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OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **MON 5775 (ESA metabolite of Alachlor):** Review of rat teratology study, mutagenicity study and metabolism studies (special metabolism and cell proliferation) with MON 5775 and a comparison of the ESA metabolite with the parent compound, Alachlor.
EPA DP Barcode D222904; EPA Submission Barcode S500250; EPA MRID No.'s 43889401, -02, -04, 43908101; EPA Pesticide Chemical Code 090501; HED Chemical No. 011; Reregistration Case# 0063

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Registrant: Monsanto Company

Action Requested: Review rat teratology study, mutagenicity study and metabolism studies (special metabolism and 2 cell proliferation) with MON 5775 and a comparison of the ESA metabolite with the parent compound, Alachlor.

Recommendations: TBII reviewed a rat teratology study, a mutagenicity study, a special metabolism study and two cell

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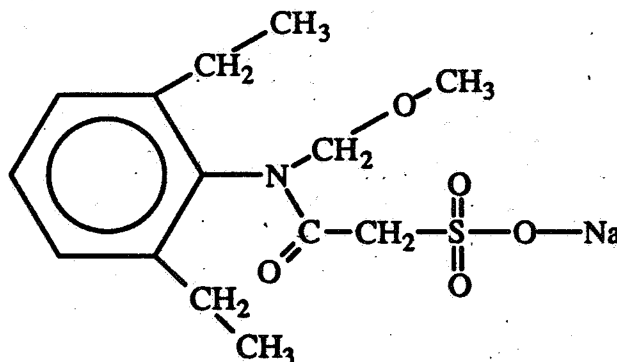
proliferation studies with MON 5775 and rereviewed the 91 day drinking water study in rats and the **IBT** 90 day subchronic rat feeding study. The conclusions from those reviews are presented in this memo. The data provided indicate that, overall, MON 5775 is less toxic than the parent Alachlor.

BACKGROUND:

The following was provided by the registrant in a document entitled *Overview of Exposure and Toxicology on MON 5775* (Project No. R.D. No. 1335, MRID# 43889405):

Chemical Nature of MON 5775 Test Material

In soil, alachlor is partly metabolized to a compound in which the chlorine atom has been replaced by a sulfonic acid moiety with the following structure:



This material has been referred to by the following names and codes:

- •MON 5775
- •CP 108065
- •sulfonic acid metabolite of alachlor
- •alachlor sulfonic acid
- •2',6'-diethyl-N-methoxymethyl-2-sulfoacetanilide, sodium salt
- •2-[(2,6-diethylphenyl)-(methoxymethyl)-amino]-2-oxoethanesulfonic acid, sodium salt
- •ESA

Although sometimes the term "sulfonic acid" is used to refer to the sulfur-bearing moiety in this molecule, the substance has always been isolated from natural matrices and synthetic preparations in its salt form for the toxicology studies, the sodium salt was always utilized

ESA Occurrence in Natural Matrices

ESA was originally identified as a metabolite of alachlor in soil (MRID 00134327). It constituted 15 - 25% of the applied radioactivity, making it the first or second most prevalent degradate. ESA has also been quantified in field soil dissipation studies following alachlor applications (MRID's 42528002, 43774701); low concentrations were detected but ESA was not found to persist or leach below 18 inches.

In water, ESA has been identified as a minor alachlor degradate in a laboratory aqueous sediment metabolism study (MRID 43774702). It has also been detected in water samples from Indiana (MRID 42479901) and Wisconsin (no MRID, submitted under FIFRA 6(a)(2)). These well water samples were taken from wells that were previously found to contain pesticide contamination. In the Indiana well water samples, ESA concentrations ranged from <1.0 - 23.0 µg/L, and in Wisconsin they ranged from <1.0 - 26.7 µg/L. Since the sampled wells were located in vulnerable hydrogeologic locations and had previous detections of pesticide residues, these results constitute an estimate of worst-case possible exposure levels.

The metabolic route leading to ESA is postulated to involve initial glutathione displacement of the chlorine atom, followed by successive degradation of the sulfur conjugated moiety through organic acid and methylsulfone intermediates, to ultimately form the sulfonic acid group as a terminal oxidative degradate.

Plant metabolism studies have been conducted with alachlor in corn and soybeans (MRID 00131424). ESA was identified as one of many alachlor degradates present in these crops. Since ESA is converted to diethylaniline (DEA) by the alachlor crop residue methodology, it has been quantified in the crop residue analyses conducted for alachlor and, as such, is included in the existing crop tolerances listed at 40 CFR § 180.249.

Animal metabolism of alachlor has been extensively investigated in rats (MRID's 00132045, 42852107, 42931101), mice (MRID 42852106) and monkeys (MRID's 00154238, 40000901). ESA has not been identified in these studies as a mammalian metabolite of alachlor. The initial chlorine displacement step involving glutathione

catalyzed by glutathione transferase has been firmly established in these mammals. Methylsulfone and sulfur-conjugated organic acids, which are also found in soil, have been shown to arise from further metabolic conversion of the glutathione adduct in rats and monkeys. Thus, although the initial metabolism of alachlor in mammals and in soil is identical oxidative conversion of the sulfur atom does not proceed to completion in mammals to produce ESA as it does in soil.

Livestock metabolism studies in support of alachlor registrations were conducted by dosing hens and goats with a mixture of alachlor plant metabolites, which included ESA as one dosed component (MRID's 00147472, 00147473). Results from these experiments demonstrated that ESA was excreted by the animals unchanged, largely via the feces (goats). It did not accumulate in edible tissues. This result provides evidence that ESA administered via the diet is not absorbed by animals nor is it extensively metabolized, in contrast to the results for alachlor itself. The conclusion from the livestock studies is reinforced by the data in the present submission on uptake, distribution, and metabolism in rats following oral administration.

DISCUSSION:

COMPARISON OF THE ESA METABOLITE OF ALACHLOR AND PARENT ALACHLOR TOXICITY:

I. Acute Toxicity:

ESA Metabolite Study:

Acute Oral Toxicity Study in Rats with MON 5775 (Springborn Laboratories, Inc. (SLS) for Monsanto Company, SLS Study No. 3044.303; Monsanto Study No. SB-92-131, 1/27/93, EPA MRID# 42701501).

Based on the data provided the acute oral LD_{50} of MON 5775 is greater than 6000 mg/kg with a toxicity category of IV.

Alachlor (parent) study:

Acute Oral Toxicity Study in Rats; Compound: Alachlor (Technical) (Bio/dynamics, Inc., for Monsanto Company, Project No. 4899-77; BD-77-433, 1979, EPA MRID# 00139383).

The acute oral LD_{50} for alachlor technical is 930 mg/kg with a toxicity category of III.

Based on these data, MON-5775 (the ESA metabolite of Alachlor) is less acutely toxic than the parent, Alachlor.

II. Subchronic Toxicity:

ESA Metabolite Study:

A 91-DAY DRINKING-WATER TOXICITY STUDY IN RATS WITH MON 5775 (Springborn Laboratories, Inc. (SLS) for Monsanto Company, SLS Study No. 3044.372, Monsanto Study No. SB-92-383, June 15, 1993, EPA MRID# 42863701). The previous review (MEMO, 1/17/95, S.Dapson to R.Taylor) came to the following conclusions:

> Systemic toxicity was noted in mid and high dose males and females in the form of effects on red blood cell parameters including decreased erythrocytes, hemoglobin, hematocrit and platelets in (mid and high dose males), with a slight increase in MCH and MCHC in the high dose males. The red cell morphology was also affected in both sexes of the high dose group. Leukocytes were increased in mid and high dose females. Other hematological parameters were unaffected. AST, ALT, albumin, urea nitrogen, creatinine, and glucose levels were slightly decreased and total bilirubin and phosphorus was slightly increased in mid and high dose males. A similar pattern was not seen in the females. With no pathology noted, the biological relevance of the above observations is unclear. Other clinical chemistry parameters were unaffected. There were clinical observations with increased incidences of decreased activity with rapid/shallow breathing (high dose), few feces and feces small in size (mid and high dose), dehydration (mid and high dose), urine staining (high dose), emaciation (high dose), hunched posture (high dose), rough coat (mid and high dose), unkempt appearance (high dose), and dark material/stain on pads of forelimb, around eyes, mouth and nose, clear and red ocular discharge, and hairloss around eyes, with the highest incidence occurring in the high dose group (may be related to eye lesions common to these strain of rats). Eye lesions noted in this study were determined not to be related to treatment or to those lesions seen with the parent compound, alachlor. There was also a decrease in body weight gain in the high dose males along with reduced food consumption and water consumption was slightly increased in high dose males. No specific histopathological observations were noted. The Systemic Toxicity LOEL is 2000 ppm and the Systemic Toxicity NOEL is 200 ppm based on hematological and clinical chemistry changes and increased incidence of clinical signs of toxicity.

Due a question on the biological relevance of hematological and clinical chemistry changes, a detailed reevaluation of the data from MRID# 42863701 was conducted and the following is the revised Executive Summary:

In a special 91-day drinking water study (MRID# 42863701), male and female Fischer CDF® F-344 Crl BR VAF/Plus® rats from Charles River Laboratories, Inc. Raleigh, NC received either 0, 200, 2000, or 10000 ppm (equal to 0, 16, 157, and 896 mg/kg/day in males and 0, 23, 207, and 1108 mg/kg/day in females) MON 5775 (90.7% & 6.6% H_2O; a metabolite of alachlor).

Systemic toxicity was observed in high dose male and female rats, with increased incidences of decreased activity with rapid/shallow breathing, few feces and feces small in size, dehydration, urine staining, emaciation, hunched posture, rough coat, unkempt appearance, and dark material/stain on pads of forelimb, around eyes, mouth and nose, clear and red ocular discharge, and hairloss around eyes. Slight decreased body weight gains (10%) was also noted in high dose male rats (decreased body weight gains were noted in all treated females; however, no dose response was noted). Several statistically significant hematological effects (decreased hemoglobin, hematocrit, red cells, increased MCH and MCHC) and clinical chemistry alterations (decreased AST, ALT, urea nitrogen, albumin, glucose, increased bilirubin and phosphorous) were observed at the mid and high dose in males and/or females, but were minor, mostly not dose related and were not considered biologically relevant, especially in the **absence** of any organ or tissue pathology at this dose. Eye lesions noted in this study were determined not to be related to treatment or to those lesions seen with the parent compound, alachlor. The clinical observations reported related to the eye are due to ocular abnormalities specific to the F-344 rat. **The Systemic Toxicity LOEL is 10,000 ppm (896 mg/kg/day in males and 1108 mg/kg/day in females) and the Systemic Toxicity NOEL is 2000 ppm (157 mg/kg/day in males and 207 mg/kg/day in females) based on increased incidence of clinical signs of toxicity in males and females, and decreased body weight gains in males.**

Alachlor (parent) Study:

90-Day Subacute Oral Toxicity of CP50144-Albino Rats (Industrial BIO-TEST Laboratories, Inc. for Monsanto Company, Project BTL-66-4, IBT No. B4477, 1966, EPA MRID# 00023658).

In a subchronic toxicity study (MRID# 00023658), male and female Charles River albino rats from Charles River Breeding Laboratories, Inc., North Wilmington, MA received either 0, 20, 200, or 2000 ppm CP50144 Technical (Alachlor) for 90 days (approximately 0, 1.5, 15, and 146 mg/kg/day for the control, low, mid and high dose groups, respectively by standard conversion factors).

Systemic toxicity was noted in the high dose animals as decreased body weights and body weight gains, decreased food consumption and efficiency and increased absolute and relative spleen weights, increased relative liver weights, increased relative to body weight kidney weights, and decreased relative gonad weights (testis and ovaries). The Systemic Toxicity NOEL is 200 ppm (15 mg/kg/day) and the Systemic Toxicity LOEL is 2000 ppm (146 mg/kg/day) based on decreased body weights, body weight gains, reduced food consumption and increased spleen, liver and kidney weights and decreased gonad weights.

This study is classified as Unacceptable and does not satisfy the guideline requirements (OPP §82-1a) for a subchronic toxicity study in rats as it is an invalidated IBT study, this study was not repeated since an adequate chronic toxicity was performed.

The chronic toxicity studies in rats with Alachlor have Systemic Toxicity NOEL's of 2.5 mg/kg/day (alachlor, epichlorohydrin free) and less than 14 mg/kg/day (alachlor with epichlorohydrin).

The subchronic data available for comparison of Alachlor with the ESA metabolite of Alachlor are **not** by the same route of administration (in the feed for the parent Alachlor and in the drinking water for the ESA metabolite of Alachlor), and the study with the parent Alachlor is an IBT study which was not validated or repeated; therefore the data may be suspect. It is important to note also that the subchronic and chronic toxicity studies with

the parent Alachlor were conducted with different strains of rats ("Charles River Albino rats" vs Long-Evans rats) than the 91 day drinking water study (Fisher 344 rats); however, the available metabolism data do not show any major differences in the handling of the compounds in the Long-Evans vs the Fisher rats.

III. Developmental (teratology) Toxicity:

ESA Metabolite Study:

A Developmental Toxicity Study of MON 5775 in Rats (Wil Research Laboratories, Inc. for Monsanto Company, R.D. No. 1335; Monsanto Report No. WI-95-068; Wil Project No. WIL-50237, December 13, 1995, EPA MRID# 43908101).

In a prenatal developmental toxicity (teratology) study (MRID# 43908101), female Sprague-Dawley Crl:CD®BR rats from Charles River Breeding Laboratories, Inc., Portage, Michigan received either 0, 150, 400, or 1000 (limit dose) mg/kg/day MON 5775 (a metabolite of Alachlor; 90.0% a.i.; Lot No.: NPD-9203-3974-T) in Mazola® corn oil by oral gavage from days 6 through 15 of gestation, inclusive. **Actual doses were 0, 135, 360, and 900 mg/kg/day based on 90.0 a.i.**

No maternal toxicity was noted in any measured parameter at the dose levels tested. **The Maternal Toxicity NOEL is equal to or greater than 900 mg/kg/day and the Maternal Toxicity LOEL is greater than 900 mg/kg/day.**

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

A 6(a)(2) letter was sent prior to the submission of this study; however, the final study report indicated that the study neither met nor exceeded any of the flagging criteria under 40 CFR 158.34 and review of the study did not indicate any toxicity.

Alachlor (parent) study:

Teratology Study in Rats (International Research and Development Corp. for Monsanto Company, Study No. IRDC No. 401-058; IR-79-020, 1980, EPA MRID# 00043645).

In a developmental (teratology) toxicity study (MRID# 00043645), Charles River rats were given 0, 50, 150 or 400 mg/kg/day of alachlor by gavage on gestation days 6-19. Both the maternal and developmental NOELs were 150 mg/kg/day. The maternal toxicity LOEL is 400 mg/kg/day, based upon increased mortality and the developmental toxicity LOEL is 400 mg/kg/day based on increased post-implantation loss, a reduced number of live fetuses, and decreased mean fetal body weight. .

