

US EPA ARCHIVE DOCUMENT

1-17-95

011389



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

JAN 17 1995

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

**MEMORANDUM**

**SUBJECT:** MON 5775 (metabolite of ALACHLOR): Review of additional data for 91 day drinking water study in rats, comments of State of Wisconsin letter and comments on minutes of meeting with registrant.  
EPA Barcode D206638, D206639; EPA Submission No. S471952, S471953; EPA MRID No: none; 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 1/13/95*  
Senior Pharmacologist, Review Section I  
Toxicology Branch II/HED (7509C)

**THRU:** Yiannakis M. Ioannou, Ph.D., D.A.B.T. *Y.M. Ioannou 1/13/95*  
Section Head. Review Section I  
and  
Marcia van Gemert, Ph.D. *M van Gemert 1/13/95*  
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 additional data for 91 day drinking water study in rats, comments of State of Wisconsin letter and comments on minutes of meeting with registrant.

**Recommendations:** Based on the additional data/explanations/statistical analysis provided by the registrant, Toxicology Branch II has determined that the effects observed in male and female rats at the low dose level (200 ppm) were minimal and probably not compound related. Thus, the Systemic Toxicity No Observable Effect Level (NOEL) for this study is 200 ppm in male and female rats, and the Lowest Observable Effect Level (LOEL) is 200 ppm in both sexes based on hematological and clinical chemistry changes and increased incidence of clinical signs of toxicity.

The study is upgraded to Core-Minimum Data and satisfies the guideline requirement (§82-1a) for a subchronic toxicity study in rats.

**Background:**

TB II previously 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 are the conclusions for the original review:

In a special 91-day drinking water study (MRID# 42863701), male and female Fischer CDF® F-344 CrI 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<sub>2</sub>O; 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 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.

01522

**Discussion:**

The registrant submitted additional data to support the above study based on a meeting on August 5, 1994 where the results of the review of the study were discussed by the Agency and the Monsanto scientist responsible for the product toxicology studies (Letter, Monsanto to R.Taylor, August 3, 1994). The main point of contention was that the NOEL for the study was based on clinical observation data, ...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.... Based on the meeting it was determined that the only clinical observations present in the low dose animals were urine stain, dark material around eye(s), ocular discharge (clear and red, and hair loss - eye. Also it was concluded that the occurrences of urine stain did not follow a dose-response relationship and therefore not related to treatment. Statistical analysis conducted by the investigators using Chi-Square with Yates Correction did not show any statistically significant differences (attached Table 1 from the investigators report) except for dark material around the right eye in high dose females.

Clinical observation data provided from the range finding study (conducted 6 weeks earlier) noted that the observations were not reproducible; dose levels up to 20000 ppm (2 times the primary study high dose). These data are provided in attached Table 2 from the investigators submission.

The dose-response assessment for the para-ocular clinical observations for total numbers of animals are provided on attached Table 3 from the investigators submission. Only in the high dose there was a slight increase in dark material around the eyes in males (may be due to marked depression in water consumption according to the investigators, which seem like a logical assumption) and possibly red ocular discharge in males. Analysis of total occurrences of these observations is presented in attached Table 4 from the investigators submission, again the high dose had a slight increase in dark material around the eyes in males and possibly red ocular discharge in males.

The time-to-observation analysis for the number of animals affected and total occurrences per group is presented in attached Table 5 of the investigators submission. There was a decrease in time-to-onset in the incidence of dark material around the eye(s) in the high dose animals. This was felt to be due to the marked decrease in water consumption during the first week, most likely

due to palatability. No other treatment related effects were noted. Also, no differences were seen in the time frame (days) when 50% of total occurrences were noted.

The investigators presented a correlation between clinical, gross and microscopic findings. They performed histopathological examinations on the eyes from all control and high dose animals as well as on the skin around the eyes of animals which were observed to have hairloss and/or dark material around the eyes at gross necropsy, this involved 1, 2, 1, and 5 males and 2, 4, 3, and 6 females from the control, low, mid, and high dose groups respectively. No treatment related effects were noted.

The investigators also supplied further information involving the spontaneous occurrence of ocular lesions in the F-344 rats. These rats are considered to have a genetic predisposition to degenerative changes and exogenous factors such as viral infection. These data were supported earlier by the Agency's contract Pathologist. The additional information is attached. The investigators state that *There were clear correlations between the occurrences of clinical observations during inlife and ocular abnormalities noted at the study conclusion: These included:*

*The overall pattern of clinical observations among groups was similar to the distribution of ocular abnormalities noted by the ophthalmologist at the end of the study. In males, many of the inlife clinical observations occurred more frequently (occurrences and/or # affected) in low dose rats than in control and mid dose rats. In females, the only ocular abnormalities were noted in the low and high dose groups, and these two groups tended to have the highest numbers of occurrences and/or animals with para-ocular clinical observations.*

*Of the 10 male rats found to have ophthalmic abnormalities at the end of the study, 9 had very high incidences of in-life findings of dark material, discharge, and hair loss. In fact in many cases, these animals accounted for the majority of findings in their entire groups. In females, the concomitant occurrences of ophthalmic findings and para-ocular clinical observations was not as pronounced. Nevertheless, it should be noted that the females diagnosed with ophthalmic abnormalities at the end of the study, especially those with dacryoadenitis, did have high occurrences of para-ocular observations during the study.*

*There was a very strong correlation between the findings of ocular abnormalities at the end of the study and the occurrence of hairloss as detected by inlife observations. In fact, every animal diagnosed with dacryadenitis upon ophthalmic examination had a high occurrence of hairloss observations during the inlife observations. Furthermore, in every case, the hairloss observations were detected only for the eye(s) in which the diagnosis of dacryadenitis was made. This correlation strongly supports the existence of a common causative factor(s) for both hairloss and dacryadenitis.*

*Overall, these observations support the likelihood that the cause of the*

ocular abnormalities also played a role in producing the para-ocular clinical observations noted during the study.

