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CASWELL FILE

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

JUN 28 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Fatty Alcohol Blend: Review of Mutagenicity Studies

FROM: Steven L. Malish, Ph.D., Toxicologist S.L. Malish 6/22/93
Tox. Branch II, Review Section IV
HED (H7509C)

TO: Bruce Sidwell, Product Manager (53)
Margarita Collantes, PM Team Reviewer
Reregistration Division (H7508W)

THRU: Jess Rowland, M.S., Acting Section Head Jess Rowland 6/23/93
Tox. Branch II, Review Section IV
HED (H7509C)

and

Marcia van Gemert, Ph.D., Branch Chief Marcia van Gemert 6/23/93
Tox Branch II; HED (H7509C)

Task Identification: Submission: S421382 DP Barcode: D180480
P.C. Code: 079029 Caswell No.: 456E

ACTION REQUESTED: Review of Mutagenicity Studies [MRID No.: 423720-01, -02, -03, -04].

Response:

Data Evaluation Reports for the above referenced studies are attached. Summaries are provided below.

1. MRID 243720-01: In vivo mammalian cytogenetics micronucleus assay in mice.

Negative for micronucleus induction in bone marrow cells of male and female CD-1 mice harvested 24 or 48 hours post-administration of three daily oral gavage doses of 500, 1000, or 2000 mg/kg/day (total dosages of 1500, 3000 or 6000 mg/kg). No overt toxicity in the treated animals or cytotoxicity in the target organ seen in any treatment group.

CORE CLASSIFICATION: Acceptable. This study satisfies the Guideline requirements (84-2b) for genetic effects, Category II, Structural Chromosome Aberrations, and is acceptable for regulatory purposes.

2. MRID 243720-02: Salmonella typhimurium/mammalian microsomal mutagenicity assay

Negative for reverse gene mutation in Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100, exposed in the presence or absence of S9 activation to six doses ranging from 1.5 ug/plate to 500 ug/plate (two independent trails). Cytotoxicity was apparent for all strains at 500 ug/plate +/- S9.

CORE CLASSIFICATION: Acceptable. This study satisfies the Guideline requirements (84-2a) for genetic effects, Category I, Gene Mutation and is acceptable for regulatory purposes.

3. MRID 243720-03: Gene mutation in cultured mammalian cells (mouse lymphoma cells)

Under the conditions of the mouse lymphoma forward mutation assay, fatty alcohol blend was tested in two independent assays with and without activation and found not to be mutagenic. In the initial assay, non-activated and S9-activated levels ranging from 9.4 ug/ml to 37.5 ug/ml were evaluated; doses ≥75 ug/ml were severely cytotoxic. In the confirmatory assay, 10-50 ug/ml -S9 and 30-70 ug/ml +S9 were evaluated. Severe cytotoxicity was observed at non-activated levels ≥60 ug/ml and at S9-activated 80 ug/ml. Based on these findings, we conclude that the fatty alcohol blend was tested to cytotoxic levels and found not to be mutagenic in this assay system.

CORE CLASSIFICATION: Acceptable. This study satisfies the Guideline requirements (84-2a) for genetic effects, Category I, Gene Mutations and is acceptable for regulatory purposes.

4. MRID 243720-04: Establishment of Methodology for the Analysis of Fatty Alcohol Blend in Dosing Solutions, Assessment of Homogeneity, Accuracy and Stability and the Routine Analysis of Dosing Solutions; the information contained in this report was incorporated in the data evaluation reports listed above.

FINAL

DATA EVALUATION REPORT

FATTY ALCOHOL BLEND

Study Type: Mutagenicity: In Vivo Micronucleus Assay with Mice

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 Kristin Jacobson, MSPH	Date 5/21/93
Independent Reviewer	Nancy E. McCarroll Nancy E. McCarroll, B.S.	Date 5/21/93
QA/QC Manager	Sharon Segal Sharon Segal, Ph.D.	Date 5/24/93

Contract Number: 68D10075
Work Assignment Number: 2-40
Clement Number: 123
Project Officer: Caroline Gordon

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MUTAGENICITY STUDIES

EPA Reviewer: Steve Malish. Ph.D. Signature: S. J. Malish
Review Section IV, Toxicology Branch II (H7509C) Date: 6/22/93
EPA Acting Section Head: Jess Rowland, M.S. Signature: Jess Rowland
Section IV, Toxicology Branch II (H7509C) Date: 6/21/93

DATA EVALUATION REPORT

CHEMICAL: Fatty alcohol blend, lot number CSI-91FA01-27

EPA IDENTIFICATION NUMBERS:

Submission No.: S421382

Caswell No.: 456E

DP Barcode: 180480

PC Code: 079029

MRID Number: 423720-01

STUDY TYPE: In vivo mammalian cytogenetics micronucleus assay in mice

SYNONYMS: None indicated

SPONSOR: Compliance Services International, Sunnyvale, CA, for: Fatty Alcohol Task Force

