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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

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MEMORANDUM

SUBJECT: Molinate: Review of Three Mutagenicity Studies

TO: Ernestine Dobbins
Product Manager (52)
Generic Chemical Support Branch/
SRRD (H7508C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 11/12/92
Toxicology Branch II Section II,
Health Effects Division (H7509C)

THRU: K. Clark Swentzel K. Clark Swentzel 11/12/92
Section II Head, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. Marcia Gemert 12/18/92
Chief, Toxicology Branch II/HFAS/HED (H7509C)

Registrant:	ICI Americas Inc.
Chemical:	Molinate; S-ethyl hexahydro-1H-azepine-1-carbothioate
Synonym:	Ordram
Submission No.:	S429440
Caswell No.:	444
Schaughnessy #:	041402
DP Barcode.:	D184697
MRID No.:	TRID #'s 460059-019, 470298-021, 470298-023

Comment: These three studies were identified as in-house but not reviewed during the preparation of Molinate for the HED Carcinogenicity Peer Review Committee. A brief review of these was performed by K. Dearfield at that time, and they have since been reviewed in depth (DER's appended).

1. Report on Mutagenicity Experiment on the Substance Molinate Tecnico [2 words illegible] the Firm Oxon Italia, S.p.A, Milan, Italy, dated July 31, 1980 (TRID # 460059-019). Molinate technical was tested at 6 dose levels (16.5 µg/plate to 500 µg/plate +/- S9 and was found to be negative in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100. Cytotoxicity was observed

at doses ≥ 500 µg/plate, without S9 activation, in a preliminary spot test, but there was no definitive evidence of cytotoxicity in the main mutation assay. The reported findings in this study do not provide an adequate basis to reach a conclusion on the mutagenicity of the test material. This study does not satisfy the guideline requirement (84-2a) for genetic effects Category I, Gene Mutations, and it is classified unacceptable.

2. ORDRAM® Technical (Lot No. WRC 4921-8-9) Mutagenicity Evaluation in Bone Marrow Micronucleus, dated November 22, 1983 (TRID # 470298-021). No conclusions can be reached from the in vivo mouse micronucleus assay conducted with 200, 400, and 600 mg/kg (♂♂)/100, 200, 400 mg/kg (♀♀) Ordram technical since (1) it could not be determined whether the increased MPE frequency in the high-dose males 48 hours posttreatment was artifactual or compound-related; (2) the data suggests that the MTD was not achieved in females; (3) there was no indication whether the slides were coded prior to analysis; and (4) purity information on the test material was not provided, and the dosing solutions were not analyzed for achieved concentrations. This study does not satisfy the guideline requirement (84-2) for genetic effects Category II, Structural Chromosomal Aberrations, and it is classified unacceptable.

3. ORDRAM® Technical (Lot No. WRC 4921-8-9) Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test Cytotoxicity Assay, dated December 2, 1983 (TRID # 470298-023). No conclusions relative to the potential of ordram technical to induce structural chromosome aberrations or sister chromatid exchange (SCE) in mouse lymphoma cells can be drawn from the series of nonactivated and S9-activated trials conducted. There were conflicting results relative to cytotoxicity (chromosomal aberration tests) and genotoxicity (chromosome aberration and SCE tests), in addition to a lack of purity information on the test material and whether the slides were coded prior to analysis. This study does not satisfy the guideline requirement (84-2) for genetic effects Category II, Structural Chromosomal Aberrations, and it is classified unacceptable.

DISCUSSION

Molinate has been tested in several mutagenicity studies considered acceptable by the Agency (discussed in the Carcinogenicity Peer Review of Molinate document, dated September 14, 1992). Guideline requirements 84-2(a) and 84-2(b) have been satisfied. As stated in the Peer Review document, because of the indicated activity for three endpoints in the mouse lymphoma assays with activation, the observed germ cell interaction of Molinate, and the positive response in a published mouse bone marrow micronucleus test, a dominant lethal study in the rat is required. Additionally, the "Other Genotoxic Effects" category has not been satisfied. It is suggested that a sister chromatid exchange assay in germ cells or a UDS assay in germ cells be performed to fulfill this category, in light of the fact that the gonads are target organs of Molinate.

The three previously unreviewed studies do not alter the Peer Review assessment of the mutagenicity aspects of Molinate.

CONCLUSION

The 3 mutagenicity studies on Molinate, identified as in-house but not reviewed, have been reviewed. Each is classified unacceptable. Additionally, none of these studies addresses the concerns emphasized by the Peer Review regarding mutagenicity. However, since guideline requirements 84-2(a) and 84-2(b) have been satisfied, and these 3 studies are from these categories, no further action with regard to these studies is required. The dominant lethal study in rat and either a sister chromatid exchange assay in germ cells or a UDS assay in germ cells to fulfill the "Other Genotoxic Effects" category, as discussed in the Peer Review document, are data requirements.

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DATA EVALUATION REPORT

MOLINATE

Study Type: Mutagenicity: Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer	Kristin Jacobson, MSPH	Date 10/30/92
Independent Reviewer	Nancy E. McCarroll, E.S.	Date 10/30/92
QA/QC Manager	Sharon Segal, Ph.D.	Date 10/30/92

Contract Number: 68D10075
Work Assignment Number: 1-134
Clement Number: 93-130
Project Officer: James Scott

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GUIDELINE §84: MUTAGENICITY
SALMONELLA

MUTAGENICITY STUDIES

Approved by: Byron T. Backus, Ph.D.
Review Section II, Toxicology Branch II
EPA Reviewer: Linda Taylor, Ph.D.
Review Section II, Toxicology Branch II
Health Effects Division (H7509C)
Acting EPA Section Head: K. Clark Swentzel
Review Section II, Toxicolcgy Branch II
Health Effects Division (H7509C)

Signature: Byron T. Backus
Date: 11/1/92
Signature: Linda Taylor
Date: 11-2-92
Signature: K. Clark Swentzel
Date: 11/2/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay

EPA IDENTIFICATION Numbers:

EPA Registration Number: 476-2107

TRID Number: 460059-019

TEST MATERIAL: Molinate technical

SYNONYM: Ordram technical

SPONSOR: Oxon Italia, Milan, Italy/ Stauffer Chemical Co., Farmington, CT

STUDY NUMBER: CRF 190/M

TESTING FACILITY: Centro Ricerca Farmaceutica S.p.A., Pomezio, Italy

TITLE OF REPORT: Report on Mutagenicity Experiment on the Substance Molinate Tecnico [*two words are illegible*] the Firm Oxon Italia, S.p.A., Milan, Italy

AUTHORS: M. Monaco and A. Nunziata

REPORT ISSUED: July 31, 1980

CONCLUSIONS--EXECUTIVE SUMMARY: Molinate technical was tested at six doses, ranging from 16.5 µg/plate to 500 µg/plate +/- S9, and was found to be nonmutagenic in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100. Cytotoxicity was observed at doses ≥500 µg/plate, without S9 activation, in a preliminary spot test assay; however, in the main mutation assay, no definitive evidence of cytotoxicity was observed. We assess, therefore, that the reported findings provide an inadequate basis to reach a conclusion on the mutagenicity of this te t compound, and that the study should be repeated.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84-2a) for genetic effects Category I, Gene Mutations.

