAP, one should easily pick up the record and find the evidence for cell death and cytotoxicity that was presented to SAP and possibly accepted. However, rather than this, a very recent and untimely debate has insued among pathologists over the questioned evidence for cytotoxicity. In my view this comes too late, and a peer review panel should take the time to review the matter properly. It should not be the duty of the Agency to now rise to explain missing cytotoxicity and cell death data so long proclaimed by the registrant, but rather to require the registrant to prove his case. Whose chemical is this, the registrant's or the Agency's? I say the registrant should defend.

17) The Agency now has a reproduction study (Doc #4) which shows a positive neoplastic response of the nasal epithelium, primarily olfactory, at the top two doses. To this I developed comments (Doc #21). I believe it to be unusual to find a neoplastic response in a reproduction study. This is an unusual finding of an exceedingly rare tumor type. In my view this study is not adequate as it stands. It raises questions left unaddressed. For examples, histopathology sections were taken for F1 and F2 pups at PND 29, but not examined. This despite the fact that nasal tumors were observed at early time point (4+ months) in F0 and F1 young animals, and they were not examined any sooner. So one question is how soon do these tumors occur, particularly in young individuals? What is the latency? The study leaves open the question of offspring susceptibility, which under FQPA must be addressed on behalf of children’s interests. I should note that as I understand, alachlor has not been similarly evaluated for nasal histopathology and carcinogenicity in a reproduction study. This finding in acetochlor poses questions about alachlor, and how it would

perform in like testing.

18) All of the work being considered in regard to *mechanism* derives from non-guideline studies, literature publications and various other reviews submitted by the registrant. There are no study protocols established by the Agency for such work, which if followed in testing and review, would be instructive in deciding whether various data should be accepted as proving a *mechanism* or *mode* of action of interest to the Agency. *How much reliance is to be placed upon non-guideline studies, journal articles, and perspectives on the subject submitted by the registrant?*

Summary

19) In my view the assessment of acetochlor is inseparable from the assessment of alachlor and indeed the chloroacetanilide class, and there is the burden of proof upon the registrant to present an unassailable case for the MOA for nasal carcinogenicity, if this is to result in the marginalization of regulatory controls on the use of these carcinogenic agents, especially since this is a public health issue. I cannot accept that this condition has been met. But if the Agency accepts the MOA as valid, I would have the added concern the data available does not provide adequate identification of the key event as spoken of in the CA risk assessment guidelines whose end points would be used to identify a POD in an MOE assessment. The registrant has claimed that quinoneimine-protein binding is the key event, but there is inadequate dose response data at doses up to and including the MTD for this binding to say where the POD lies. ***The NOEL for the tumor response should not be used as the POD in calculating the MOE.***

END

Exhibit X

William Hirzy <whirzy@american.edu>
04/20/04 11:07 AM

To: Brian Dementi/DC/USEPA/US@EPA
cc:
Subject: Re: MTARC meeting

J. William Hirzy
Chemist in Residence
Phone: 202-885-1780

-----Forwarded by William Hirzy/whirzy/AmericanU on 04/20/2004 11:06AM -----

To: bdementi@earthlink.net
From: Makris.Susan@epamail.epa.gov
Date: 04/18/2004 11:54AM
cc: whirzy@american.edu, Carley.John@epamail.epa.gov, Protzel.Alberto@epamail.epa.gov
Subject: Re: MTARC meeting

Brian, I understand your concerns, but the acetochlor CARC and MTARC meetings cannot be delayed any further. The decision on whether or not to conduct a cumulative risk assessment for the chloracetanilides rests on the outcome of the MTARC meeting, and the completion date for this cumulative assessment was established in response to a lawsuit by NRDC. Other decisions and processes that are critical to completion of the acetochlor risk assessment, such as the EFED water assessment, cannot proceed until the cancer meeting is held.

I believe that Alberto's request to Dr. Pletcher to focus his attention on the problem issue (i.e., the evidence of cytotoxicity/cell death) is appropriate. Dr. Pletcher has already been involved in the discussions,

as you are aware. I did not think that Alberto was suggesting that Dr. Pletcher write a position paper, but simply that he be prepared to discuss his thoughts at the meeting. The data package, including correspondence memos, was distributed at least three weeks prior to the scheduled meeting, which should have provided sufficient time for all meeting participants to be familiar with the issues.