TESTING FACILITY: Inveresk Research International, Ltd., Tranent, Scotland

TITLE OF REPORT: Fatty Alcohol Blend Micronucleus Test in Bone Marrow of CD-1 Mice

AUTHORS: M. Holmstrom and D. Innes

STUDY NUMBERS: IRI Project No. 751943; IRI Report No. 8568

REPORT ISSUED: February 11, 1992

CONCLUSIONS--EXECUTIVE SUMMARY: Negative for micronucleus induction in bone marrow cells of male and female CD-1 mice harvested 24 or 48 hours post-administration of three daily oral gavage doses of 500, 1000, or 2000 mg/kg/day (total dosage 1500, 3000, or 6000 mg/kg). No overt toxicity in the treated animals or cytotoxic effects on the target organ were seen in any treatment group.

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2b) for genetic effects Category II, Structural Chromosome Aberrations, and is acceptable for regulatory purposes.

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A. MATERIALS

1. Test Material: Fatty alcohol blend

Description: Colorless liquid
Identification number: Lot no. CSI-91FA01-27
Purity: Total average weight % alcohol: 96.6% (55.3% decanol, 40.7% octanol, 0.4% dodecanol, and 0.2% hexanol)
Receipt date: October 24, 1991
Stability: Not reported
Contaminants: None listed
Vehicle used: Corn oil
Other provided information: The test material was stored at room temperature in the dark. Analytical determinations were performed on test solutions used in the micronucleus assay. The frequency of dosing solution preparation was not reported; however, the stability of the dosing solutions was verified. The methodology used for the stability testing was not provided.

2. Control Materials:

(a) Acute dose-range finding study:

Vehicle/route of administration: Mice received corn oil via oral gavage once a day for 3 days at a dosing volume of 10 ml/kg

(b) Cytogenetic assay:

Vehicle/route of administration: Corn oil was administered by oral gavage once a day for 3 days at a dosing volume of 10 mL/kg.

Positive/final concentration/route of administration: Cyclophosphamide (CP) was dissolved in distilled water and administered by oral gavage once a day for 3 days at a final concentration of 40 mg/kg/day and a dosing volume of 10 mL/kg.

3. Test Compound:

Route of administration: Three daily administrations by oral gavage

Volume of test substance administered: 10 mL/kg

Dose levels used:

- Preliminary toxicity test: 400, 800, 1200, 1600, and 2000 mg/kg/day
- Main toxicity test: 2000 mg/kg/day
- Micronucleus assay: 500, 1000, and 2000 mg/kg/day

Note: Dosing was based on individual body weights determined immediately before compound administration. In the micronucleus

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assay, animals were assigned to dose groups using a computer-generated randomization.

4. Test Animals:

(a) Species: Mouse Strain: CD-1 Age: ≈5-8 weeks (at dosing)
Weight Range:

- Preliminary toxicity test: 26-31 g (males); 20-23 g (females)
- Main toxicity test: 27-30 g (males); 18-23 g (females)
- Micronucleus assay: 26-36 g (males); 19-27 g (females)

Sex: Male and female Source: Charles River, Ltd., Kent, UK.

(b) Number of animals used (per dose, per sacrifice time):

- Preliminary toxicity test: 2 males, 2 females
- Main toxicity test: 5 males, 5 females
- Micronucleus assay:

Treatment groups:	5 males	5 females
Positive control:	5 males	5 females
Vehicle control:	5 males	5 females

(c) Were test animals properly maintained? Yes. Although humidity in the animal rooms (ranging from 29 to 48%) was outside the range recommended by NIH guidelines (40-70%), this deviation was judged to have not adversely affected the outcome of the study.

B. TEST PERFORMANCE:

1. Range-finding Studies: Animals received three administrations of the selected doses of the test material and were observed for signs of toxicity and/or mortality on the day of dosing and daily thereafter for 5-6 days. At study termination, animals were killed by CO_2 asphyxiation and gross necropsies were performed on all treated animals.

2. Micronucleus Assay:

Treatment and sampling times:

(a) Test compound:
Dosing: _____ once _____ twice
__x__ other: three administrations (24 hours apart)

Sampling (after last dose): _____ 6 hours _____ 12 hours
__x__ 24 hours __x__ 48 hours _____ 72 hours

Note: Sampling was performed on the low- and mid-dose animals only at 24 hours.

