7-21-94

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **MON 5775 (metabolite of ALACHLOR):** Review of 91 day drinking water study in rats.
EPA Barcode D205429; EPA Submission No. S469774; EPA MRID No: 428637-00 and -01; EPA Pesticide Chemical Code 090501 (Alachlor), Toxicology Chemical No. 011 (Alachlor).

TO: Robert Taylor/Vickie Walters, PM 25
Herbicide-Fungicide Branch
Registration Division (7505C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/20/94
Senior Pharmacologist, Review Section I
Toxicology Branch II/HED (7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y.M. Ioannou 7/20/94
Section Head, Review Section I
and
Marcia van Gemert, Ph.D. Marcia van Gemert 7/21/94
Chief, Toxicology Branch II
Health Effects Division (7509C)

Registrant: Monsanto Company, Suite 1100, 700 14th Street, N.W., Washington, D.C. 20005

Action Requested: Review of a 91 day drinking water study with MON 5775, the ethane sulfonate metabolite of alachlor.

Recommendations: TB II reviewed the report entitled *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). The following is the Executive Summary from the review:

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, 300, 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 noted in all dosed males and females in the form of increased incidences of decreased activity, rapid/shallow breathing, few feces, feces small in size, dehydration, urine staining, emaciation, hunched posture, rough coat, unkempt

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appearance, 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 in most cases. There was a decrease in body weight gain in the high dose males and all treated females had reduced weight gains (however, they were not dose related) along with reduced food consumption and water consumption was slightly increased in high dose males. There appears to be an effect on red blood cell parameters in the form of decreased erythrocytes, hemoglobin, hematocrit and platelets in the 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. No specific histopathological observations were noted. 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 Systemic Toxicity LOEL is equal to or less than 200 ppm and the Systemic Toxicity NOEL is less than 200 ppm based on the increased incidence of clinical signs of toxicity.**

This study is classified as Core-Supplementary Data and does not satisfy the guideline requirement (§82-1a) for a subchronic toxicity study in rats due to the lack of a No Observable Effect Level.

Subchronic toxicity data in rats with the parent compound Alachlor indicates a Systemic Toxicity NOEL of 200 ppm (IBT data), while the chronic toxicity studies in rats have Systemic Toxicity NOEL's of 2.5 mg/kg/day (alachlor, epichlorohydrin free) and less than 14 mg/kg/day (alachlor with epichlorohydrin).

TBII previously reviewed the report entitled *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, MRID No. 427015-01); the following are the conclusions of the review:

Based on the data provided the **acute oral LD_{50} of MON 5775 is greater than 6000 mg/kg.** The study is classified as **Core Guideline Data** with a **Toxicity Category of IV. This study satisfies the guideline requirements (§81-1) for an acute oral toxicity study in rats.** The acute oral LD50 for alachlor technical is 0.93 g/kg with a toxicity category of III; therefore MON-5775 is less acutely toxic than the parent chemical.

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I. Toxicology Profile for Alachlor (40 CFR 180.249)

Technical: Alachlor
Use Pattern: food and non-food

This compound is a registered active ingredient. The following data are required for technical lachlor. 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	NO[1]
§82-1(a)90 day feeding study - rat	Yes	NO[2]
§82-1(a)90 day feeding study - rat/metabolite	Yes	Yes
§82-1(b)90 day feeding study - nonrodent	Yes	NO[3]
§82-1(b)90 day feeding study - nonrodent/met.	Yes	NO[3]
§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[4]

[1] = study received and is presently under review
[2] = satisfied by 2-year chronic feeding study in the rat
[3] = satisfied by 6 month subchronic feeding study in the dog
[4] = based on human and monkey data submitted to the agency

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II. Data Gaps

The database for technical Alachlor is not complete:

581-6 Dermal sensitization - guinea pig, this study has been received and is presently under review.

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.

A. New toxicology Data on Alachlor

Discussed above on cover page (DER attached).

