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

MEMORANDUM

JUN 23 1983

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Robert Taylor (25)
Registration Division (TS-767)

THRU: William L. Burnam, Chief
Toxicology Branch/HED (TS-769)

SUBJECT: Alachlor (Technical); EPA Reg.#524-316 and Lasso EC (45% a.i.) EPA Reg.#524-314. Review of Acute Inhalation and Mutagenicity Studies; Report MSL-2403, R.D.#433, 7/27/82. Accession#248053. CASWELL#11

Requested Action:

Monsanto Company submitted the following studies for review:

A. Acute Inhalation Studies

1) An Acute Inhalation Study with Alachlor in Rats, BD-81-183, by Bio/dynamics, Inc. (#81-7502).

2) An Acute Inhalation Study with Lasso Herbicide in Rats, BD-81-184, by Bio/dynamics, Inc. (#81-7501).

B. Mutagenicity Studies

1) Microbial Mutagenicity Assays with Alachlor, ET-80-0101, January 1980. A study sponsored by Monsanto and performed by Shirasu, et al., at the Institute of Environmental Toxicology in Japan.

2) Gregory S. Probst, et al., Chemically-Induced Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures: A Comparison with Bacterial Mutagenicity Using 218 Compounds," Environmental Mutagenesis, 3, 11-32 (1981.)

3) Y. Shirasu, et al., "Mutagenicity Screening of Pesticides in the Microbial System," Mutation Research, 40, 19-30 (1976.)

4) S.J. Eisenbeis, et al., "The Ames Mutagen Assay Tested Against Herbicides and Herbicide Combination," Soil Science, 131, January, 1981, p. 44.

Study #1 above, the "Microbial Mutagenicity Assays", is a replacement for IBT #8536-8850 and #8536-8852.

Recommendations:

A. Acute Inhalation Studies in Rats

1) The LC_{50} for Alachlor (techncial) was higher than 5.1 mg/L (gravimetric concentration) $\underline{=}$ 1.04 mg/L (analytical concentration).

2. The LC_{50} for Lasso EC (45% a.i.) was higher than 6.51 mg/L (gravimetric concentration) $\underline{=}$ 0.62 mg/L (analytical concentration).

The registrant indicated that the test material concentration used in each of the above 2 studies was the highest possible under the experimental conditions of these studies; however, it is not possible for this reviewer to evaluate this statement.

The registrant was contacted (by telephone in February, 1983) to provide an adequate explanation for the noted large difference between the gravimetric and analytical concentration values in each of the above two studies. Awaiting a response from the registrant concerning this issue, these two studies are classified as Core Supplementary Data.

B. Mutagenicity Studies

Only one of the four submitted studies was classified as acceptable. All the other three studies were unacceptable due to the fact that these studies did not provide adequate quantitative data; hence, they could not be adequately evaluated (i.e., all three published studies are summary data).

The acceptable study is the "Microbial Mutagenicity Assays", sponsored by Monsanto at the Institute of Environmental Toxicology in Japan, Report#ET-80-0101, January, 1980. The study used a 99.9% pure Alachlor and was negative with or without activation (see also the reviewer's discussion on the submitted mutagenicity studies, under Section B of the attached reviews).

Consequently the following mutagenicity data gaps should be filled within a reasonable time:

1. In vitro mammalian cell point mutation (L5178Y (TK); or CHO (HGPRT); or V79 (HGPRT).

2. In vitro cytogenetic damage: both chromosomal aberration and SCE (in CHO cells; or human lymphocytes; or other rodent/human cell lines/strains.

3. In vitro/in vivo primary hepatocyte repair for UDS testing both in vivo and in vitro exposure of cells to Alachlor.

4. In vivo cytogenetics test for chromosomal aberrations using bone marrow preparations of rats.

5. Dominant lethal test in rats or mice.

Review:

All the studies submitted in this action were reviewed for Alachlor Registration Standard by Dynamac Corporation. The reviews were acceptable to the Toxicology Branch and copies of these reviews are attached to this memo.

Amal Mahfouz 6/9/83 6/9/83

Amal Mahfouz, Ph.D.
Toxicology Branch/HED (TS-769)

Attachments

6/22/83

TS-769:th:TOX/HED:AMahfouz:6-7-83:card 6

REVIEWS

A. ACUTE INHALATION

1. Study Type: Acute inhalation studies with alachlor technical (95.3% ai).

Accession Number: 248053.

MRID Number: 000GS-01.

Sponsor: Monsanto Co., St. Louis, MO.

Contracting Laboratory: Bio/dynamics, Inc., East Millstone, NJ.

Project No.: 81-7502 (BD-81-183).

Date of Initiation: November 19, 1981

Date of Termination: December 3, 1981

Responsible Professionals: James C. Eschbach, B.A., Study Director
Geoffrey K. Hogan, Ph.D., V.P., Toxicology

Core Classification: This study is classified as supplementary until the registrant satisfactorily explains the five-fold difference between the gravimetric and analytical concentrations.