(b) Vehicle control:
Dosing: _____ once _____ twice
__x__ other: three administrations (24 hours apart)
Sampling (after last dose): __x__ 24 hours __x__ 48 hours
_____ 72 hours

(c) Positive control:
Dosing: _____ once _____ twice
__x__ other: three administrations (24 hour apart)
Sampling (after last dose): __x__ 24 hours _____ 48 hours
_____ 72 hours

3. Tissues and Cells Examined:

__x__ bone marrow _____ other (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: 1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: Number observed while scoring 300 erythrocytes (PCEs + NCEs)

4. Details of Cell Harvest and Slide Preparation: At 24 and 48 hours after the final administration of the high dose (2000 mg/kg/day) of the test material or the vehicle control, the appropriate groups of animals were sacrificed by cervical dislocation. Animals in the low- and mid-dose groups (500 and 1000 mg/kg/day) and in the positive control group were sacrificed 24 hours postexposure. Bone marrow cells from one femur of each animal were flushed into coded tubes containing fetal calf serum and 0.8% trisodium citrate in Sorenson's buffer (1:1 solution), centrifuged, resuspended and spread onto slides. Slides were air-dried, fixed in methanol, stained with 1% May-Grunwald, counterstained with 15% Giemsa, cleared with Histo-Clear® and coverslipped. Two slides were prepared per animal, and the "better" of the two coded slides was scored for micronucleated PCEs (MPEs) and the PCE:NCE ratio.

5. Statistical Methods: The data were not analyzed statistically.

6. Evaluation Criteria: Using the criteria of Salamone et al. (1980)[1] and assuming a background frequency of MPEs in CD-1 mice of 0.122% for both sexes combined (per Tamura et al. (1990)[2]), the test material was considered positive if, in a group of five animals, there were >14 (>0.28%) MPEs. For the combined results of groups of ten animals (5/sex), a positive response required >24 (>0.24%) MPEs.

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[1]Salamone, M.F., Heddle, J.A., and Katz, M. (1980). The mutagenic activity of 41 compounds in the in vivo micronucleus assay. In: J. Ashby and F. deSerres (eds.). Proceedings of the International Program for the Evaluation of Short-Term Tests for Carcinogenicity, Elsevier, Amsterdam, pp. 686-697.

[2]Tamura, R.N., Garriott, M.L., and Parton, J.W. (1990). Pooled inference across sexes for the in vivo micronucleus assay. Mutat Res 240:127-133.

Note: The cumulative historical incidence of the performing laboratory for micronuclei in CD-1 mice treated with distilled water, corn oil, or 0.5% carboxymethyl cellulose was reported to be 0.121%.

7. Protocol: Not provided

C. REPORTED RESULTS:

1. Range-finding Studies: In the preliminary toxicity test, two animals per sex were exposed to doses ranging from 400-2000 mg/kg/day for three consecutive days. No mortality occurred. The only clinical signs that were noted were piloerection (all dose levels in both sexes) and reduced activity (one animal at 1200 mg/kg/day); neither the incidence nor duration of these symptoms was reported. There were no observable macroscopic changes at necropsy.

   Based on these results, five animals per sex were exposed to 2000 mg/kg/day for 3 consecutive days. There were no deaths, adverse effects, or gross changes reported in any treated animals. The study authors, therefore, determined that the MTD was >2000 mg/kg/day and, as directed by the sponsor, selected dose levels of 500, 1000, and 2000 mg/kg/day for the micronucleus assay.

2. Micronucleus Assay: In agreement with the findings of the two toxicity tests, there were no deaths or adverse clinical signs in animals exposed to 2000 mg/kg/day for three consecutive days in the micronucleus assay. There were also no appreciable increases in MPEs for any treated animals or sexes combined (see Attachment A). In addition, no cytotoxicity was observed (i.e., there were no appreciable reductions in mean PCE:NCE ratios). By contrast, the positive control (40 mg/kg/day CP) induced marked increases in the frequency of both MPEs and micro-nucleated NCEs.

3. Analytical Determination: Analysis of the dosing solutions revealed that the low-dose solution (target concentration 50 mg/mL) was 13.6-14.4% below the target; the achieved concentrations for the two higher dose groups were within 5% of the target concentrations. The deviation in the low-dose solution was judged not to have affected the outcome of the study.

Based on the overall results, the study authors concluded that fatty alcohol blend (lot no. CSI-91FA01-27) was not genotoxic in this in vivo mouse micronucleus assay.

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GUIDELINE SERIES 84: MUTAGENICITY
MICRONUCLEUS

D. REVIEWERS' DISCUSSION/CONCLUSIONS: In bone marrow erythrocytes of male and female mice exposed to 500, 1000, or 2000 mg/kg/day fatty alcohol blend (lot no. CSI-91FA01-27) by oral gavage for three consecutive days, there were no appreciable increases in the frequency of micronucleated cells. No signs of overt toxicity in the animals or cytotoxicity in the target organ were seen, and the total high dosage delivered to the animals (6000 mg/kg) exceeded the limit dose for short term testing. It was, therefore, concluded that the test material was adequately evaluated and failed to induce a genotoxic response in this in vivo test system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement, indicating that the report was audited and that the in-life phase of this type of study is inspected on a pre-determined schedule, was signed and dated June 16, 1992.)