It is not, however, appropriate to invite Dr. Genter to participate in the HED CARC and/or MTARC meetings. The contents of these meetings are internal and deliberative, allowing HED scientists to have frank, candid conversations about the subject matter; although of course the resulting decision documents are made publicly available through various processes. Dr. Genter's work is not being critiqued, nor is it being disregarded. On the contrary, her work is cited prominently in the weight-of-evidence discussions on the mechanism of action. I would hope that during the course of the MTARC meeting, you will help identify and articulate any critical issues, needed clarifications, uncertainties, etc. that arise during the discussions, not only in regard to Dr. Genter's work, but more broadly as well. I suggest that we make a point to meet "off-line" during the proceedings (e.g., during breaks or at lunchtime) to discuss whether your concerns are being adequately addressed. (Please note that I have a schedule conflict on Wednesday morning, but will arrive as soon as possible in the afternoon of that day.) Sue

Susan Makris
USEPA/OPPTS/OPP/HED/TOX (7509C)
1200 Pennsylvania Ave., NW
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P.S. I think you need to check the internal clock on your computer, since your message appears to have been sent on 04/17/04, not 08/07/03.

brian dementi <bdementi@earthli To: Susan

Makris/DC/USEPA/US@EPA
nk.net> cc:
whirzy@american.edu
Subject: MTARC meeting

08/07/03 06:29
AM
Please respond
to
bdementi

Susan, April 16, 2004

As a follow-up to the meeting we had yesterday afternoon (5/14) to discuss the upcoming meetings (MTARC/CARC) [present were Jessica Kidwell, Bill Burnam, Nancy McCarroll, Alberto Protzel, Jess Rowland (late arrival), and the two of us], I have concerns over the manner of review of pathology evidence for cytotoxicity (cell death) of the nasal olfactory epithelium that is taking place at this late date prior to the meeting. It is my opinion that whatever is to be said by our pathologist(s) be fairly commented on by others in the public place who may have already been drawn into the issue. I made it clear at our meeting that Dr. Mary Beth Genter, University of Cincinnati, be accorded the opportunity to confirm/refute or otherwise comment on that which may be being said of her work by our staff. She and her colleagues have performed historic work on alachlor, so much in fact that the registrant quotes her (their) work, even though her views in various ways do not align with the registrant?s perspectives.

Also, as I understood, Alberto has requested our staff pathologist (Dr. Pletcher I believe) to develop something on the subject. Whatever our pathologist may have to say should be available well before the meeting

to afford fair opportunity for persons such as myself to comment. But there isn?t enough time between now and 4/21 for comment. I acknowledge that I am not a pathologist, but I am a well educated scientist assigned to this project who can ask questions and expect that science as presented by others should be reasonable. Furthermore, where pathology issues are concerned, there are certain outside experts with whom I discuss such issues. Basically, what I am saying is that the question of cytotoxicity and cell death as elements of the registrant?s hypothesized MOA are now under discussion, largely brought about by the registrant?s acknowledgment this past January 21, that the he has no data showing cytotoxicity and/or cell death for acetochlor, and does not have data showing cell death for alachl

The entire collection of evidence needs to laid out, and then reasonable time accorded individuals such as myself to evaluate it. I do not consider there to be adequate time for this prior to the meetings.

Therefore, I must request that the hearings be stalled, i.e. postponed until such time as all persons concerned have had a reasonable period of time to review all that is currently being said. Evidence for cytotoxicity is too central to the validity of the MOA for a cursory review of the evidence for its existence in the neoplastic process.

Best Wishes,

Brian Dementi, Ph.D., DABT

Exhibit XI

"KRONENBERG, JOEL M [AG/1000]" <joel.m.kronenberg@monsanto.com>
04/27/04 04:46 PM

To: Larry Chitlik/DC/USEPA/US@EPA
cc: Susan Makris/DC/USEPA/US@EPA, Alberto Protzel/DC/USEPA/US@EPA, Brian Dementi/DC/USEPA/US@EPA, Linnea Hansen/R9/USEPA/US@EPA, "DIRKS, RICHARD C [AG/1000]" <richard.c.dirks@monsanto.com>, "HEYDENS, WILLIAM F [AG/1000]" <william.f.heydens@monsanto.com>, "FEE-WHITE, DAWN M [AG/1000]" <dawn.m.fee-white@monsanto.com>, "'Juberg, Daland (DAS)'" <drjuberg@dow.com>, "'Kaminski, Barb (DAS)'" <BJKaminski@dow.com>
Subject: RE: Need for additional Acetochlor data

Larry:

This is a follow-up to our phone conversation on April 15 regarding your request for additional information to support the pathology results of the acetochlor chronic rat and mouse studies. As I indicated, Monsanto no longer owns a toxicology laboratory or employs any pathologists. However, in response to your inquiries on these studies, I have spoken with Darryl Thake, the former Director of Pathology for Monsanto, and Jerry Hardisty, the EPL pathologist who managed the pathology peer reviews and Pathology Working Groups that were conducted on these studies. I have also spoken with Dow AgroSciences (DAS), which has replaced Zeneca as Monsanto's partner in the Acetochlor Registration Partnership (ARP), and which currently owns one of the two mouse studies in question (MRID 41565119).