CONCLUSIONS

1. The LC_{50} was greater than 5.1 mg/l, the highest attainable gravimetric concentration (equivalent to an analytical concentration of 1.04 mg/l). No deaths occurred among 10 animals (5/sex) exposed for 4 hours and observed 14 days.

2. All symptoms (see review) were reversible after 14 days in both males and females, except for reduced body weight gains in 2/5 females. At necropsy, however, 3/5 females had scattered gray foci on the lungs, and 1/5 males had a slightly dilated renal pelvis.

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COMMENTS

- The gravimetric concentration was five times greater than the analytical concentration. No explanation was provided for this difference.
- The concentration was much less than 20 mg/l, as required by EPA guidelines.
- The report stated that the test concentration was the highest possible under the test conditions; however, no evidence was provided to support this statement.
- The experiment was not designed to assess the possible effects caused by the solvent used to dilute the active ingredient; i.e., no solvent controls.

PROTOCOL

Animals: Sprague-Dawley rats; 5/sex.

Test Article: Alachlor technical (95.3% ai) diluted [redacted] to yield 80% ai.

Test Chamber: 100-liter Plexiglas.

Nominal Concentration: 18.6 mg/l.

Analytical Concentration: 1.04 mg/l.

Gravimetric Concentration: 5.1 mg/l.

Mean Particle Size: 2.93 um.

Exposure Time: 4 hours.

Observation Period: 17 days preexposure, 0-8 hours and 1-14 days postexposure (individually housed). Body weights were recorded on days 0 (preexposure), 1, 2, 4, 7, and 14.

Necropsy: On day 14, the animals were exsanguinated. Kidneys, livers, lungs (at least two lobes and mainstem bronchi) and gross lesions were preserved in formalin for possible histopathology.

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RESULTS

An LC_{50} determination could not be derived from the available data.

The most common observations immediately after exposure were lacrimation, nasal discharges (mucoid or red), salivation, dry or moist rales, swollen eyelids, and reduced righting reflex. There were no deaths during the 14-day postexposure observation period. All symptoms decreased in frequency. The incidence of lacrimation increased to five animals on days 5 and 6, and decreased again. During the last 3 days, only three animals exhibited lacrimation or nasal discharge on any day.

Body weights decreased the first two days after exposure, and then increased through day 14. The body weight gains for 2/5 females were reduced, however, compared to those for the other animals.

At necropsy, 3/5 females had scattered gray or red foci on the lungs, and 1/5 males had a slightly dilated renal pelvis.

6.

ALACHLOR

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Department Director
Dynamac Corporation

Signature John R. Strange
Date 8 March 1983

Richard L. Hebert, M.S.
Project Scientist
Dynamac Corporation

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EPA Scientist

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ALACHLOR

2. Study Type: Acute inhalation studies with LASSO (EC, 45% ai).

Accession Number: 248053.

Sponsor: Monsanto Co., St. Louis, MO.

Contracting Laboratory: Bio/dynamics, Inc., East Millstone, NJ.

Date of Initiation: September 11, 1981

Project No.: 81-7501 (BD-81-184).

Date of Termination: September 25, 1981

Responsible Professionals: James C. Eschbach, B.A., Study Director
Geoffrey K. Hogan, Ph.D., V.P., Toxicology

Core Classification: This study is classified as supplementary until the registrant satisfactorily explains the ten-fold difference between the gravimetric and analytical concentrations.

CONCLUSIONS

1. The LC_{50} was greater than 6.51 mg/l, the highest attainable gravimetric concentration (equivalent to an analytical concentration of 0.62 mg/l). No deaths occurred among 10 animals (5/sex) exposed for 4 hours and observed 14 days.

2. All symptoms (see review) were reversible after 14 days in both males and females. At necropsy, however, 5/5 males had scattered red or gray foci on the lungs, and 1/5 males had a discolored kidney.

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COMMENT

- The gravimetric concentration was about ten times greater than the analytical concentration. No explanation was provided for this difference.
- The concentration was much less than 20 mg/l, as required by EPA guidelines.
- The report stated that the test concentration was the highest possible under the test conditions; however, no evidence was provided to support this statement.
- The experiment was not designed to assess the possible effects caused by the solvent(s) used to dilute the active ingredient; i.e., no solvent controls.

PROTOCOL

Animals: Sprague-Dawley rats; 5/sex.

Test Article: Lasso (EC, 45% ai).

Test Chamber: 100-liter Plexiglas.

Nominal Concentration: 9.97 mg/l.

Analytical Concentration: 0.62 mg/l.

Gravimetric Concentration: 6.51 mg/l.

Mean Particle Size: 2.11 um.

Exposure Time: 4 hours.

Observation Period: 17 days preexposure, 0-8 hours and 1-14 days postexposure (individually housed). Body weights were recorded on days 0 (preexposure), 1, 2, 4, 7, and 14.