Based on these data, the ESA metabolite of alachlor is less toxic than the parent, Alachlor in terms of developmental (teratology) toxicity.

IV. Mutagenicity:

ESA Metabolite Studies:

AMES MUTAGENICITY STUDIES ON FIVE COMPOUNDS REPRESENTATIVE OF MAJOR CLASSES OF ALACHLOR METABOLITES [Alachlor Metabolite CP 108065: Ames Salmonella Mutagenicity Assay, Project No. ML-84-037, Study No. 840013, 5/18/84] (Monsanto Environmental Health Laboratory for Monsanto Agricultural Products Company, Study No.s RD# 583 and MSL# 4507, 2/12/85, Accession Number 256736); the following are the conclusions of the review:

Under the conditions of two independent assays. 0.01 to 10 mg/plate CP108065 (metabolite of Alachlor) did not cause increases in the reversion of four *S. typhimurium* strains to histidine prototrophy in either the presence or absence of S9 activation.

Mouse Bone Marrow Micronucleus Assay of MON 5775 (Environmental Health Laboratory, Monsanto Company, Study No. ML-95-069, September 29, 1995, EPA MRID# 43889403).

In a mouse micronucleus assay (MRID# 43889403), groups of five male CD-1 mice received single oral gavage administrations of 500, 1000 or 2000 mg/kg MON 5775 (90.7%). The test material was delivered to the animals in deionized water. Animals were sacrificed at 24 and 48 hours postadministration; bone marrow cells were harvested and 2000 erythrocytes per male were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs).

No overt toxicity for the treated animals or cytotoxicity for the target organ was observed up to the currently recommended limit dose (2000 mg/kg). The positive control induced the expected high yield of MPEs in the treated males. **There was, however, no evidence that the test material induced a clastogenic or aneugenic effect at any dose or sacrifice time.**

Alachlor (parent) Studies:

Alachlor: Microbial Mutagenicity Study (Institute of Environmental Toxicology, Japan for Monsanto Company, Study No. ET-80-0101, 1980, EPA MRID# 00109563).

Alachlor was shown to be negative in an Ames *Salmonella* assay [MRID# 00109563].

Rat Bone Marrow Micronucleus Assay of Alachlor (Environmental Health Laboratory, Monsanto Company, Study No. EHL 91102: 1156, 1992, EPA MRID# 42651303).

Alachlor was negative in a micronucleus assay in Long-Evans rats [MRID# 42651303] conducted with a single intraperitoneal injection of 150, 300, or 600 mg/kg and 24-, 48-, and 72-hour sacrifice times. Two males and one female receiving the high dose died, and clinical signs of toxicity were observed in males at all doses and in mid- and high-dose females. A separate experiment in the same study with radiolabeled Alachlor provided evidence that the test

material reached the target organ when administered intraperitoneally.

Other mutagenicity studies with Alachlor are as follows:

Urine from Alachlor treated rats tested in an Ames *Salmonella* assay resulted in a weak mutagenic response in strain TA98 in the presence of ß-glucuronidase. A weak mutagenic response was also observed in strain TA1537 in the presence of both ß-glucuronidase and metabolic activation [MRID# 00155392]. Additional Ames *Salmonella* assays with metabolites of Alachlor showed that of five metabolites tested (t-hydroxysulfone [rat, mouse, goat, hen, rotation crops metabolite], *sec*- amide p-hydroxy methylsulfone [rat metabolite], t-sulfinyllacetic acid [corn metabolite], t-oxanilic acid [soil, water, soybean metabolite], and t-sulfonic acid [corn, soil, soybeans, water metabolite]), only the t-hydroxysulfone metabolite was observed to be mutagenic (strain TA100 at 3 and 10 mg/plate in the presence and absence of metabolic activation). Alachlor was positive in an *in vivo/in vitro* UDS assay at 1000 mg/kg, a dose approximating the LD_{50} in rats [MRID# 00014061]. Alachlor was negative in a recombinant/ conversion assay in bacteria, a DNA damage/repair assay in rat hepatocytes, an *in vitro* cytogenetics assay in rat bone marrow, and in an HGPRT gene mutation assay in CHO cells.

Alachlor was negative in an Ames *Salmonella typhimurium* mammalian microsome plate incorporation assay [MRID# 42651301], conducted in the absence of S9 and with S9 prepared from uninduced rat, mouse, or monkey nasal turbinates, at concentrations ranging from 50 to 5000 µg/plate. Tester strains TA98, TA100, TA1535, and TA1537 were used.

Alachlor was positive for inducing unscheduled DNA synthesis (UDS) in hepatocytes [MRID# 42651302] recovered from male Fischer-344 rats at 12 hours after oral gavage administration of 1000 mg/kg. The average number of net nuclear grains increased by >5 compared with the controls, and >10% of the cells were in repair. Similarly, a comparison of the individual data from treated animals and the vehicle control group showed that hepatocytes recovered from 3 of 5 animals were positive for UDS, cells from one animal showed a borderline positive response, and liver cells

from the remaining animal was negative. Based on Mirsalis et al. (1982) and the reviewer's experience, these data are suggestive of a genotoxic response, but it is noted that the dose at which a positive response was observed **approximates the LD_{50}** of Alachlor in rats. There was no indication of UDS activity at 12 hours after oral gavage administration of lower doses (50, 200, or 500 mg/kg) or at 2 hours following gavage with 1000 mg/kg.

Alachlor was fed to rats and mice in the diet for up to 60 days and cell proliferation was assessed by 3H-thymidine labeling *in vivo* [MRID# 42852102]. Dose levels ranged from 0.5 to 260 mg/kg/day. Cell proliferation was seen at doses of 126 and 260 mg/kg/day in the olfactory epithelium of the nasal turbinates of rats but not mice. The response in rats was reversible. The NOEL for cell proliferation in the olfactory epithelium of the nasal turbinates in rats was 46 mg/kg/day and the LOEL was 154 mg/kg/day.

Related to these studies is a study entitled *Study of the Effects of Alachlor on Cellular Stress Response Genes in Rat Nasal Turbinate Tissue* (Monsanto Company, The Agricultural Group, Environmental Health Laboratory for Monsanto Company, Monsanto Study# ML-94-160, Monsanto EHL Study# EHL94081, February 28, 1995, EPA MRID# 43590002) where two stress response genes were examined, the heat shock protein 70 (hsp70) and the NAD(P)H: quinone oxidoreductase also known as NAD(P)H: menadione oxidoreductase 1 (nmo). Long Evans rats were treated with Alachlor in the diet at 1600 to 2450 ppm (equivalent to 226 mg/kg/day) for 30 or 60 days. The respiratory and olfactory epithelia from the nasal turbinates were harvested for the examinations. The investigators found that hsp70 and nmo are expressed constitutively in nasal turbinate tissue of the Long Evans rats. Treatment of these rats with 126 mg Alachlor/kg body weight/day for 60 days caused a statistically significant induction of nmo in both respiratory and olfactory epithelia and a statistically significant induction of hsp70 in the olfactory epithelia. They state that the stress gene induction observed in Alachlor treated nasal turbinates indicates that the nasal turbinate cells are under physiological stress. Cell damage resulting from such stress may lead to increased cell turnover in these tissues, which may in turn lead to promotion of nasal tumors.

Overall, the **registrant** suggests that the submitted mutagenicity data provides evidence that Alachlor itself is not genotoxic, and that of all the Alachlor metabolites tested, few metabolites, including diethylaniline and t-hydroxysulfone demonstrate mutagenic activity. The mutagenic activity demonstrated by these metabolites occurs only at higher concentrations of test material and the data indicate only weakly positive effects in the *Salmonella* assays. The UDS activity observed occurs only at a dose approximating the LD_{50} in rats. These data are supportive of a non-genotoxic mechanism of action in induction of tumors of the nasal epithelium, gastric mucosa, and thyroid from administration of Alachlor.

These data indicate that MON 5775 (ESA metabolite of Alachlor) has no mutagenic activity compared to the parent Alachlor which was weakly mutagenic.

V. Metabolism:

ESA Metabolite Studies:

Elimination, Absorption, Tissue Distribution and Metabolism of Alachlor Ethane Sulfonate (MON 5775) in Long-Evans Rats Following Oral Administration (EPA MRID# 43889404).

In a special metabolism study (MRID# 43889404), two groups of male and female Long-Evans rats (two/sex/group) were administered alachlor ethane sulfonate at a dose of 70 mg/kg by gavage. Group 1 rats were sacrificed 24 hours after treatment and Group 2 animals at 5 days after treatment. Disposition of alachlor ethane sulfonate was determined by collection of excreta and by whole-body autoradiography. Metabolism was assessed by HPLC analysis of processed urine and feces samples. The major route of excretion for alachlor ethane sulfonate at 70 mg/kg was the feces, with between 71-82% of the administered dose excreted by this route. Excretion was rapid with the majority of radioactivity excreted by 24 hours post-dose. HPLC analysis of urine and feces showed alachlor ethane sulfonate to be the major component in both urine and feces, with three other components isolated but not identified, each comprising less than 2% of the dose.

Autoradiographic data on ESA derived radioactivity at 14 hours postdose showed the major areas of localization were stomach contents, cecum, intestinal contents and urinary bladder. The data indicate that alachlor ethane sulfonate is poorly absorbed, rapidly excreted, and undergoes minor metabolism. This study provides information on the disposition of alachlor ethane sulfonate in Long-Evans rats.

Effect of MON 5775 on Cell Proliferation in the Nasal Tissue of Male F-344 Rats (EPA MRID# 43889401).

In a special study (MRID# 43889401), the proliferating cell nuclear antigen technique (PCNA) was utilized to determine the effect of treatment with 2000 ppm Alachlor (157 mg/kg/day for 91 days) on cell proliferation in the olfactory region at the second palatial ridge (Level III), where Alachlor-induced tumors are found. Mean nasal cell proliferation values (number of labelled cells per mm of mucosal length) showed no statistically significant increases in cell proliferation in either the olfactory septum or turbinates of male Fischer 344 rats administered MON 5775 in drinking water for 91 days. This study provides limited information on the nasal proliferative response from administration of MON 5775 to male Fischer 344 rats.

Evaluation of Cell Proliferation and Measurement of Mucosal Thickness in Gastric Fundi of Rats From Study SB-92-383 (EPA MRID# 43889402).

In a special study (MRID# 43889402), glandular stomach tissue from female Fischer 344 rats treated with MON 5775 in drinking water at a dose of 10,000 ppm for 91 days was evaluated using the proliferating cell nuclear antigen for evidence of a proliferative response or changes in mucosal thickness. A significant increase in the percentage of labelled cells in the fundic neck region was observed in treated rats, but there were no significant changes in labelling of the fundic base nor in mucosal thickness.

Alachlor (parent) Studies:

A FIFRA rat metabolism study (Guideline #85-1) was conducted to examine the metabolism of Alachlor in Long-Evans rats. The summary of this review is presented for comparison to the available data on metabolism of ESA.

The disposition and metabolism of Alachlor was examined in male and female Long-Evans rats after oral and intravenous administration in the four studies listed above. In MRID # 42651306, female Long-Evans Crl:CD(LE)BR rats six to nine weeks of age were used in five dose groups. The first 3 dose groups consisted of 33 rats in each group, which received single oral doses of radiolabeled alachlor (uniformly labeled in the phenyl ring with 14-C, and enriched with 13-C at the C-2 carbon) at target doses of 7, 70, and 700 mg/kg. Group 4 consisted of 21 rats which received 15 consecutive daily doses of radiolabeled alachlor at 700 mg/kg/day. Group 5 consisted of 6 rats which received a single oral dose of radiolabeled alachlor at 700 mg/kg for the purpose of obtaining plasma samples at 2, 4, and 6 hours post-dosing. In MRID # 42852107, metabolites of alachlor in urine, feces, and tissues collected from the rats used in MRID # 42651306 were characterized. In MRID #'s 42651308 and 42852108, disposition and metabolism of alachlor following intravenous administration at 7 and 70 mg/kg was investigated. Absorption at the 7 and 70 mg/kg dose levels was essentially complete, with a slight decrease in absorption at the 700 mg/kg dose level. Repeated oral dosing at 700 mg/kg had no significant effect on absorption. Residual radioactivity did not exceed 5% of the administered dose at any of the dose levels in this study. On a µg/g basis, the residual radioactivity in the non-glandular stomach was higher than in the glandular stomach except at 4 hours post-dose at the 700 mg/kg dose level. Decreasing the dose decreased the percentage of the dose in the non-glandular stomach but not in the glandular stomach. Nasal turbinates showed a secondary peak of radioactivity at 8 hours post-dose at the 700 and 70 mg/kg dose levels in contrast to other tissues. Excretion of alachlor derived radioactivity was approximately equivalent between urine and feces, with between 30-47% excreted in urine and 41-45% excreted in feces at single oral doses of 7, 70, and 700 mg/kg. Intravenous dosing at 7 or 70 mg/kg resulted in a similar excretion profile, while repeated oral dosing at 700 mg/kg resulted in a slight

increase in fecal excretion of radioactivity. In urine, the *sec*-amide hydroxymethyl sulfone metabolite (metabolite **F5**) of alachlor was the predominant urinary metabolite after oral and intravenous administration, ranging from 2.1-7.4% of the dose. Repeated oral dosing resulted in the appearance of several additional metabolites, but it is not known whether these additional metabolites are unique to repeated oral administration of alachlor. In feces, the *tert*- amide mercapturic acid and the disulfide appeared to be the major metabolites after single oral doses of alachlor. Increasing the dose appeared to increase the percentage of these 2 metabolites in feces.

The available in vivo metabolism data indicate that in comparison to Alachlor, ESA is poorly absorbed and metabolized to only a minor degree. Although the products of ESA metabolism were not identified, the occurrence of these metabolites was at such a low level that they would not be of any toxicological concern.

Autoradiography Studies

For comparative purposes the results of autoradiography studies with Alachlor in rats are presented below.

In an autoradiography study, female Long-Evans rats (2/dose, 35-81 days of age) were administered single oral doses of 14-C Alachlor at 7, 70, or 700 mg/kg, and single dermal doses of 7 and 70 mg/kg alachlor. Female CD-1 mice 46 days of age and male squirrel monkeys aged 4-6 months were given single oral doses of 7, 70, and 700 mg/kg alachlor. At 24 or 120 hours after dosing, animals were euthanized and radioactivity localization visualized by WBA. At 24 hours post-dose, labelled material was present in blood of all species, but significant amounts at 5 days post-dose were evident in the blood of only rat and mouse. At 24 hours, the liver, kidney, nasal vibrissae, body hair, surfaces of the mouth and tongue, surfaces of roots and teeth, and periorbital fat were observed with detectable radioactivity in all species. At 5 days post-dose, tissue levels of labelled material in the monkey appeared less than in rat or mouse. Accumulation of labelled

material was significant in the nasal turbinates of the rat, less in the mouse, and absent in the monkey. There were no apparent changes in the distribution of labelled material with a change in dose levels.