The slides from the four chronic studies you cited are stored in the archives of either Monsanto or Dow AgroSciences and thus could probably be reviewed within a month of authorization. However, none of the individuals I spoke to believe that such a review is warranted for the following reasons:

A. Nasal Olfactory Epithelium

You have requested that we re-examine the slides of the nasal turbinates from 4 of the 5 chronic studies to confirm that olfactory tissue was indeed present. If I understood you correctly, the primary concerns were

(1) to ensure that the apparent species-specific nasal tumor response was not a result of failure to examine olfactory tissue from mouse nasal turbinates, and (2) to ensure that the sex-related difference in response observed in the first chronic rat study was not a result of failure to examine olfactory tissue from females.

The ARP believes that re-evaluation of the nasal turbinate slides is not necessary to resolve either of these concerns for the following reasons:

1. Even if the first Monsanto mouse study with acetochlor (MRID 00131089) is discounted, there are more than sufficient data available demonstrating that neither acetochlor nor alachlor produce olfactory tumors in mice:

(a) Olfactory tissue was specifically included in the protocol list of tissues to be evaluated for all animals in the second mouse study with acetochlor (MRID 41565119).

(b) No nasal tumors were noted in either of two Monsanto chronic mouse studies with alachlor, which is a more potent nasal carcinogen than acetochlor.

(c) No nasal tumors were reported following chronic administration of alachlor to two strains of mice by Mary Beth Genter (March 2004 SOT presentation). Dr. Genter is a leading academic expert on alachlor-induced olfactory tumors and has been consulted by EPA (Brian Dementi) on several occasions.

2. We cannot explain the low olfactory tumor response in females in the 1983 Monsanto rat study with acetochlor. However, this was not a result of failure to examine olfactory tissue since these tissues were re-examined in 1986 to specifically look for nasal olfactory tumors (MRID 40484801). No significant sex-difference was observed in either of the other two acetochlor rat studies, or with alachlor, so the apparent sex-specific response in this one study is considered to be aberrant.

B. Other Potential Target Organs

Based on your email and our phone conversation, it appears that your request for a "slide/tissue inventory" for the other potential target organs was a result of a concern that there may have been a number of

autolyzed/not readable tissues that were not properly accounted for in the pathology tables. If so, you indicated that this would affect the denominator and could alter the cancer slope factor (Q1*) in a quantitative oncogenic risk assessment. We believe that conducting such a "slide/tissue inventory" for this purpose is unnecessary for the following reasons:

1. All five of the acetochlor chronic studies were conducted under GLPs and were subjected to QA inspections and audits. In addition, at least two of the studies (the 1983 rat and mouse studies) were subject to an in-depth audit in 1985 by Dr. Adrian Gross and four other EPA auditors. Therefore, it is highly unlikely that there would be a significant discrepancy between the numbers of tissues examined and the numbers listed in the final reports. It is possible that there may be some very minor discrepancies in some of the more fragile tissues, particularly for the two earliest studies that were conducted in the early days of GLPs, but it is highly unlikely that these would have any significant impact on either the study conclusions or risk assessment calculations. Any discrepancies would be even less likely in the later studies because of the use of computers for data collection and compilation, as well as the more established GLP practices.

2. Autolysis was sometimes a significant problem in older studies (i.e., 1970's). However, this occurred primarily in smaller tissues from animals (particularly mice) that were found dead during the study. This was generally not a significant problem in studies conducted in the mid-1980's, particularly for animals that were sacrificed moribund or killed at scheduled interim or final sacrifices.

3. Questions regarding the number of tissues evaluated and the possible impact of autolysis were raised by the Agency in 1988 relative to the second Monsanto rat study (MRID 40077601). However, this was apparently due to lack of clarity in the format Monsanto then used for our histopathology tables as this information was actually included. This was clarified by Dr. Dennis Ward (Monsanto) in discussions with Stephen Dapson and in a letter to the Agency dated July 29, 1988. The Agency agreed and confirmed the adequacy of the data in a letter from Drs. Dapson, Rowe and Van Gemert dated January 17, 1989.