F. ATTACHMENTS: Attachment A, Summarized Study Results, Report p. 25.

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2b) for genetic effects Category II, Structural Chromosome Aberrations, and is acceptable for regulatory purposes.

ATTACHMENT A

SUMMARIZED STUDY RESULTS

Study Report p. 25

TABLE 1

Fatty Alcohol Blend: Micronucleus Test in Bone Marrow of CD-1 Mice

Treatment	Time of Dosing (h)	Time of Sampling (h)	Sex	No. of Dosed Mice (No. of Surviving Mice)	Erythrocytes				
					Normochromatic Cells (NCE)	Polychromatic Cells (PCE)			PCE/NCE Mean ± S.D.
					No. of MN-NCE	PCE Analysed	No. of MN-PCE	% MN-PCE	
10 ml Corn oil.kg^{-1}.day^{-1}	0+24+48	72	♂	5 (5)	2	5000	6	0.12	0.89 ± 0.23
			♀	5 (5)	1	5000	8	0.16	0.97 ± 0.07
			♂♀	10 (10)	3	10000	14	0.14	0.93 ± 0.16
		96	♂	5 (5)	2	5000	3	0.06	1.05 ± 0.15
			♀	5 (5)	2	5000	7	0.14	0.98 ± 0.24
			♂♀	10 (10)	4	10000	10	0.10	1.02 ± 0.19
40 mg Cyclophosphamide.kg^{-1}.day^{-1}	0+24+48	72	♂	5 (5)	41ϕ	5000	81*	1.62	0.39 ± 0.18
			♀	5 (5)	50ϕ	5000	69*	1.38	0.53 ± 0.11
			♂♀	10 (10)	91ϕ	10000	150*	1.50	0.46 ± 0.16
500 mg Fatty Alcohol Blend.kg^{-1}.day^{-1}	0+24+48	72	♂	5 (5)	2	5000	4	0.08	0.90 ± 0.19
			♀	5 (5)	3	5000	5	0.10	1.10 ± 0.23
			♂♀	10 (10)	5	10000	9	0.09	1.00 ± 0.23
1000 mg Fatty Alcohol Blend.kg^{-1}.day^{-1}	0+24+48	72	♂	5 (5)	3	5000	6	0.12	1.01 ± 0.10
			♀	5 (5)	4	5000	3	0.06	0.86 ± 0.23
			♂♀	10 (10)	7	10000	9	0.09	0.94 ± 0.18
2000 mg Fatty Alcohol Blend.kg^{-1}.day^{-1}	0+24+48	72	♂	5 (5)	2	5000	4	0.08	0.84 ± 0.29
			♀	5 (5)	6	5000	4	0.08	0.87 ± 0.13
			♂♀	10 (10)	8	10000	8	0.08	0.86 ± 0.21
		96	♂	5 (5)	5	5000	12	0.24	0.78 ± 0.18
			♀	5 (5)	3	5000	12	0.24	1.02 ± 0.09
			♂♀	10 (10)	8	10000	24	0.24	0.90 ± 0.18

PCE = Polychromatic erythrocytes
NCE = Normochromatic erythrocytes
* = Positive response in PCE

MN-PCE = Micronucleated PCE
MN-NCE = Micronucleated NCE
ϕ = Positive response in NCE

FINAL

DATA EVALUATION REPORT

FATTY ALCOHOL BLEND

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 Date 5/21/93
Kristin Jacobson, MSPH

Independent Reviewer Nancy E. McCarroll Date 5/21/93
Nancy E. McCarroll, B.S.

QA/QC Manager Sharon Segal Date 5/24/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-40
Clement Number: 124
Project Officer: Caroline Gordon

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MUTAGENICITY STUDIES

EPA Reviewer: Steven Malish, Ph.D. Signature: S.J. Malish
Section IV, Toxicology Branch II (H7509C) Date: 6/22/93
EPA Acting Section Head: Jess Rowland, M.S. Signature: Jess Rowland
Review Section IV, Toxicology Branch II (H7509C) Date: 6/24/93

DATA EVALUATION REPORT

CHEMICAL: Fatty alcohol blend, lot number CSI-91FA01-27

EPA IDENTIFICATION NUMBERS:

Submission No.: S421382

Caswell No.: 456E

DP Barcode: 180480

PC Code: 079029

MRID Number: 423720-02

STUDY TYPE: Salmonella typhimurium/mammalian activation gene mutation assay

SYNONYMS: None indicated

SPONSOR: Compliance Services International, Sunnyvale, CA, for Fatty Alcohol Task Force

TESTING FACILITY: Inveresk Research International, Ltd., Tranent, Scotland

TITLE OF REPORT: Fatty Alcohol Blend Lot No.: CSI-91FA01-27 — Testing for Mutagenic Activity with Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100

AUTHORS: D.M. Dillon and M.A. McCartney

STUDY NUMBERS: IRI Project No. 751938; IRI Report No. 8604

REPORT ISSUED: January 31, 1992

CONCLUSIONS--EXECUTIVE SUMMARY: Negative for reverse gene mutations in Salmonella typhimurium TA 1535, TA1537, TA1538, TA98, and TA100 exposed in the presence or absence of S9 activation to six doses ranging from 1.5 µg/plate to 500 µg/plate (two independent trials). Cytotoxicity was apparent for all strains at 500 µg/plate +/- S9.