The available autoradiography data indicate that in comparison to Alachlor, MON 5775 (ESA metabolite of Alachlor) does not show any significant localization to the nasal cavity, thyroid and glandular stomach (gastric mucosa).

Cell Proliferation

For comparison purposes the results of cell proliferation studies with Alachlor are presented below.

The study (MRID# 42852102) examined cell proliferation in Long-Evans rats. Alachlor was administered in the diet at dose levels of 0.5 to 252 mg/kg/day for up to 60 days and cell proliferation was assessed by 3H-thymidine labeling in vivo. Alachlor produced a dose-related increase in cell proliferation in the nasal mucosa of Long-Evans rats. An increase in cell proliferation was produced in the other tissues studied but only at the highest dose levels tested (up to 252 mg/kg/day). The response was found to be reversible after cessation of Alachlor treatment. No consistent effects were noted in the glandular stomach or thyroid.

Another study examined the effects of Alachlor on hepatotoxicity and cell proliferation in the F344 rat (MRID# 43504101). This study was performed as a result of previous UDS assays which showed a weak UDS response at a dose of 1000 mg/kg alachlor. This weak UDS response might represent a secondary effect related to hepatotoxicity, or might be related to induction of cell proliferation in the liver by alachlor. To investigate these hypotheses, 5 rats/dose were administered alachlor in corn oil at 50, 200, 500, and 1000 mg/kg. Rats were sacrificed at 12 hours post-dose, which had been selected as a time point based on dosing of 2 other groups in this study. At sacrifice, blood was analyzed for serum enzyme activities, while the liver was analyzed for

histopathology and hepatocellular proliferation analysis as well as GSH levels. At 12 hours post-dose, liver GSH levels were decreased by 10-56% over the dose range used (50-1000 mg/kg). Elevations in serum ALT, AST, and LDH were observed at 500 and 1000 mg/kg alachlor. Microscopic lesions were evident in increased incidence at 500 and 1000 mg/kg alachlor, and consisted of centrilobular cytoplasmic eosinophilia, centrilobular inflammation, and centrilobular hepatocellular degeneration/necrosis. These lesions were found in increased incidence mainly in the median and right lateral lobe. There did not appear to be an increase in cell proliferation in alachlor-treated rats. The results of this study suggest that the weak UDS response observed at a 1000 mg/kg dose of alachlor is related to hepatotoxicity at this dose.

The available cell proliferation data indicate that in comparison to Alachlor, MON 5775 (ESA metabolite of Alachlor) does not induce cell proliferation.

VI. Registrant's Conclusions:

•The amount of toxicological information available on ESA is more than that for nearly any other pesticide degradate and is certainly more than that available for most trace environmental contaminants. This amount of information is equivalent to that which EPA has previously required to exclude certain pesticide degradates from a tolerance expression based on a lack of toxicological significance.

•The results from these toxicological and metabolic studies uniformly demonstrate that ESA behaves very differently in living organisms from alachlor. The acute, subchronic, developmental, and aquatic toxicity of ESA is significantly lower than that of the parent material. This reduced toxicity is rational and expected, because ESA is a polar ionic compound that has completely different pharmacodynamic properties from alachlor. Furthermore, the chlorine substituent, which serves as the primary metabolic target in alachlor, is not present in ESA. As a result, the cascade of metabolic conversions and tissue-specific responses that have been extensively studied with alachlor could not occur

with ESA, even if is were systemically available.

•It is especially noteworthy that ESA does not accumulate in nasal tissue, a site of substantial binding and tumor development following alachlor administration. The specific preneoplastic responses that are believed to be involved in the production of rat nasal and stomach tumors following alachlor administration do not occur when animals are exposed to ESA. There is no basis for presuming that ESA would be carcinogenic in rodent chronic studies, and indeed there is evidence to support the contrary conclusion.

•There are sufficient data to estimate the possible exposure of humans to ESA. Since ESA is not a commercial material, there is no opportunity for exposure during manufacture, formulation, shipping, or application. There is no opportunity for exposure from accidental spillage or spray drift. The sole route of possible exposure to ESA is by consumption of alachlor-treated crop commodities or from drinking water that could contain trace ESA levels. Legal ESA residues in alachlor-treated commodities have been included in alachlor tolerances for decades.

•As an extreme worst-case estimate, if a 70-kg human consumed 2 liters of water per day containing 26.7 µg/L of ESA, the highest level ever measured in the above cited well water samples, this would constitute a daily dose of 0.00076 mg/kg/day. Comparison of this exposure to the lowest NOEL observed in any of the toxicology studies (20 mg/kg/day in the 91-day drinking water study), provides a 26,315-fold safety factor. This margin of safety is more than sufficient to guard against any health effects of ESA, especially considering the extreme assumptions used in the exposure estimate.

VII. EPA Conclusions:

The data provided indicate that MON 5775 has less toxic potential than the parent Alachlor.

I. Toxicology Profile for Alachlor (40 CFR 180.249)

Date: 3/96
Technical: Alachlor
Use Pattern: food and non-food

This compound is a registered active ingredient. The following data are required for technical alachlor. This chemical is on **LIST A** for reregistration.

THIS INFORMATION DOES NOT NECESSARILY REFLECT THE DATA REQUIREMENTS FOR REREGISTRATION.

	Required	Satisfied
§81-1 Acute oral toxicity in rats	Yes	Yes
§81-2 Acute dermal toxicity in rabbits	Yes	Yes
§81-3 Acute inhalation toxicity in rats	Yes	Yes
§81-4 Primary eye irritation in rabbits	Yes	Yes
§81-5 Primary dermal irritation in rabbits	Yes	Yes
§81-6 Dermal sensitization - guinea pig	Yes	Yes
§82-1(a) 90 day feeding study - rat	Yes	NO[1]
§82-1(b) 90 day feeding study - nonrodent	Yes	NO[2]
§82-2 21 day dermal - rabbit	Yes	Yes
§83-1(a) 2-year feeding - rodent	Yes	Yes
§83-1(a) 2-year feeding - rodent/stabilized	Yes	Yes
§83-1(b) 2 year feeding - nonrodent	Yes	Yes
§83-2(a) Carcinogenicity - rat	Yes	Yes
§83-2(a) Carcinogenicity - rat/stabilized	Yes	Yes
§83-2(b) Carcinogenicity - mouse	Yes	Yes
§83-2(b) Carcinogenicity - mouse/stabilized	Yes	Yes
§83-3(a) Teratology - rat	Yes	Yes
§83-3(b) Teratology - rabbit	Yes	Yes
§83-4 Multigeneration reproduction-rat	Yes	Yes
§84-2(a) Mutagenicity - Gene Mutation	Yes	Yes
§84-2(b) Muta - Struct.Chromosome Aberr.	Yes	Yes
§84-4 Muta - Other Genotoxic Effects	Yes	Yes
§85-1 General metabolism - rat	Yes	Yes
§85-2 Dermal penetration (absorption)	Yes	Yes[3]

1 = satisfied by 2-year chronic feeding study in the rat
2 = satisfied by 6 month subchronic feeding study in the dog
3 = based on human and monkey data submitted to the agency

Alachlor Metabolite-MON 5775 (also called CP108065)

§81-1 Acute oral toxicity in rats	Yes	Yes
§82-1(a)91 day drinking water study - rat	Yes	Yes
§83-3(a)Teratology - rat	Yes	Yes
§84-2 Mutagenicity - Ames	Yes	Yes
§84-2 Mutagenicity - Micronucleus	Yes	Yes
§85-1 General metabolism - rat	Yes	Yes

II. Data Gaps

The database for technical Alachlor is complete.

There are acute toxicity study data gaps with the registered formulations. These must be resolved before further additional permanent food use tolerances are granted.

III. Actions Being Taken to Obtain Additional Information or Clarification

None needed at this time.

IV. Reference Dose

The RfD is 0.01 mg/kg/day based on the chronic feeding study in the dog with a NOEL of 1 mg/kg/day and an uncertainty factor (UF) of 100.

V. Pending Regulatory Actions

None at this time.

VI: Toxicological Issues Pertinent to this Request

This chemical was a registration standard in 1983 and is on **LIST A** for reregistration, RED candidate.

A. New toxicology Data on Alachlor

Data on ESA metabolite of Alachlor (MON 5775) discussed above (DERs attached).

B. Carcinogenicity

The Health Effects Division Carcinogenicity Peer Review Committee (HED/CPRC) met on September 27 and October 4, 1995 and January 3, 1996 to discuss and reevaluate the weight-of-the-evidence on Alachlor with particular reference to its carcinogenic potential, based on mechanistic and other data provided by the registrant. These data were not requested by the Agency but were provided by the registrant in support of their chemical. The classification of Alachlor at that time was a Group B2 - probable human carcinogen, with a recommendation that a low dose extrapolation model be applied to the animal data for the quantification of human risk (Q_1*).

New data provided by the registrant consisted of a new mouse carcinogenicity study, additional mutagenicity studies, mechanistic data, and additional metabolism studies and toxicology data from a related compound, Butachlor. Upon evaluation of all of the submitted data regarding the carcinogenicity potential of Alachlor and consideration of the full weight-of-the-evidence, the Health Effects Division Carcinogenicity Peer Review Committee could not reach a consensus as to the classification of Alachlor as a carcinogen. Therefore the CPRC recommended to defer the carcinogenicity classification of Alachlor and reconsider the classification in the near future using the new Cancer Assessment Guidelines when such guidelines are in effect. In addition, the CPRC recommended not to utilize the linear low dose approach, but to utilize the Margin of Exposure (MOE) methodology for the estimation of human risk. The CPRC concluded that the data in support of the mechanism for the nasal turbinates is indicative of a rat specific response. Although the rat and human were recognized to possess the same enzyme(s) involved in production of

the putative toxic species from Alachlor, it was also recognized that the activity of these enzymes was substantially greater in the rat compared to the human. Thus, the model of rat nasal tumorigenesis may not be relevant for human cancer assessment. Thyroid tumors have been proposed 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. The mechanistic data for thyroid tumor formation meet the criteria established by the Agency and the use of the MOE approach for human cancer assessment is consistent with Agency policy. The CPRC stated that the stomach tumor formation is a direct contact effect, non-genotoxic mechanism which parallels human pathological conditions. These tumors result from an indirect response to change in pH. The use of the MOE approach for human cancer assessment is consistent with Agency policy.

Primary Review by: Stephen C. Dapson, Ph.D. *Stephen C. Dapson 3/1/96*
Senior Pharmacologist, Review Section I, TBII/HED (7509C)

Secondary Review by: James N. Rowe, Ph.D. *James N. Rowe 3/1/96*
Section Head, Review Section III, TBII/HED (7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Prenatal Developmental Toxicity Study
Species: Rat; Guideline: OPPTS 870.3700, OPP §83-3a

EPA ID No.s: EPA MRID No. 43908101
EPA DP Barcode D222904
EPA Submission Barcode S500250
EPA Pesticide Chemical Code 090501
Toxicology Chemical Code 011

Test Material: MON 5775

Synonyms: CP 108065; sulfonic acid metabolite of alachlor; alachlor sulfonic acid; ESA; 2',6'-diethyl-N-methoxymethyl-2-sulfoacetanilide, sodium salt; 2-[(2,6-diethylphenyl)-(methoxymethyl)-amino]-2-oxoethanesulfonic acid, sodium salt

Sponsor: Monsanto Company
800 N. Lindbergh Blvd., St. Louis, Mo 63167

Testing Facility: Wil Research Laboratories, Inc.
A Subsidiary of Great Lakes Chemical Corporation, Ashland, OH 44805-9281

Title of Report: A Developmental Toxicity Study of MON 5775 in Rats

Study Number(s): R.D. No. 1335; Monsanto Report No. WI-95-068; Wil Project No. WIL-50237

Author(s): Joseph F. Holson, Ph.D.

Report Issued: December 13, 1995

Executive Summary: In a prenatal developmental toxicity (teratology) study (MRID# 43908101), female Sprague-Dawley Crl:CD®BR rats from Charles River Breeding Laboratories, Inc., Portage, Michigan received either 0, 150, 400, or 1000 (limit dose) mg/kg/day MON 5775 (a metabolite of Alachlor; 90.0% a.i.; Lot No.: NPD-9203-3974-T) in Mazola® corn oil by oral gavage from days 6 through 15 of gestation, inclusive. **Actual doses were 0, 135, 360, and 900 mg/kg/day based on 90.0 a.i.**

No maternal toxicity was noted in any measured parameter at the dose levels tested. **The Maternal Toxicity NOEL is equal to or greater than 900 mg/kg/day and the Maternal Toxicity LOEL is greater than 900 mg/kg/day.**

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

This study is classified as Acceptable and satisfies the guideline requirements (OPPTS 870.3700; OPP §83-3a) for a prenatal developmental toxicity (teratology) study in rats.

Compliance

A signed and dated STATEMENT OF NO CONFIDENTIALITY CLAIMS, a signed and dated COMPLIANCE STATEMENT (40 CFR Part 160), a signed and dated FLAGGING STATEMENT (40 CFR 158.34; according to the investigators the study neither meets nor exceeds any of the applicable criteria) and a signed and dated QUALITY ASSURANCE UNIT STATEMENT was provided.

A. Materials and Methods A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: MON 5775
Purity: 90.0% a.i.
Description: Light purple powder
Lot No.: NPD-9203-3974-T
Receipt date: 4/19/95 and 6/22/95
other provided information: Supplier - Monsanto Research Center, St. Louis, MO

Vehicle(s): 100% Pure Mazola Corn Oil
Description: Clear yellow viscous liquid

Test Animal(s): Species: Female rat
Strain: Sprague-Dawley Crl:CD®BR
Source: Charles River Breeding Laboratories, Inc., Portage, Michigan
Age: 75 days old at start of study
Body Weight: Mean body weights of females ranged from 244-248 g on gd 0.
Resident males of the same strain were used

B. Study Design

This study was designed to assess the developmental toxicity potential of MON 5775 when administered by oral gavage on gestation days 6 through 15, inclusive. MON 5775 is a degradate of alachlor formed through microbial breakdown in soil. This substance has the chemical structure shown below, and has been referred to under the following names:

- CP 108065
- sulfonic acid metabolite of alachlor
- alachlor sulfonic acid
- ESA
- 2', 6'-diethyl-N-methoxymethyl-2-sulfoacetanilide, sodium salt
- 2-[(2, 6-diethylphenyl)-(methoxymethyl)-amino]-2-oxoethanesulfonic acid, sodium salt

Mating Procedure

From page 19 of the study report: *Positive evidence of mating was confirmed by the presence of a copulatory plug or sperm in a vaginal smear. Each mating pair was examined daily. The day on which evidence of mating was identified was termed day 0 of gestation and the animals were separated.*

Animal Husbandry

Animals were kept under standard animal care conditions and received Purina® Certified Rodent Chow® #5002 and municipal water by an automatic system *ad libitum*.