Necropsy: On day 14, the animals were exsanguinated. Kidneys, livers, lungs (at least two lobes and mainstem bronchi) and gross lesions were preserved in formalin for possible histopathology.

RESULTS

An LC_{50} determination could not be derived from the available data.

The most common observations immediately after exposure were lacrimation, nasal discharges (mucoid or red), salivation, dry or moist rales, labored or irregular breathing, reduced righting reflex, and chromodacryorrhea. There were no deaths during the 14-day postexposure observation period. All symptoms decreased in frequency. After day 10, only three total symptoms were reported: nasal discharge, dry rales, and yellow anogenital fur.

Body weights decreased the first two days after exposure, and then increased through day 14.

At necropsy, 5/5 males had scattered gray or red foci on the lungs, and 1/5 males had pale green-brown kidneys with red cortico-medullary borders.

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ALACHLOR

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DISCUSSIONS: B. MUTAGENICITY OF ALACHLOR (LASSO^R)

The Genetic Toxicology Program of OPTS (Gene Tox) provides a minimum standard for testing mutagenicity and was chosen as a guide for judging the adequacy of the data presented for the registration of Alachlor (LASSO^R), as a technical grade active ingredient. These guidelines of Gene Tox recommended a battery of assays capable of detecting various genetic endpoints because this approach makes it more likely to demonstrate if the test material has an intrinsic capacity for causing mutations or otherwise damaging chromosome. The assays which one has selected to meet Gene Tox "suggest guidelines: should have reasonably demonstrated this capacity for an in vivo and an in vitro mammalian system, and, if positive results were obtained, they should have provided guidance in suggesting whether the test materials' effect(s) was/were relevant to a risk of cancer, teratogenesis, or some other heritable defect. The proposed guidelines 40-CFR-158 published in the Federal Register Vol. 47, No. 227, November 24, 1982 states "For each test substance, a battery of tests is required to assess the potential to cause gene mutations, structural chromosome observations or other genotoxic effects as listed below." Since these guidelines follow Gene Tox, and because Gene Tox has had considerable expert review, Gene Tox will be used as the basis for evaluating the mutagenicity data available to support registration of Alachlor. The discussions which follows compare the recommended requirements of Gene Tox to the data which has been submitted for the purpose of assessing the mutagenicity of Alachlor (LASSO^R).

The three "suggested requirements" of Gene Tox are as follows: Gene Tox Requirement 1. Two different tests that are sensitive for point mutations, including one for a mammalian system (in vivo or in vitro). Gene Tox Requirement 2. At least two different tests for chromosome damage (in vivo or in vitro). Gene Tox Requirement 3. At least one auxiliary test for direct DNA damage or a test appropriate for other mechanisms of mutagenicity.

Under the Gene Tox Requirement 1, there were three potentially qualifying studies designed to determine the induction of point mutations. These included the report by Shirasu et al. (ET-80-0101, 1980, MRID No. 000GS-03), the report by Eisenbeis et al. (1981) on the formulation, and the report by Shirasu et al. (1976, MRID No. 000GS-05). Of these, the latter two were judged to be inconclusive. The report of Eisenbeis et al. (1981) tested the formulation, Lasso EC, alone and in combination with other herbicides and the data was simply summarized along with that of 19 other herbicides. The negative response is not supported with quantitative data reflecting dose responses and is therfore inconclusive. The report of Shirasu et al. (1976, MRID No. 000GS-05) is likewise a summary of mutagenicity assays performed on 166 pesticides, in which negative results were reported for Alachlor in E. coli and S. typhimurium reverse mutation assays. There was no quantitative data

presented and therefore the data was considered inconclusive. The report by Shirasu et al. (ET-80-0101, 1980, MRID No. 000GS-03) presented data for reverse mutation using the E. coli WP-2 system and the (Ames system) at six concentrations (range = 10- 50,000 ug/plate), both with and without S9 activation. This assay is acceptable and the authors concluded from the results that Alachlor lacked mutagenicity. This reviewer agrees with this assessment, but adds the notation that a weak but questionably positive response was seen in only S. typhimurium strain TA1535 at a concentration of 5,000 ug per plate. However, the response was not repeated for consecutive doses.

To make the data satisfactory for meeting requirement one, at least one mammalian point mutation test should be performed so that it can be reasonably ascertained if Alachlor has a mutagenic potential in mammals. This result could further clarify the questionable "weak positive result" obtained by Shirasu (ET-80-0101, 1980, MRID No. 000GS-03).

Under Gene Tox Requirement 2, there were no qualifying reports to test for chromosome damage. Therefore, it is recommended by this reviewer that an in vivo assay, such as the micronucleus test and a cytogenetic test using mammalian cells in culture e.g., sister chromatid exchange or metaphase plate analysis with CHO cells, be performed.