B. Carcinogenicity

This chemical has been classified as a Group B2 Carcinogen (Probable Human Carcinogen) by the HED Peer Review Committee (PRC) and the Science Advisory Panel (SAP). This is based on the evidence that administration of alachlor was associated with an increased incidence of benign and malignant tumors in male and female rats in multiple experiments to an unusual degree and at an unusual site (nasal turbinates) and of benign lung tumors in female CD-1 mice. The risk assessment determined a Q1* of 8.0 x 10-2 (mg/kg/day)-1 (in human equivalents) using the nasal turbinate tumors.

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Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/19/94
Senior Pharmacologist, Review Section I, TBII (7509C)

Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. 7/19/94
Section Head, Review Section I, TBII (7509C)

DATA EVALUATION RECORD

Study Type: Subchronic Oral (Drinking Water) Toxicity
Species: Rat Guideline: §82-1

EPA Numbers: EPA MRID# 42863701
EPA Pesticide Chemical Code 090501 (Alachlor)
Toxicology Chemical No. 011 (Alachlor)
EPA DP Barcode D205429
EPA Submission Barcode S469774

Test Material: MON 5775 (90.7% & 6.6% H_2O; a metabolite of alachlor)

Synonyms: Ethane sulfonic metabolite of Alachlor, ESA

Title of Report: A 91-DAY DRINKING-WATER TOXICITY STUDY IN RATS WITH MON 5775

Sponsor: Monsanto Company
800 N. Lindbergh Boulevard, St. Louis, MO 63167

Testing Facility: Springborn Laboratories, Inc. (SLS)
Life Sciences Division, 640 N. Elizabeth,
Spencerville, OH 45887

Study Number: SLS Study No. 3044.372
Monsanto Study No. SB-92-383

Author(s): Joseph C. Siglin, M.S., D.A.B.T.

Report Issued: June 15, 1993

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 noted in all dosed males and females in the form of increased incidences of decreased activity, rapid/shallow breathing, few feces, feces small in size, dehydration, urine staining, emaciation, hunched posture, rough coat, unkempt appearance, dark material/stain on pads of forelimb, around eyes, mouth and nose, clear and red ocular discharge, and

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hairloss around eyes, with the highest incidence occurring in the high dose group in most cases. There was a decrease in body weight gain in the high dose males and all treated females had reduced weight gains (however, they were not dose related) along with reduced food consumption and water consumption was slightly increased in high dose males. There appears to be an effect on red blood cell parameters in the form of decreased erythrocytes, hemoglobin, hematocrit and platelets in the 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. No specific histopathological observations were noted. 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 Systemic Toxicity LOEL is equal to or less than 200 ppm and the Systemic Toxicity NOEL is less than 200 ppm based on the increased incidence of clinical signs of toxicity.**

This study is classified as Core-Supplementary Data and does not satisfy the guideline requirement (§82-1a) for a subchronic toxicity study in rats due to the lack of a No Observable Effect Level.

A. Materials and Methods: A copy of the Materials and Methods section from the investigators report is attached.

1. **Test compound:** **MON 5775**
   Description - Pinkish white powder
   Lot # - NPD-9203-3974-T
   Purity - 90.7% & 6.6% H_2O
   Received - 4/28/92 & 10/13/92
   Contaminants - none reported

2. **Vehicle(s):** Distilled water or water purified by reverse osmosis (RO water)

3. **Test animals:** Species: Male and female rats
   Strain: Fischer CDF® F-344 Crl BR VAF/Plus®
   Age: 8 weeks at initiation
   Weight: at initiation-Males:173-190 g;
   Females:120-134 g
   Source: Charles River Laboratories, Inc.
   Raleigh, NC

4. **Animal assignment**

Animals were assigned by *a computer randomization program* (based on day-1 body weights) to the following test groups:

Test Group	Conc. in water (ppm)	Main Study Male	Female
1 Control	0	10	10
2 Low (LDT)	200	10	10
3 Mid (MDT)	2000	10	10
4 High (HDT)	10000	10	10

5. **Preparation of Drinking water solutions**

Drinking water solutions were prepared weekly during weeks 1-7 and then biweekly thereafter; the solutions were stored at room temperature. Samples of the drinking water solutions were analyzed for stability (4, 8, and 10 days post-preparation, also end of study week 1), homogeneity (prior to study initiation) and concentration (at weeks 1, 2, 3, 4, 8, and 12). Further, additional analyses were carried out to evaluate the precipitate formation that was noted in the higher concentrations. Dose levels were based on *A Preliminary Range-Finding Toxicity Study in Rats with MON 5775* (provided), where 5 animals per sex per dose group received either 0, 700, 2000, 7000, or 20000 ppm in the drinking water for 28 days (equivalent to 0, 69, 183, 656, and 2217 mg/kg/day, respectively). Two animals died in the 20000 ppm dose group and adverse clinical sings were observed in this group.

There were also significant decreases in body weight and body weight gain and food consumption in the 20000 ppm dose group. Initially there was decreased water consumption in the 2000, 7000, and 20000 ppm dose groups, but this was not noted following week 1. Dose levels of 200, 2000, and 10000 ppm were chosen for the primary study.

6. Animal husbandry

Animals were kept under standard animal care conditions and were acclimated to the laboratory conditions for 12 days prior to study initiation. They received Purina Certified Rodent Meal® #5002 and water (distilled or RO water either untreated for controls or the solutions containing MON 5775 for the treated animals) *ad libitum*.

7. Observations

Toxicity/Mortality

Animals *were observed daily for clinical signs of toxicity, including physical and behavioral abnormalities*. Mortality checks were conducted twice daily.

Body weight

Animals were weighed weekly.

Water consumption and compound intake

Water consumption was determined once or twice weekly and compound intake was calculated based on water consumption measurements.

8. Ophthalmological examination

Ophthalmological examinations *were performed on all animals* on study days -6 and 90 using biomicroscopic and indirect ophthalmoscopic techniques.

9. Hematology and clinical chemistry

Blood was collected *on the day of scheduled sacrifice* after an overnight fast via ocular bleeding (lightly anesthetized). The following parameters were examined.

a. **Hematology**

Erythrocyte count (RBC)*
Hematocrit (HCT)*
Hemoglobin concentration (HGB)*
Mean corpuscular HGB (MCH)
Mean corpuscular HGB concentration (MCHC)
Mean corpuscular volume (MCV)
Platelet count*
Reticulocyte count
Leukocyte count (WBC)*
Leukocyte differential count*

* Required for subchronic studies

b. **Clinical Chemistry**

Serum alanine aminotransferase (also SGPT)*
Albumin*
Albumin/globulin ratio (calculated)
Alkaline phosphatase (ALK)
Serum aspartate aminotransferase (also SGOT)*
Calcium*
Cholesterol*
Chloride*
Blood creatinine*
Glucose (fasting)*
Globulin (calculated)
Phosphorous*
Potassium*
Sodium*
Total bilirubin
Total serum protein (TP)*
Triglycerides
Blood urea nitrogen*

* Required for subchronic studies

10. **Urinalysis**

Urinalysis was not conducted and is not required for subchronic toxicity studies.

11. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to gross pathological examination, this *included evaluation of the external surfaces of the body and all internal viscera.* The following tissues were collected for histological examination (preserved in 10% neutral buffered formalin) and the bolded organs, in addition, were weighed.