SALMONELLA

A. MATERIALS:

1. Test Material: Molinate technical

Description: Not provided
Identification number: Sample code CRF 190/M
Purity: 97% (See Appendix A)
Receipt date: June 16, 1980
Stability: Not provided
Contaminants: See Appendix A
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: Storage conditions of the test material were not provided. There was no indication in the report that dosing solutions were analyzed for actual concentrations.

2. Control Materials:

Negative: Untreated cells

Solvent/final concentration: DMSO/0.1 mL/plate

Positive:

Nonactivation:

Ethyl methanesulfonate	500 µg/plate TA1535
9-Aminoacridine	25 µg/plate TA1537
Icantone	25 µg/plate TA1538, TA98
Methyl methanesulfonate	100 µg/plate TA100

Activation:

2-Anthramine	1.25 µg/plate all strains

3. Activation: S9 derived from 200-g male Sprague-Dawley

___ Aroclor 1254	_x_ induced	_x_ rat	_x_ liver
___ phenobarbital	___ noninduced	___ mouse	___ lung
___ none		___ hamster	___ other
_x_ other		___ other	

Rats received intraperitoneal injections of phenobarbital and β-naphthoflavone as follows:

Inducing Agent	Dose (mg/kg)	Day
Phenobarbital	30	1
	60	2
	60	3
	60	4
β-naphthoflavone	80	3

NOTE: The combined injection of phenobarbital and β-naphthoflavone is considered a safe and effective alternative to Aroclor 1254 induction.[1]

One day posttreatment, following a 12-hour fast, rats were sacrificed and their livers were removed. The S9 fraction was prepared by the performing laboratory and prior to use, the S9 fraction was characterized for metabolic activity with 2-anthramine. In addition, the total protein and aminopirine demethylase activity were evaluated. Results of these determinations were not reported. Neither the composition of the S9 mixture nor the percentage S9 per plate were provided. The report indicated, however, that the study was conducted in accordance with the method of Ames et al. (1975)[2].

4. Test Organism Used: S. typhimurium strains

___ TA97	_x_ TA98	_x_ TA100	___ TA102	___ TA104
_x_ TA1535	_x_ TA1537	_x_ TA1538		

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

(a) Preliminary solubility and cytotoxicity assays: Solubility of the test material was evaluated by streaking 2 mL of top agar, containing 0.1 mL of test substance solution, onto minimal media. The concentrations assayed ranged from 1250 µg/plate to 5000 µg/plate; no precipitate formed at any of these dose levels.

The spot test was used in the preliminary cytotoxicity assay, without S9 activation; all tester strains were evaluated. Initially, test material concentrations ranging from 1500 µg/plate to 5000 µg/plate were evaluated; since all three concentrations produced areas of inhibition in all tester strains, the test was repeated at doses of 500, 750, and 1000 µg/plate. Single plates were used per strain, per dose.

(b) Mutation assay: A standard plate incorporation assay was conducted using 16.5, 32.5, 75, 125, 250, and 500 µg/plate +/- S9, with all tester strains. Triplicate plates were prepared per strain, per dose, per condition.

[1]Maron, D.M., and Ames, B.N. (1983). Revised methods for the Salmonella mutagenicity test. Mutat Res 113: 173-215.
[2]Ames, B.N., McCann, J., and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella mammalian microsome mutagenicity test. Mutat Res 31: 347-364.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
   - x Standard plate test
   - ___ Pre-incubation (_____) minutes
   - ___ "Prival" modification
   - ___ Spot test
   - ___ Other (describe)

2. Preliminary Cytotoxicity Assay: Two-milliliter volumes of top agar, containing 0.1 mL of the appropriate tester strain, 0.5 mM histidine, 0.5 mM biotin, and 0.5 mL phosphate buffer (0.2 M), were poured over minimal media, and allowed to harden. A volume of 0.1 mL of each test material solution was added to a paper disc, which was then placed on the hardened top agar surface. Plates were incubated for 48 hours at 37°C, and zones of growth inhibition were measured.

   Mutation Assay: The appropriate tester strain (0.1 mL) from 16-hour nutrient broth cultures was added to 2 mL of top agar containing 0.5 mL S9 mix (S9-activated test) or 0.5 mL phosphate buffer (nonactivated test), and 0.1 mL of the selected test material dose, the solvent or the positive controls. The contents of each tube were mixed and poured over minimal agar medium. Plates were incubated at 37°C for 48 hours and scored for the number of revertants per plate. Means and standard deviations were determined.

3. Evaluation Criteria: The test material was considered positive if it caused a reproducible, dose-related, approximately two-fold increase in the number of revertants per plate of at least one strain.

4. Protocol: Not provided

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: In the spot test, zones of inhibition were observed for all strains at test material concentrations ≥750 µg/plate. At 500 µg/plate, zones of inhibition measuring 2 mm and 1 mm were also noted for strains TA98 and TA100, respectively. Cytotoxicity in the presence of S9 was not evaluated. Based on these results, doses selected for the plate incorporation mutation assay ranged from 16.25 µg/plate to 500 µg/plate +/- S9.

2. Mutation Assay: Representative results from the nonactivated and S9-activated mutation assay with molinate technical are presented in Table 1. As shown, the test material was neither cytotoxic nor mutagenic in any strain at any dose in either the presence or absence of S9 activation. Although the study authors stated that "atossic effects" were observed in strains TA98 and TA100 at 500 µg/plate, the provided data do not support this statement. By contrast, all strains responded to the mutagenic action of the appropriate nonactivated or S9-activated positive control compound.

From the overall results, the study authors concluded that molinate technical was not mutagenic in this microbial test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the reported findings provide an inadequate basis to reach a conclusion on the potential mutagenicity of molinate technical. There was no indication of a mutagenic response at any dose with or without S9 activation. However, in contrast to the preliminary cytotoxicity assay (spot test) findings, in which the 500-µg/plate dose level produced cytotoxicity in two tester strains, no clear evidence of cytotoxicity was observed at the highest dose tested (500 µg/plate +/- S9) in any tester strains in the mutation assay. In addition the report provided no evidence that quality assurance measures were taken to ensure the integrity of the study. Based on the above considerations, we conclude that the study is unacceptable and should be repeated.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Not known. A Quality Assurance statement was not included in the report.