4. Formal Pathology Working Group (PWG) evaluations have been conducted on a number of the tissues listed in your email, including rat and mouse liver, mouse lung, mouse kidney, and mouse uterus (as part of histiocytic

sarcoma). As indicated in the PWG reports, the first step in these PWGs was to have an independent pathology peer review of all of these tissues for all animals. In a recent phone conversation, Dr. Hardisty indicated that although the EPL reports for these PWGs specified only the numbers of animals in each group, not the number of tissues that were actually re-examined, he would have disclosed any significant discrepancies in either the tables or text of the report. Dr. Hardisty has now reviewed his files for these studies and concluded that "very few missing slides were noted during the Peer Review/PWG reviews of these studies".

5. No formal PWG was conducted on the thyroid follicular tumors. However, it has been demonstrated that acetochlor, alachlor and butachlor induce thyroid tumors as a consequence of induction of hepatic UDPGT. This is a well-known threshold-mediated mechanism to which the rat is especially sensitive and as such would not be subject to quantitative oncogenic risk assessment. Thus, even in the highly unlikely event of a significant discrepancy in the number of thyroids evaluated, this would not have any impact on the risk assessment calculations.

Because of the reasons outlined above, the ARP respectfully requests that the Agency reconsider its request for reexamination of the slides from the chronic studies. We do not believe that the information obtained by performing this work would have any significant impact on the Agency's cancer classification or risk assessment decisions. We believe that the data from five chronic studies (especially the last three) are more than adequate to assess the potential oncogenicity of acetochlor, particularly when considered together with the data previously evaluated for alachlor.

I will be present at the acetochlor SMART meeting on May 19 and would welcome the opportunity to discuss this further.

Regards,

Joel

Joel Kronenberg, Ph.D., D.A.B.T.
Team Lead, Food & Chemical Toxicology
Monsanto Company

-----Original Message-----
From: Chitlik.Larry@epamail.epa.gov
[mailto:Chitlik.Larry@epamail.epa.gov]
Sent: Friday, April 02, 2004 8:54 AM
To: joel.m.kronenberg@monsanto.com
Cc: Makris.Susan@epamail.epa.gov; Protzel.Alberto@epamail.epa.gov; Dementi.Brian@epamail.epa.gov; Hansen.Linnea@epamail.epa.gov
Subject: Need for additional Acetochlor data

Dear Dr. Kronenberg,

As a result of our re-examination of the Acetochlor mouse oncogenicity and rat chronic/oncogenicity studies relative to the upcoming CARC meeting, some issues have recently been identified that we hope to resolve prior to this meeting. If we can resolve these issues, this should expedite the peer review process. We would like you to provide a slide/tissue inventory for each study and tissue as specified below. We must confirm the number of tissues actually present on those slides for each specified tissue in order to produce a meaningful risk assessment. Specifically, please note the following:

For the high dose group of each study (both sexes), please provide the total number of tissues on the slides which were examined. Subtotals should include tissues from interim sacrifices, early deaths and moribund sacrifices and those sacrificed at term. Exclude from this total the number of missing or autolyzed unreadable tissues. If any differences are noted as compared to the submitted report, examination of these tissues from the lower dose groups and controls will be necessary. If differences persist in other lower dose groups, a slide inventory for all tissues and all groups might then be necessary. I believe that if there are few differences noted at the high dose level, this task will not be very time consuming.

The CARC meeting is scheduled three weeks from today. Please note that this request is based upon a consensus of toxicologists currently reviewing Acetochlor toxicology data. As noted above, if this task can be completed prior to the ucoming meeting, we believe that this would greatly expedite the review process. I will be out of the office during some of the next few weeks and if you have questions, please follow them up with Dr. Brian Dementi. Thanks

Please provide a slide/tissue inventory based upon a re-examination of the actual slides as noted below:

1983 Mouse Oncogenicity Study, (MRID 00131089)

Olfactory region of the nasal epithelium, thyroid, lung, uterus, kidney, ovary and liver

1989 Mouse Oncogenicity Study (MRID 41565119)

Olfactory region of the nasal epithelium, kidney, lung, thyroid, adrenal, liver, testis and ovary

1986 Rat Study (MRID 40077601)

Olfactory region of the nasal epithelium, lung, thyroid/ parathyroid, and liver.

Relative to this study, I am aware that a similar question was raised in the original review with a
response by Monsanto (Dennis Ward) of 7/25/88. Unfortunately, the response did not include an actual tissue slide inventory.