Accessory genital organs (Epididymides, Seminal vesicles, Prostate or Uterus* & Vagina)
Adrenal gland*
All gross lesions and masses
Aorta*
Brain*+ (including medullar/pons, cerebellar cortex & cerebral cortex)
Cecum*
Colon*
Duodenum*
Esophagus*
Exorbital lachrymal gland#
Eyes (optic n.)*#
Bone*# (Femur including articular surface)
Heart*
Ileum*
Jejunum*
Kidneys*+
Liver *+
Lungs*
Mammary gland*#
Mesenteric lymph node*
Nasopharyngeal turbinates
Pancreas*
Peripheral nerve*# (sciatic)
Pituitary*
Rectum*
Salivary glands*
Skeletal muscle*# (thigh)
Spinal cord (cervical, midthoracic & lumbar)*#
Spleen
Sternum with bone marrow*
Stomach*
Testes*+/**Ovaries***+
Thymus*
Thyroid*/**Parathyroid***
Trachea*
Urinary bladder*

* Required for subchronic studies.
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
\+ Organ weight required in subchronic studies.

Microscopic pathology

According to the investigators: *Tissues collected at necropsy from control animals, high-dose animals and all animals found dead during the study, as well as the lungs, liver, kidneys and gross lesions from all low and mid-dose animals were processed in paraffin, sectioned and stained with hematoxylin and eosin.* Histology was conducted by EPL.

12. Statistics

The following procedures were utilized in analyzing the numerical data (from the investigators report:

Continuous data, including body weights, weight gain, food consumption, water consumption, organ weights and clinical pathology, were analyzed by One Way Analysis of Variance (ANOVA) [2]. When significance was observed with ANOVA, control to treatment group comparisons were performed using Dunnett's Test [3] or a modified version of Dunnett's Test [4]. All tests were two-tailed with a minimum significance level of 5%.

13. Compliance

A signed and dated STATEMENT OF NO CONFIDENTIALITY CLAIMS was provided.

A signed and dated COMPLIANCE STATEMENT for compliance with GLP described under 40 CFR Part 160 was provided.

A signed and dated FLAGGING STATEMENT FOR POTENTIAL ADVERSE EFFECTS was provided. According to the investigators, the study neither met nor exceeded the applicable criteria.

A signed and dated QUALITY ASSURANCE STATEMENT was provided.

B. Results:

1. Test compound solution analyses

According to the investigators: *...MON 5775 was homogeneous and stable in aqueous solution following room temperature storage for up to 10 days.* They also determined that the formation of the precipitate *...had no detrimental effect on target concentrations on MON 5775.* They also stated that the solutions were *...accurately prepared* based on the concentration analyses. These statements are supported by the provided data.

2. Observations

Mortality

One high dose male rat was found dead on study day 13.

Clinical Observations

The investigators provided group mean and individual animal data. The following table presents selected observations:

Clinical Observations (occurrence/# animals affected)a

# animals/sex examined		Control 10	Low 10	Mid 10	High 10
Observation					
Decreased activity, rapid/shallow breathing					
	M	0/0	0/0	0/0	4/2
	F	0/0	0/0	0/0	2/1
Few feces	M	0/0	0/0	5/1	34/5
	F	0/0	0/0	0/0	13/7
Feces small in size	M	0/0	0/0	5/1	31/6
	F	0/0	0/0	0/0	22/7
Dehydration	M	0/0	0/0	4/1	37/5
	F	0/0	0/0	0/0	34/7
Urine stain	M	23/1	0/0	3/1	13/4
	F	69/5	70/5	72/2	156/10
Emaciation	M	0/0	0/0	0/0	21/5
	F	0/0	0/0	0/0	10/2
Hunched posture	M	0/0	0/0	0/0	5/2
	F	0/0	0/0	0/0	5/2
Rough coat	M	0/0	0/0	2/1	34/5
	F	0/0	0/0	0/0	3/2
Unkempt appearance	M	0/0	0/0	0/0	11/2
	F	0/0	0/0	0/0	2/1
Dark material/stain - pads of forelimb					
	M	0/0	0/0	0/0	6/3

Continued

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MON 5775

91-DAY DRINKING WATER STUDY IN RATS

Clinical Observations (occurrence/# animals affected)[a] cont.