Group Arrangement:

Test Group	Dose Level (mg/kg) Target	Actual	Number Assigned
Control	0	0	25
Low Dose	150	135	25
Mid Dose	400	360	25
High Dose	1000	900	25

Dose levels were based on a preliminary range-finding study (WIL-50236) which was not provided; however, the study tested to e approximately the "limit" dose.

Dose Administration:

All doses were administered in a volume of 5 ml/kg of body weight/day prepared once during the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on the most recent body weight.

The low dose concentration was within 96.8% of target and the high dose was within 96.2% of target. Homogeneity was 103% (99.9-107%) of target for the low dose, 98.4% (95.6-101%) of the target for the mid dose and 102% (98.6-104%) of the target for the high dose. No degradation was noted in dosing solutions.

Observations

From pages 20-23 of the study report: *All rats were observed twice daily for moribundity and mortality. Detailed clinical observations were recorded for each animal from days 0 through 20 of gestation (prior to compound administration during the dosing period). Animals were also observed for signs of toxicity at the time of dosing and approximately one hour following dosing; all significant findings were recorded.*

Individual maternal body weights for all females were recorded on gestation days 0, 6, 9, 12, 16, 18 and 20. A group mean body weight was calculated for each of these days. Mean body weight changes were calculated for each corresponding interval and also for days 6-16, 16-20 and 0-20.

Gravid uterine weight was collected and net body weight (the day 20 body weight minus the weight of the uterus and contents) and net body weight change (the day 0-20 body weight change minus the weight of the uterus and contents) were calculated and presented for each gravid female at the scheduled laparohysterectomy.

Individual food consumption was recorded on days 0, 6, 9, 12, 16, 18 and 20 of gestation. Food intake was calculated as g/animal/day and g/kg/day for the corresponding body weight change intervals.

All maternal animals were euthanized by carbon dioxide inhalation on gestation day 20. The thoracic, abdominal and pelvic cavities were opened by a ventral midline incision and the contents were examined. In all instances, the post mortem findings were correlated with the ante mortem comments and any abnormalities were recorded. The uterus and ovaries were excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened and the number and location of all fetuses, early and late resorptions and the total number of implantation sites were recorded. The individual uterine distribution of implantation sites was documented using the following procedure. All implantation sites, including resorptions, were numbered in consecutive order beginning with the left distal to the left proximal uterine horn, noting the position of the cervix, and continuing from the right proximal to the right distal uterine horn.

Uteri with no macroscopic evidence of nidation were excised, opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss as described by Salewski[3]. Maternal tissues were retained only as deemed necessary by the gross findings.

The liver, kidneys and spleen from each dam were excised, trimmed and weighed, and all findings were recorded. The carcasses of the dams, including the liver, kidneys and spleen, were then discarded.

Each fetus was sexed, weighed and tagged for identification. Fetal tags contained the WIL study number, the female number and the fetus number. A detailed external examination of each fetus was conducted to include, but was not limited to, the eyes, palate and external orifices and each finding was recorded. Crown-rump measurements were recorded (as appropriate) for late resorptions and the tissues were discarded. The sex of each fetus was verified by an internal examination. Each fetus was examined viscerally by

a modification of the Stuckhardt and Poppe[4] fresh dissection technique to include the heart and major vessels. Fetal kidneys were examined and graded for renal papillae development by a method described by Woo and Hoar[5].

Heads from approximately one-half of the fetuses from each female were placed in Bouin's fixative for subsequent soft-tissue examination by the Wilson[6] sectioning technique. The heads from the remaining one-half of the fetuses were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol. Following fixation in alcohol, each fetus was macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described in Dawson[7]. The skeletal examination was conducted utilizing low power magnification provided by a stereomicroscope. External, visceral and skeletal findings were recorded as developmental variations or malformations. The fetal developmental findings were summarized by: 1) presenting the incidence of a given finding both as a percentage of the no. of fetuses and the no. of litters available for examination in the group; and 2) considering the litter as the basic unit for comparison and calculating the no. of affected fetuses in a litter on a proportional basis.

Historical control data were provided to allow comparison with concurrent controls.

Statistical analysis

The following statistical analysis methods were employed (from page 23 of the study report):

All analyses were conducted using two-tailed tests for a minimum significance level of 5% comparing each treated group to the vehicle control group. Each mean was presented with the standard deviation (S.D.) and the number of animals (N) used to calculate the mean. The following statistical tests were performed by a Digital® MicroVAX® 3400 computer (with appropriate programming) in this laboratory and are referenced on the report tables:

STATISTICAL TEST	PARAMETER
-Chi-square test[8] with Yates' correction factor	Fetal Sex Ratios
-Fisher's Exact test[8]	Malformations and Variations
-Mann-Whitney U-test[8]	Early and Late Resorptions, Dead Fetuses, Postimplantation Losses

-ANOVA (two-tailed) with Dunnett's test[8]	Corpora Lutea, Total Implantations, Viable Fetuses, Fetal Body Weights, Maternal Body Weights and Weight Changes, Maternal Net Body Weight Changes and Gravid Uterine Weights, Maternal Food Consumption, Organ Weights
-Kruskal-Wallis test[8]	Litter Proportions of Intrauterine Data (Considering the Litter, Rather than the Fetus, as the Experimental Unit)

C. Results

1. Maternal Toxicity:

Mortality

No animals were reported to have died.

Clinical Observations

The only observation of note was high dose females with rales in 5 animals during the middle of the dosing periods on 1 to 3 occasions.

Body Weight

The investigators supplied the following group mean and individual animal data. The following tables present body weight and body weight gains:

Table I: Body Weights (grams)[a]

Group:	Day: 0	6	16	20	20-GUW[1]
Control	244	274	324	382	303.1
LDT	248	275	324	382	306.2
MDT	247	276	319	375	300.5
HDT	248	279	322	382	301.6

[1] = 20-GUW = Gestation Day 20 Body Weight minus Gravid Uterine Weight.
[a] = Data extracted from WIL-50237, Tables 5 and 7, pages 41 and 43.

Table II: Body Weight Gains (grams)[a]

Group:	Days: 0-6	6-16	16-20	6-20[1]	0-20	0-20-GUW[2]
Control	30	49	58	108	138	59.4
LDT	27	49	58	107	134	58.2
MDT	28	43	56	99	128	53.2
HDT	31	43	60	103	135	54.1

[1] = calculated by the reviewer from mean data; [2] = 0-20-GUW = Gestation Day 0-20 Body Weight Gain minus Gravid Uterine Weight.
[a] = Data extracted from WIL-50237, Tables 5-7, pages 41-43.

No treatment related effects were noted in the above data.

Food Consumption

The investigators supplied group mean and individual animal data. The following tables present food consumption and food efficiency data for similar periods as for body weight gain data:

Table III: Food Consumption Data (g/rat/day)[a]

Group:	Day: 0-6	6-16	16-20	6-20[1]	0-20
Control	20	19	25	21	21
LDT	20	19	25	21	21
MDT	20	19	25	21	20
HDT	21	19	25	21	21

[1] = calculated by the reviewer from mean data
[a] = Data extracted from WIL-50237, Table 8, page 44.

Table IV: Food Efficiency Data (%)[a]

Group:	Day: 0-6	6-16	16-20	6-20	0-20
Control	25	26	58	37	33
LDT	23	26	58	36	32
MDT	23	23	56	34	32
HDT	26	23	60	35	32

[a] = calculated by reviewer

No treatment related effects were noted in the above data.

Gross Pathological Observations

The investigators supplied group summary and individual animal data for gross observations and group mean and individual animal data for organ weights (liver, kidney and spleen). No treatment related effects were noted.

Cesarean Section Observations

The investigators provided group mean and individual animal data. The following table presents the cesarean section observation data:

Table V: Cesarean Section Observations[a]

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	25	25	25	25
#Animals Mated/Inseminated	25	25	25	25
#Animals Pregnant	23	24	24	24
Pregnancy Rate (%)	92	96	96	96
Maternal Wastage				
#Died	0	0	0	0
#Died/pregnant	0	0	0	0
#Non pregnant	2	1	1	1
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total# litters available	23	24	24	24
Total Corpora Lutea	358	370	390	425
Corpora Lutea/dam	16.3±2.27	16.1±2.89	16.3±1.98	17.7±3.29
Total Implantations	339	352	350	372
Implantations/Dam	14.7±1.71	14.7±3.46	14.6±2.70	15.5±2.02
Total Live Fetuses	324	332	332	354
Live Fetuses/Dam	14.1±1.56	13.8±3.31	13.8±2.68	14.8±2.23
Total Resorptions	15	20	18	18
Early	15	19	18	17
Late	0	1	0	1
Resorptions/Dam (E/L)	0.7/0.0	0.8/0.2	0.8/0.0	0.7/0.2
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm)	3.6±0.21	3.5±0.20	3.4*±0.31	3.5±0.25
Preimplantation Loss(%)1	5.3	4.9	10.3	12.5
Postimplantation Loss(%)1	4.4	5.7	5.1	4.8
Sex Ratio (Male/Female)	161/163	165/167	185/147	200/154

* = significantly different from control at 0.05 level.
a = Data extracted from WIL-50237, Tables 1 and 11, pages 36 and 47.

No treatment related effects were noted in the above data. The statistically significant difference noted in mid dose fetal body weights is a chance occurrence and not dose related.

2. Developmental Toxicity

External Examinations

The investigators provided group summary and individual animal data. No treatment related effects were noted. Observations recorded were 1 control fetus with mandibular micrognathia and aglossia; 1 low dose fetus with unilateral microphthalmia; 1 mid dose fetus with mandibular micrognathia, microglossia and unilateral microphthalmia and another mid dose fetus with shorter than normal body, micromelia and cleft palate. No observations were noted in the high dose group.

Visceral Examinations

The investigators provided group summary and individual animal data. No treatment related effects were noted. Observations recorded were 1 low dose fetus with distended ureter and red areas of the adrenal gland in a high dose fetus.

Skeletal Examinations

The investigators provided group summary and individual animal data. No treatment related effects were noted. Observations recorded for skeletal malformations (investigator classification) were 1 low dose fetus with absent right thoracic arch and half of centrum #11, anterior situated right thoracic arch #12 and proximally fused right ribs #10 and 11. The following table presents the skeletal variation observations (investigator classification):

Table VI: Skeletal Variations[a]

Observation:	Control	Low	Mid	High
#fetuses/#litters examined	324/23	332/24	332/24	354/24
Cervical centrum#1 ossified	62/17	68/20	66/17	67/18
Bent rib(s)	3/2	0/0	1/1	1/1
14th rudimentary rib(s)	5/5	3/2	2/2	1/1
27 presacral vertebrae	1/1	0/0	0/0	0/0
7th cervical rib(s)	6/4	5/3	2/2	10/8
Sternebra(e) #5 &/or #6 unoss.	9/6	17/7	13/7	11/9
Reduced ossif. - 13th rib(s)	8/6	6/6	5/5	11/4
Hyoid unossified	3/2	0/0	3/2	3/3
Pubis unossified	0/0	0/0	1/1	0/0
Sternebra(e) #1,#2,#3&/or#4 unoss.	1/1	0/0	0/0	1/1
25 presacral vertebrae	0/0	1/1	0/0	0/0
Sternebra(e) malaligned	0/0	2/2	0/0	0/0

a = Data extracted from WIL-50237, Table 15, page 53.

D. <u>Discussion/Conclusions</u>

a. <u>Maternal Toxicity</u>:

No treatment related effects were noted.

b. <u>Developmental Toxicity</u>:

i. **Deaths/Resorptions:**

No treatment related effects were noted.

ii. **Altered Growth:**

No treatment related effects were noted.

iii. **Developmental Anomalies:**

No treatment related effects were noted.

iv. **Malformations:**

No treatment related effects were noted.

E. <u>Study Deficiencies</u>: None

F. <u>Core Classification</u>: **Acceptable**

Maternal Toxicity NOEL => 900 mg/kg/day
Maternal Toxicity LOEL > 900 mg/kg/day
Developmental Toxicity NOEL => 900 mg/kg/day
Developmental Toxicity LOEL > 900 mg/kg/day

APPENDIX I:

WIL-50237; Monsanto Company; Sponsor No.: WI-95-068

IV. EXPERIMENTAL PROCEDURES

A. INTRODUCTION

The experimental phase of the study was initiated with the assignment of mated rats to study groups on June 20, 1995, and concluded with the last laparohysterectomy on July 14, 1995. The dosing period initiated on June 26, 1995, and concluded on July 9, 1995. Dose levels were selected based on the results from a preliminary range-finding study (WIL-50236[1]).

B. TEST AND CONTROL ARTICLES

1. TEST ARTICLE IDENTIFICATION

MON 5775 was received from the Monsanto Research Center, St. Louis, Missouri, in two shipments as follows:

Label Identification	No. of Containers Received	Description	Date Received
Toxicology Test Sample Tare: 44.79 Net: 250 g Gross: 294.86 Test Material: MON 5775 Lot #: NPD-9203-3974-T Expiration Date: 02/01/96 Storage Conditions: 35-100 Degrees F Monsanto Study No.: WI-95-067 WI-95-068	1 Bottle Gross Weight: 295.5 g	Light Purple Powder	4/19/95
Toxicology Test Sample Test Material: MON 5775 Lot#: NPD-9203-3974-T Expiration Date: 02/01/96 Storage Conditions: 35-100 Degrees F Monsanto Study No.: WI-95-067 WI-95-068	1 Bottle Gross Weight: 143.6 g	Light Purple Powder	6/22/95

Stability and purity data were provided by the sponsor. The bulk test article was 90.0% pure. However, for purposes of dose calculations, the test article was considered to be 100% MON 5775. The test article was considered stable when stored in a sealed container at room temperature. Approximate one gram reserve samples of the bulk test article were collected from each shipment and stored at WIL Research Laboratories, Inc.