1983 Rat Chronic/Oncogenicity Study (MRID 00131088 and 40484801)

Olfactory region of the nasal epithelium, pituitary, thyroid, testis, uterus, pancreas and liver

COMMENTS ON 8/5/04 ACETOCHLOR CARC DRAFT

Brian Dementi 08/16/04 04:18 PM

To: William Burnam/DC/USEPA/US@EPA
cc:
Subject: August 5 Acetochlor CARC report; sign off related comments

William Burnam
Chairman, CARC
HED

Dear William,

In my May 19, 2004 "Dissenting Views on the April 21-22, 2004 Assessment of Acetochlor", I presented much that is of concern to me regarding the validity of the hypothesized MOA for nasal tumor induction. I remain of the opinion that were nasal tumors recognized as the most sensitive indicator of carcinogenicity, it would be inappropriate to depart from the quantitative risk assessment based on these lesions. I will not attempt to reiterate that which I have presented before in the May 19 document. However, in order for me to sign the CARC report (Parts 1 and 2), it is necessary that I make a few additional observations, which must be included as an addendum to my May 19, 2004 dissenting views as appended to the CARC report. I believe other committee members should be made aware of my comments.

I) In reference to Part 1, concerning nasal tumors in offspring in the reproduction study as mentioned on p. 2, I do not accept that the evidence for decreased latency can be dismissed as reflective of earlier sacrifice time. This requires confirmation, as offspring susceptibility is such an important issue. As it stands, the proper interpretation is that this study evidences to a degree not adequately explored, heightened vulnerability of offspring to this neoplastic response. Also, this finding should now serve as a directive that additional study be conducted with acetochlor to more firmly characterize offspring versus adult latency and magnitude of response. Furthermore, since the toxicology of alachlor is so heavily wedded to the assessment of acetochlor as an analogue under the common mechanism assessment, then conversely, the acetochlor study informs that an analogous reproduction study be required in the case of *alachlor,* to include a yet more rigorous assessment of nasal tissue responses (latency and magnitude of response).

Additionally, the acetochlor reproduction study DER (TXR No. 0050658; MRID 45357503) indicates that nasal passages from PND day 29 offspring were preserved in formalin, but not examined. It is important that these tissues be examined histopathologically, in both the F1 and particularly the F2 generations.

On p. 58 where the CARC report compares the nasal tumor incidence in the three rat bioassays, a preferred statement in reference to cancer study 1, would read as follows: "Incidence in females *at 500 (study 1), 1000 (study 2), 1500 (study 1), 1750 (study 3) and 5000 (study 1) ppm* was 0%, 28%, 3%, 57% *and 0%."* A statement such as this serves to emphasize that study 1 was a negative study in females, even at 5000 ppm, and that had a 500 ppm dose level been included in either study 2 or 3, 500 ppm likely would have been quite positive, particularly in view of the rarity of the nasal tumors in control rats. However, given the situation, we can only conclude there has been inadequate testing for nasal tumors in this critical dose range. This in essence constitutes a dose deficiency in the testing of acetochlor for nasal neoplasia, and should be acknowledged in this report.

II) In reference to Part 2 (MOA report), when developing my comments for this meeting, the subject of the possible role of lipofuscin in nasal tissue neoplasia had not been a topic of consideration by anyone I know of, including the registrant. However, the subject came up just immediately prior to the April 21-22 meeting, and much issue was then made of it, having been found in the reproduction study, in support of the nasal tissue MOA. It is cited in at least ten places in the Part 2 manuscript. So, all of a sudden, this finding has assumed a key place in the arguments wagered in support of cytotoxicity as a key element in the nasal tissue neoplastic response.

I had no opportunity as toxicologist reviewer to consider the subject at that time just before the meeting, but have a few comments to offer here. First, I get the impression that much weight is assigned to the finding, which I do not view as merited. For example, Part 2 says: "Lipofuscin pigment was observed to increase in a dose related manner in the nasal olfactory epithelium of rats that show nasal olfactory tumors at the high dose. Lipofuscin pigment is associated with oxidative damage to lipids and lipoproteins, which is consistent with the redox alterations known to be produced by quinones and quinone imines." (p. 2) This statement is made while there is no evidence to say that quinones or quinoneimines are responsible for the effect. This is but an association that unjustifiably implies a cause and effect relationship. As indicated, similar statements appear through out the manuscript as if this were the long awaited evidence upon which to sustain an argument in support of an oxidative mechanism for cytotoxicity, heretofore not recognized, even by the registrant.