# animals/sex examined		Control 10	Low 10	Mid 10	High 10
Observation					
Dark material around eye(s)	M	58/2	91/4	83/4	254/9
	F	36/9	189/8	158/8	230/10
Ocular discharge - clear	M	0/0	4/1	2/2	9/31
	F	1/1	11/5	7/2	15/6
Ocular discharge - red	M	12/2	9/3	6/2	59/6
	F	8/4	51/6	28/4	55/4
Dark material:					
around mouth	M	0/0	0/0	0/0	18/5
	F	0/0	0/0	0/0	15/3
around nose	M	1/1	0/0	1/1	12/3
	F	0/0	0/0	1/1	3/2
Hairloss - eye:					
right	M	28/1	40/3	0/0	36/3
	F	5/1	58/4	35/4	117/8
left	M	32/1	14/1	37/2	87/3
	F	31/4	103/5	55/2	95/6
The following are not related to animal observations:					
Several pinkish colored crystals in water bottle	M	0/0	0/0	31/10	439/9
	F	0/0	0/0	28/9	492/10

[a] - Data extracted from SLS Study No. 3344.372, Table 1.

There were treatment related observations in all dosed males and females in the form of increased incidences of decreased activity, rapid/shallow breathing, few feces, feces small in size, dehydration, urine staining, emaciation, hunched posture, rough coat, unkempt appearance, 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 in most cases.

3. Body weight

The investigators provided group mean and individual animal data. The following table presents selected body weight gain data:

Body Weight Gains (g)[a]

Dose:		Control	Low	Mid	High
Days:					
1-43	M	102	102	98(96)[1]	84(82)
	F	50	39(78)	45(90)	41(82)
1-91	M	151	155	155	136(90)
	F	70	61(87)	64(91)	60(86)

[1] = percent of control

[a] = Calculated by reviewer from means from SLS Study No. 3044.372, Table 2.

The males appeared to have a dose related decrease in body weight gain while all treated females had reduced weight gains; however, they were not dose related.

4. Food and water consumption and compound intake

Food consumption

The investigators provided group mean and individual animal data. The following table presents selected food consumption data:

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

Dose:		Control	Low	Mid	High
Days:					
1-43	M	18.0	18.3	17.7(98)[1]	16.3(91)
	F	13.7	13.3(97)	13.5(99)	12.8(93)
1-91	M	18.0	18.4	17.9(99)	17.2(96)
	F	13.5	13.1(97)	13.1(97)	12.9(96)

[1] = percent of control

[a] = Calculated by reviewer from means from SLS Study No. 3044.372, Table 4.

Food consumption was slightly reduced in the high dose males and females in the first 43 days on study.

Water consumption

The investigators provided group mean and individual animal data. The following table presents selected water consumption data:

Water Consumption Data (g/kg/day)[a]

Dose:		Control	Low	Mid	High
Days:					
1-43	M	94.7	94.8	89.3	101.0
	F	124.5	119.8	110.2	117.0
1-91	M	80.9	81.5	78.8	88.1
	F	112.8	113.9	103.5	110.8

a = Calculated by reviewer from means from SLS Study No. 3044.372, Table 4.

Water consumption was slightly increased in high dose males.

Compound Intake

Based on water consumption data, according to the investigators the *Average mean test article consumption for the 200, 2000, and 10000 ppm males was 16, 157, and 896 mg/kg/day, respectively...*and for the females...*23, 207, and 1108 mg/kg/day respectively.* This was supported by the provided data.

