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

CASWELL FILE

DEC 13 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Methyl isothiocyanate: Review of Mutagenicity Data.

Submission: S478165
P.C. Code: 068103

MRID #'s 412214-11 and 412214-12
DP Barcode: D216666

FROM: Timothy F. McMahon, Ph.D., Pharmacologist 12/6/95
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

TO: Mark Wilhite / Leonard Ryan - PM 51
Special Review and Reregistration Division (7508W)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head
Review Section I, Toxicology Branch II
Health Effects Division (7509C)
12/7/95

and

Stephanie Irene, Ph.D., Acting Branch Chief
Toxicology Branch II
Health Effects Division (7509C)
12/11/95

Registrant: DeGussa Corporation

Action Requested: Review of an in vitro chromosome aberration assay in human lymphocytes and a sister chromatid exchange assay in Chinese hamster V79 cells.

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I. General

The registrant (DeGussa Corporation) has submitted two mutagenicity studies in fulfillment of the Subdivision F guideline for mutagenicity testing. These two studies were reviewed by Nancy McCarroll, Toxicology Branch II, the results of which are presented below in executive summary format.

II. Review of Data

A) **MRID # 412214-11: Methyl Isothiocyanate: Chromosome aberration assay in human lymphocytes with methyl isothiocyanate technical (MITC).**

In an in vitro cytogenetic assay, human lymphocytes derived from a single healthy donor were exposed to 3.0 or 5.0 µg/ml methyl isothiocyanate (MITC) technical in either the presence or absence of S9 activation for 4 hours. Cells were harvested at 24 or 48 hours after initiation of treatment and scored for structural chromosome aberrations. The S9 was derived from Aroclor 1254-induced rat livers, and the test material was delivered in serum-free Dulbecco's Modified Eagle Medium (DMEM/F12).

Cytotoxicity, as indicated by a slight to moderate reduction in the mitotic index, was noted at 5.0 µg/ml +/- S9. The positive controls induced the expected high yield of cells with abnormal chromosome morphology. **There was, however, no evidence of a clastogenic effect at either MITC dose with or without S9 activation or at either cell harvest time.**

Classification: **acceptable**

The study is classified as **Acceptable** and **satisfies** the requirements (§84-2) for an in vitro cytogenetic assay.

B) **MRID # 412214-12: Methyl Isothiocyanate: Sister chromatid exchange assay in Chinese hamster V79 cells with methyl isothiocyanate technical (MITC).**

In two independently performed in vitro sister chromatid exchange (SCE) assays, Chinese Hamster V79 cells were exposed to nonactivated doses of 0.1, 2.0, or 3.5 µg/ml or S9-activated doses of 0.1, 2.5, or 5.0 µg/ml methyl isothiocyanate technical (MITC) for 4 hours in both trials. The S9 was derived from Aroclor 1254-induced rat livers, and the test material was delivered in serum-free Minimal Essential Medium (MEM).

Due to severe cytotoxicity at doses ≥ 2 µg/ml -S9 or ≥ 2.5 µg/ml +S9 when the test substance was prepared in dimethyl sulfoxide, the solvent was changed to MEM. A slight delay in cell cycling was seen at 3.5 µg/ml -S9 and at 5.0 µg/ml +S9 in the initial trial; the effect was not reproduced in the confirmatory trial. **The combined results of both trials did, however, provide reliable evidence that MITC technical did not induce a genotoxic response in this test system.** The results obtained with the positive controls were adequate to demonstrate assay sensitivity.

Classification: **acceptable**

The study is classified as **Acceptable** and **satisfies** the requirements (§84-4) for an in vitro SCE assay.