Under Gene Tox Requirement 3, there were three potentially qualifying studies designed to test for direct DNA damage. These were included in the reports by Probst et al. (1981, MRID No. 000GS-04), Shirasu et al. (1976, MRID No. 000GS-05) and Shirasu et al. (ET-80-0101, 1980, MRID No. 000GS-03). The first report (Probst et al. 1981, MRID No. 000GS-04) stated that Alachlor did not induce unscheduled DNA synthesis in primary rat hepatocyte cultures. However, it was intended to be a survey study for over 200 compounds, so the quantitative data was only reported for positively responding chemicals. If the raw data on Alachlor from this study could be presented, it might help satisfy requirement three, but the data as presented can only be considered inconclusive. The rec-assay performed on B. subtilis H17 (rec^+) and M45 (rec^-) was reported for Alachlor along with the results for many other pesticides. Although it was considered negative, the results in a survey study like this one must be considered inconclusive. However, the results from Shirasu et al. (1980, MRID No. 000GS-03) showed that six concentrations of Alachlor between 20 and 2,000 ug/plate failed to cause DNA-damage as judged by the differential inhibition of B. subtilis strains M45 and H17.

Although the B. subtilis rec-assay performed by Shirasu et al. (1980, MRID No. 000GS-03) meets the minimal requirements for Gene Tox Requirement 3, it is suggested by this reviewer that the raw data from Probst et al. (1981, MRID No. 000GS-4) be solicited so that the results might be assessed from a quantitative standpoint.

In summary, the total data presented from four documents indicated that Alachlor is either non-mutagenic or perhaps weakly mutagenic. However, there are serious data gaps which include 1) a mammalian point mutation assay, and 2) an assessment of induced chromosomal aberrations.

This reviewer recommends that an in vitro mammalian test, preferably the mouse lymphoma or Chinese hamster ovary assay be performed to satisfy the point mutation requirement. To adequately assess the capacity of Alachlor to induce chromosomal aberrations, the in vivo micronucleus assay and an in vitro test such as the sister chromatid exchange (SCE) assay are suggested. It is preferable that a procedure combining the micronucleus and SCE assays be performed so that the results can be correlated. Of course, comparable tests may be used as alternatives for the assays recommended provided that they are properly justified for adequately testing the following requirements: (a) in vitro or in vivo mammalian point mutation induction and (b) in vivo and in vitro induction of chromosome aberrations.

The lack of substantial data with mammalian systems is a serious one, which in the opinion of this reviewer must be overcome in order to properly assess the mutagenic potential of Alachlor.

Summary Table of Mutagenicity Studies with Alachlor

Study / Lab / Study Date	Material	MRID No.	Results	Core Classification/ Evaluation
1. Alachlor: Microbial Mutagenicity study, Shirasu, et al. Institute of Environmental Toxicology, Kodaira, Tokyo 187, Japan. (Report No. ET-80-0101) January, 1980.	Alachlor (99.9%)	000GS-03	A rec-assay was conducted at 6 concentrations (20-2,000 ug/plate) in B. subtilis strains H45 and H17 showed no evidence of test compound-induced inhibition. The reverse mutation assay conducted at 6 concentrations (10-5,000 ug/plate) in E. coli strain WP2 hcr and S. typhimurium strains TA1538, TA1537, TA1535, TA98 and TA100, with and without S9 metabolic activation, was also negative.	Acceptable
2. The Ames Mutagen Assay Tested Against Herbicides and Herbicide Combinations. Eisenbeis, et al. Soil Science 131(1):44-47. 1981.	Alachlor (Lasso®) Formulation: 48% w/v	N/A	The test compound alone and in combination with other herbicides was negative for mutagenic activity in S. typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100, not differing significantly from the spontaneous reversion rate. Tests were performed with and without S9 metabolic activation. However, quantitative data was not presented. Summary data. Adequate quantitative data were not submitted.	Unacceptable *
3. Mutagenicity Screening of Pesticides in the Microbial System. Shirasu, Y., et al. Mutation Research 40:19-30. 1976.	Alachlor (unspecified purity)	000GS-05	An initial screen at an unspecified dose or dosages of Alachlor produced negative results in the rec-assay with B. subtilis strains H17 and H45. Reverse mutation for E. coli WP2 and S. typhimurium (Ames series) was also negative in the spot assay without S9 activation. Summary data. Adequate quantitative data were not submitted.	Unacceptable *
4. Chemically-Induced Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures: A comparison with Bacterial Mutagenicity using 218 compounds. Probst, G.S., et al. Environmental Mutagenesis 3:11-32 (1981).	Alachlor (unspecified purity)	000GS-04	Alachlor was assayed at 8 concentrations ranging from 0.5 to 1,000 nmoles/ml with primary cultures of adult rat hepatocytes. An Ames assay was also conducted in 8 histidine auxotrophs of S. typhimurium and 2 tryptophan auxtrophs of E. coli in a gradient concentration range of 10,000 fold with and without metabolic activation (S9). Results of both assays were negative. Summary data. Adequate quantitative data were not submitted.	Unacceptable *

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* the reviewer classified these studies as inconclusive. However, since the classification was based on the fact that these studies were summary data and that adequate

1. DATA EVALUATION RECORD

(1) CHEMICAL: Alacholor (99.9% pure).