F. CBI APPENDICES: Appendix A, Certificate of Purity, CBI p. 14; Appendix B, Materials and Methods, CBI pp. 7-10

CORE CLASSIFICATION: Unacceptable; the study does not satisfy Guideline requirements (§84-2a) for genetic effects Category I, Gene Mutations.

Table 1: Representative Results of the Salmonella typhimurium/Mammalian Microsome Mutation Assay with Molinate Technical

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Negative Control							
Untreated cells	--	-	10.8±1.24	7.6±1.20	12.0±1.92	25.8±1.82	228.4±6.80
	--	+	15.2±0.73	8.6±1.20	18.4±1.77	32.8±2.81	185.4±4.34
Solvent Control							
Dimethyl sulfoxide	0.1 mL	-	10.8±1.24	6.6±0.50	12.0±1.92	25.8±1.82	228.4±6.80
	0.1 mL	+	15.2±0.73	8.6±1.20	18.4±1.77	32.8±2.81	185.4± 4.34
Positive Controls							
EMS	500 µg	-	59.6±2.84	--	--	--	--
9-Aminoacridine	25 µg	-	--	42.3±2.40	--	--	--
Icantone	25 µg	-	--	--	235.0±4.93	176.0±4.0	--
MMS	100 µg	-	--	--	--	--	370.0±8.66
2-AA	1.25 µg	+	60.3±2.07	72.6±3.17	656.3±8.45	513.3±12.57	372.0±12.16
Test Material							
Molinate technical	250 µg[b]	-	14.3±2.18	6.0±1.15	14.0±2.08	20.3±2.60	200.3±1.85
	500 µg	-	14.3±1.20	5.0±1.52	13.3±2.84	20.3±3.92	164.6±31.66
	250 µg[b]	+	11.3±1.85	9.3±0.66	25.3±1.76	32.0±3.51	210.0±10.0
	500 µg	+	15.3±2.02	6.3±1.33	17.3±1.45	29.3±2.33	253.6±3.75

EMS = Ethyl methanesulfonate; MMS = Methyl methanesulfonate; 2-AA = 2-Anthramine

[a]Means and standard deviations of the counts from three plates
[b]Results for lower doses (16.5, 32.5, 75 or 125 µg/plate +/- S9) did not suggest a mutagenic effect.
Note: Data were extracted from the study report, CBI p. 12.

APPENDIX A

ANALYSIS OF PURITY
CBI p. 14

Page _____ is not included in this copy.

Pages 12 through 17 are not included in this copy.

The material not included contains the following type of information:
Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s)

_____ The document is not responsive to the request.

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

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DATA EVALUATION REPORT

MOLINATE

Study Type: Mutagenicity: In Vivo Micronucleus Assay in Mice

Prepared for:

009862

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer _____ Date 11/2/92
Nancy E. McCarroll, B.S

Independent Reviewer _____ Date 11/2/92
Lynne Haber, Ph.D.

QA/QC Manager _____ Date 11/2/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-134
Clement Number: 93-131
Project Officer: James Scott

GUIDELINE § 84: MUTAGENICITY MICRONUCLEUS

MUTAGENICITY STUDIES

Approved by: Byron Backus, Ph.D.
Review Section II,
Toxicology Branch II/HED (H-7509C)

Signature: Byron T. Backus
Date: 11/6/92

EPA Reviewer: Linda Taylor, Ph.D.
Review Section II,
Toxicology Branch II/HED (H-7509C)

Signature: Linda Taylor
Date: 11-9-92

EPA Section Head: Clark Swentzel
Review Section II,
Toxicology Branch II/HED (H-7509C)

Signature: K. Clark Swentzel
Date: 11/12/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo micronucleus assay in mice

EPA IDENTIFICATION Numbers:

EPA Registration Number: 476-2107

TRID Number: 470298-021

TEST MATERIAL: Ordram Technical

SYNONYMS/CAS NUMBER: Molinate

SPONSOR: Stauffer Chemical Co., Farmington, CT.

STUDY NUMBER: Report Number T-11820

TESTING FACILITY: Stauffer Chemical Co., Farmington, CT.

TITLE OF REPORT: ORDRAM® Technical (Lot No. WRC 4921-8-9) Mutagenicity Evaluation in Bone Marrow Micronucleus

AUTHORS: Majeska, J.B., and Matheson, D.W.

REPORT ISSUED: November 22, 1983

CONCLUSIONS--EXECUTIVE SUMMARY: No conclusions can be reached from the in vivo mouse micronucleus assay conducted with 200, 400, and 600 mg/kg (males) and 100, 200, and 400 mg/kg (females) Ordram technical for the following reasons:

1. Owing to the wide variation in the frequency of micronucleated polychromatic erythrocytes (MPEs) in the vehicle control males (0.14–0.40%), we are not able to determine whether the increased MPE frequency in high-dose males 48 hours posttreatment (0.60%) was artifactual or a compound effect. However, the frequency in this treatment group was ≈2.3-fold higher than the combined value for vehicle controls males (0.26%).

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2. There was no evidence of a genotoxic response in females at any dose or harvest time; however, the lack of overt toxicity in the animals and cytotoxic effects in the target organ (i.e., bone marrow) indicates that the maximum tolerated dose (MTD) was not achieved. Based on the results of the preliminary range-finding study, a level higher than 400 mg/kg should have been assayed in the females.

3. The report did not indicate whether slides were coded prior to analysis.

4. Purity information on the test material was not provided and dosing solutions were not analyzed for actual concentrations.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84-2) for genetic effects Category II, Structural Chrc osomal Aberrations.

A. MATERIALS:

1. Test Material: Ordram technical

Description: Amber liquid
Identification numbers: Lot number: WRC 4921-8-9; EHC-0525-19; T-11820
Purity: Not listed
Receipt date: July 28, 1983
Stability: Stable under ambient temperature and pressure; expiration date--February 1984
Contaminants: None listed
Vehicle used: Corn oil
Other provided information: It was assumed that the test material was stored at room temperature. The frequency of dose solution preparation was not reported; analytical determinations were not performed on dosing solutions.

2. Control Materials:

Negative/route of administration: None

Vehicle/final concentration/route of administration: Corn oil was administered by oral gavage at a dosing volume of 0.5 mL/animal.

Positive/final concentration/route of administration: Cyclophosphamide (CP) was dissolved in distilled water and administered by oral gavage at 50 mg/kg (males) and 80 and 100 mg/kg (females).

3. Test Compound:

Route of administration: Oral gavage

Dose levels used:

- Preliminary toxicity test: 200 (two treatments), 300, 400 (one treatment or two treatments), 600 800, 1000, and 1200 mg/kg

Note: Dose selection for the preliminary toxicity test was based on the reported information stating that the acute oral LD_{50} for mice was 795-1260 mg/kg.