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METHYL ISOTHIOCYANATE

MAMMALIAN CELLS IN CULTURE
SISTER CHROMATID EXCHANGE

MUTAGENICITY STUDIES

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED (7509C)
Signature: Nancy S McCarroll
Date: 12/5/95

EPA Secondary Reviewer: Tim McMahon, Ph.D.
Review Section I,
Toxicology Branch II/HED (7509C)
Signature: Tim McMahon
Date: 12/5/95

DATA EVALUATION REPORT

STUDY TYPE: Mammalian cells in culture sister chromatid exchange (SCE) assay in Chinese hamster V79 cells

DP BARCODE: D216666

SUBMISSION NO.: S489060

PC CODE: 068103

MRID NUMBER: 412214-12

TEST MATERIAL: Methyl isothiocyanate (MITC)

SYNONYM(S): None

STUDY NUMBER(S): TB 88008

SPONSOR: NOR-AM Chemical Co./Schering AG, Berlin, Germany

TESTING FACILITY: CCR Cytotest Cell Research GmbH & Co. KG, Darmstadt, Germany

TITLE OF REPORT: T102 Methyl Isothiocyanate: Sister chromatid exchange assay in Chinese hamster V79 cells with methyl isothiocynanate technical (MITC)

AUTHOR(S): A. Heidemann

REPORT ISSUED: October 24, 1988

EXECUTIVE SUMMARY: In two independently performed in vitro sister chromatid exchange (SCE) assays (MRID No. 412214-12), Chinese hamster V79 cells were exposed to nonactivated doses of 0.1, 2.0 or 3.5 μg/ml or S9-activated doses of 0.1, 2.5 or 5.0 μg/ml methyl isothiocyanate technical (MITC) for 4 hours in both trials. The S9 was derived from Aroclor 1254-induced rat livers, and the test material was delivered in serum-free Minimal Essential Medium (MEM).

Due to severe cytotoxicity at doses ≥2 μg/ml -S9 or ≥2.5 μg/ml +S9 when the test substance was prepared in dimethyl sulfoxide, the solvent was changed to MEM. A slight delay in cell cycling was seen at 3.5 μg/ml -S9 and at 5.0 μg/ml +S9 in the initial trial; the effect was not reproduced in the confirmatory trial. The combined results of both trials did, however, provide reliable

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evidence that MITC technical did not induce a genotoxic response in this test system. The results obtained with the positive controls were adequate to demonstrate assay sensitivity.

STUDY CLASSIFICATION: Acceptable.

The study is classified as Acceptable and satisfies the guideline requirements (§84-4) for an in vitro SCE assay.

A. MATERIALS:

1. Test Material: Methyl isothiocyanate (MITC)

Description: Slightly yellow "solidified melt"
Lot/ batch number: 370983
Purity: Technical grade (the percentage of the active ingredient was not provided)
Receipt date: Not reported
Stability: Expiration date (neat substance): May 9, 1989; solutions prepared in water were listed as stable for 3 days
CAS number: 573
Solvent used: MEM (assumed by our reviewers to be Minimal Essential Medium) without fetal calf serum
Other provided information: The test material was stored at < 28°C, protected from light and moisture. The frequency of dosing solution preparation was not reported and doses used in the study were not verified analytically.

2. Control Materials:

Negative: None

Solvent/final concentration: Dimethyl sulfoxide (DMSO) at 1% -- preliminary cytotoxicity test; MEM without fetal calf serum (FCS)-- main assays.

Positive:

Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in MEM to yield a final concentration of 630 μg/ml (5.0 mM).

Activation: (Concentration, solvent): Cyclophosphamide (CP) was prepared in MEM to yield a final concentration of 1.4 μg/ml (5.0 μM)

3. Activation: S9 derived from 8-12 week old male, 150-200 g Wistar

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

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The S9 fraction was prepared by the performing laboratory and the protein content was determined prior to use. The composition of the S9 mix was:

NADP	5 mM
Glucose-6-phosphate	5 mM
Magnesium chloride	8 mM
Potassium chloride	33 mM
Sodium-ortho-phosphate buffer, pH 7.4	100 mM
S9 homogenate (protein content)	0.3 mg/ml (final concentration)

4. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Two trials of the preliminary cytotoxicity test were performed with the following test material doses dissolved in DMSO:

Trial 1: Nine levels (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 and 10.0 μg/ml +/-S9) were evaluated using duplicate cultures per dose per condition.