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2a) for genetic effects Category I, Gene Mutation, and is acceptable for regulatory purposes.

A. MATERIAL:

1. Test Material: Fatty alcohol blend lot no. CSI-91FA01-27

Description: Clear colorless liquid
Identification number: Lot no. CSI-91FA01-27
Purity: 96.6% alcohol (55.3% decanol, 40.7% octanol, 0.4% dodecanol, and 0.2% hexanol)
Receipt date: October 24, 1991
Stability: Not reported
Contaminants: None listed
Solvent used: Acetone[a]
Other provided information: The test material was stored at room temperature in the dark. The frequency of dosing solution preparation was not reported. Dosing solutions were analyzed by the performing laboratory.

2. Control Materials:

Negative: None

Solvent/final concentration: Acetone/50 µL/plate

Positive:

Nonactivation:		
Sodium azide	1	µg/plate TA 1535, TA 100
9-Aminoacridine	80	µg/plate TA 1537
2-Nitrofluoride	1	µg/plate TA 1538, TA 98
Activation:		
2-Aminoanthracene (2-AA)	2	µg/plate TA 1535, TA 1537
	0.5	µg/plate TA 1538, TA 98, TA 100

3. Activation: S9 derived from adult male Fischer 344

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

Two batches of the rat liver S9 homogenate identified as Batch Numbers FLI063 and FLI064 were prepared by the testing laboratory and used in this study. Prior to use, the two batches were characterized for total protein (27.3 and 25.7 mg/mL for Batch Nos. FLI063 and FLI064, respectively), and cytochrome P450/P_1-450 and N-demethylase activity. The ability to metabolize 2-AA, 2-acetylaminofluorene, 4-acetylaminofluorene, benzo(a)pyrene, and dimethylaminoazobenzene was evaluated with strain TA 1538.

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[a]Dimethyl sulfoxide (DMSO) was used as the solvent in the preliminary cytotoxicity test. The study authors reported that acetone rather than DMSO was used in the mutation assay because high levels of toxicity "could not be detected accurately in DMSO during analysis."

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The S9 mix contained the following components:

Component:	Final Concentration
Phosphate buffer (pH 7.4)	50 mM
Glucose-6-phosphate	25 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	33 mM
S9	10%

Note: The phosphate buffer and cofactors were filter-sterilized prior to the addition of the S9 homogenate.

4. Test Organism Used: S. typhimurium strains
   ____ TA 97 __x__ TA 98 __x__ TA 100 ____ TA 102 ____ TA 104
   __x__ TA 1535 __x__ TA 1537 __x__ TA 1538
   list any others:

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

5. Test Compound Concentrations Used:

   (a) Preliminary cytotoxicity assay: Six dose levels (33, 100, 333, 1000, 3333, and 10,000 µg/plate +/- S9) were evaluated in strain TA 100, using a single plate per dose, per condition.

   (b) Mutation assay: In two independent assays, six levels (1.5, 5, 15, 50, 150, and 500 µg/plate +/- S9) were evaluated in the five tester strains, using triplicate plates per dose, per strain, per condition.

B. TEST PERFORMANCE:

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

2. Cytotoxicity/Mutation assays: Similar procedures were used for the cytotoxicity and mutation assays. A mixture containing 0.1 mL of the appropriate concentration of test material, solvent or positive control, 0.1 mL of a 16-hour oxoid broth culture (≈$2x10^9$ cells/mL) of the appropriate tester strain, and 0.5 mL of the S9 mix or phosphate buffer was added to 2 mL of top agar. The contents of the tubes were mixed and poured onto minimal Vogel-Bonner medium supplemented with 0.05 mM L-histidine and 0.05 mM biotin. Following incubation at 37°C for 48 hours, revertant colonies were counted, and means and standard deviations were determined for the mutation assays. In addition, the background lawn of growth was evaluated.

3. Evaluation Criteria:

(a) Valid assay: The assay was considered valid if (1) the appropriate genetic markers were verified for each tester strain; (2) the number of spontaneous revertants on at least two of the three plates for each strain was within the stated expected range; (3) there was a ≥1.5-fold increase in revertant colonies for the positive controls for strain TA 100, and a ≥2-fold increase in the positive controls for the other four tester strains (a value of ≥10 revertant colonies in the appropriate solvent control was assumed); (4) for each strain, there were at least four noncytotoxic dose levels without contamination; and (5) in cases where a mutagenic response was observed, "no more than one dose level was discarded before the dose which gave the highest significant mean [revertant] colony number."