2. VEHICLE CONTROL ARTICLE IDENTIFICATION

The vehicle control article utilized in preparation of the test mixtures was Mazola® corn oil received from Best Foods Division, CPC International, Inc., Engelwood Cliffs, New Jersey, on November 2, 1994, as follows:

Label Identification	No. of Containers	Description	Expiration Date
100% Pure Mazola Corn Oil	6-one gallon containers	Clear yellow viscous liquid	8/22/95

3. PREPARATION

An appropriate amount of the vehicle, Mazola® corn oil, was dispensed into a properly-labeled storage container for administration to the control group. The vehicle formulation was homogenized on a Polytron® PT 6000 laboratory mixer. An appropriate amount of the test article, MON 5775, was weighed for each group into a tared, precalibrated, properly-labeled storage container. A sufficient amount of vehicle was added to attain an appropriate volume for mixing. The preparations were homogenized on a Polytron® PT 6000 laboratory mixer for approximately five minutes to ensure proper mixing.

Dosing preparations were formulated once on June 23, 1995, and were stored at room temperature. The control and test article preparations were stirred each day using a magnetic stir bar and plate throughout the sampling and dosing procedures. The dosing preparations were visually inspected for homogeneity prior to initiation of dosing. All formulations were visually homogeneous and acceptable for use.

4. ADMINISTRATION

The test mixtures were administered orally by gavage, via a 16-gauge stainless steel gavage cannula (Popper and Sons, Inc., New Hyde Park, New York 11040), once daily for 10 consecutive days initiating on gestation day 6 and continuing up to and including

day 15 of gestation. A dosage volume of 5 ml/kg was used for all dosage levels. The control animals received Mazola® corn oil on a comparable regimen of 5 ml/kg. Individual dosages were based on the most recent body weights to provide the correct mg/kg/day dose. The following diagram presents the study group assignment:

Group Number	Test Article	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (ml/kg)	Number of Females
1	Mazola® corn oil	0	0	5	25
2	MON 5775	150	30	5	25
3	MON 5775	400	80	5	25
4	MON 5775	1000	200	5	25

5. ANALYSES OF DOSING PREPARATIONS

Samples of the dosing preparations from all groups were analyzed at WIL Research Laboratories, Inc., for concentration prior to the initiation of dosing. Dosing samples from the low and high dose groups were also analyzed for homogeneity. Samples were collected from the low and high dose groups prior to the initiation of dosing and analyzed to verify stability following the termination of dosing.

C. ANIMAL RECEIPT AND ACCLIMATION

One hundred twenty-five sexually mature, virgin female rats, Sprague-Dawley Crl:CD®BR, were received in good health from Charles River Breeding Laboratories, Inc., Portage, Michigan on June 8, 1995. The animals were approximately 75 days old upon receipt. Upon receipt, each animal was observed by a qualified technician. The animals were weighed on the day following receipt. All animals were uniquely identified by a metal eartag displaying the animal number and housed for 12 days for acclimation purposes. During this period, the animals were observed twice daily for mortality and moribundity.

D. ANIMAL HOUSING

Upon arrival and until pairing, all animals were individually housed in clean, wire-mesh cages suspended above cage-board. The animals were paired for mating in the home cage of the male. Following positive identification of mating, the females were returned to an individual suspended wire-mesh cage; nesting material was not required as the females were euthanized prior to the date of expected parturition. Animals were maintained in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals"[2]. The animal facilities at WIL

Care and Use of Laboratory Animals"[2]. The animal facilities at WIL Research Laboratories, Inc., are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

E. DIET. DRINKING WATER AND MAINTENANCE

The basal diet used in this study was Purina® Certified Rodent Chow #5002. This diet is a certified feed with appropriate analyses performed and provided by the manufacturer and provided to WIL Research Laboratories, Inc. Municipal water supplying the facility is sampled for contaminants according to Standard Operating Procedures. The results of these analyses are maintained at WIL Research Laboratories, Inc. Contaminants present in animal feed or water were not expected to interfere with the objectives of this study. Drinking water delivered by an automatic watering system and the feed were provided ad libitum throughout the acclimation period and during the study.

F. ENVIRONMENTAL CONDITIONS

All animals were housed throughout the acclimation period and during the study in an environmentally-controlled room. Controls were set to maintain a temperature at 72° + 3°F and a minimum relative humidity of 40%. Room temperature and relative humidity were recorded daily. Temperature ranged from 71° to 72°F and relative humidity ranged from 42% to 58% during the study period. Light timers were calibrated to provide a 12-hour light/12-hour dark photoperiod. Air handling units were set to provide approximately 10 fresh air changes per hour.

G. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS AND BREEDING PROCEDURES

At the conclusion of the acclimation period, all available females were weighed and examined in detail for physical abnormalities. At the discretion of the study director, animals judged to be in good health and meeting acceptable body weight requirements (a minimum of 220 g) were placed in suspended wire-mesh cages with resident males from the same strain and source for breeding. Resident males were untreated, sexually mature rats utilized exclusively for breeding. These rats were maintained under similar laboratory conditions as the females. A breeding record containing the male and female identification numbers and the dates of cohabitation was prepared. The selected females were approximately 12 weeks old when paired for breeding.

Positive evidence of mating was confirmed by the presence of a copulatory plug or sperm in a vaginal smear. Each mating pair

was examined daily. The day on which evidence of mating was identified was termed day 0 of gestation and the animals were separated.

The experimental design for WIL-50237 consisted of three MON 5775 treated groups and one control group. The bred females were consecutively assigned in a block design to groups containing 25 rats each by the following randomization procedure. The first mated female and the appropriate gestation day 0 designation were recorded and the female was assigned to group 1, the second mated female was assigned to group 2, and the third to group 3, etc. This process was continued daily until 25 females had been placed into each group. Body weight values ranged from 210 g to 278 g on day 0 of gestation.

H. MATERNAL OBSERVATIONS DURING GESTATION

1. CLINICAL OBSERVATIONS AND SURVIVAL

All rats were observed twice daily for moribundity and mortality. Detailed clinical observations were recorded for each animal from days 0 through 20 of gestation (prior to compound administration during the dosing period). Animals were also observed for signs of toxicity at the time of dosing and approximately one hour following dosing; all significant findings were recorded.

2. BODY WEIGHTS AND GRAVID UTERINE WEIGHTS

Individual maternal body weights for all females were recorded on gestation days 0, 6, 9, 12, 16, 18 and 20. A group mean body weight was calculated for each of these days. Mean body weight changes were calculated for each corresponding interval and also for days 6-16, 16-20 and 0-20.

Gravid uterine weight was collected and net body weight (the day 20 body weight minus the weight of the uterus and contents) and net body weight change (the day 0-20 body weight change minus the weight of the uterus and contents) were calculated and presented for each gravid female at the scheduled laparohysterectomy.

3. FOOD CONSUMPTION

Individual food consumption was recorded on days 0, 6, 9, 12, 16, 18 and 20 of gestation. Food intake was calculated as g/animal/day and g/kg/day for the corresponding body weight change intervals.

I. GESTATION DAY 20 LAPAROHYSTERECTOMY

All maternal animals were euthanized by carbon dioxide inhalation on gestation day 20. The thoracic, abdominal and pelvic cavities were opened by a ventral midline incision and the contents were examined. In all instances, the *post mortem* findings were correlated with the *ante mortem* comments and any abnormalities were recorded. The uterus and ovaries were excised. The number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened and the number and location of all fetuses, early and late resorptions and the total number of implantation sites were recorded. The individual uterine distribution of implantation sites was documented using the following procedure. All implantation sites, including resorptions, were numbered in consecutive order beginning with the left distal to the left proximal uterine horn, noting the position of the cervix, and continuing from the right proximal to the right distal uterine horn.

Uteri with no macroscopic evidence of nidation were excised, opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss as described by Salewski[3]. Maternal tissues were retained only as deemed necessary by the gross findings.

Intrauterine data was summarized using two methods of calculation. An example of each method of calculation follows:

1. Group Mean Litter Basis:

$$\text{Postimplantation Loss/Litter} = \frac{\text{No. Dead Fetuses, Resorptions (Early/Late)/Group}}{\text{No. Gravid Females/Group}}$$

2. Proportional Litter Basis:

$$\text{Summation per Group (\%)} = \frac{\text{Postimplantation Loss/Litter (\%)}^{a}}{\text{No. of Litters/Group}}$$

$$a = \frac{\text{No. Dead Fetuses, Resorptions (Early/Late)/Litter}}{\text{No. Implantation Sites/Litter}} \times 100$$

J. ORGAN WEIGHTS - SCHEDULED NECROPSY

The liver, kidneys and spleen from each dam were excised, trimmed and weighed, and all findings were recorded. The carcasses of the dams, including the liver, kidneys and spleen, were then discarded.

K. FETAL MORPHOLOGICAL EXAMINATION

Each fetus was sexed, weighed and tagged for identification. Fetal tags contained the WIL study number, the female number and the fetus number. A detailed external examination of each fetus was conducted to include, but was not limited to, the eyes, palate and external orifices and each finding was recorded. Crown-rump measurements were recorded (as appropriate) for late resorptions and the tissues were discarded. The sex of each fetus was verified by an internal examination. Each fetus was examined viscerally by a modification of the Stuckhardt and Poppe[4] fresh dissection technique to include the heart and major vessels. Fetal kidneys were examined and graded for renal papillae development by a method described by Woo and Hoar[5].

Heads from approximately one-half of the fetuses from each female were placed in Bouin's fixative for subsequent soft-tissue examination by the Wilson[6] sectioning technique. The heads from the remaining one-half of the fetuses were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol. Following fixation in alcohol, each fetus was macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described in Dawson[7]. The skeletal examination was conducted utilizing low power magnification provided by a stereomicroscope. External, visceral and skeletal findings were recorded as developmental variations or malformations. The fetal developmental findings were summarized by: 1) presenting the incidence of a given finding both as a percentage of the no. of fetuses and the no. of litters available for examination in the group; and 2) considering the litter as the basic unit for comparison and calculating the no. of affected fetuses in a litter on a proportional basis as follows:

$$\text{Summation per Group}(\%) = \frac{\sum \text{Viable Fetuses Affected/Litter}(\%)^a}{\text{No. of Litters/Group}}$$

$$a = \frac{\text{No. Viable Fetuses Affected/Litter}}{\text{No. Viable Fetuses/Litter}} \times 100$$

L. STATISTICAL ANALYSES

All analyses were conducted using two-tailed tests for a minimum significance level of 5% comparing each treated group to the vehicle control group. Each mean was presented with the standard deviation (S.D.) and the number of animals (N) used to calculate the mean. The following statistical tests were performed by a Digital® MicroVAX® 3400 computer (with appropriate programming) in this laboratory and are referenced on the report tables:

STATISTICAL TEST	PARAMETER
-Chi-square test[8] with Yates' correction factor	Fetal Sex Ratios
-Fisher's Exact test[8]	Malformations and Variations
-Mann-Whitney U-test[8]	Early and Late Resorptions, Dead Fetuses, Postimplantation Losses
-ANOVA (two-tailed) with Dunnett's test[8]	Corpora Lutea, Total Implantations, Viable Fetuses, Fetal Body Weights, Maternal Body Weights and Weight Changes, Maternal Net Body Weight Changes and Gravid Uterine Weights, Maternal Food Consumption, Organ Weights
-Kruskal-Wallis test[8]	Litter Proportions of Intrauterine Data (Considering the Litter, Rather than the Fetus, as the Experimental Unit)

M. DATA RETENTION

The sponsor will have title to all documentation records, raw data, specimens or other work product generated during the performance of the study. All work product including raw paper data and specimens will be retained in the Archives at WIL Research Laboratories, Inc., as specified in the study protocol.

Raw data in magnetic form, retention samples of the test article and the original final report will be retained at WIL Research Laboratories, Inc., in compliance with regulatory requirements.

IX. REFERENCES

1. WIL-50236 - A Dose Range-Finding Developmental Toxicity Study of MON 5775 in Rats. WIL Research Laboratories, Inc., Ashland, Ohio.

2. NIH (1985) Guide for the Care and Use of Laboratory Animals. United States Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH Publication No. 86-23, 83 pages.

3. Salewski (Koln), V.E. (1964) Farbemethode zum makroskopischen Nachweis von Implantationstellen am Uterus der Ratte. Naunyn - Schm. Archiv. fur Exper. Pathologie und Pharm. 247:367.

4. Stuckhardt, J.L. and Poppe, S.M. (1984) Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. Teratogenesis, Carcinogenesis and Mutagenesis 4:181-188.

5. Woo, D.C. and Hoar, R.M. (1972) Apparent hydronephrosis as a normal aspect of renal development in late gestation of rats: The Effect of Methyl Salicylate. Teratology 6:191-196.

6. Wilson, J.G. (1965) Embryological consideration in teratology. In: Teratology: Principles and Techniques. (Wilson, J.G. and Warkany, J., eds.) The University of Chicago Press, Chicago, Illinois, pp. 251-277.

7. Dawson, A.B. (1926) A note on the staining of the skeleton of cleared specimens with Alizarin Red S. Stain Technol. 1:123-124.

8. BMDP (1979) Biomedical Computer Programs. (Dixon, W.J. and Brown, M.B., eds.) University of California Press, Berkeley, CA, pp. 612, 780, 781.

ALACHLOR (ANSI) **MICRONUCLEUS (84-2)**

Principal Reviewer: Nancy E. McCarroll Signature: Nancy E. McCarroll
Review Section III, Toxicology Branch Date: 2/29/96
II/HED (7509C)
Secondary Reviewer: Byron T. Backus, Ph.D. Signature: Byron T. Backus
Review Section II,
Toxicology Branch II/HED (7509C) Date: 2/29/96

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mouse micronucleus assay; OPPTS 870.5395 [§84-2]

DP BARCODE: D222904 SUBMISSION NO.: S500250

PC CODE: 090501 TOX. CHEM. NO.: MRID NO: 43889403

TEST MATERIAL (PURITY): MON 5775 (90.7%)

SYNONYM(S): Sulfonic acid metabolite of alachlor; 2',6'-diethyl-N-methoxymethyl-2-sulfoacetanilide, sodium salt; 2-[(2,6-diethylphenyl)-(methoxymethyl)-amino]-2-oxoethanesulfonic acid, sodium salt

CITATION: Stegeman, S.D., Kier, L.D., Garrett, S.L. and Mc Adams, J.G. (1995) Mouse Bone Marrow Micronucleus Assay of MON 5775; Environmental Health Laboratory, Monsanto Co.; Study No. ML-95-069; Study Completion Date: September 29, 1995. (Unpublished) MRID NUMBER: 43889403

SPONSOR: Monsanto Company, St. Louis, MO

EXECUTIVE SUMMARY: In a mouse micronucleus assay (MRID No: 43889403), groups of five male CD-1 mice received single oral gavage administrations of 500, 1000 or 2000 mg/kg MON 5775 (90.7%). The test material was delivered to the animals in deionized water. Animals were sacrificed at 24 and 48 hours postadministration; bone marrow cells were harvested and 2000 erythrocytes per male were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs).