Trial 2: Six doses without S9 activation (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 μg/ml) and six doses with S9 activation (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 μg/ml) were assayed.

(b) SCE assay: Two independent trials were conducted; doses used are listed below:

(1) Initial trial: A series of eight levels (0.1-3.5 μg/ml -S9 and 0.1-5.0 μg/ml +S9) were assayed. Cells exposed to 0.1, 2.0 or 3.5 -S9 or 0.1, 2.5 or 5.0 μg/ml +S9 were scored.

(2) Confirmatory trial: As above for the initial SCE trial.

5. Test Cells: Chinese hamster V79 cells, were obtained from an unspecified source but were reported to be maintained as large stocks held in liquid nitrogen at the CCR cell bank. Cells used in this study were grown for 48 hours prior to use in complete MEM (+10% FSC).

Properly maintained? Yes.
Cell line or strain periodically checked for mycoplasma contamination? Not reported.
Cell line or strain periodically checked for karyotype stability? Not reported.

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B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound, solvent or positive controls for: 4 hours (nonactivated) 4 hours (activated)

(b) Cells were washed and reincubated in medium containing 20 μM BrdU for 17.5 hours (nonactivated and S9-activated conditions).

(c) Colcemid (0.2 μg/ml) was added and cells were harvested 2.5 hours after mitotic arrest (nonactivated and S9-activated conditions).

2. Preliminary Cytotoxicity Assay: The report stated that the preliminary cytotoxicity assay was carried out in a manner similar to that described for the SCE assay. Cytotoxicity, as indicated by a reduction in plating efficiency (PE) was, however, not assessed in the SCE assay. Based on the results, the highest dose causing an ≈ 20-50% reduction in the PE was selected for evaluation in the SCE assay.

3. Sister Chromatid Exchange (SCE) Assay:

(a) Exposure: Two-day old exponentially growing cells were seeded into Quadriperm dishes containing slides (2 chambers/dish) at a density of 0.5×10^5 cells per chamber. After 48 hours of incubation, the culture medium (MEM+10% FCS) was replaced with serum-free MEM and cells were exposed to the selected test material doses, the solvent (culture medium) or positive controls (EMS -S9; CP +S9) with and without S9 activation for 4 hours. Cells were washed, refed medium containing BrdU (20 μM) and incubation was continued for an additional 19.5 hours. Colcemid was added to all cultures for the final 2.5 hours of incubation.

(b) Preparation of chromosomes: Cells attached to the slides were treated with 0.4% KCl, fixed with absolute methanol:glacial acetic acid (3:1), and differentially stained using the fluorescent-plus-Giemsa technique of Perry and Wolff[1]. Slides were coded prior to scoring.

(c) Slide analysis: At least 50 well-defined metaphases (25/culture) containing 22±1 chromosomes were scored for SCEs. Cell cycle delay was assessed by determining the percentage of differentially stained metaphases per 100 mitotic cells.

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[1]Perry, P.E. and Wolff, S. (1974) New Giemsa method for the differential staining of sister chromatids. Nature 251, 156-158.

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4. Statistical Analysis: The data were evaluated for statistical significance using the nonparametric Mann-Whitney test at $p<0.01$.

5. Evaluation Criteria: The test material was considered positive if it induced either a statistically significant and dose-related increase in the number of SCEs or a reproducible and significant response for at least one dose group.

6. Compliance: Was the test performed under GLPs? Yes. (A Quality Assurance Statement was signed and dated October 12, 1988.)