5. Ophthalmological examinations

The investigators stated that *There was no test article-related ophthalmological changes in the study animals. Keratopathies were noted in most animals at the pretest examination, and in all animals at the final examination. At the final examination, the range of keratopathies varied, but the general characteristics were comparable to findings noted at the pretest evaluation.* The following table presents the ophthalmological observations at 90 days:

Ophthalmological Observations (at 90 days)[a]

Observation		Control	Low	Mid	High
Dacryoadenitis					
	M	1	2	1	4
	F	0	1	0	3[1]
Chorioretinopathy					
	M	0	2	0	1
	F	0	0	0	2
Keratopathies					
	M	all[2]			
	F	all			

[1] = one animal bilateral, [2] = one animal unilateral

a = Data extracted from SLS Study No. 3044.372, Appendix I.

The ophthalmologic observation data was submitted to the HED Contract Expert Pathology Consultant, Lucas H. Brennecke, D.V.M. for review to determine whether the eye lesions observed were treatment related and if the lesions are related to those observed

with the parent compound, alachlor, in long term studies. The evaluation is attached as an appendix.

The Expert Pathology Consultant felt that the lesions observed were clearly not treatment related and the incidences of dacryoadenitis were most likely due to either Sialodacryoadenitis (S.D.A.) virus or the orbital bleeding techniques used. Further, the lesions observed in this study are not related to those observed in the alachlor long term study.

6. Hematology and Clinical Chemistry

a. Hematology

The investigators provided group mean and individual animal data. The attached Table 7 presents the provided data. There appears to be an effect on red blood cell parameters in the form of decreased erythrocytes, hemoglobin, hematocrit and platelets in the high dose males and to a lesser extent in the mid 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.

b. Clinical Chemistry

The investigators provided group mean and individual animal data. The attached Table 8 presents the provided data. AST, ALT, albumin, urea nitrogen, creatinine, and glucose levels were slightly decreased and total bilirubin and phosphorus were 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.

7. Pathology

a. Gross pathology

The investigators provided group summary and individual animal data. They stated that *Gross necropsy findings for animals which survived were generally unremarkable.*

b. Organ weight

The investigators provided group summary and individual animal data. They stated that *There were no significant differences in absolute or relative organ weight data among the groups.* Attached Tables 10 and 11 present the absolute and organ

weight relative to brain weight ratio data. The investigators did not calculate organ to body weight ratios; however, this reviewer calculated the ratios (hand written on Table 10), and no relevant differences were noted between groups.

c. Microscopic pathology

The investigators provided a pathology report from EPL (Experimental Pathology Laboratories, Inc.) and individual animal data. The following table presents the incidences of selected lesions and their severity (several eye observations have been discussed previously under ophthalmic observations):

Histopathological Observations (incidence)a

		Control	Low	Mid	High
# animals/sex examined		10	10	10	10
Observation					
Adrenal: vacuolation, cortical					
total	M	9			9
	F	0			0
minimal	M	6			9
	F	0			0
slight/mild	M	3			0
Bone marrow, sternum: cellularity					
total	M	10			10
	F	10			10
minimal	M	0			1
	F	0			0
slight/mild	F	0			3
moderate	M	8			7
	F	1			7
moderately severe	M	2			2
	F	9			0
Eye w/optic nerve:					
Hemorrhage, periorbital					
total	M	7			1
	F	6			6
minimal	M	0			1
	F	2			1
slight/mild	M	7			0
	M	4			4
moderate	F	0			1
Retinal dystrophy, unilateral					
total	M	1			1
	F	0			1
slight/mild	F	0			1
moderate	M	1			1