(b) Positive response: The test material was considered positive if it caused a ≥1.5-fold dose-related, reproducible increase in the revertant colonies of strain TA 100, or a ≥2-fold dose-related, reproducible increase in the revertant colonies of any of the other four strains. A value of ≥10 revertants in the corresponding solvent control was assumed.

4. Protocol: Not provided

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: No compound precipitation was observed at concentrations up to 10,000 µg/plate +/- S9. With strain TA 100, doses ≥1000 µg/plate +/- S9 were severely cytotoxic, and nonactivated 333 µg/plate resulted in 50% cytotoxicity. The remaining levels (33 and 100 µg/plate +/- S9) were not cytotoxic. Accordingly, concentrations selected for the mutation assay ranged from 1.5 µg/plate to 500 µg/plate +/- S9 and the solvent was changed from DMSO to acetone.

2. Mutation Assays: In agreement with the preliminary cytotoxicity assay, cytotoxicity was observed during both trials at 500 µg/plate +/- S9 in the majority of tester strains. There were, however, no appreciable increases in revertants in any strain in either trial, in the presence or absence of S9 activation. By contrast, the positive controls induced the expected responses in the appropriate tester strains, with or without S9 activation. Summarized findings from the initial trial, which are representative of the overall study, are shown in Table 1.

Based on these findings, the study authors concluded that fatty alcohol blend (lot no. CSI-91FA01-27) was not mutagenic in this bacterial test system.

Table 1: Representative Results of the Initial Salmonella typhimurium/Mammalian Microsome Mutation Assay with Fatty Alcohol Blend (Lot no. CSI-91FA01-27)

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA 1535	TA 1537	TA 1538	TA 98	TA 100
Solvent Control							
Acetone	50 µL	-	18 ± 2	10 ± 3	23 ± 5	26 ± 2	124 ± 13
		+	23 ± 5	14 ± 2	19 ± 4	39 ± 8	132 ± 14
Positive Controls							
Sodium azide	1 µg	-	245 ± 26	--	--	--	747 ± 47
9-Aminoacridine	80 µg	-	--	1334 ± 16	--	--	--
2-Nitrofluorene	1 µg	-	--	--	206 ± 21	186 ± 21	--
2-Aminoanthracene	0.5 µg	+	--	--	569 ± 21	522 ± 21	598 ± 24
	2 µg	+	250 ± 5	150 ± 10	--	--	--
Test Material							
Fatty alcohol blend	150 µg[b]	-	14 ± 1	8 ± 4	13 ± 4	23 ± 4	119 ± 11
	500 µg[c]	-	10 ± 4	2 ± 2	3 ± 2	7 ± 1	58 ± 16
	150 µg[b]	+	19 ± 6	16 ± 1	20 ± 7	27 ± 3	118 ± 12
	500 µg[c]	+	13 ± 1	6 ± 2	7 ± 4	20 ± 4	89 ± 8

[a]Means and standard deviations of the counts from three plates
[b]Results for lower doses (1.5, 5, 15, and 50 µg/plate +/ -S9) did not suggest a mutagenic effect.
[c]Highest dose tested; thinning of the background lawn of growth was reported for all strains at this concentration with or without S9 activation.

Note: Data were extracted from the study report, pp. 23-24.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study authors' interpretation of the data was correct. Doses of 1.5-500 µg/plate +/- S9 did not induce a mutagenic response. Levels as high as 10,000 µg/plate +/- S9 were soluble, and cytotoxicity was observed in the majority of strains at 500 µg/plate +/- S9. In addition, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the results obtained with the positive controls. We conclude, therefore, that fatty alcohol blend (lot no. CSI-91FA01-27) was tested over an appropriate range of concentrations and was negative in this bacterial assay system.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLP? Yes. (A quality assurance statement, indicating that the report was audited and that this type of short-term study is only inspected according to a predetermined schedule, was signed and dated June 16, 1992.)

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2a) for genetic effects Category I, Gene Mutation, and is acceptable for regulatory purposes.

FINAL

DATA EVALUATION REPORT

FATTY ALCOHOLS

Study Type: Mutagenicity: Gene Mutation in Cultured Mammalian Cells (Mouse Lymphoma Cells)

Prepared for:

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

Prepared by

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9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Lynne Haber — Date 6/7/93
Lynne Haber, Ph.D.