C. REPORTED RESULTS:

1. Cytotoxicity Assays: The test material was prepared in DMSO for both trials of the preliminary cytotoxicity assay. In Trial I, doses of 0.5-10.0 µg/ml +/-S9 were tested. No cells survived treatment with nonactivated levels ≥2.0 µg/ml or S9-activated doses ≥4.0 µg/ml. Survival relative to the solvent control (RPE) for the remaining nonactivated treatment groups ranged from 60.9% at 1.0 µg/ml to 100.8% at 0.5 µg/ml. In the presence of S9 activation, RPE was also dose-related, ranging from 6.7% at 3.0 µg/ml to 101.1% at 0.5 µg/ml. Based on these findings, Trial II was conducted with lower test material levels (0.1-1.0 µg/ml -S9; 0.5-3.0 µg/ml +S9). Without S9, 59.6% of the cells were viable after the 4-hour treatment with 1.0 µg/ml; RPEs for the lower doses ranged from 68.6 to ≥86.9% at 0.8 to ≤0.6 µg/ml, respectively. The two highest S9-activated doses (2.5 and 3.0 µg/ml) were severely cytotoxic. Approximately 44.2 or 52.3% of the cells were recovered at 2.0 or 1.0 µg/ml +S9, respectively. Based on the overall data, the doses chosen for the SCE assay were 0.1-3.5 µg/ml without S9 and 0.1-5.0 µg/ml +S9. Owing to the severe cytotoxicity and the low dose ranges selected for further study, the solvent was changed from DMSO to serum-free culture medium (MEM).

2. SCE Assays:

(a) Initial assay: A slight delay in cell cycling was noted only at the high dose with or without metabolic activation. Accordingly, metaphases from cultures exposed to 0.1, 2.0 or 3.5 µg/ml -S9 or 0.1, 2.5 or 5.0 µg/ml +S9 were scored for SCE induction. As the summary data presented in Study Report Table 4 (see attachment) indicate, slight but significant ($p<0.01$) elevations in the SCE frequencies were noted for all nonactivated treatment groups. The response was, however, neither dose related nor approached a doubling over the solvent control frequency. Slight but nonsignificant and nondose-dependent elevations in SCEs per cell were seen in the S9-activated phase of testing. Our reviewers concluded that these slight increases were not indicative of a genotoxic effect, and resulted from the relatively low SCE frequencies in the solvent control cultures.

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(b) Confirmatory trial: Using a comparable range of test substance doses, cell replication was not suppressed at any level in the confirmatory trial. Similarly, the significantly elevated SCE frequencies observed under nonactivated conditions in the initial trial were not reproduced. There was also no significant induction of SCEs at any S9-activated concentration (see attachment, Study Table 5).

By contrast, the positive controls (630 μg/ml EMS -S9 or 5.0 μg/ml CP +S9) induced clear genotoxicity in both trials. From the overall findings, the study author concluded that MITC technical did not increase the frequency of SCEs in the V79 Chinese hamster cell line.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: The evidence of an adverse effect on cell cycle kinetics seen in the preliminary assays and the first trial of the cytogenetic evaluation was not reproduced in the confirmatory trial. Nevertheless, we believe that there were sufficient reliable data from both the two nonactivated and two S9-activated trials to conclude that MITC technical was not genotoxic in this in vitro mammalian cell SCE assay. In addition, the ability of the test system to detect genotoxicity was adequately demonstrated in both trials by the results obtained with the nonactivated (EMS) and S9-activated (CP) positive controls. Based on the above considerations, we conclude that the study provided acceptable evidence that the test material was not genotoxic in this assay system.

E. STUDY DEFICIENCIES: While the percent active ingredient in the technical grade of MITC was not listed, the evidence of cytotoxicity indicates that the negative response was not caused by an inability of the test material to penetrate cellular membranes and interact with genetic material. Therefore, the requirement for test material purity information can be waived.

METHYL ISOTHIOCYANATE

MAMMALIAN CELLS IN CULTURE
CYTOGENETICS

MUTAGENICITY STUDIES

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED (7509C)
EPA Secondary Reviewer: Tim McMahon, Ph.D.
Review Section I,
Toxicology Branch II/HED (7509C)

Signature: Nancy S. M. Carroll
Date: 12/5/95

Signature:
Date: 12/5/95

DATA EVALUATION REPORT

STUDY TYPE: In vitro chromosome aberrations in human lymphocytes

DP BARCODE: D216666

SUBMISSION NO.: S489060

PC CODE: 068103

MRID NUMBER: 412214-11

TEST MATERIAL: Methyl isothiocyanate (MITC)