Continued

# animals/sex examined		Control 10	Low 10	Mid 10	High 10
Observation					
Kidney:					
Concretions, tubular					
total	M	10	10	10	10
	F	10	10	10	10
minimal	M	6	7	4	10
	F	9	10	10	10
slight/mild	M	4	2	6	0
	F	1	0	0	0
moderately severe	M	0	1	0	0
Regenerative epithelium					
total	M	9	9	8	7
	F	0	1	0	1
minimal	M	8	9	8	7
	F	0	1	0	1
slight/mild	M	1	0	0	0
Lung w/bronchi: Mononuclear infiltrate, peribronchial					
total	M	10	10	10	10
	F	10	10	10	10
minimal	M	0	0	2	1
	F	2	1	3	0
slight/mild	M	9	8	5	9
	F	6	9	6	8
moderate	M	1	2	3	0
	F	2	0	1	2
Nasal turbinate: Mononuclear infiltrate					
total	M	10			8
	F	10			9
minimal	M	1			4
	F	1			2
slight/mild	M	9			4
	F	9			7
Stomach:					
ectopic squamous epithelium					
total	M	0		1(1)[1]	0
	F	0		1(1)	0
	M	0		1	0
	F	0		1	0
erosion, mucosal					
moderate	M	0		0	1

[1] = number of animals examined

[a] = Data extracted from SLS Study No. 3044.372, Appendix O.

The investigators stated that:

> No histopathologic alterations were observed in any of the tissues evaluated which could be specifically attributed to administration of the test material. Reduced spermatogenic activity,

classified as hypospermatogenesis, was observed in 2/10 high dose male rats. One of those animals, however, was a found dead animal which, in addition to associated hypospermia in the epididymis, also showed evidence of debility in the bone marrow, spleen and stomach. Evidence of minimal to mild inflammation was noted in sections of nasal turbinate from a single high dose male and in both control and treated females. The change consisted of generally focal subepithelial collections of primarily mononuclear inflammatory cells. Discrete accumulations of mononuclear cells, classified as mononuclear infiltrate, were also present in sections of nasal turbinate from most control and treated rats. The change classified as nonsuppurative inflammation, while slightly more common in high dose females, was not prominent or consistent enough to be considered treatment related.

Changes which were also considered to be spontaneous or incidental in occurrence were noted in several of the remaining organs and tissues evaluated. Cortical vacuolation was observed in sections of adrenal from most control and high dose male rats. Incidental changes in sections of eye included periorbital hemorrhage, chronic focal keratitis and unilateral retinal dystrophy. Focal nonsuppurative myocarditis and hemorrhage occurred in sections of heart with essentially comparable incidence in control and treated animals. Spontaneous kidney alterations included tubular concretions, mononuclear infiltrate and regenerative tubular epithelium. Mononuclear infiltrate, focal necrosis and bile duct proliferation occurred in sections of liver as incidental observations. The remaining individual or rarely described alterations, or those which occurred with comparable incidence between control and treated animals, were likewise considered to be spontaneous or incidental in occurrence.

No treatment related effects were noted in the presented data; although erosion of the stomach and 2 incidences of ectopic squamous epithelium were noted (only 2 animals examined) in the mid dose, this does not appear to be dose related as at the high dose with all animals examined, no incidences were noted. Alachlor (parent) is noted to cause lesions of the stomach. The eye lesions are discussed as follows. In a lifetime chronic study in Long-Evans rats with alachlor (containing epichlorohydrin) ocular lesions were noted in the form of uveal degeneration syndrome, a molting of the retinal pigmentation (*In its mildest form the syndrome was characterized by free floating iridial and choroidal pigment in the ocular chambers and pigment deposition on the cornea and lens. In its most severe form, the syndrome was characterized by bilateral degeneration of the iris and diminution of the size of the ocular globe with secondary total cataract formation.*). A second study (2-year feeding) with alachlor (epichlorohydrin-free) in Long-Evans rats, no ocular lesions were noted. In a special study to evaluate the ocular lesions, Long-Evans rats were exposed to alachlor stabilized with epoxidized soybean oil for 2-years; uveal degeneration syndrome was noted. In this last study, chronic keratitis was noted in 3/29 males of the control group and 8/13 males of the high dose group (126 mg/kg/day), no females presented with this observation; this was not considered to be related to treatment. A human exposure study was conducted prompted by the uveitis noted in the rat study. The volunteers were exposed to alachlor for 5-8 years with no evidence of a similar syndrome to that of the rat study. Therefore, the keratitis noted does not seem to be related to treatment with MON 5775 (metabolite of alachlor). It must also be noted that the eye lesions with the parent compound were noted in Long-Evans rats and the present study uses Fischer 344 rats.