- Micronucleus assay: 200, 400, and 600 mg/kg--males
  100, 200, and 400 mg/kg--females

4. Test Animals:

(a) Species: mouse Strain: $B6C3F_1$ Age (at initiation): 6-8 weeks
Weight range (at initiation): 18-25 g
Source: Charles River Breeding Laboratories

(b) Number of animals used per dose:

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- Preliminary toxicity test: 10 animals (5 males and 5 females)

- Micronucleus assay: 15 males; 15 females (treatment and vehicle control groups)
  10 males; 10 females (positive control groups)

Dosing was based on mean body weights calculated separately for males and females; these data were not provided.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

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

(b) Vehicle control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): x 24 hr x 48 hr
x 72 hr

(c) Positive control:
Dosing: x once _____ twice (24 hr apart)
_____ other (describe): _____
Sampling (after last dose): x 24 hr x 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

   x bone marrow _____ others (list):
   Number of polychromatic erythrocytes (PCEs) examined per animal: 1000
   Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: 1000

3. Details of Slide Preparation: Bone marrow cells were harvested from animals 72 hours following administration of selected test doses in the preliminary toxicity test and 24, 48, and 72 hours after administration of the test material or vehicle control in the micronucleus assay. Sacrifice time for the positive control groups was 24 and 48 hours.

   At the specified intervals after administration of the test material, the appropriate groups of animals were sacrificed by cervical dislocation. Bone marrow cells were either aspirated or flushed from both tibiae into fetal calf serum (FCS) and centrifuged. Supernatants were removed; cell pellets were resuspended in FCS and spread onto slides. Prepared slides were fixed in absolute methanol, stained with 2% Giemsa, and scored. The report did not specify whether slides were coded prior to scoring.

4. Statistical Methods: The results were evaluated for statistical significance at $p<0.01$ using the Kastenbaum-Bowman tables.

5. Evaluation Criteria: The test material was considered positive for micronuclei induction if a statistically significant ($p<0.01$) increase in the number of micronucleated polychromatic erythrocytes (MPEs) over the vehicle control group was observed.

C. REPORTED RESULTS:

1. Preliminary Toxicity Assay: No males or females survived exposure to the three highest doses (800, 1000, or 1200 mg/kg). One male receiving a single dose of 600 mg/kg, one male receiving two doses of 400 mg/kg, and three females administered 600 mg/kg succumbed to treatment (Table 1). No deaths were reported in the remaining dose groups. The evaluation of bone marrow cells harvested from surviving males and females in the 400-(two treatments) and 600-mg/kg groups revealed a reduction in PCEs compared to the vehicle control in both sexes. Based on these findings, 200, 400, and 600 mg/kg were chosen for administration to male mice. The study authors stated that since the preliminary results suggested that females were more sensitive to the toxic effects of the test material, lower levels (100, 200, and 400 mg/kg) were administered to the females.

2. Micronucleus Assay: No deaths or other signs of clinical toxicity were reported for males or females receiving the selected doses of Ordram technical. Representative results from the micronucleus assay are presented in Table 2. As shown, the percentage of PCEs per NCEs-

TABLE 1. Summarized Results of the Preliminary Range-Finding Study in Mice Treated with Ordram Technical

Substance	Dose	Number of Treatments[a]	Number Dead/Number Treated	% Relative PCE[b] Frequency
		Males		
Vehicle Control				
Corn oil	0.5 mL	2[c]	0/5	100
Test Material				
Ordram technical	400 mg/kg[d]	1	0/5	NE[e]
	400 mg/kg	2	1/5	59
	600 mg/kg	1	1/5	38
	800 mg/kg[f]	1	5/5	NE
		Females		
Vehicle Control				
Corn oil	0.5 mL	2	0/5	100
Test Material				
Ordram technical	400 mg/kg[d]	1	0/5	NE
	400 mg/kg	2	0/5	50
	600 mg/kg	1	3/5	78
	800 mg/kg[f]	1	5/5	NE

[a]Animals were sacrificed ~72 hours after the oral gavage administration of the selected test material dose or vehicle control.
[b]PCE = Polychromatic erythrocytes
[c]The two treatments were separated by 24 hours.
[d]No deaths occurred in lower dose groups (two treatments with 200 or one treatment with 300 mg/kg); PCEs were not evaluated in these treatment groups.
[e]NE = Not evaluated
[f]No animals survived exposure to 800, 1000, or 1200 mg/kg of the test material.

Note: Data were extracted from the study report; CBI p. 9

TABLE 2. Representative Results of the Micronucleus Assay in Mice Treated with Ordram Technical

Substance	Dose/kg	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group	Number of MPEs per Group	MPEs per 1000 PCEs[b]	Percent MPEs per Group[b]	Percent PCEs per NCEs[b]
Vehicle Control									
Corn oil	0.5 mL	24	M	5	5000	7	1.4	0.14	47.0
	0.5 mL	24	F	5	10000	27	2.7	0.27	49.2
	0.5 mL	48	M	5	5000	20	4.0	0.40	45.4
	0.5 mL	48	F	5	5000	12	2.4	0.24	40.5
	0.5 mL	72	M	5	5000	12	2.4	0.24	54.6
	0.5 mL	72	F	5	5000	13	2.6	0.26	64.8
Positive Control									
Cyclophosphamide	50 mg[c]	24	M	5	5000	100*	20.0	2.00	59.1
	80 mg[c,d]	24	F	5	5000	152*	30.4	3.04	48.6
Test Material									
Ordram technical	600 mg[e]	24	M	5	5000	10	2.0	0.20	40.3
		48	M	5	5000	30	6.0	0.60	30.3
		72	M	5	5000	12	2.4	0.24	35.2
	400 mg[e]	24	F	5	10000	24	2.4	0.24	50.1
		48	F	5	5000	1	0.2	0.02	25.1
		72	F	5	5000	12	2.4	0.24	52.7

[a]Time after compound administration by oral gavage
[b]Calculated by our reviewers
[c]Significant ($p<0.01$) results were also obtained at the 48-hour harvest.
[d]Additional females were treated with 100 mg/kg; significant ($p<0.01$) micronuclei induction was observed at both the 24- and 48-hour harvests.
[e]Results for lower doses (200 and 400 mg/kg in males and 100 and 200 mg/kg in females) did not suggest a genotoxic effect.

*Significantly higher ($p<0.01$) than the corresponding vehicle control by Kastenbaum-Bowman tables.

Abbreviations used:

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes.

Note: Data were extracted from the study report; see CBI p. 9.

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for males exposed to 600 mg/kg was low, particularly at the 48- and 72-hour posttreatment harvests, which suggests a slight cytotoxic effect on bone marrow stem cells. Although, the PCE:NCE ratio was also low for high-dose females (400 mg/kg) at the 48-hour sampling time, there was no time or dose-related trend. The data are, therefore, insufficient to conclude that Ordram technical adversely affected hematopoiesis in the treated females.