SYNONYM(S): None

STUDY NUMBER(S): TB 88007

SPONSOR: NOR-AM Chemical Co./Schering AG, Berlin, Germany

TESTING FACILITY: CCR Cytotest Cell Research GmbH & Co. KG, Darmstadt, Germany

TITLE OF REPORT: T103 Methyl Isothiocyanate: Chromosome aberration assay in human lymphocytes with methyl isothiocyanate technical (MITC)

AUTHOR(S): A. Heidemann

REPORT ISSUED: October 31, 1988

EXECUTIVE SUMMARY: In an in vitro cytogenetic assay (MRID No. 412214-11), human lymphocytes derived from a single healthy donor were exposed to 3.0 or 5.0 µg/ml methyl isothiocyanate (MITC) technical in either the presence or absence of S9 activation for 4 hours. Cells were harvested at 24 or 48 hours after initiation of treatment and scored for structural chromosome aberrations. The S9 was derived from Aroclor 1254-induced rat livers, and the test material was delivered in serum-free Dulbecco's Modified Eagle Medium (DMEM/F12).

Cytotoxicity, as indicated by a slight to moderate reduction in the mitotic index, was noted at 5.0 µg/ml +/-S9. The positive controls induced the expected high yield of cells with abnormal chromosome morphology. There was, however, no

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evidence of a clastogenic effect at either MITC dose with or without S9 activation or at either cell harvest time.

STUDY CLASSIFICATION: Acceptable.

The study is classified as Acceptable and satisfies the guideline requirements (§84-2) for an in vitro cytogenetic assay.

A. MATERIALS:

1. Test Material: Methyl isothiocyanate (MITC)

   Description: Slightly yellow "solidified melt"
   Lot/ batch number: 370983
   Purity: Technical grade (the percentage active ingredient was not provided)
   Receipt date: Not reported
   Stability: Expiration date (neat substance): May 9, 1989; solutions prepared in water were listed as stable for 3 days
   CAS number: 573
   Solvent used: DMEM/F12 (assumed by our reviewers to be Dulbecco's Modified Eagle Medium) without fetal calf serum (FCS)
   Other provided information: The test material was stored at < 28°C, protected from light and moisture. Dosing solutions were prepared on the day of used; doses used in the study were not verified analytically.

2. Control Materials:

   Negative: None

   Solvent/final concentration: DMEM/F12 without FCS

   Positive:

   Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in DMEM to yield a final concentration of 720 µg/ml (5.76 mM).

   Activation: (Concentration, solvent): Cyclophosphamide (CP) was prepared in DMEM to yield a final concentration of 19.8 µg/ml (70.0 µM)

3. Activation: S9 derived from 8-12 week old male, 150-200 g Wistar

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

The S9 fraction was prepared by the performing laboratory and the protein content was determined prior to use. The composition of the S9 mix was:

NADP	5 mM
Glucose-6-phosphate	5 mM
Magnesium chloride	8 mM
Potassium chloride	33 mM
Sodium-ortho-phosphate buffer, pH 7.4	100 mM
S9 homogenate (protein content)	0.3 mg/ml (final concentration)

4. Test Compound Concentrations Used:

Nonactivated conditions: Seven levels (0.1, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 μg/ml) were evaluated using duplicate cultures per dose per condition with cell harvests at 24 and 48 hours. Cultures treated with 3 or 5 μg/ml were selected for metaphase analysis.

S9-activated conditions: As above for the nonactivated test.

5. Test Cells: Human lymphocytes were obtained from the blood of a single healthy volunteer; no further details were provided. Lymphocyte cultures were initiated (within 24 hours of collection) in DMEM/F12 medium containing 15% fetal calf serum, heparin, antibiotics and phytohemagglutinin (concentration not specified). Cells were cultured at 37°C for 48 hours prior to use.

Properly maintained? Yes.

Cell line or strain periodically checked for mycoplasma contamination? Not applicable.