D. Discussion and Conclusions

Systemic toxicity was noted in all dosed males and females in the form of increased incidences of decreased activity, rapid/shallow breathing, few feces, feces small in size, dehydration, urine staining, emaciation, hunched posture, rough coat, unkempt appearance, 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 in most cases. There was a decrease in body weight gain in the high dose males and all treated females had reduced weight gains (however, they were not dose related) along with reduced food consumption and water consumption was slightly increased in high dose males. There appears to be an effect on red blood cell parameters in the form of decreased erythrocytes, hemoglobin, hematocrit and platelets in the 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. No specific histopathological observations were noted. Eye lesions noted in this study were determined not to be related to treatment or to those lesions seen with the parent compound, alachlor.

SYSTEMIC TOXICITY NOEL < 200 PPM
SYSTEMIC TOXICITY LOEL ≤ 200 PPM

61136

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June 2, 1994

SUBJECT: Evaluation of Eye Lesion Data from MON 5775 (91 Day Study)

FROM: Lucas H. Brennecke, D.V.M.
Expert Pathology Consultant
Health Effects Division

TO: Stephen C. Dapson, Ph.D.
Senior Pharmacologist, Review Section I
Toxicology Branch II /Health Effects Division (H7509C)
Office of Pesticide Programs
USEPA

Registrant: Monsanto, The Agricultural Group

Action Requested: (1) Review eye lesion data in "A 91-Day Drinking-Water Toxicity Study in Rats with MON 5775" (6/15/93) with reference to the eye lesions observed in the studies with the parent, alachlor. (2) Review additional alachlor study data (in Long Evans rats) and human exposure data submitted by Monsanto.

Conclusion: There is no correlation between the lesions in F344 rats noted by either the ophthalmologist (Dr. Riis) or the pathologist (Dr. Ferrell) in the 91-Day Drinking Water Study and those lesions described in the two-year or lifetime studies in Long Evans rats or in the Human Exposure report (Monsanto Company, 7/30/80).

Discussion: The ophthalmologist who performed the pretest as well as the final examination indicated that the lesions noted were clearly not treatment-related and, in fact, were consistent with lesions caused by either Sialodacryoadenitis (S.D.A.) virus or orbital bleeding techniques. Dacryoadenitis is an inflammation of the lachrymal glands, not an ocular lesion. S.D.A. virus is the most common cause of dacryoadenitis in the rat. The dark material noted around the eye(s) is indicative of secretion by the lacrimal gland, most likely as a result of the inflammation. The fact that no histopathologic findings were noted in the exorbital lacrimal glands does not rule out dacryoadenitis in that the infraorbital lacrimal glands (Harderian glands) appear to be most often affected. The infraorbital glands were apparently not evaluated microscopically.

Chronic focal keratitis (inflammation of the cornea) was noted with equal frequency in the control and high dose groups. This is a common finding in F344 rats, particularly males.

The periorbital hemorrhage noted in many of the rats (more control than treated) was due to the bleeding procedure. It was not associated with the treatment with the test article.

Unilateral retinal dystrophy was noted in an equal number of control and high dose males (1/10) and in only one of ten high dose females. This lesion has been shown to be associated with S.D.A. virus. In addition, there is no significant difference between treated and control groups.

In addition to the absence of treatment-related effects in the 91-Day study and the fact that the lesions which were seen were most likely attributable to S.D.A. virus, orbital bleeding procedures, and commonly occurring degenerative changes in F344 rats, there is no evidence linking any of these lesions to those noted in studies using alachlor. The lesions noted in the human study were not of a similar nature to those seen in the 91-day study.

Alachlor

RIN 4446-96

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