The incidence of MPEs in males dosed with corn oil was erratic and ranged from 0.14% (24-hour harvest) to 0.40% (48-hour harvest). Since MPE frequencies for vehicle control animals should be relatively constant, regardless of the harvest time, and the study authors did not furnish historical control ranges, our reviewers combined the data from the three harvest intervals and evaluated the test material results by sex relative to the combined spontaneous frequency of 0.26% MPEs (males) or 0.26% (females). Using this approach, the MPE frequency for high-dose males at the 48-hour harvest time (0.60%) was ≈2.3-fold higher than the combined value for vehicle control males. The increase, while confined to the high-dose group and the 48-hour harvest interval, was also outside of the range for the vehicle control males. Results for females did not suggest a genotoxic effect. However, there was no convincing evidence that the maximum tolerated dose (MTD) was achieved. Significant ($p<0.01$) increases in micronuclei induction were observed in males treated with 50 mg/kg CP and females treated with 80 or 100 mg/kg CP at both harvest times.

Based on the overall results, the study authors concluded that Ordram technical was negative in the in vivo mouse micronucleus assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: Several factors preclude acceptance of the findings of the in vivo mouse micronucleus assay with Ordram technical as valid evidence of a negative response:

1. The increased incidence of MPEs in high-dose males of the 48-hour sacrifice group was not sufficient to conclude that Ordram technical was genotoxic; however, the findings are suspect. Owing to the wide variation in control data, we are unable to determine whether the increase was artifactual or related to treatment.

2. While there was no evidence that the test material caused micronuclei induction in the females, our reviewers have concerns regarding the selected doses. Based on the findings from the preliminary toxicity study showing that 3 of 5 females treated with 600 mg/kg died and no deaths occurred in groups receiving either one or two daily administrations of 400 mg/kg, the expected LD_{50} would fall within a dose range of 400 to 600 mg/kg. Therefore, an intermediate dose should have been chosen for the micronucleus assay. Additionally, the selection of 400 mg/kg as the high dose for females was not consistent with the reporting laboratory's rationale for dose selection (i.e., the MTD). As defined by the study authors, the MTD would be considered to be a dose that reduces the frequency of PCEs relative to

control values. Based on the preliminary data, two administrations of 400 mg/kg (total dose = 800 mg/kg) caused a reduction in PCEs; animals dosed once with 400 mg/kg were not evaluated for cytotoxic effects on bone marrow cells. The lack of overt signs of toxicity or cytotoxicity following exposure to the high dose (400 mg/kg), supports our assessment that a higher level of Ordram technical should have been tested in females.

3. Slides were not coded prior to analysis.

4. Purity information on the test material was not provided.

Based on the above considerations, we conclude that the study is unacceptable and should be repeated.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated November 22, 1983.)

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI p. 6; Appendix B, Protocol, CBI pp. 11-12.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§§ [illegible]) for genetic effects Category II, Structural Chromosomal Aberrations.

APPENDIX A

MATERIALS AND METHODS
CBI p. 6

Page _____ is not included in this copy.

Pages 28 through 31 are not included in this copy.

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Identity of product inert ingredients.

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✓ FIFRA registration data.

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

DOC 730213

FINAL

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DATA EVALUATION REPORT

MOLINATE

Study Type: Mutagenicity: Chromosomal Aberration and Sister Chromatid Exchange (SCE) Assays in Mouse Lymphoma Cells

Prepared for:

009562

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

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Fairfax, VA 22031-1207

Principal Reviewer _____ Date _____
Nancy E. McCarroll, B.S

Independent Reviewer _____ Date 11/2/92
Lynne Haber, Ph.D.

QA/QC Manager _____ Date 11/2/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-134
Clement Number: 93-132
Project Officer: James Scott

GUIDELINE § 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE CYTOGENETICS

MUTAGENICITY STUDIES

Approved by: Byron Backus, Ph.D
Review Section II,
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Signature: Byron T. Backus
Date: 11/6/92

EPA Reviewer: Linda Taylor
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Signature: Linda Lee Taylor
Date: 11-9-92

EPA Section Head: Clark Swentzel
Review Section II,
Toxicology Branch II/HED (H-7509C)

Signature: K. Clark Swentzel
Date: 11/12/92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Chromosomal aberration and sister chromatid exchange (SCE) assays in mouse lymphoma cells

EPA IDENTIFICATION Numbers:

EPA Registration Number: 476-2107

MRID Number: 470298-023

TEST MATERIAL: Ordram technical

SYNONYM/CAS NUMBER: Molinate

SPONSOR: Stauffer Chemical Co., Farmington, CT.

STUDY NUMBER: Report Number T-11856

TESTING FACILITY: Stauffer Chemical Co., Farmington, CT.

TITLE OF REPORT: ORDRAM® Technical (Lot No. WRC 4921-8-9) Mutagenicity Evaluation in Mouse Lymphoma Multiple Endpoint Test Cytogenetic Assay

AUTHORS: Majeska, J.B. and [illegible]theson, D.W.

REPORT ISSUED: December 2, 1983

CONCLUSIONS--EXECUTIVE SUMMARY: No conclusions relative to the potential of ordram technical to induce structural chromosome aberrations or sister chromatid exchange (SCE) in mouse lymphoma cells can be drawn from the series of nonactivated and S9-activated trials that were conducted. The data from the nonactivated assays performed with doses ranging from 0.0125 to 0.2 µL/mL of the test material were negative; higher nonactivated doses (0.3 µL/mL) were cytotoxic. However, the results from the three S9-activated chromosome aberration (dose range: 0.0025-0.1 µL/mL) and SCE (dose range: 0.0025-0.04 µL) assays produced conflicting results relative to cytotoxicity (chromosome aberration tests) and genotoxicity (chromosome aberration and SCE tests). For

a detailed discussion see Sections C and D (Reported Results and Reviewers' Discussion/Conclusion). Additionally, purity information on the test material was not provided and the report did not indicate whether slides were coded prior to analysis. We conclude, therefore, that the study is unacceptable. This study classification is not upgradeable.

STUDY CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84-2) for genetic effects Category II, Structural Chromosomal Aberrations.

A. MATERIALS:

1. Test Material: Ordram technical

Description: Amber liquid
Identification number: Lot numbers: WRC 4921-8-9; EHC-0525-19; T-11856
Purity: Not listed
Receipt date: July 28, 1983
Stability: Expiration date--February 1984
Contaminants: None listed
Vehicle used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored at ambient temperature and humidity protected from light. The frequency of dose solution preparation was not reported; analytical determinations were not performed on the dosing solutions.