This rationale should incorporate certain qualifying remarks. For example, the CARC (Report 1) says: "Increased lipofuscin of the olfactory mucosa was observed in all dose groups of females (almost all animals affected at 600 and 1750 ppm) *but was not considered adverse due to minimal to slight severity and lack of this finding*

in the chronic rat studies (emphasis added). (pp 28-29). Similarly, the reproduction study DER says: "While the dose-response nature suggests that these findings are related to treatment, they were characterized as minimal to slight in severity and *were not clearly associated with the purported mechanism of action for tumor induction in the nasal epithelium* (emphasis added). The weight of the evidence therefore suggests that the increased incidence of lipofuscin in female rats at 200 ppm was not an adverse event, and should not be used as the basis of the LOAEL in this study." (p. 21) Now Part 2 of the CARC report not only ignores these qualifying statements, but says: "Minimally increased brown pigment (lipofuscin) was observed in the olfactory mucosa, mainly in the lamina propria and occasionally in the basal epithelium in most animals receiving 600 and 1750 ppm in both F0 and F1 generations and also in F1 females at the 200 ppm dose level; however, this finding was not." (p. 36) Not what, one might ask? The statement is obviously incomplete. Actually, the full rendition appears in the DER for the acetochlor reproduction study and reads exactly as above, but more completely, thusly: "*.....; however, this finding was not considered to be of toxicological significance* (emphasis added)." (p. 2) This is a most significant qualifying statement in the reproduction study that appears to have been left off of what should be a statement cited in its completion and referenced as a quotation from the reproduction study DER.

Given the qualifying remarks in the CARC, Part 1, report and reproduction study DER, regarding the character of the lipofuscin finding, I dissent from the employ of this finding as carrying the weight newly assigned to it as supporting the cytotoxicity element of the proposed MOA for nasal neoplasia. This is a critical issue to the veracity of the MOA. If the CARC members are prepared to accept this new meager evidence for cytotoxicity as somehow explaining or undergirding cancer causation, *the committee must own acknowledgment of these qualifying remarks.* Also, this issues of the role of lipofuscin has come to fore so suddenly, there has been no serious study of its possible meaning in carcinogenicity. Precisely because this finding has been assigned so much significance in Part 2 in CARC's assertion of cytotoxicity, attests to the real shallowness of the evidence for the nasal cytotoxicity that is so central to the MOA. This issue of the role of lipofuscin should go before outside peer reviewers.

Further, if indeed lipofuscin is playing a critical role in neoplasia as Part 2 appears to claim, the incidences of this effect should be viewed as "key events" as defined in the 1999 Cancer Risk Assessment Guidelines, and treated as such in extending the neoplastic dose response. The finding suggests enhanced offspring susceptibility. And it would need to be treated as a LOAEL in the reproduction study, requiring a revision to the DER.

Part 2 says "Most of the benign tumors exhibited ciliation of the olfactory epithelial cells and were associated with **respiratory metaplasia of adjacent olfactory mucosa."**(p. 23) It would be helpful to your audience to cite the very documents and pages within those documents that would support of this claim.

Part 2 says: "Rats administered the sulfdoxide metabolite of acetochlor (a proximate precursor of the toxic metabolite, the quinoneimine) show nasal olfactory mucosa adenomas after 26 weeks of treatment (MRID 46081801)." (p. 60) It should be recognized that this particular MRID is the registrant's so-called "white paper", which HED requested of the registrant, and regarding this particular one-year study of the sulfoxide, the white paper presents the data in Appendix 3, while claiming that: "This report is currently in draft form and will be submitted in the near future." So to my knowledge this report has not been submitted, and much less reviewed. Suitable qualification is needed here in making claims about what the study showed.

Report 2 claims: "The weight of the evidence in support for the mode of action evaluated in this document is *high* (emphasis added). Rationale for this claim is offered. (p. 3). By contrast, the report claims on p. 60: "Thus, the confidence on this mode of action is *'moderate to high*'"(emphasis added), where essentially the same rationale for this level of confidence is offered as also on p. 3. What is the true level of confidence in the mind of the CARC for this MOA?

Report 2 says: "Re-reading of the slides for the 1988 acetochlor rat chronic study (MRID 41592004) and for butachlor and alachlor studies indicated that most of the benign tumors were associated with respiratory metaplasia of the adjacent olfactory epithelium." (p. 48) Needed at this point in Part 2, for the benefit of the reader, is a reference citation wherein this claim of metaplasia is to be found in support of this very important claim. MRID 41592004 itself does not make this claim regarding metaplasia, as I examined the document.

Best Wishes,
Brian Dementi, Ph.D., DABT
Toxicologist

RESPONSE TO COMMENTS

Hi Brian,

We have had a chance to look over your latest memo (8/16/04 email to William Burnam) containing your additional comments on the acetochlor CARC/MTARC document. We appreciate your bringing to our attention a few editorial errors or statements needing clarification in each of our documents which had been overlooked during the final review.