(2) CITATION: Shirasu, Y.; Moriya, M.; and Ohta, T. (January 1980) Alachlor: Microbial Mutagenicity Study (Unpublished report #ET-80-0101 prepared by the Institute of Environmental Toxicology, Kodaira, Tokyo 187, Japan submitted by Monsanto Company).

(3) SPONSOR: Monsanto Company.

(4) REVIEWED BY:

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Department Director
Dynamac Corporation

Signature: John R. Strange
Date: 11 March 1983

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Project Scientist
Dynamac Corporation

Signature: I. Cecil Felkner
Date: March 11, 1983

(5) APPROVED BY:

Amal Mahfouz, Ph.D.
EPA Scientist

Signature: Amal Mahfouz
Date: 3/15/83

(6) STUDY TYPE: Mutagenicity.

(7) ACCESSION NO.: 248053

(8) MRID NO.: 000GS-03.

(9) PROTOCOL:

1. Alachlor, chemical name 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetamilide, lot NBP 1511174, and purity 99.9%, served as the test compound.

2. Bacilluis subtilis, strains H17 (rec^+) and M45 (rec^-) were used for the rec-assay.

   Escherichia coli, strain WP2 hcr, and Salmonella typhimurium, strains TA1535, TA1537, TA1538, TA100, and TA98

3. The test compound was dissolved in dimethyl sulfoxide (DMSO) and concentrations of 20, 100, 200, 500, 1,000, and 2,000 ug in 0.02 ml aliquots were applied to filter paper discs for the rec-assay.

   For the reverse mutation assay, 10, 50, 100, 500, 1,000, and 5,000 ug/plate of the test compound, respectively were added to top agar.

4. The rec-assay was conducted by streaking prepared cultures onto B-2 agar plates, applying a disc containing the test compound, incubating overnight at 37° C, and measuring the zones of inhibition.

   The reverse mutation assay was performed by the pour plate method. Top agar containing the bacterial syspension and the test compound, with or without S9 metabolic activator (prepared from rats pretreated with 500 mg/kg Aroclor 1,254) was poured onto minimal agar plates, and incubated at 37° C for 2 days. The revertant colonies were then scored.

5. Control plates were assayed with Kanamycin, used as the positive control for antibiotic bacteriostasis. Mitomycin C served as the positive DNA-damaging control and DMSO was the negative or solvent control.

   In the reverse mutation assay, AF-2(2-[2-furyl]-3-[5-nitro-2-furyl]acrylamide); b-propiolactone; 9-aminoacridine; 2-nitro fluorene; and 2-amino-antracene served as positive controls, while a solvent control (DMSO) was also assayed.

(10) RESULTS

In the rec-assay (Table 1 of original paper), the test compound concentrations assayed failed to produce an inhibition zone of 1 mm in all cases with either strain, while the positive control, mitomycin C caused an 8.5 mm zone of inhibitor in strain M45 and a 2 mm zone in strain H17. Kanamycin, the other positive control, produced a 6 mm zone in strain M45 and a 5 mm zone in strain H17. The results of the reverse mutation assay are presented in Table 2 (compiled by reviewer). The authors state that there was no significant increase in the numbers of revertant colonies for any strain in the presence or absence of S9 metabolic activator, while the positive controls demonstrated induction of reverse mutations. Toxicity was observed at the highest dose level (3,000 ug/plate) with strain TA1535 and TA1537 with metabolic activation (S9), and also in strain TA1537 without activation.

(11) CONCLUSIONS

The rec-assay with Alachlor at 6 concentrations ranging from 20-2,000 ug/plate in B. subtilis strains H17 and M45 showed no evidence of inhibition, while positive controls gave the appropriate positive, inhibitory effect. Kanamycin was reported to be a negative control. It is the reviewer's opinion that this compound serves as a positive control for metabolic (bacteriostatic or bacteriocidal) inhibition, unrelated to DNA damage. Mitomycin C, reported as the positive control, serves as the positive control for DNA damage.

The reverse mutation assay conducted at 6 concentrations ranging from 10-5,000 mg/plate in the E. coli and Salmonella strains also produced negative results. No criteria was given for judging a positive responses. A positive mutagenic effect should be assessed using the twofold rule (twofold increase in the number of revertant colonies relative to the solvent control or spontaneous rate for at least one strain indicates a positive response) using this criterion a mutagenic response was produced for all positive control compounds (except 2-aminoanthracene without S9). This demonstrates that the assay was capable of producing the appropriate response. The test compound produced negative responses in all cases, with the possible exception of strain TA1535. A doubling was produced at 1,000 ug/plate with S9 activation. This response was not confirmed at the 5,000 ug/plate level probably due to cytotoxicity. However, this single result was judged by the reviewer to be a questionable or +/- result.

(12) CORE CLASSIFICATION/EVALUATION: Acceptable.