No overt toxicity for the treated animals or cytotoxicity for the target organ was observed up to the currently recommended limit dose (2000 mg/kg). The positive control induced the expected high yield of MPEs in the treated males. **There was, however, no evidence that the test material induced a clastogenic or aneugenic effect at any dose or sacrifice time.**

The study is classified as Acceptable. The study contained major guideline deficiencies (i.e, use of a single sex, only 5 males/dose/sampling time and no 72-hour posttreatment sacrifice). However, these requirements were waived for the following reasons:

- The parent compound, alachlor, is not active in the mouse bone marrow micronucleus assay.

- Adequate justification for the use of males only was provided.

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- Variations within and among treatment groups were minimal; hence, the findings with the smaller than recommended sample size are considered valid.
- The uniformly negative response in conjunction with the absence of an effect on cell cycling suggest that sampling cells 72 hours after compound administration would not have altered the outcome of the study.

Based on these considerations, it was concluded that the study satisfies the requirements for FIFRA Test Guideline 84-2 for in vivo cytogenetic mutagenicity data.

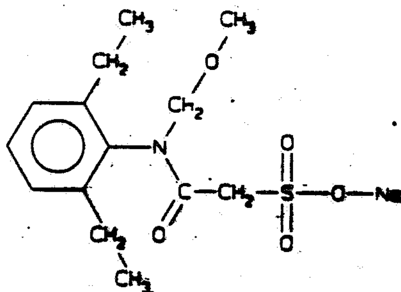
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. **MATERIALS AND METHODS**

A. MATERIALS:

1. Test Material: MON 5775

Description: Pinkish white powder
Lot/batch number: NPD-9203-3974-T
Purity: 90.7% MON 5775 + 6.6% H_2O
Receipt date: Not provided
Stability: Stable under the conditions of use.
CAS number: Not listed
Structure:



Vehicle used: Corn oil (initial range-finding assay); deionized water (DH_2O) --repeat range-finding and micronucleus assays

Other provided information: The test material was stored at room temperature. Dosing solutions were prepared on the day of use and samples of the high dosing solution (micronucleus assay) were analyzed for achieved concentration, stability and homogeneity.

2. Control Materials:

Negative/route of administration: None

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Vehicle/final concentration/route of administration: Corn oil was used as the vehicle for the initial range-finding study. DH_2O, at a dosing volume of 10 mL/kg administered by oral gavage, was used as the vehicle for the repeat range-finding and micronucleus assays.

Positive/final concentration/route of administration: Cyclophosphamide (CP) was prepared in DH_2O and administered by oral gavage at 40 mg/kg.

3. Test Compound:

Route of administration: Oral gavage

Dose levels used:

- Range-finding toxicity test I: 500, 1000 or 2000 mg/kg administered to groups of three males and three females.
- Range-finding toxicity test II: 2000 mg/kg administered to three males and three females.
- Micronucleus assay: 500, 1000 or 2000 mg/kg (5 males per dose, per sacrifice time).

4. Test Animals:

(a) Species mouse Strain CD-1 Age 9-14 weeks
Weight range at dosing (micronucleus assay) : 30.8-39.4 g (males)
Source: Charles River Laboratories, Inc., Portage, MI

(b) No. animals used per dose:

Treatment and vehicle control: 10 males; -- females
Positive control: 5 males; -- females

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound and vehicle:
Dosing: x once ____ twice (24 hr apart)
____ other (describe): ____
Sampling (after last dose): ____ 6 hr ____ 12 hr
x 24 hr x 48 hr ____ 72 hr

(b) Positive control:
Dosing: x once ____ twice (24 hr apart)
____ other (describe): ____
Sampling (after last dose): x 24 hr ____ 48 hr

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2. Tissues and Cells Examined:

   __x__ bone marrow _______ others (list):
   Number of polychromatic erythrocytes (PCEs) examined per animal: 2000
   Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: 1000 (PCEs + NCEs)

3. Details of Slide Preparation: At 24 and 48 hours after administration of the test material or the vehicle control, the appropriate groups of animals were sacrificed by CO_2 asphyxiation. Sacrifice time for the positive control group was 24 hours. Bone marrow cells were flushed from both femurs with fetal bovine serum and centrifuged. Supernatants were discarded and cells were spread onto slides. Slides were stained with Wright-Giemsa solution, coded, and scored.

4. Statistical Methods: Micronucleated PCE (MPEs) frequencies were transformed to the square root and evaluated for statistical significance at $p \leq 0.05$ using Dunnett's t-test. Untransformed PCE/NCE ratios were also evaluated using Dunnett's t-test at $p \leq 0.05$.

5. Evaluation Criteria: If a significant increase in MPEs was observed, the response was evaluated relative to dose- and/or time-dependency, historical control values, and the positive control results.

C. REPORTED RESULTS:

1. Preliminary Toxicity Test: Two preliminary studies were performed. In Trial I, 500, 1000 or 2000 mg/kg MON 5775, suspended in corn oil, were administered orally to groups of three male and three female mice; survivors were sacrificed 48 hours postdosing. For Trial II, three males and three females received a single oral gavage dose of MON 5775 (2000 mg/kg, prepared in DH_2O). One animal per sex was sacrificed at 24 hours; bone marrow cells were harvested, stained as described under Section B.3 (Details of Slide Preparation), and scanned to determine whether PCE:NCE ratios could be scored. All survivors were sacrificed 48 hours posttreatment. Data, combined for both trials, indicated that MON 5775 was not toxic to either sex up to 2000 mg/kg. Based on these results, levels of 500, 1000 and 2000 mg/kg were selected for further study. Owing to the lack of a sex-specific toxic response, only male mice were evaluated in the micronucleus assay.

2. Micronucleus Assay:

   Analytical Determinations: Results from the analytical determinations showed that the high dosing solution of the test substance used in the micronucleus assay was accurately prepared (within 3% of target). The data also indicate that the test material was uniformly distributed in the vehicle and stable under the conditions of use.

   Animal Observations: Animals were observed for toxic effects and deaths on the day of dosing and daily, thereafter, until sacrifice. Body

weights were recorded prior to treatment and at termination. No deaths or clinical signs of toxicity were observed in animals receiving MON 5775. There was also no effect on the body weight of the treated males.

Bone Marrow Analysis: Summarized results for PCE:NCE ratios and MPEs/1000 PCEs are presented in Study Report Tables 3 and 4, pp 25 and 26, respectively, (see Attachments). As shown, treatment with MON 5775 did not adversely affect the PCE:NCE ratios for any group at either sacrifice interval. Similarly, no significant increases in the frequency of MPEs were noted at any dose or sacrifice time. By contrast, the positive control (40 mg/kg CP) induced a significant ($p \leq 0.01$) genotoxic response.

Based on the overall results, the study authors concluded that MON 5775 was negative in the mouse micronucleus assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: The study as performed has several major guideline deficiencies (i.e., use of a single sex, only 5 animals/dose/sacrifice time, and no 72-hour cell harvest). Nevertheless, we believe that the findings provide valid evidence of a negative response in this test system for the following reasons:

- The parent compound, alachlor, is not active in the mouse bone marrow micronucleus assay.
- The presented data provided adequate justification for the use of males only.
- Variations within and among treatment groups were minimal; hence, the results with the smaller than recommended sample size are considered valid.
- The uniformly negative results in conjunction with the absence of an effect on cell cycling suggest that sampling cells 72 hours after compound administration would not have altered the outcome of the study.

Additionally, the sensitivity of the test system to detect a genotoxic response induced by an unknown compound was adequately demonstrated by the significant ($p \leq 0.01$) results obtained with the positive control. We conclude, therefore, that MON 5775 was tested up to the currently recommended limit dose for *in vivo* genetic toxicology studies (2000 mg/kg) and failed to induce a toxic response in the treated animals or a cytotoxic or genotoxic effect on the target organ. Based on these considerations, we assess that the study provided acceptable evidence of a negative response in this whole animal test system.

E. STUDY DEFICIENCIES: This study has been classified as Acceptable despite the above cited deficiencies. We caution, however, that major departures from guidelines are not encouraged. The scientific as well as regulatory implications of such departures are considered strictly on a case by case basis.

Alachlor

RIN 4446-96

Page_____ is not included in this copy.

Pages 51 through 52 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) __________.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

EPA Reviewer: Timothy F. McMahon, Ph.D. Date: 3/6/96
Section I, Toxicology Branch II (7509C)
EPA Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Date: 3/7/96
Section I, Toxicology Branch II (7509C)

Data Evaluation Record

Study type: Metabolism (Special Study) - rat non-guideline

DP Barcode: D222904 Submission: S500250
P.C. Code.: 090501

Study Number: Monsanto Study Number ML-95-066; Laboratory Project Number: EHL 95031/MSL 14489

Test Material: Alachlor Ethane Sulfonate; purity: 98.18%

Synonyms: MON 5775

Citation: Kraus, L.J., Hopkins, W.E., Kinnett, M.L., and Wilson, A. (1995): Elimination, Absorption, Tissue Distribution and Metabolism of Alachlor Ethane Sulfonate (MON 5775) in Long-Evans Rats Following Oral Administration. Study dated December 28, 1995

Testing Facility: Ceregen, a unit of Monsanto Company

Sponsor: Monsanto Company

Executive Summary:

In a special metabolism study (MRID # 438894-04), two groups of male and female Long-Evans rats (two/sex/group) were administered alachlor ethane sulfonate at a dose of 70 mg/kg by gavage. Group 1 rats were sacrificed 24 hours after treatment and Group 2 animals at 5 days after treatment. Disposition of alachlor ethane sulfonate was determined by collection of excreta and by whole-body autoradiography. Metabolism was assessed by HPLC analysis of processed urine and feces samples. The major route of excretion for alachlor ethane sulfonate at 70 mg/kg was the feces, with between 71-82% of the administered dose excreted by this route. Excretion was rapid with the majority of radioactivity excreted by 24 hours post-dose. HPLC analysis of urine and feces showed alachlor ethane sulfonate to be the major component in both urine and feces, with three other components isolated but not identified, each comprising less than 2% of the dose. The data indicate that alachlor ethane sulfonate is poorly absorbed, rapidly excreted, and undergoes minor metabolism. This study is classified as **supplementary** and provides information on the disposition of alachlor ethane sulfonate in Long-Evans rats.

Compliance

A signed statement of no data confidentiality claims was provided.

A signed statement of GLP compliance (40 CFR 160.35) was provided

A signed statement of quality assurance was provided.

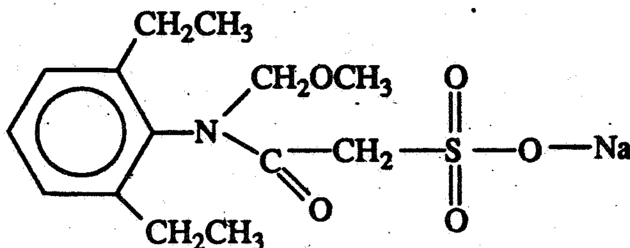
A. MATERIALS

1. Test Materials:

   a) Unlabelled Alachlor Ethane Sulfonate, purity not stated
      Lot No. NPD-9203-3974-T

   b) Labelled Alachlor Ethane Sulfonate, radiochemical purity 98.18%

Chemical Structure:



Alachlor Ethane Sulfonate

2. Vehicle: 5% water / Corn oil

3. Test Animals: Species: rat, male and female
Strain: Long-Evans
Age at dosing: 44 days (males); 56 days (females).
Weight at dosing: 202-221 grams (males); 181-212 grams (females)
Source: Charles River Breeding Laboratory, Portage, MI
Housing: Roth-type metabolism cages
Diet: PMI Certified Rodent Diet # 5002, *ad libitum*
Water: St Louis public water supply, *ad libitum*

Environmental Conditions: A twelve hour light/dark cycle was provided in an animal room under conditions of controlled temperature (70-74 ^{o}F) and humidity (40-60%). Rats were quarantined for 3 days in stainless steel cages before being placed on study. Rats were acclimated to the Roth-type cages approximately 24 hours prior to dosing.

4. Preparation of Dosing Solutions

Test material was first dissolved in water. After addition of corn oil, the solution was shaken vigorously. The dosing solution was aliquoted by weight, diluted, and weighed portions were counted by liquid scintillation counting to determine concentration. Actual doses administered were calculated by weighing the dosing syringes plus the needle before and after dosing. Target dose volume was 4 mL/kg body weight.

B. Study Design

1. Group Arrangements

This study utilized two groups of rats. Each group consisted of 2 male and 2 female Long-Evans rats. Both groups received a single oral dose of 70 mg/kg alachlor ethane sulfonate. Group 1 animals were sacrificed 24 hours after dosing, and Group 2 animals were sacrificed 5 days after dosing.

2. Sample Collection

Urine, feces, and cage wash samples were collected 24 hours after dosing and daily thereafter until sacrifice. Urine and feces samples were kept chilled by collection in to vials held within stainless steel cooling jackets through which a chilled water solution continuously circulated. At each collection period, the metabolism cages were rinsed with distilled water. At termination, each

cage was rinsed first with distilled water and then with 70% acetonitrile in distilled water. Urine samples were analyzed directly by LSC. Feces was homogenized with water using a Tekmar tissuemizer. Duplicate aliquots of the fecal homogenates were combusted in a Packard System 387 Sample Oxidizer, using a scintillation cocktail mixture of Permaflour and Carbosorb. Urine samples were analyzed directly after addition of approximately 15 mL of Ultima Gold counting cocktail.

a. Pharmacokinetic Studies

There were no pharmacokinetic experiments performed in this study.

b. Metabolite Characterization

HPLC coupled with radioactive flow monitoring and subsequent fraction collection was used. It was not stated whether urine samples were analyzed individually or not. For feces, pooled fecal samples were prepared for each animal in Group II representing the 0-48 hour period following dosing. For Group I, the 0-24 hour fecal samples were used.

c. Autoradiographic Studies

Rats were sacrificed at the appropriate time by exposure to CO_2 and were rapidly frozen in a hexane/dry ice bath (approximately -70 ^{o}C). Animals were left in the cryostat for several hours to allow the hexane to evaporate off. Following this, the fur was shaved and the limbs and tail removed. The frozen animal (-20 ^{o}C) was then embedded in carboxymethylcellulose and frozen in the dry ice/hexane bath until completely frozen. The embedded animal was then mounted in a Leica Cryomacrocut and sectioned sagitally at 40 µm. Sections were dehydrated for at least 24 hours and then pressed onto x-ray film. Following exposure, films were developed. All films were compared visually for darkening produced by the presence of radioactive material. An assessment of the relative level of darkening was made by comparison of the darkening observed in various regions of the body.