Although the major issues that you disagree with will not change (acceptance of the proposed MOA for nasal tumors, use of linear low-dose extrapolation for the cancer risk assessment of acetochlor instead of the nasal tumors), the following changes have been made to the CARC/MTARC documents to reflect your comments:

I. CARC Part 1

(1) on pp. 28-29, discussion of the nonneoplastic findings of the multigeneration reproductive toxicity study just below Table 15, sentence beginning "Increased lipofuscin of the olfactory mucosa.": the phrase ..."but was not considered adverse due to minimal to slight severity and lack of this finding in the chronic rat studies" was removed and instead it was added that the finding was considered to be treatment-related.

The issue of the relevance of lipofuscin accumulation (observed in the rat multigeneration reproductive toxicity study) to the proposed nasal tumor MOA was indeed a subject that came to be reconsidered shortly prior to the meeting. This became more apparent while revisiting the database in preparation for the CARC/MTARC meeting, and from the expert opinions of the two consulting veterinary pathologists at the meeting. This finding is not the only, or even the major support for the mechanism, but rather is consistent with oxidative damage to the olfactory epithelial cells by the quinoneimines and the subsequent responses of respiratory metaplasia and hyperplasia/tumor formation. The draft DER for the rat reproductive toxicity study stated that the finding was probably not relevant to the MOE, but should be modified to indicate that it is likely to be an early event secondary to oxidative damage.

(2) As suggested, in the WOE discussion (p. 58 under Bullet 2, "Nasal olfactory epithelium"), the sentence giving the incidence of nasal tumors has been revised to include the study number in parentheses after each dose level. The 5000 ppm dose was not included in that sentence because the dose caused excessive

toxicity and was not considered to be relevant to the cancer WOE. In order to provide more clarity to that discussion, a sentence was added at the end of the paragraph stating that fact, but also providing the incidence at the excessive dose (see CARC 1, p. 58). It is noted here that although in females there was a surprisingly low incidence of nasal tumors at the higher doses, incidence in males including at 500 ppm showed a clear dose response. In the repro study, 200 and 600 ppm doses were also included (one adenoma at 600 ppm, none at 200 ppm), and testing is considered adequate when all the studies are evaluated.

II. MTARC Part 2.

Your comments for Part 2 (MTARC) have been addressed as follows (in order of occurrence):

Comment No 1:
"Now Part 2 of the CARC report not only ignores these qualifying statements, but says: "Minimally increased brown pigment (lipofuscin) was observed in the olfactory mucosa, mainly in the lamina propria and occasionally in the basal epithelium in most animals receiving 600 and 1750 ppm in both F0 and F1 generations and also in F1 females at the 200 ppm dose level; however, this finding was not." (p. 36) Not what, one might ask? The statement is obviously incomplete. Actually, the full rendition appears in the DER for the acetochlor reproduction study and reads exactly as above, but more completely, thusly: "....................; *however, this finding was not considered to be of toxicological significance* (emphasis added)." (p. 2) This is a most significant qualifying statement in the reproduction study that appears to have been left off of what should be a statement cited in its completion and referenced as a quotation from the reproduction study DER".

Reply: The phrase " this finding was not." (P.36) has been eliminated. It was left there by oversight. The finding of lipofucsin is of toxicological significance in so far as it has been seen associated with oxidative damage. This was acknowledged during the CARC meeting by the pathologists. The Repro study DER will be revised to indicate that the lipofucsin pigment has a toxicological significance. Whether and how it affects the NOAEL for reproductive effects will have to be discussed when the endpoints for risk assessment are discussed.

The statement " "Minimally increased brown pigment (lipofuscin) was observed in the olfactory mucosa, mainly in the lamina propria and occasionally in the basal epithelium in most animals receiving 600 and 1750 ppm in both F0 and F1 generations and also in F1 females at the 200 ppm dose level; however, this finding was not.", now reads:
"Minimally increased brown pigment (lipofuscin) was observed in the olfactory

mucosa, mainly in the lamina propria and occasionally in the basal epithelium in most animals receiving 600 and 1750 ppm in both F0 and F1 generations and also in F1 females at the 200 ppm dose level."

Comment No. 2

Part 2 says "Most of the benign tumors exhibited ciliation of the olfactory epithelial cells and were associated with **respiratory metaplasia of adjacent olfactory mucosa."**(p. 23) It would be helpful to your audience to cite the very documents and pages within those documents that would support of this claim.