Independent Reviewer Nancy E. McCarroll — Date 6/7/93
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QA/QC Manager Sharon Segal — Date 6/7/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-40
Clement Number: 125
Project Officer: Caroline Gordon

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GUIDELINE SERIES 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE GENE MUTATIONS

MUTAGENICITY STUDIES

EPA Reviewer: Steven Malish, Ph.D.
Review Section IV,
Toxicology Branch II/HED H7509C
Signature: Steven Z. Malish
Date: 6/14/93

Secondary Reviewer: Jess Rowland, M.S.
Acting Section Head, Review Section IV,
Toxicology Branch II/HED H7509C
Signature: Jess Rowland
Date: 6/22/93

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Gene mutation in cultured mammalian cells (mouse lymphoma cells)

EPA IDENTIFICATION Numbers:

Submission: S421382

DP Barcode: D180480

Caswell Number: 456E

P.C. Code: 079029

MRID Number: 423720-03

TEST MATERIAL: Fatty alcohol blend

SYNONYM: None reported. However, the composition of the test material (average percent weight alcohol) was listed as: hexanol, 0.1629%; octanol, 40.7239%; decanol, 55.2961%; and dodecanol, 0.4196%.

SPONSOR: Compliance Services International, Sunnyvale, CA

STUDY NUMBERS: Project number: 751985; Report number: 8715

TESTING FACILITY: Inveresk Research International, Ltd., Tranent, Scotland

TITLE OF REPORT: Fatty Alcohol Blend Mouse Lymphoma Gene Mutation Assay

AUTHORS: Cattanach, P.J. and Riach, C.G.

REPORT ISSUED: February 17, 1992

CONCLUSIONS-EXECUTIVE SUMMARY: Under the conditions of the mouse lymphoma forward mutation assay, fatty alcohol blend was tested in two independent assays with and without activation and found not to be mutagenic. In the initial assay, nonactivated and S9-activated levels ranging from 9.4 µg/mL to 37.5 µg/mL were evaluated; doses ≥75 µg/mL were severely cytotoxic. In the confirmatory assay, 10-50 µg/mL -S9 and 30-70 µg/mL +S9 were evaluated.

Severe cytotoxicity was observed at nonactivated levels ≥60 µg/mL and at S9-activated 80 µg/mL. Based on these findings, we conclude that fatty alcohol blend was tested to cytotoxic levels and found not to be mutagenic in this assay system.

CORE CLASSIFICATION: Acceptable. This study satisfies Guideline requirements (§84-2a) for genetic effects, Category I, Gene Mutations and is acceptable for regulatory purposes.

A. MATERIALS:

1. Test Material: Fatty alcohol blend

Description: Colorless liquid
Identification number: Batch number: CSI-91FA01-27
Purity: 96.6%
Receipt date: October 24, 1991
Stability: Not reported
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO) for the preliminary cytotoxicity assay and acetone for the mutagenicity assay[1]
Other provided information: The test material was stored at room temperature in the dark. The frequency of test solution preparation was not reported. Analytical determinations were performed on all dosing solutions to verify actual concentrations.

2. Control Materials:

Negative: None

Solvent/final concentration: Acetone at a final concentration of 10 µl/mL.

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in acetone to yield a final concentration of 250 µg/mL.

Activation (concentrations, solvent): 3-Methylcholanthrene (3-MC) was prepared in acetone to yield a final concentration of 2.5 µg/mL.

3. Activation: S9 derived from male Fischer-344 (average weight 223 g)

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

The S9 liver homogenate (batch FLI 067) was prepared by the study laboratory on October 30 1991; the expiration date was listed as April 30, 1992. The total protein concentration was determined to be

[1]The solvent was reportedly changed due to impurity peaks in the gas chromatograms, as decribed in IRI Project 353278/Report No. 8818.

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26.6 mg/mL, and the cytochrome P450/$P_1$450 concentration was 8.85 nmol/mL. N-demethylase activity was determined to be 643 nmol HCHO/mL/min. Additionally, the ability of the S9 to convert several promutagens (2-aminoanthracene, 2-acetylaminofluorene, 4-acetylaminofluorene, benzo(a)pyrene, and dimethylaminoazobenzene) to forms mutagenic in Salmonella typhimurium strain TA1538 was also assessed.

S9 mix composition:

Component	Concentration in S9 mix
NADP	4 mM
Glucose 6-phosphate	25 mM
S9 homogenate	10%

4. Test Cells: Mammalian cells in culture

__x__ mouse lymphoma L5178Y cells
_____ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Not reported.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

__x__ thymidine kinase (TK)
Selection agent: (give concentration)
_____ bromodeoxyuridine (BrdU)
_____ fluorodeoxyuridine (FdU)
3 µg/mL trifluorothymidine (TFT)

_____ hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
Selection agent: (give concentration)
_____ 8-azaguanine (8-AG)
_____ 6-thioguanine (6-TG)

_____ Na^+/K^+ATPase
Selection agent: (give concentration)
_____ ouabain

_____ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

(a) Cytotoxicity assay: Five doses (0.4, 4.3, 43.2, 432, and 4320 µg/mL) were evaluated in the presence and absence of S9 activation.