III. RESULTS

1. Dosing

A summary of the doses received in this study was presented on page 47 of the report. The actual doses received were very close to the nominal dose (i.e. range of 69.5-73.1 mg/kg) for all animals

2. Excretion of Radioactivity

A summary of the excretion of radioactivity for Group I and Group II rats was presented on pages 23 and 24 of the report. These data are shown below

Table 1

Excretion of 14-C Derived Alachlor Ethane Sulfonate Radioactivity
% Administered Dose

	Group I		Group II	
	Males	Females	Males	Females
Urine	9.90±3.30	13.08±0.94	4.11±2.51	17.95±1.14
Feces	71.02±10.42	73.24±15.30	84.27±1.44	82.45±1.80
Cage Wash	0.92±0.71	1.03±0.02	1.06±0.56	2.45±0.52
Total	81.86±6.39	87.35±16.25	89.45±4.52	102.78±0.131

As shown in the above table, the feces was the major route for excretion of alachlor ethane sulfonate derived radioactivity. At 24 hours, approximately 70% of the administered dose was excreted by male and female rats, and by 5 days post-dose, 82-84% of the dose was excreted by this route. The majority of the remaining radioactivity was excreted via the urine. In male rats from Group I, it is observed that approximately 20% of the administered dose was not accounted for by 24 hours post-dose. In addition, it is observed that in male rats from Group II, urinary excretion as a percent of the dose was less than that reported at 24 hours. However, it is also noted that an increase was observed in the percentage of the dose excreted in feces in Group II males, which would largely explain the missing radioactivity at 24 hours post-dose.

3. Metabolite Characterization

Data on characterization of urinary metabolites of alachlor ethane sulfonate were presented in Table 4, page 26 of the report. Table 5 presented the urinary data as a percentage of the administered dose. As a percentage of the dose, the parent chemical was observed in the greatest percentage for both male and female rats. Three metabolites of alachlor ethane sulfonate

were separated by HPLC, but not one of these metabolites exceeded 2% of the dose. In all but one case, these metabolites represented less than 1% of the administered dose in urine. Summary of these data is made below:

Urinary Metabolites of Alachlor Ethane Sulfonate at 24 Hours Post-Dose
(% Administered Dose)

	Group I		Group II	
	Male	Female	Male	Female
Metabolite 1	1.22	0.07	0.40	0.10
Metabolite 2	1.16	0.17	0.31	0.305
Metabolite 3	2.69	0.505	0.94	0.685
Parent	3.78	11.97	1.87	16.20

As indicated, the parent chemical was the major urinary metabolite detected as a percentage of the administered dose. As a percentage of urinary excreted radioactivity, the parent chemical represented 40.95% of the radioactivity in males, and 92.03% of the radioactivity in females. It is observed from the above data that male rats in Group I appeared to show higher levels of the three metabolites than female rats in either Group I or Group II. There was a similarity in the percentages of the three metabolites for female rats in both groups (as would be expected), but for male rats, Group I showed higher urinary percentages of the three metabolites. The explanation for this is not readily apparent.

The recovery of fecal radioactivity from extraction of samples was presented on page 29 of the report, and showed that for all samples, recovery was 100% or greater. The distribution of fecal metabolites as a percentage of the dose was presented in Table 9, page 31 of the report. These data are shown below:

Fecal Metabolites of Alachlor Ethane Sulfonate
(% Administered Dose)

	Group I[a]		Group II[b]	
	Male	Female	Male	Female
Metabolite 1	0.415	0.05	1.11	0.20
Metabolite 2	1.98	0.34	2.32	0.77
Metabolite 3	1.23	0.36	1.25	0.51
Parent	65.31	71.71	73.65	78.07

a - 24 hour collection   b - 24 and 48 hour collection

As with urine, the major metabolite observed in feces was the parent chemical, which was found to compose between 68-72% of the administered dose, and between 91-95% of the fecal radioactivity. The three metabolites observed were again found to be in very low percentage in feces, and were not identified. In contrast to the urinary profile for male rats, the fecal profile did not show differences in the percentages of the three metabolites between Group I and Group II male rats. This suggests that there may have been a technical problem in reporting the percentages of urinary metabolites for male rats, although this cannot be proven from the available data.

4. Whole Body Autoradiography

Representative autoradiographs were presented within the study report, but cannot be reliably reproduced here. From examination of the autoradiographs at 24 hours (1 male and 1 female rat each), the major areas of localization were reported to be the stomach contents, cecum, intestinal contents, and urinary bladder. Very slight localization was observed in the surface of the tongue and esophagus. It is noted that there was no localization to the liver at 24 hours post-dose, attesting to the poor absorption of the test material. No significant differences were observed between males and females.

Autoradiograms for the 5 day post-dosing sacrifice group showed no localization of radioactivity. It appeared as if all of the radioactivity had been eliminated in male and female rats by this time.

IV. DISCUSSION

This study examined the disposition of Alachlor ethane sulfonate, a metabolite of Alachlor observed in corn, soybeans, soil, and water. Two groups of male and female Long-Evans rats (two/sex/group) received alachlor ethane sulfonate at a target dose level of 70 mg/kg. Disposition of radiolabelled test chemical as well as the metabolites of the test chemical were monitored in excreta. From the data in this study, it is evident that the majority of the radioactivity is excreted via the feces, and that this represents unabsorbed test chemical. Metabolite analysis of urine and feces showed that the ethane sulfonate parent chemical was the major chemical entity present in both urine and feces, with three very minor metabolites which were not further identified. Autoradiography indicated that at 24 hours post-dose, the areas showing the highest concentration of radioactivity were the stomach contents, cecum, intestinal contents, and urinary bladder. Very slight localization was observed in the surface of the tongue and esophagus. It is noted that there was no localization to the liver at 24 hours post-dose, further attesting to the poor absorption of the test material. In conclusion, the data indicate poor absorption of alachlor ethane sulfonate after oral administration to rats, with rapid excretion. Metabolism appears to be minor.

Classification: supplementary

EPA Reviewer: Timothy F. McMahon, Ph.D. Date: 3/4/96
Section I, Toxicology Branch II (7509C)
EPA Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. Date: 3/7/96
Section I, Toxicology Branch II (7509C)

Data Evaluation Record

Study type: Nasal Tissue Cell Proliferation - rat (non-guideline)

DP Barcode: D222904 Submission: S500250
P.C. Code.: 090501

Test Material: Alachlor Ethane Sulfonate; purity: not stated

Synonyms: MON 5775

Citation: Hotz, Kathy J. (1995): Effect of MON 5775 on Cell Proliferation in the Nasal Tissue of Male F-344 Rats. Monsanto Study # MI-95-070. EHL Study # 95034.

Testing Facility: Monsanto Company, Environmental Health Laboratory

Sponsor: Monsanto Company

Executive Summary:

In a special study (MRID # 438894-01), the proliferating cell nuclear antigen technique (PCNA) was utilized to determine the effect of treatment with 2000 ppm Alachlor (157 mg/kg/day for 91 days) on cell proliferation in the olfactory region at the second palatal ridge (Level III), where Alachlor-induced tumors are found. Mean nasal cell proliferation values (number of labelled cells per mm of mucosal length) showed no statistically significant increases in cell proliferation in either the olfactory septum or turbinates of male Fischer 344 rats administered MON 5775 in drinking water for 91 days. This study is classified as **supplementary** and provides limited information on the nasal proliferative response from administration of MON 5775 to male Fischer 344 rats.

Compliance

A signed statement of no data confidentiality claims was provided with this study.

A signed statement of GLP compliance (40 CFR 160.35) was provided.

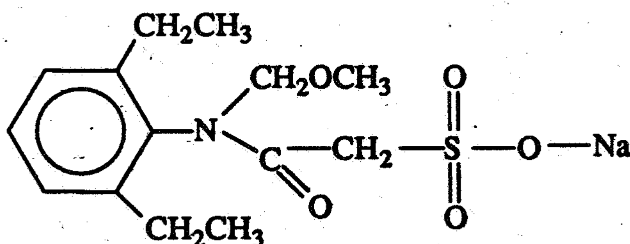
A signed statement of quality assurance was provided.

A. MATERIALS

1. Test Materials:

a) Unlabelled Alachlor Ethane Sulfonate, purity not stated
Lot No. NPD-9203-3974-T

Chemical Structure:



Alachlor Ethane Sulfonate

According to the report, the results of the present study are based upon analysis of tissue blocks or wet tissue of the nasal cavity from male Fischer 344 rats who were given MON 5775 in drinking water for 91 days at doses of 0, 200, 2000, or 10,000 ppm. This study (study # SB-92-383, MRID # 42863701) had been previously conducted at Springborn Laboratories, Inc., Spencerville, Ohio.

2. Vehicle: drinking water

3. Test Animals: Species: rat, male
Strain: Fischer 344
Age at dosing: approximately 8 weeks.
Weight at dosing: 173-190 grams (males)
Source: Charles River Laboratory, Raleigh, N.C.
Other information on animal husbandry is presumably within the Springborn Laboratory report.

B. Study Design

The original paraffin blocks of nasal tissue from the control group were used in the present analysis for nasal cell proliferation. Wet tissue from the rats treated with 2000 ppm MON 5775 were decalcified and two sections trimmed - one at the level of the incisive papilla and the other at the second palatal ridge. The tissue sections were processed and then embedded in paraffin. The paraffin blocks from both the control and treated animals were sectioned at 4 µm for cell proliferation analysis. As mentioned, cell proliferation was measured using the PC 10 antibody to PCNA followed by biotinylated anti-mouse IgG antibodies and streptavadin-horseradish peroxidase. The staining reaction was then detected by the use of the chromogen, diaminobenzidine

The nasal septum and turbinates were evaluated separately without knowledge of the dose group from which they originated. A standard pattern was used to count the labelled nuclei which included 10 sites for the septum and 16 sites for the turbinates. It was stated in the report (page 10) that "due to tissue inadequacy or poor staining quality, some of the animals from the control and 2000 ppm dose group could not be used. Five of the ten animals in the control group were analyzed for cell proliferation. Only nine animals were analyzed in the 2000 ppm dose group." Further, the report stated (page 9) that "the 2000 ppm dose group was chosen to be evaluated due to tissue inadequacy in most of the tissue blocks at the 10,000 ppm dose level."

Statistical evaluation was done by use of Student's t-test ($p < 0.05$ or $p < 0.01$, two-sided).

III. RESULTS

Cell proliferation results are presented in the following table, as copied from the report (page 10):

Cell Proliferation in the Nasal Tissues of MON 5775 Treated Male Fischer Rats

Dose Group	No. Animals	Dose (ppm)	Olfactory Region[a] Septum	Olfactory Region[a] Turbinates	Overall Mean
Control	5	0	7.13±3.07[b]	10.83±4.05	9.29±2.82
Treated	9	2000	9.58±3.70	7.57±3.77	8.40±3.53

a- values are expressed as the number of labelled cells / mm of mucosal length.
b - mean of four animals.

According to the above data, there were no statistically significant increases in cell proliferation in either the olfactory septum or turbinates of male Fischer 344 rats given 2000 ppm (157 mg/kg/day) MON 5775 in drinking water for 91 days.

IV. DISCUSSION

The purpose of the present study was to evaluate cell proliferation in the nasal tissue of male Fischer 344 rats who had been treated for 91 days with 2000 ppm MON 5775 in drinking water. The tissue samples used in the present study were obtained from a previously conducted study (Springborn Laboratories study # SB-92-383, MRID # 42863701) in which MON 5775 was administered in drinking water for 91 days at doses of 0, 200, 2000, and 10,000 ppm. The evaluation of nasal tissue at the 2000 ppm dose level was felt to be adequate for assessment of cell proliferation, as this dose level (equivalent to 157 mg/kg/day) was similar to the dose level at which alachlor-induced nasal tumors are observed (126 mg/kg/day). The results of this study, using the proliferating cell nuclear antigen technique, indicated no statistically significant increase in cell proliferation in either the septum or turbinate region of the olfactory epithelium in treated rats. It must be kept in mind that this is a limited data set, i.e. 9 male rats, which is based upon a limited concurrent control data set, i.e. 5 male rats, although in other studies, nasal tumors were not observed in concurrent control groups. It should also be pointed out that the route of administration of the test material is different in this study than in the long-term studies conducted with Alachlor. This difference in the route of administration could or could not have a significant influence on the delivered dose of test chemical, as, for example, has been observed with chloroform. It could also be pointed out that the duration of exposure in this study was less than in the previous data demonstrating nasal tumors from Alachlor administration. The data suggested some increase in proliferation in the nasal septum at the 2000 ppm dose level, but this increase (from 7.13 to 9.58 cells per mm of mucosal length) was not statistically significant. It would be of interest to know whether longer term exposure resulted in any further increases. It would also be of interest to determine the effect of administration in drinking water vs administration in the diet for this test chemical. In support of the conclusions of this study, it is known that oral administration of alachlor ethane sulfonate results in poor absorption (MRID # 438894-04). Based on such poor absorption, nasal localization would not be expected, and thus a proliferative response would not result. The data in this study tend to support the conclusion that alachlor ethane sulfonate is without significant effect on the proliferative response of nasal tissue in rats. There may be some effect, as indicated by the data, but longer term exposures at higher doses might be necessary to show an actual effect.

Classification: supplementary

This study provides limited information on the nasal tissue proliferative response from administration of MON 5775 to male Fischer 344 rats. The data appear to indicate no significant effect of treatment, but further study of this chemical would provide more definitive conclusions.

011861

EPA Reviewer: Timothy F. McMahon, Ph.D. [signature] Date: 3/6/96
Section I, Toxicology Branch II (7509C)
EPA Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. [signature] Date: 3/7/96
Section I, Toxicology Branch II (7509C)

Data Evaluation Record

Study type: Gastric Fundi Cell Proliferation and Mucosal Thickness - rat (non-guideline)

DP Barcode: D222904 Submission: S500250
P.C. Code.: 090501

Test Material: Alachlor Ethane Sulfonate; purity: not stated

Synonyms: MON 5775

Citation: Iatropoulos, M.J. and Wang, C.X. (1995): Evaluation of Cell Proliferation and Measurement of Mucosal Thickness in Gastric Fundi of Rats From Study SB-92-383. Monsanto Report # AH-95-071. Study dated July 28, 1995.

Testing Facility: American Health Foundation, Valhalla, New York.

Sponsor: Monsanto Company, St. Louis, MO.

Executive Summary:

In a special study (MRID # 438894-02), glandular stomach tissue from female Fischer 344 rats treated with MON 5775 in drinking water at a dose of 10,000 ppm for 91 days was evaluated using the proliferating cell nuclear antigen for evidence of a proliferative response or changes in mucosal thickness. A significant increase in the percentage of labelled cells in the fundic neck region was observed in treated rats, but there were no significant changes in labelling of the fundic base nor in mucosal thickness. This study is classified as **supplementary** data.

Compliance

A signed statement of no data confidentiality claims was provided with this study.

A signed statement of GLP compliance (40 CFR 160.35) was provided.