2. Control Materials:

Negative: Untreated cells grown in either RPMI 1640 or Fischer's medium supplemented with 10% horse serum, 2 mM glutamine, 22 mg/mL sodium pyruvate, 50 mg/mL pluronic, and antibiotics.

Solvent/concentration: 1% DMSO

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in an unspecified solvent to yield a final concentration of 0.5 µL/mL.

Activation (concentrations, solvent): Dimethylnitrosamine (DMN) was prepared in an unspecified solvent to yield a final concentration of 0.05 µL/mL.

3. Activation: S9 derived from male Sprague-Dawley

__x__ Aroclor 1254	__x__ induced	__x__ rat	__x__ liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The S9 homogenate was prepared by the performing laboratory and was identified as lot number EHC-0476-11.

The composition of the S9 mix per 10 mL of treatment medium was as follows:

S9 mix composition:

Component	Amount/10 mL Culture
NADP	240 µg
Isocitrate acid	450 µg
S9	1 mL

4. Test Compound Concentration Used:

(a) Cytotoxicity assay: Cytotoxicity was assessed in parallel with the initial chromosome aberration and SCE tests. Six nonactivated doses (0.0125, 0.025, 0.05, 0.1, 0.2, and 0.3 µL/mL) and nine S9-activated doses (0.00125, 0.0025, 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, and 0.1 µL/mL) were evaluated.

(b) Cytogenetic assays:

- Chromosome aberration test: One nonactivated and three S9-activated trials were conducted; doses were as follows:

  Nonactivated: 0.0125, 0.025, 0.05, 0.1, and 0.2 µL/mL

  S9-activated: 0.0025, 0.005, 0.01, 0.02, and 0.04 µL/mL (Trial 1)
  As above for the first S9-activated trial (Trial 2)
  0.02, 0.04, 0.06, 0.08, and 0.1 µL/mL (Trial 3)

- SCE test: One nonactivated and three S9-activated trials were conducted; doses were as follows:

  Nonactivated: As above for the nonactivated chromosome aberration test

  S9-activated: 0.0025, 0.005, 0.01, 0.02, and 0.04 µL/mL (Trial 1)
  As above for the first S9-activated SCE test (Trial 2)
  0.02 and 0.04 µL/mL (Trial 3)

5. Test Cells: The mouse lymphoma cell line L5178Y ($TK^{+/-}$) was obtained from Dr. Donald Clive, Burroughs Wellcome Co., Research Triangle Park, NC.

Properly maintained? Yes.

Cell line or strain periodically checked for mycoplasma contamination? Yes.

Cell line or strain periodically checked for karyotype stability? Not reported.

B. TEST PERFORMANCE:

1. Cell Treatment: Cells, seeded at a density of $6x10^5$ cells/mL, were exposed to the selected nonactivated or S9-activated doses of the test material, the negative control (medium) or solvent control (DMSO) for 4 hours. Cultures were assessed for viability, the percentage of first division metaphases, and the relative percent staining index (i.e., percent second and third division metaphases in the treatment group relative to the vehicle control group). Based on the findings, doses were selected for continuation in the chromosome aberration and SCE tests.

   Cells exposed to the selected doses were resuspended in culture medium containing 10^{-4} mM BrdU, incubated for 21 hours, and dosed with 0.1 µg/mL colcemid for three hours. Metaphase cells were collected, treated with a hypotonic solution, and fixed in Carnoy's fixative. Slides were stained with Giemsa (chromosome aberrations) or with Hoechst's stain, exposed to dark light, and counterstained with Giemsa (SCEs).

2. Metaphase Analysis: The first 50 well-defined metaphases per culture were scored for chromosome aberrations. The mitotic index (MI) was determined by counting the number of mitoses/500 cells. For the evaluation of SCEs, 20 to 40 cells per culture were scored. The report did not indicate whether slides were coded prior to analysis.

3. Statistical Analyses: Structural aberrations were analyzed using Student's T-test (one-tailed). The SCE frequency/cell was analyzed by Student's t-test (two-tailed).

4. Evaluation Criteria:

   (a) Assay acceptability: The assays were considered valid if (1) the solvent and positive control results were within the historical range established by the performing laboratory (Note: Historical negative and solvent control data were not provided) and (2) a sufficient number of cells were available for analysis in the "control" and at least three treatment groups.

   (b) Positive response: A test material was considered positive for either chromosome aberrations or SCE induction if the results were significantly different ($p<0.01$) from the solvent control data.

C. REPORTED RESULTS:

1. Chromosome Aberration Test: No rationale was provided for the selection of doses. The cytotoxicity assessment was conducted in parallel with the initial chromsome aberration assay.

   (a) Nonactivated conditions: In the nonactivated system, relative percent growth (RPG) was dose related and ranged from 26% at the highest concentration (0.3 µL/mL) to 70% at the lowest dose (0.0125 µL/mL). No mitotic cells were recovered at the highest dose; however, mitotic delay was not apparent at the remaining concentrations. Based on these results, cultures exposed to 0.0125, 0.025, 0.05, 0.1, and 0.2 µL/mL were selected for the cytogenetic evaluation.

   As shown in Table 1, the MI at the highest dose (0.2 µL/mL) was increased relative to the negative and vehicle controls. There was also no indication that ordram technical was clastogenic over the assayed concentration. By contrast, the nonactivated positive control (0.5 µL/mL EMS) induced a significant ($p<0.01$) increase in the percentage of cells with structural aberrations.

   (b) S9-activated conditions: In the presence of S9 activation, the test material was more cytotoxic as indicated by the marked reduction in survival (≤50%) at doses (0.04, 0.06, 0.08, and 0.1 µL/mL) lower than those investigated without S9 activation. Similarly, no metaphases were recovered from cultures treated with concentrations ≥0.06 µL/mL. At lower doses, no clear indication of interference with cell-cycle kinetics was seen. Based on these results, cultures exposed to 0.0025, 0.005, 0.01, 0.02, and 0.04 µL/mL were assessed for chromosome damage. In the first S9-activated trial, ordram technical was not clastogenic (Table 1) and the expected results were obtained with the positive control (0.05 µL/mL DMN +S9).