Table 1 Rec-assay with B. subtilis M45 and H17

Compound	µg/disk	Inhibitory zone (mm) M45	H17	Difference (mm)
Control (DMSO)		0	0	0
Alachlor	20	0	0	0
	100	0	0	0
	200	0	0	0
	500	0	0	0
	1000	<1	<1	<1
	2000	<1	<1	<1
Kanamycin	10	6	5	1
Mitomycin C	0.1	8.5	2	6.5

Table 2. Mean Revertant Colony Counts Produced in the Reverse Mutation Assay
(compiled by the reviewer)

Compound	Tester Strain / S9 Metabolic Activation / Concentration (ug/plate)	WP2 hcr +	WP2 hcr -	TA1535 +	TA1535 -	TA100 +	TA100 -	TA1537 +	TA1537 -	TA1538 +	TA1538 -	TA98 +	TA98 -
DMSO (solvent control)	0	9	12	7	8	130	132	9	9	10	14	25	25
Alachlor	10	12	11	12	12	109	139	6	7	15	15	24	23
	50	13	11	12	5	123	124	10	7	12	11	21	19
	100	16	15	9	8	145	125	9	2	14	12	19	18
	500	9	8	10	6	141	121	8	5	15	13	14	18
	1000	15	12	14	5	133	131	7	5	15	11	19	14
	5000	12	16	5*	6	100	82	*	*	7	5	13	12
Positive Controls: 2-aminoanthracene	10	62	11	410	11	3000	160	400	15	3000	25	3000	42
Other [a-f]	a-f		1541[a]		1292[b]		714[c]		>10000[d]		>3000[e]		390[f]

[a] AF-2 (0.25 ug/plate).
[b] b-propiolactone (50 ug/plate).
[c] AF-2 (0.05 ug/plate).
[d] 9-AA (200 ug/plate).
[e] 2-NF (50 ug/plate).
[f] AF-2 (0.1 ug/plate).
*Toxic

2. DATA EVALUATION RECORD

(1) CHEMICAL: Alachlor (unspecified purity).

(2) CITATION: Probst, Gregory S.; McMahon, Robert E.; Hill, L.E.; Thompson, Christina Z.; Epp, J.K.; and Neal, S.B. (1981) Chemically Induced Unscheduled DNA Synthesis in Primary Rat Hepatocyte Cultures: A Comparison with Bacterial Mutagenicity Using 218 Compounds. Environmental Mutagenesis 3:11-32.

(3) REVIEWED BY:

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(4) APPROVED BY:

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EPA Scientist

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(5) STUDY TYPE: Mutagenicity (Unscheduled DNA Synthesis (UDS) and Ames Assay).

(6) ACCESSION NO.: 248053

(7) MRID NO: 000GS-04.

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(8) PROTOCOL:

1. Alachlor (2',chloro-N-(2,6-diethylphenyl)-N-methoxymethyl-acetamide served as the test compound was tested as supplied (by Chem Service Inc., West Chester, PA) without further purification.

2. Species/Strain: UDS - Primary cultures of adult rat hepatocytes were prepared by in situ perfusion of the liver of male Fischer 344 rats. Ames assay - eight histidine auxotrophs of Salmonella typhimurium (G46, TA1535, TA1000, C3076, TA1537, D3052, TA1538, and TA98) and two tryptophan auxotrophs of Escherichia coli (WP2 and WP2 uvrA$^-$) were used.

3. Concentration: UDS - Eight concentrations covering a range from 0.5 to 1,000.0 nmoles/ml were used for UDS. The highest concentration of DMSO (solvent) did not exceed 1%. Ames assay - concentrations were not specified for Ames assay. Gradient plates were used (see Experimental Methods).

4. Procedure: Unscheduled DNA Synthesis (UDS) - Primary cultures of adult rat hepatocytes were prepared and the HPC-DNA repair test was conducted essentially as described by Williams (1977a and b). Incubation was conducted at 37° C and following an attachment period of 90 minutes, the cells were washed and the appropriate test compound dilution was added to each culture. After incubation for five hours (Exposure Period), the medium was replaced and incubation was continued for an additional 18 to 20 hours (Chase Period). Compounds showing a negative response for UDS were retested as above, except that the exposure period was increased to 20 hours and the chase period was deleted. Cultures were washed, fixed and stained, then autoradiograms were examined by oil immersion microscopy. UDS was quantified by counting the number of silver grains over the nucleus using a Biotran II automated colony counter.

   Ames assay - Bacterial mutagenesis was measured by a modificaion of the Ames test (Ames et al., 1975) utilizing concentration gradient plates as described by Cline and McMahon (1977) and McMahon et al. (1979). Each compound was incorported into four gradient plates to give a tenfold concentration range per plate, thus providing a 10,000-fold concentration range for the test. For metabolic activation, an S9 fraction, derived from the liver of Aroclor 1254 pretreated rats, was mixed with appropriate co-factors and included in an agar overlay on the gradient plates. Each compound was tested with and without metabolic activation.