Reply: Modified to read:

Most of the benign tumors exhibited ciliation of the olfactory epithelial cells and were associated with **respiratory metaplasia of adjacent olfactory mucosa.** Many lesions were close to the olfactory-respiratory epithelial junctions (See pp. 14-16 in MRID 44496214).

Additional references to the alachlor and butachlor studies themselves appear below Table 6 as follows:

"To determine if there was a similarity in morphology, origin, and location of proliferative lesions , the Registrant conducted a review (See MRID 44496214) of hematoxylin/eosin slides of nasal tissue of rats treated with acetochlor, alachlor or butachlor in previously conducted long-term oral studies. In the case of acetochlor, slides from study #3 (88/SUC017/0348, MRID 41592004) of Table 1 were used. In the case of alachlor, slides from an alachlor special rat chronic study (EHL 93049, MRID 43590001); and in the case of butachlor, slides from a butachlor rat chronic study (Biodynamics 79-2388, MRID 00088984) were used."

Comment No. 3

"Part 2 says: "Rats administered the sulfoxide metabolite of acetochlor (a proximate precursor of the toxic metabolite, the quinoneimine) show nasal olfactory mucosa adenomas after 26 weeks of treatment (MRID 46081801)." (p. 60) It should be recognized that this particular MRID is the registrant's so-called "white paper", which HED requested of the registrant, and regarding this particular one-year study of the sulfoxide, the white paper presents the data in Appendix 3, while claiming that: "This report is currently in draft form and will be submitted in the near future." So to my knowledge this report has not been submitted, and much less reviewed. Suitable qualification is needed here in making claims about what the study showed."

Reply: Modified to read:

Rats administered the sulfoxide metabolite of acetochlor (a proximate precursor of the toxic metabolite, the quinone imine) show nasal olfactory mucosa adenomas after 26 weeks of treatment (MRID 46081801). It is noted these results on the sulfoxide metabolite were submitted to the Agency as a brief preliminary report, part of an ARP's position paper (MRID 46081801). The full report, presently in draft form, has not yet been submitted to the Agency to undergo a full review.

Comment No. 4

"Report 2 claims: "The weight of the evidence in support for the mode of action evaluated in this document is *high* (emphasis added). Rationale for this claim is offered. (p. 3). By contrast, the report claims on p. 60: "Thus, the confidence on this mode of action is *'moderate to high*'"(emphasis added), where essentially the same rationale for this level of confidence is offered as also on p. 3. What is the true level of confidence in the mind of the CARC for this MOA? "

Reply: The statement in page 60 " ...moderate to high... " was meant to be removed, so as to read as in p. 3. The statement in p. 60 reads now as in p 3.:

The weight of the evidence in support for the mode of action evaluated in this document is high. The evidence would have been strengthened if corroborative experiments, such as prevention or reversal of a precursor event (e.g. cell proliferation) by appropriate administration of a chemical (e.g. N-acetylcysteine) known to interfere with a key step (e.g. formation of quinone imine), had been available. Although dimethylaniline (DMA) and diethylaniline (DEA) [analogs of ethylmethyl aniline (EMA)] have been found to form *in vivo* DNA adducts in rat nasal mucosa, concerns about a genotoxic mechanism for acetochlor are mitigated by several factors. These include absence of formation of DNA adducts in nasal mucosa in parallel experiments in rats using alachlor and the reversibility of cell proliferation of olfactory epithelium observed with the analog alachlor.

Comment No. 5.

Report 2 says: "Re-reading of the slides for the 1988 acetochlor rat chronic study (MRID 41592004) and for butachlor and alachlor studies indicated that most of the benign tumors were associated with respiratory metaplasia of the adjacent olfactory epithelium." (p. 48) Needed at this point in Part 2, for the benefit of the reader, is a reference citation wherein this claim of metaplasia is to be found in support of this very important claim. MRID 41592004 itself does not make this claim regarding metaplasia, as I examined the document.

Reply: The above is replaced with:

Re-reading of the slides (See MRID 44496214) for the 1988 acetochlor rat chronic study (MRID 41592004) and for butachlor (MRID 00088984) and alachlor (MRID 43590001) studies indicated that most of the benign tumors were associated with respiratory metaplasia of adjacent olfactory epithelium. This effect implies disappearance (death) of olfactory epithelium and replacement with respiratory epithelium.

Your 8/16/04 memo may therefore be amended to reflect these changes prior to inclusion in the final CARC document on acetochlor so that your final comments are consistent with our documents. Again, thanks for taking notice of these points that needed our attention.

Alberto and Linnea