   No explanation was provided for the performance of a second S9-activated trial with doses equivalent to those used in Trial 1 (0.0025, 0.005, 0.01, 0.02, and 0.04 µL/mL). As the data presented in Table 2 show, MIs at all test doses were either higher or comparable to the negative and solvent control results. In contrast to the findings from Trial 1, however, significant ($p<0.05$) increases in the percentage of cells with aberrations were noted at 0.01 and 0.02 µL/mL. At both levels, simple (chromatid breaks and/or acentric fragments) and complex (triradials, quadriradials, and/or translocations) structural aberrations were seen. The assay was, therefore, repeated with doses of 0.02, 0.04, 0.06, 0.08, and 0.1 µL/mL. The MI at 0.08 and 0.1 µL/mL was higher than both the negative and solvent controls. The slight reductions in MIs at lower concentrations (i.e., ≤15% reduction compared to the vehicle control) were insufficient to conclude that the test material had an adverse effect on the mouse lymphoma cells. Since the study authors did

TABLE 1 Representative Results of the Initial Chromosomal Aberration Assay in Mouse Lymphoma Cells with Ordram Technical

Substance	Dose/mL	S9 Activation	Percent Mitotic Index	Total Number of Cell Cell Scored	Total Number Aberrations	No. Aberrations/Cell	% Cells with Aberrations	% Cells with >2 Aberrations	Biologically[a] Significant Aberrations No./type
Negative Control									
Medium	--	-	8.0	100[b]	2	0.02	2	0	1TB; 1T
	--	+	6.9	100[b]	0	0.00	0	0	--
Solvent Control									
Dimethyl sulfoxide	0.01 mL	-	6.9	100[b]	1	0.01	1	0	1T
	0.01 mL	+	9.0	100[b]	0	0.00	0	0	--
Positive Control									
Ethyl methanesulfonate	0.5 µL	-	11.2	50	26	0.52	32*	14	6TB; 1F; 2AF; 11T; 4TR; 2CR
Dimethylnitrosamine	0.05 µL	+	10.0	50	24	0.48	40*	10	3TB; 2TD; 4F; 2T; 9TR; 3QR; 1CR
Test Material									
Ordram technical	0.2 µL[c]	-	9.6	50	1	0.02	2	0	1F
	0.04 µL[c]	+	12.2	50	0	0.00	0	0	--

[a]Abbreviations Used:

TB = Chromatid Break
TD = Chromatid Deletion
F = Fragment
AF = Acentric Fragment
TR = Triradial
QR = Quadriradial
CR = Complex Rearrangement
T = Translocation

[b]Duplicate cultures were prepared for the negative and solvent control groups; average values for these groups were calculated by our reviewers.

[c]Higher doses (0.3 µL/mL -S9 and 0.06, 0.08, and 0.1 µL/mL +S9) were cytotoxic. Results for lower levels (0.0125 0.025, 0.05, and 0.1 µL/mL -S9 and 0.0025, 0.005, 0.01, and 0.02 µL/mL +S9) did not suggest a clastogenic effect.

*Significantly higher ($p<0.01$) than the corresponding solvent control by Student's t-test.

Note: Data were extracted from the study report; see CBI pp. 41-42

not comment on the condition of the cells, cell survival, or the availability of mitotic cells, our reviewers assume that ordram technical was not cytotoxic up to 0.1 µL/mL. This finding conflicts with the data from Trial 1 indicating that no mitotic cells were recovered from cultures treated with 0.06, 0.08, or 0.1 µL/mL +S9. As further shown in Table 2, the presence of rare complex structural aberrations in the negative (2 translocations) and vehicle (3 translocations) control groups, confounds the interpretation of the test material results. The relevance, therefore, of similar aberrations in three treatment groups (0.04, 0.08, and 0.1 µL/mL) and a triradial in the 0.06-µL/mL group can not be determined. There were, however, no significant increases in the frequency of structural chromosome aberrations at any dose.

2. SCE Assays: A similar approach (i.e., one complete nonactivated and S9-activated trial and two additional S9-activated trials) was used for the SCE assays. Dose selection for the initial SCE assay was governed by the cytotoxicity assessment described for the first nonactivated and S9-activated chromosome aberration tests.

(a) Trial 1: Nonactivated doses ranging from 0.0125 to 0.2 µL/mL were neither cytotoxic nor genotoxic (Table 3). Similarly, the results from the first S9-activated trial provided no convincing evidence that the selected doses of ordram technical (0.0025, 0.005, 0.01, 0.02, or 0.04 µL/mL) were either cytotoxic or genotoxic. Presumably, the S9-activated phase of testing was repeated because of the significant ($p<0.05$) increase in SCEs/cell observed at the lowest assayed concentration.

(b) Additional S9-activated trials: Significant but not dose related increases in SCEs/cell were noted at concentrations ranging from 0.0025 to 0.02 µL/mL in the second S9-activated trial (Table 4). SCE frequencies for the highest level (0.04 µL/mL) were slightly lower than the solvent control. It was noteworthy, that significant clastogenic effects were also obtained in the second S9-activated chromosome assay (Table 2) at comparable levels.

It was of additional note that both the second SCE and chromosome aberration assays were initiated on the same day (see CBI pp. 40 and 47). For the third trial, 0.02 and 0.04 µL/mL +S9 were assayed. At both doses, the test material was not genotoxic (Table 4). No explanation was provided for counting fewer cells (48 total cells) in the high-dose group. Although this would tend to suggest that fewer than 80 metaphases were available for analysis, only 49 cells were counted in the negative control cultures. We are, therefore, unable to determine whether cytotoxicity was achieved at 0.04 µL/mL +S9. Based on a comparative evaluation of the S9-activated chromosome aberration tests and the S9-activated SCE assays, it appears unlikely that 0.04 µL/mL was cytotoxic.

TABLE 2. Results of the Additional S9-Activated Chromosomal Aberration Assays in Mouse Lymphoma Cells with Ordram Technical

Substance	Dose/mL	Percent Mitotic Index	Total Number of Cells Scored	Total Number Aberrations	No. Aberrations/Cell	% Cells with Aberrations	% Cells with >2 Aberrations	Biologically[a] Significant Aberrations No./type
Negative Control								
Medium	--	8.2[b]	100[d]	0	0.00	0	0	--
	--	8.3[c]	100[d]	5	0.05	3	2	2TB; 2T; 1F
Solvent Control								
Dimethyl sulfoxide	0.01 mL	6.7[b]	100[d]	2	0.02	2	0	2TB
	0.01 mL	11.3[c]	100[d]	5	0.05	5	0	1TB; 3T; 1F
Positive Control								
Dimethylnitrosamine	0.05 µL	11.2[b]	10	12	1.20	50**	50	1TB; 2F; 5T; 3TR; 1CR
	0.05 µL	9.6[c]	50	51	1.02	46**	22	16TB; 2F; 17T; 5TR; 4QR; 6CR; 1R
Test Material								
Ordram technical	0.0025 µL	9.6[b]	50	1	0.02	2	0	1TB
	0.005 µL	10.8	50	2	0.04	4	0	1AF; 1PU
	0.01 µL	8.2	50	4	0.08	8*	0	1F; 1AF; 1T; 1TR
	0.02 µL	11.6	50	4	0.08	8*	0	1TB; 1AF; 1TR; 1QR
	0.04 µL	11.2	50	1	0.02	2	0	1TB
	0.02 µL	10.3[c]	100[d]	2	0.02	2	0	1TB; 1F
	0.04 µL	10.1	150[d]	3	0.02	1.3	0.7	1AF; 1F; 1T
	0.06 µL	9.5	50	1	0.02	2	0	1TR
	0.08 µL	11.5	73[d]	3	0.04	2.7	1.4	1TB; 1T; 1F
	0.1 µL	15.8	50	5	0.01	6	2	2TB; 2T; 1F

[a]Abbreviations Used:

TB = Chromatid Break
F = Fragment
AF = Acentric Fragment
TR = Triradial
QR = Quadriradial
CR = Complex Rearrangement
T = Translocation
R = Ring
PU = Pulverized Chromosome

[b]Results from trial 2

[c]Results from trial 3

[d]Duplicate cultures were prepared for the negative and solvent control groups and the indicated test material dose groups; average values for these groups were calculated by our reviewers.