5. Controls: DMSO (dimethylsulfoxide) was used as control in UDS (See Compound Preparation below). Solvent or control were not reported for Ames assay.

   2-AAF (2-acetylaminofluorine) and MNNG (N-methyl-N-nitro-N-nitrosoguanidine) were used as positive controls for UDS. No positive controls were reported for Ames assay.

(9) RESULTS

Alachlor was found to be inactive both in the HPC-DNA repair test and in the Ames test. In the case of UDS, 10 nmoles/ml was the highest concentration that did not produce cytotoxicity. Nuclear silver grain counts, which represent counts of nuclei from from 20 morphologically unaltered cells, were 0.6 ± 1.0 (mean ± S.D.) and 0.0 ± 1.6 for treated and control groups respectively.

The procarcinogen 2-AAF and the direct carcinogen MNNG, which served as positive controls in these tests, produced dose-dependent autoradiographic responses. The highest levels of UDS occurred at the highest chemical concentration that did not produce pronounced cytotoxicity. The degree of UDS activity decreased in a dose-related fashion at lower doses. The presence of DMSO, the solvent for most test compounds, had no effect on the induction of UDS.

Results for Ames assay were reported to be negative. However, no quantitative data were given in this report.

(10) CONCLUSIONS

Alachlor was tested for mutagenicity along with 217 other compounds. Chemically-induced unscheduled DNA synthesis (UDS) was studied in rat hepatocyte cultures and the mutagenicity results were compared with those obtained from Ames assay in Salmonella typhimurium (eight strains).

Based on the information presented in this study, it appears that Alachlor does not induce DNA repair synthesis in the autoradiographic assay for UDS in the primary cultures of rat hepatocytes. However, without the supportive data for UDS and appropriate tables for Ames assays from which the numbers of replicates, number of revertants, etc. can be evaluated, the conclusions presented can not be properly evalutated.

(11) CORE CLASSIFICATION/EVALUATION: Inconclusive.*

* this study was classified as inconclusive based on the fact that it is a summary, and adequate quantitative data were not submitted for review. Hence, the Toxicology Branch classify this study as Unacceptable

Sarah Mahfouz (Tox. Br.)

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3. DATA EVALUATION RECORD

March 11, 1983

(1) CHEMICAL: Alachlor (unspecified purity).

(2) CITATION: Shirasu, Y.; Moriya, M.; Kato, K.; Furuhashi, A.; and Kada, T. (1976) Mutagenicity Screening of Pesticides in the Microbial System. Mutation Research 40:19-30.

(3) REVIEWED BY:

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Date: 11 March 1983

I. Cecil Felkner, Ph.D.
Project Scientist
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Date: March 11, 1983

(4) APPROVED BY:

Amal Mahfouz, Ph.D.
EPA Scientist

Signature: Amal Mahfouz
Date: 3/15/83

(5) STUDY TYPE: Mutagenicity.

(6) ACCESSION NO: 248053

(7) MRID NO: 000GS-05.

(8) PROTOCOL:

1. Alachlor, 2-chloro-2'-6'-diethyl-N-(methoxymethyl) acetanilide, served as the test compound and was obtained through the courtesy of Dr. T. Suzuki of the Agricultural Chemicals Inspection Station of the Ministry of Agriculture and Forestry Kodaira-shi, Japan.

2. Strains used: Rec-assay - Bacillus subtilis H17 Rec^+ and M45 Rec^-.

3. Samples were dissolved in DMSO at a concentration of 1 mg/ml and 0.02 ml solution of sample was applied to a paper disc, which was placed on the cultured agar plate.

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4. B. subtilis H17 Rec$^+$ and M45 Rec$^-$) were grown overnight in B-2 broth. Two cultures were streaked on the "dry" surface of B-2 agar and the "starting points" were covered with a paper disk (10 mm diameter) containing 0.02 ml solution of each sample. All the plates were incubated for 24 h at 37° C and the length of inhibition zones for each streak was measured.

(9) RESULTS

Alachlor was nonmutagenic in rec-assay and therefore it was not subjected to further reversion assays with auxotrophic strains of Escherichia coli and Salmonella typhimurium. Detailed data on pesticides giving negative results in mutagenicity tests were not reported.

(10) CONCLUSIONS

Alachlor was one of 166 pesticides subjected to mutagenicity screening. The screening procedure consisted of : "(a) prescreening of DNA-damage chemicals by the rec-assay, followed by, (b) determination of mutation specificities by reversion assays on plates." Metabolic activation was not provided in these tests of positive agents.

Based on the information presented in this study, it appears that Alachlor was not DNA-damaging in the rec-assay. However, without the supportive data e.g., the extent of the inhibitory zones produced by various dose levels and the control data, the conclusions presented can not be properly evaluated.