(b) Mutation assay:

(1) Initial assay: Six doses (9.4, 18.8, 37.5, 75, 150, and 300 µg/mL) were assayed in the presence and absence of S9

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activation; cultures treated with levels ≥75 µg/mL +/-S9 were severely cytotoxic and were not cloned.

(2) Confirmatory assay:

- Nonactivated assay: Eight doses (10, 20, 30, 40, 50, 60, 70, and 80 µg/mL) were assayed. Cultures treated with concentrations ≤50 µg/mL were cloned.

- S9-activated assay: Eight doses (10, 20, 30, 40, 50, 60, 70, and 80 µg/mL) were assayed. Cultures treated with concentrations ≥30 µg/mL and ≤70 µg/mL were cloned.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells were exposed to the test compound or negative, solvent, or positive controls
__4__ hours (nonactivated) __4__ hours (activated).

(b) After washing, cells were cultured for __2__ days (expression period) before cell selection.

(c) After expression, cells seeded at $2x10^5$ cells/plate (3 plates with 5 mL each) were cultured for __11-14__ days in selection medium to determine the numbers of mutants; cells seeded at 200 cells/plate (3 plates) were cultured for __11-14__ days without selection medium to determine cloning efficiency (CE).

2. Statistical Methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if (1) the mean CE of the solvent control cultures was ≥50%, (2) the mutation frequency (MF) of the solvent control culture was $\geq 12x10^{-6}$ and $<100x10^{-6}$, and (3) the MF of the positive control cultures was at least double the value of the solvent control and $\geq 100x10^{-6}$ at a concentration where the CE was >10%.

(b) Positive result: The test material was considered positive if it induced a reproducible ≥1.7-fold increase in the mutation frequency (MF) compared to the solvent control at test material levels with a CE ≥10%, and the effect was dose related or associated with an increase in mutant numbers.

4. Protocol: Not provided; however, a copy of the primary data was included in the study report.

C. REPORTED RESULTS:

1. Cytotoxicity Assay: The preliminary cytotoxicity test was conducted with five concentrations of fatty alcohol blend ranging from 0.4 µg/mL to 4320 µg/mL +/-S9 activation. No cells survived treatment with S9-activated and nonactivated levels ≥432 µg/mL. At 43.2 µg/mL, relative suspension growth (RSG) was 53 and 55% in the absence and presence of S9, respectively. There was no appreciable cytotoxicity at doses ≤4.32 µg/mL +/-S9. Based on these results, the initial mutation assay was conducted with 9.4-300 µg/mL +/-S9 activation.

2. Analytical Determination: All dosing solutions were analyzed to verify actual concentrations. All solutions used in the initial assay were within 10% of the nominal concentrations, and all solutions used in the confirmatory assay were within 2% of the intended level.

3. Mutation Assays: In the initial assay, levels ≥75 µg/mL +/-S9 were severely cytotoxic and were not plated for mutants and survivors (Table 1). At 37.5 µg/mL, RSG was >80% in both the presence and absence of S9 activation. However, there was no evidence of mutagenicity at any dose. Therefore, the confirmatory assay was performed with nonactivated and S9-activated doses ranging from 10 µg/mL to 80 µg/mL. Concentrations ≥60 µg/mL -S9 and 80 µg/mL +S9 were also severely cytotoxic and not plated. RSG at the highest levels plated for mutant selection (50 µg/mL -S9 and 70 µg/mL +S9) was 40.8 and 28.4%, respectively (Table 2). Presumably because a sufficient number of higher doses were available, cultures exposed to S9-activated 10 and 20 µg/mL were not cloned. In agreement with earlier findings, there was no evidence of mutagenicity at any dose. The nonactivated (EMS at 250 µg/mL) and S9-activated (3-MC at 2.5 µg/mL) positive controls induced marked increases in MFs and mutant colonies. Based on the overall results, the study authors concluded that fatty alcohol blend was not mutagenic in the mouse lymphoma assay.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that the study authors correctly concluded that fatty alcohol blend was not mutagenic in the mouse lymphoma mutation assay. We further agree that the test material was adequately tested to cytotoxic levels, and that the steep cytotoxicity curve made it difficult to obtain mutagenicity data at RSG ≤20%. In addition, the sensitivity of the test system to detect a mutagenic response was adequately demonstrated by the results achieved with the positive controls. We, therefore, conclude that this study is acceptable.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated June 16, 1992. However, the laboratory phase of the study was not inspected by quality assurance personnel.)

F. CBI APPENDICES: Appendix A, Materials and Methods, CBI pp. 9-20.

CORE CLASSIFICATION: Acceptable. This study satisfies Guideline requirements (§84-2a) for genetic effects, Category I (Gene Mutations) and is acceptable for regulatory purposes.

Page_____ is not included in this copy.

Pages 26 through 27 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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APPENDIX A

MATERIALS AND METHODS
CBI pp. 9-20

Page_____ is not included in this copy.

Pages 29 through 40 are not included.

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_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

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