*Significantly higher ($p<0.05$) than the corresponding solvent control by Student's t-test.
**Significantly higher ($p<0.01$) than the corresponding solvent control by Student's t-test.

Note: Data were extracted from the study report; see CBI pp. 43-44

TABLE 3 Representative Results from the Initial Sister Chromatid Exchange Assay in Mouse Lymphoma Cells with Ordram Technical

Substance	Dose/mL	S9 Activation	Number of Cells Counted[a]	Number of Chromosomes[a]	Number of SCE[a]	Number of SCE/ Chromosome[a]	Number of SCE/Cell[a]
Negative Control							
Medium	--	-	80	3164	801	0.25	10.0
	--	+	80	3159	762	0.24	9.5
Solvent Control							
Dimethylsulfoxide	0.01 mL	-	80	3160	702	0.22	8.7
	0.01 mL	+	80	3151	687	0.22	8.6
Positive Controls							
Ethyl methanesulfonate	0.5 µL	-	40	1598	1971	1.23	49.3*
Dimethylnitrosamine	0.05 µL	+	40	1581	2122	1.34	53.1*
Test Material							
Ordram technical	0.2 µL[b,c]	-	40	1571	352	0.22	8.8
	0.0025 µL[d]	+	40	1577	452	0.29	11.3*
	0.04 µL[b]	+	40	1575	326	0.21	8.2

[a]Duplicate cultures were prepared for the negative and solvent control groups; average values for these groups were calculated by our reviewers.
[b]Higher doses (0.3 µL/mL -S9 and 0.06, 0.08, and 0.1 µL/mL +S9) were cytotoxic.
[c]Results for the lower doses (0.0125, 0.025, 0.05, or 0.1 µL/mL) did not suggest a genotoxic effect.
[d]Lowest assayed level; results for intermediate concentrations (0.005, 0.01, or 0.02 µL/mL) did not suggest a genotoxic effect.

*Significantly higher ($p<0.001$) than the solvent control by Student's t-test.

Note: Data were extracted from the study report; see CBI p. 46

TABLE 4 Results from the Additional S9 Activated Sister Chromatid Exchange Assays in Mouse Lymphoma Cells with Ordram Technical

Substance	Dose/mL	Number of Cells Counted[a]	Number of Chromosomes[a]	Number of SCE[a]	Number of SCE/ Chromosome[a]	No. of SCE/Cell[a]
Negative Control						
Medium	-- --	80[b] 49[c]	3169 1906	840 484	0.27 0.25	10.5 9.9
Solvent Control						
Dimethylsulfoxide	0.01 mL 0.01 mL	80[b] 80[c]	3171 3141	795 852	0.25 0.27	10.0 10.7
Positive Controls						
Dimethylnitrosamine	0.05 µL 0.05 µL	40[b] 40[c]	1569 1572	2348 2108	1.50 1.34	58.7** 52.7**
Test Material						
Ordram technical	0.0025 µL 0.005 µL 0.01 µL 0.02 µL 0.04 µL	40[b] 40 40 40 40	1581 1572 1562 1586 1586	457 452 519 502 417	0.29 0.29 0 33 0 32 0.26	11.4* 11.3* 13.0** 12.6** 10.4
	0.02 µL 0.04 µL	80[c] 48	3093 1875	743 386	0.24 0.21	9.3 8.0

[a]Duplicate cultures were prepared for the negative, solvent, and two treatment groups; average values for these groups were calculated by our reviewers.
[b]Results from trial 2
[c]Results from trial 3

*Significantly higher ($p<0.05$) than the solvent control by Student's t-test.
**Significantly higher ($p<0.001$) than the solvent control by Student's t-test.

Note: Data were extracted from the study report; see CBI pp. 47-48

From the overall results, the study authors concluded that "Ordram Technical is neither clastogenic nor an inducer of SCE in L5178Y mouse lymphoma cells when tested directly or in the presence of an Aroclor 1254 induced rat liver activation system."

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that no conclusion relative to the potential of ordram technical to induce genotoxic effects in mouse lymphoma cells can be reached from this series of chromosome aberration or SCE assays. The overall study was seriously compromised for the following reasons:

1. The inability to reproduce reasonably comparable cytotoxic effects in the S9-activated chromosome aberration trials casts doubts on the overall performance of the study. Since all trials were conducted (September to December 1983) before the expiration date of the test material (February 1984), we assume that test material stability was not a problem. It is, therefore, conceivable that dosing errors account for the difference in cytotoxicity between the trials (i.e., 0.04 µL/mL was the highest dose that could be assayed in the first trial because no mitotic cells were recovered following treatment with doses >0.06 µL/mL; however, at doses up to 0.1 µL/mL in Trial 3, a sufficient number of metaphases were found). It is acknowledged that the range of S9-activated doses that was used in the chromosome aberration assays (0.0025–0.1 µL/mL) was narrow, however, the marked difference in the cytotoxicity response between the first and final S9-activated trials cannot be overlooked. The issue was not resolved by the results of Trial 2 since 0.04 µL/mL was the highest assayed dose.

2. Of additional concern were the significant increases in chromosome aberration yield and SCE induction in the second S9-activated trial. The results were not confirmed in the subsequent experiments. However, the chromosome aberration data from Trial 3 could not be interpreted because of confounding negative and solvent control results. Similarly, an insufficient number of doses were investigated in the third SCE trial. Given the concerns regarding dose solution preparation and the lack of analytical data to verify actual concentrations, there is no assurance that equivalent doses were assayed for SCE induction in Trial 3.

3. Purity information on the test material was not provided.

Based on the above considerations, we conclude that the study is unacceptable and should be repeated.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated December 2, 1983.)

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F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 48. Appendix B Protocol, CBI pp. 49-54.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84-2) for genetic effects Category II, Structural Chromosomal Aberrations.

APPENDIX A

MATERIALS AND METHODS
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Page _____ is not included in this copy.

Pages 46 through 53 are not included in this copy.

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