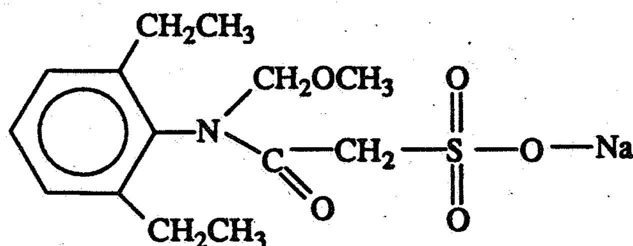
A signed statement of quality assurance was provided.

A. MATERIALS

1. Test Materials:

a) Unlabelled Alachlor Ethane Sulfonate, purity not stated
Lot No. NPD-9203-3974-T

Chemical Structure:



Alachlor Ethane Sulfonate

In this study, glandular stomach tissues from female Fischer 344 rats were labeled with an antibody to Proliferating Cell Nuclear Antigen to provide an analysis of the stomach's proliferative response to MON 5775. In addition, the thickness of the fundic gastric mucosa was also measured. The tissues were received in block form from Springborn Laboratories and were from the control and high dose groups of study # SB-92-383. (MRID # 42863701) .

2. Study Design

Proliferating cells in stomach mucosa were stained using immunohistochemical techniques for Proliferating Cell Nuclear Antigen (PCNA) published in the literature as well as in house methodology. Staining reactions were visualized with 3,3' diaminobenzidine tetrahydrochloride. Immunohistochemically-treated tissues were counterstained with hematoxylin. Labeled cells of the glandular stomach were quantitated by using a square graticule with 25 equal subdivisions. Each side of the graticule was confirmed by measurement with a second micrometer as 0.125mm, providing a square counting area that contains between 50 and 90 cells in the fundus at 400X magnification. All labelled nuclei were counted for PCNA. In the fundus, two regions were scored: the neck region and the basal mucosa. The method employed resulted in between 450 and 1000 fundic nuclei counted per fundic level per slide.

Fundic mucosal thickness was measured by measuring the full depth of gastric mucosa from foveolar surface to the base of the gastric glands. The limit of measurement was 0.1 of the graticule width, or 0.0125 mm.

III. RESULTS

Summary data on fundic mucosal thickness and labelling were provided in the report (page 13), and are summarized below:

Proliferative Activity in MON 5775 Treated Glandular Stomach of Female F344 Rats

		Labelling Indices (%)		
Group	No. Animals	Fundic Neck	Fundic Base	Mucosal Thickness (mm)
Control	10	16.53±1.97	0.94±0.50	0.569±0.061
Treated	10	20.80±2.68*	1.12±0.49	0.561±0.052

* $P < 0.001$ vs control by t-test.

As indicated, examination of the fundic mucosa provided evidence of an increased cell proliferation in the fundic neck region which was statistically significant. Cell proliferation in the fundic base region was not significantly affected, nor was the thickness of the fundic mucosa.

IV. DISCUSSION

The present data were reported to provide evidence on the ability of MON 5775, a metabolite of alachlor, to induce proliferative changes in the fundic mucosa of female Fischer 344 rats. Rats in this study were exposed to MON 5775 in drinking water for 91 days at dose levels of 0, 200, 2000, and 10,000 ppm. As shown, there was a statistically significant increase in the percentage of labelled cells in the fundic neck region of the stomach of female rats treated at the 10,000 ppm dose level. Of interest would be the response observed at 2000 ppm, which was the dose level reported in the study on nasal cell proliferation (MRID # 438894-01). In that study, there was no significant response, although a numerical increase was noted. It could be speculated that a significant response would be observed in the nasal turbinate epithelium at 10,000 ppm had this dose level been investigated (it is unclear why stomach tissue but not nasal tissue could be examined at the 10,000 ppm dose level; the stated reason of 'tissue inadequacy' of the nasal tissue at the 10,000 ppm dose level is not readily explainable).

The report noted that both alachlor and the analog butachlor (both chloroacetanilide herbicides) produce glandular stomach tumors in female rats after lifetime administration of dose levels of approximately 126 and 190 mg/kg/day. The mechanism proposed for these compounds involves stimulation of cell proliferation driven by aggressive fundic mucosal atrophy, with changes noted as early as 30-60 days after the start of treatment. In contrast, only a minimal increase in cell proliferation and no decrease in mucosal thickness was observed after treatment with MON 5775 at 10,000 ppm (1108 mg/kg/day). The report concludes that these data support the conclusion that the potential of MON 5775 to induce glandular stomach tumors in rats is much less than that of the parent material alachlor.

Based on the data presented, it would appear that a significant response with respect to glandular stomach cell proliferation is observed at 1108 mg/kg/day, a much higher dose than observed with studies on alachlor. The present data do tend towards the conclusion that MON 5775 is much less potent at causing cell proliferation in the stomach than is the parent chemical alachlor. It might also be concluded that MON 5775 does possess some activity of its own, although the consequences of this are not known. As this metabolite is formed in soil, it is possible for animal species to be exposed to this metabolite directly. As stated, the consequences of this exposure are not known at this time, but tend towards the conclusion that the response would be much less than that observed with alachlor. The fact that some response is observed in the stomach while little or no response is observed in the nasal tissue is supported by the poor absorption of the ethane sulfonate metabolite.

Classification: supplementary

011861

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 3/21/96
Senior Pharmacologist, Review Section I, TBII/HED (7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. YM Ioannou 3/21/96
Section Head, Review Section III, TBII/HED (7509C)

DATA EVALUATION RECORD

NOTE: THIS IS AN IBT STUDY

Study Type: Subchronic Toxicity (Feeding) Study
Species: Rat; Guideline: OPP §82-1a

EPA ID No.s: EPA MRID No. 00023658
EPA DP Barcode None
EPA Submission Barcode none
EPA Pesticide Chemical Code 090501
Toxicology Chemical Code 011

Test Material: CP50144 Technical
Synonyms: Alachlor

Sponsor: Monsanto Company
800 N. Lindbergh Blvd., St. Louis, Mo 63167

Testing Facility: Industrial BIO-TEST Laboratories, Inc.

Title of Report: Report to Monsanto Company: 90-Day Subacute Oral Toxicity of CP50144 - Albino Rats

Study Number(s): Project BTL-66-4, IBT No. B4477

Author(s): C. Wolf

Report Issued: 1966

Executive Summary: In a subchronic toxicity study (MRID# 00023658), male and female Charles River albino rats from Charles River Breeding Laboratories, Inc., North Wilmington, MA received either 0, 20, 200, or 2000 ppm CP50144 Technical (Alachlor) for 90 days (approximately 0, 1.5, 15, and 146 mg/kg/day for the control, low, mid and high dose groups, respectively by standard conversion factors).

Systemic toxicity was noted in the high dose animals as decreased body weights and body weight gains, decreased food consumption and efficiency and increased absolute and relative spleen weights, increased relative liver weights, increased relative to body weight kidney weights, and decreased relative gonad weights (testis and ovaries). The Systemic Toxicity NOEL is 200 ppm (15 mg/kg/day) and the Systemic Toxicity LOEL is 2000 ppm (146

mg/kg/day) based on decreased body weights, body weight gains, reduced food consumption and increased spleen, liver and kidney weights and decreased gonad weights.

This study is classified as Unacceptable and does not satisfy the guideline requirements (OPP §82-1a) for a subchronic toxicity study in rats as it is an invalidated IBT study, this study was not repeated since an adequate chronic toxicity was performed.

Compliance

None provided.

A. Materials and Methods: A copy of the materials and methods sections from the investigators report is attached.

1. **Test compound:** CP50144 Technical (Alachlor)
   Description: not provided
   Batch# not provided
   Purity: not provided
   Vehicle: corn oil used in mixing in diet

2. **Test animals:** Species: Albino rat
   Strain: Charles River strain
   Age: weanlings
   Weight: males-96 g & females-97 g
   Source: Charles River Breeding Labs, North Wilmington, MA

B. Study Design:

1. **Animal assignment**

Animals were assigned to the following test groups:

Test Group	Dose in Diet (ppm)	# animals assigned male	female
1 Control	0	10	10
2 Low (LDT)	20	10	10
3 Mid (MDT)	200	10	10
4 High (HDT)	2000	10	10

2. **Diet preparation**

Diet was prepared weekly and no indication of storage temperature was provided. There was no indications if samples of treated food were analyzed for stability and concentration.

3. **Animal Husbandry**

No information was provided. Food was Wayne Lab Blox, pulverized (Allied Mills, Inc., Chicago, IL).

4. **Observations**

Body weights were recorded prior to study initiation and then weekly. Food consumption was measured weekly. Clinical observations were made daily for mortality and abnormal behavior. Hematological measurements conducted prior to study initiation, at 30 days and study termination consisted of determinations of hemoglobin concentrations, erythrocyte counts, hematocrit values and both total and differential leukocyte counts (platelet counts

were not measured as required for subchronic studies). Clinical chemistry measurements included determinations of blood urea nitrogen, serum alkaline phosphatase and iso-citrate dehydrogenase (Calcium, Chloride, Phosphorous, Potassium, Sodium, Albumin, Blood creatinine, Cholesterol, Glucose, Total Serum Protein, Serum alanine aminotransferase and Serum aspartate aminotransferase were not measured as required for subchronic studies). Urinalysis included albumin concentrations, glucose concentrations, presence of microscopic elements, pH determination and specific gravity (these parameters are not required for subchronic studies). Gross autopsy was conducted on all animals with a *complete set of tissues and organs removed...for histopathologic study*. This included esophagus, stomach (cardia, fundus and pylorus), small intestine (duodenum, jejumen and ileum), cecum, colon powell, liver, kidney, spleen, pancreas, urinary bladder, pituitary gland, adrenal gland, testis, ovary, thyroid gland, parathyroid gland heart, lung, mesenteric lymph node, bone marrow, skeletal muscle, uterus, seminal vesicle, trachea, prostate, submaxillary salivary gland, spinal cord, eye, optic nerve and brain (cerebrum, cerebellum and pons) (Aorta, Thymus, Rectum, Parathyroids, and Lung were not examined as required from subchronic studies). Organ weights were recorded for liver, kidneys, spleen, gonads, heart and brain (thyroids and parathyroids were not weighed as required for subchronic studies).

5. Statistics

The following procedures were utilized in analyzing the numerical data:

Analysis of variance and "t"-tests.

C. RESULTS:

1. Observations:

A low dose and two high dose animals died, deaths were not attributable to treatment. The investigators reported no behavioral changes attributable to treatment.

2. Body weight

The investigators presented only group mean data. The following table presents body weight and body weight gain data for selected intervals.

Body Weights and Body Weight Gains (g)

Group	Day: 0	35	70	90	BW Gains
Control	96/97[1]	324/228	461/278	490/290	394/193
LDT	96/97	316/231	443/287	473/305	377/208
MDT	96/97	319/229	446/286	479/301	383/204
HDT	96/97	304/210	416/253	436/264	340**/167

[1] = Males/Females; ** = $p < 0.01$

A treatment related effect was noted in high dose males and females as decreased body weights and body weight gains.

3. Food consumption and compound intake

The investigators presented only group mean data. The following table presents food consumption data for selected intervals (similar to body weight data).

Food Consumption (g/rat/week)

Group	Week: 1	5	10	13	Total (g)
Control	105/95[1]	162/120	162/111	135/95	2026/1463
LDT	105/97	160/122	158/123	129/102	1943/1514
MDT	97/91	161/120	170/122	129/104	1996/1509
HDT	93/86	150/112	152/115	126/90	1813/1413

[1] = Males/Females

Food consumption was decreased in the high dose groups. Related food efficiency was 19.5, 19.4, 19.2, and 18.8% for males and 13.2, 13.7, 13.5, and 11.8% for females of the control, low, mid and high dose groups, respectively. This indicated reduced food efficiency in the high dose groups, an indicator of toxicity. Compound intake was not provided, using standard conversion factors, approximate compound intake was 0, 1.5, 15, and 146 mg/kg/day for the control, low, mid and high dose groups, respectively.

4. Hematology and Clinical Chemistry

Total leukocyte counts were increased in high dose males (only control and high dose groups were measured) at 30 days and at study termination, no other parameter was affected. The biological relevance of this difference is unclear since no related pathological changes were noted.

5. Urinalysis

No treatment related effects were noted.

6. Sacrifice and Pathology

a. Organ weights

The investigators only provided mean organ weights. The following table presents absolute and relative (to body weight and brain weight) weight data.

Organ Weight Data (g)

Organ		Control	20	200	2000
Liver	A[1]	21.4/12.3[2]	20.3/12.2	21.6/11.6	21.6/12.6
	RBW[3]	4.37/4.21	4.27/3.97	4.49/3.85*	4.93**/4.77**
	RBrW[4]	10.6/6.37	10.5/6.33	10.7/5.96	10.8/6.67
Kidney	A	3.85/2.28	3.84/2.49	3.98/2.41	3.76/2.34
	RBW	0.788/0.787	0.813/0.817	0.835/0.808	0.862/0.886**
	RBrW	1.90/1.19	1.99/1.29	1.98/1.24	1.89/1.24
Spleen	A	0.723/0.519	0.708/0.503	0.876/0.569	0.842/0.582
	RBW	0.147/0.179	0.150/0.165	0.183**/0.189	0.192**/0.221
	RBrW	0.357/0.269	0.367/0.261	0.434/0.293	0.422/0.308
Gonads	A	3.50/0.110	3.31/0.117	3.43/0.127	2.93/0.129
	RBW	0.714/0.0378	0.706/0.0385	0.719/0.0425	0.664/0.0497**
	RBrW	1.73/0.0570	1.72/0.0609	1.70/0.0652	1.46/0.0684**

[1] = A = Absolute; [2] = males/females; [3] = RBW = Relative to body weights; [4] = RBrW = Relative to brain weights; * = $p < 0.05$; ** = $p < 0.01$

The above data show that there were increased relative liver weights, relative to body weight kidney weights in high dose animals. The absolute and relative to body weight spleen weights were increased in mid and high dose animals. Also there were decreased relative gonad weights (testis and ovaries) in high dose animals. The effect on the spleen weight in mid dose males is significant since body weights were unaffected at this dose, however, there was no related pathology reported.

b. Gross pathology

No treatment related effects were noted.

c. Microscopic pathology

No treatment related effects were noted.

D. Discussion:

Systemic toxicity was noted in the high dose animals as decreased body weights and body weight gains, decreased food consumption and efficiency and increased absolute and relative spleen weights, increased relative liver weights, increased relative to body weight kidney weights, and decreased relative gonad weights (testis and ovaries).

Systemic Toxicity NOEL = 200 ppm (15 mg/kg/day)
Systemic Toxicity LOEL = 2000 ppm (146 mg/kg/day)

Alachlor

RIN 4446-96

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