(11) CORE CLASSIFICATION/EVALUATION: Inconclusive.*

*This study was classified as inconclusive based on the fact that it is a summary, and adequate quantitative data were not submitted for review. Hence, the Toxicology Branch classify this study as Unacceptable

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Ames B.N., McCann J, Yamasaki E. 1975. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31:347-364.

Cline J.C., McMahon R.E. 1977. Detection of chemical mutagens: Use of concentration gradient plates in a high capacity screen. Res. Commun. Chem. Pathol. Pharmacol. 16:523-533.

McMahon R.E., Cline J.C., Thompson C.A. 1979. The assay of 855 test chemicals in 10 tester strains employing a new modification of the Ames test for bacterial mutagens. Cancer Res. 39:682-693.

Wiliams G.M. 1977. Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. Cancer Res. 37:1845-1851.

Williams G.M., Bermudez R., Scaramuzzino D. 1977. Rat hepatocyte primary cultures. III. Improved dissociation and the ehnancement of survival by culture medium. In vitro 13:809-817.

3-11-83

4. DATA EVALUATION RECORD

(1) CHEMICAL: Alachlor (formulation = 48% w/v).

(3) CITATION: Eisenbeis, S. J.; Lynch, D. L.; and Hampel, A. E. (1981) The Ames Mutagen Assay Tested Against Herbicides and Herbicide Combinations. Soil Science 131(1):44-47.

(3) REVIEWED BY:

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(5) STUDY TYPE: Mutagenicity.

(6) ACCESSESION NO.: 248053

(7) ~~MRID NO.~~: N/A

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(8) PROTOCOL:

1. Alachlor (Lasso) and combinations listed below (by trade names) served as the test compound.

   Amiben + Lasso
   Banvel + Lasso
   Dyanap + Lasso
   Furloe + Lasso
   Lasso + Atrazine
   Lasso + Bladex
   Lorox + Lasso
   Modown + Lasso
   Paraquat + Atrazine + Lasso
   Paraquat + Lorox + Lasso
   Premerge + Lasso
   Sencor + Lasso

2. Strains Used: Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 were used.

3. Suspected mutagens (herbicides), positive controls, and controls containing solvent were added as crystals or microdrops (10 ul) to the hardened top agar surface. The herbicide concentrations used were those recommended in the Illinois Agronomy Handbook (1978) and the Thirtieth Illinois Custom Spray Operators Training School Manual (1978). Herbicides were tested at full strength in a diluted water mixture approximating a tank mix delivering 15 gal/acre of recommended herbicide concentrations.

4. Procedure: 0.1 ml of a 14-hour culture of tester strain, and 0.5 ml of S-9 mix (if utilized) were added to the top agar, mixed, and poured over the surface of the bottom agar plate. Suspected mutagens and controls were added as crystals or microdrops (10 ul) to the hardened top agar surface. All suspected mutagens and controls were tested against all five tester strains, both with and without S-9 mix. The metabolic activation system (S-9 mix), was derived from male rats induced with a single i.p. injection of a polychlorinated biphenyl mixture, Aroclor 1254.

5. Positive Control: N-methyl-N-nitro-N-nitrosoguanidine, 9-amino-acridine, aflatoxin B1, and 2-aminofluorene were used as positive controls. Concentrations and solvent used were not reported.

(9) RESULTS

The mutagenicity tests with Lasso and its mixtures with other herbicides was not not increased over the background, at either full strength or as an aqueous solution. This was true for all five tester strains: TA1535, 1537, 1538, 98, and 100, with and without metabolic activation. The report stated that differences in revertant colonies per plate was never significantly different from the background spontaneous rate per plate: 20(TA1535), 7(TA1537), 25(TA1538), 160(TA100), and 40(TA98). This reviewer was not able to confirm this statement as detailed data were not reported in this manuscript.

Positive controls confirmed the sensitivity of the tester strains (all reversion rates were greater than 2000 revertants per plate). Average number of revertants in the control (negative) fell within the expected limits set by Ames et al., (1975). Slightly higher reversion rates were obtained when S-9 mix was used.

(10) CONCLUSION

Based on the information presented in this study, it appears that Alachlor (Lasso) is nonmutagenic in this test system. However, without appropriate tables from which the numbers of replicates, number of revertants, etc. can be evaluated, the conclusions presented can not be properly evaluated nor statistically verified. The results from this study do not provide an adequate basis for judging the mutagenicity of Alachlor by itself or in combination with other herbicides because of the following reasons:

1. Supportive data on the results of the mutagenicity tests were not presented. Only summary observations and conclusions were presented. Statistical analyses, if any, were not reported.

2. The solubility of the compound and its mixtures in the solvent (water) was not reported.

(11) CORE CLASSIFICATION/EVALUATION: Inconclusive.

This study was classified as inconclusive based on the fact that it is a summary, and adequate quantitative data were not submitted for review. Hence, the Toxicology Branch classify this study as Unacceptable

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Ames, B.M., J. McCann, and E. Yamasaki, 1975. Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test. Mut.Res. 31:347-364.

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