Further: It is particularly noteworthy in this study that all para-ocular changes observed in the treatment groups were also observed in untreated control animals (males and/or females). The most dramatic example was 'dark material around eye(s)', which was observed in 9-of-10 control females. The occurrences of these para-ocular changes would not be expected in 'normal' untreated animals.

As noted by Dr. Brennecke, the HED Expert Pathology Consultant, the most likely cause of the ocular discharge from the lacrimal glands is inflammation in or around the glands. The red discharge itself, which contains porphyrins, is irritating and caustic to the periocular tissue; this results in hairloss in the area contacted by the draining discharge. The hairloss may also have been exacerbated by normal preening activity of the rats in an effort to reduce the unpleasant sensation related to the inflammation. The drying of the red discharge accounts for the observation of dark material around the eye. Thus, it is highly probable that these clinical observations are all part of the same syndrome resulting from a common cause.

Some clinical observations, such as 'dark material around the eyes' of males, may have been elevated in high dose animals. However, interpretation of this finding is complicated by the fact that the same symptoms are observed in animals that have been stressed by water deprivation, which occurred in this study due to decreased palatability. In any case, none of the para-ocular observations were significantly elevated ( $p > 0.14$ ) in low and mid-dose animals, and the incidences did not follow any dose-response patterns in these groups. Likewise, there was no dose-related decrease in time to onset. Finally, there were no correlating histopathological lesions, thus indicating no underlying test article-related pathology in ocular or surrounding tissues. Therefore, the data do not support a causal relationship between test material administration and the clinical signs observed.

Spontaneous ocular problems are common in the F-344 rat due to genetic and exogenous factors, such as viral infection. These ocular problems can result in the types of symptoms observed in this study. It was noted that several animals in this study had dacryoadenitis, the most common cause of which is the S.D.A. virus. It stands to reason, therefore, that occurrences of ocular clinical signs are most likely due to one or more of the incidental factors which are active in the F-344 rat. This is especially true in the absence of treatment-related effects as discussed previously.

These statements are supported by the previously mentioned attached tables and the attached Tables 6 and 7 which present the occurrences of the para-ocular observations in males diagnosed with and without ocular abnormalities and occurrences of hairloss in animals diagnosed with dacryoadenitis and/or chorioretinopathy, respectively.

The provided additional information and data support the conclusion that the clinical observations reported are not due to treatment with MON 5775 but due to ocular abnormalities specific to the F-344 rat. Based on this, the following is the revised conclusions for the 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):

In a special 91-day drinking water study (MRID# 42863701), male and female Fischer CDF® F-344 CrI 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% & 5.6% H<sub>2</sub>O; a metabolite of alachlor).

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.

This study is classified as Core-Minimum Data and

011389

satisfies the guideline requirement (§82-1a) for a subchronic toxicity study in rats.

**Comparison of Metabolite and Parent Compound Toxicity:**

Subchronic toxicity data in rats with the parent compound Alachlor indicate 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<sub>50</sub> 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 930 mg/kg with a toxicity category of III; therefore MON-5775 is less acutely toxic than the parent chemical.

TBII previously reviewed the report entitled *AMES MUTAGENICITY STUDIES ON FIVE COMPOUNDS REPRESENTATIVE OF MAJOR CLASSES OF ALACHLOR METABOLITES* [Alachlor Metabolite CP 108065: Ames *Salmonella* Mutagenicity Assay, Project No. ML-24-037, Study No. 840013, 5/18/84] (Monsanto Environmental Health Laboratory for Monsanto Agricultural Products Company, Study No.s ED# 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. The study is classified as Acceptable and satisfies the guideline requirement (§84-2) for an Ames assay. Most tests with alachlor technical were negative; however, there was evidence in one study of a weakly mutagenic response with Alachlor. Overall, there is no indication that the ESA metabolite was more toxic than the parent in terms of mutagenic response.



## 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 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	NO <sup>1</sup>
§82-1(a)90 day feeding study - rat	Yes	NO <sup>2</sup>
§82-1(b)90 day feeding study - nonrodent	Yes	NO <sup>3</sup>
§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 <sup>4</sup>

<sup>1</sup> = study received and is presently under review

<sup>2</sup> = satisfied by 2-year chronic feeding study in the rat

<sup>3</sup> = satisfied by 6 month subchronic feeding study in the dog

<sup>4</sup> = 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)90 day feeding study - rat	Yes	Yes
§84-2(a)Mutagenicity Gene Mutation	Yes	Yes

## II. Data Gaps

The database for technical Alachlor is not complete:

§81-6 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, RED candidate.

### 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  $8.0 \times 10^{-2}$  (mg/kg/day)-1 (in human equivalents) using the nasal turbinate tumors.