Cell line or strain periodically check for karyotype stability? Not applicable.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound, solvent or positive controls for: 4 hours (nonactivated) - 4 hours (activated)

(b) Cells harvested 24 and 48 hours after initiation of treatment (nonactivated)

Cells harvested 24 and 48 hours after initiation of treatment (activated)

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Note: Cells exposed to the positive controls were only collected at 24 hours.

(c) Colcemid 0.2 µg/ml was added 3 hours before harvest (nonactivated and S9-activated conditions).

3. Cytogenetic Assay:

(a) Treatment: Forty-eight hours after lymphocyte initiation, duplicate cultures were exposed to the selected test material doses, the solvent (culture medium) or positive controls (EMS -S9; CP +S9) in both the presence and absence of S9 activation. Under both nonactivated and S9-activated conditions, lymphocytes were treated for 4 hours. Cultures were centrifuged, resuspended in fresh culture medium and reincubated for an additional 20 or 44 hours. Colcemid (final concentration, 0.2 µg/ml) was added 3 hours before all cultures were harvested.

(b) Cell preparation: Metaphase cells were collected, swollen in 0.075 M KCl, fixed in glacial acetic acid:absolute methanol (1:3) and stained with Giemsa. Slides were not coded prior to scoring for chromosome aberrations.

(c) Metaphase analysis: Approximately 1000 lymphocytes per group (500 cells/culture) were counted to determine the mitotic index (MI). The level of the test material that caused an ≈50% depression in mitotic activity was selected as the high dose for the metaphase analysis.

Two hundred metaphase plates (100 cells/ culture) from each dose group, the solvent, and positive control groups were scored for chromosome aberrations; gaps were recorded and aberration frequencies were presented with and without gaps.

4. Statistical Analysis: The data were not evaluated for statistical significance.

5. Evaluation Criteria: The test material was considered positive if it induced a statistically significant increase in the frequency of aberrant cells at one or more dose levels.

6. Compliance: Was the test performed under GLPs? Yes. (A Quality Assurance Statement was signed and dated October 12, 1988.)

C. REPORTED RESULTS:

Seven dose of the test material (0.1 to 5.0 µg/ml +/-S9) were initially investigated for cytotoxic effects. Without S9 activation, the high dose caused slight reductions in the MI (61.4 and 53.9% of control) at 24 and 48 hours, respectively. In the presence of S9 activation, the relative MIs 24

or 48 hours postexposure to 5.0 μg/ml were 28.8 or 50.5%, respectively. Mitotic suppression at lower levels (≤4.0 μg/ml +/-S9) was not clearly dose related or indicative of cytotoxicity. Based on these results, cultures exposed for 4 hours to 3.0 or 5.0 μg/ml +/-S9 were evaluated for structural chromosome aberrations at 24 and 48 hours posttreatment.

As the summary data presented in Tables 3 and 4 (see attachment) indicate, MITC did not induce a clastogenic effect at either concentration with or without S9 activation in lymphocytes harvested 24 or 48 hours postdosing. By contrast, both positive controls (720 μg/ml EMS -S9; 19.8 μg/ml +S9) caused marked increases in the yield of cells with structural chromosome damage (sampling only at 24 hours).

From the overall results, the study author concluded that MITC did not induce structural chromosome aberrations in human lymphocytes in vitro.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We concur with the study author's conclusion that MITC was not clastogenic in cultured human lymphocytes. We note that the results are in relatively good agreement with the data from the sister chromatid exchange assay in Chinese hamster V79 cells (see MRID No. 412214-12) showing that MITC doses of 3.5 -S9 and 5.0 μg/ml +S9 caused slight cell cycle delay but no genotoxicity. In addition, the ability of the test system to detect clastogenesis was adequately demonstrated by the results obtained with the nonactivated (EMS) and S9-activated (CP) positive controls. We conclude, therefore, that study the provided acceptable evidence that the test material was not genotoxic in this test system.

E. STUDY DEFICIENCIES: The following study deficiencies were noted:

- While the percent active ingredient was not listed for MITC, the evidence of cytotoxicity indicates that the negative response was not caused by an inability of the test material to penetrate cellular membranes and interact with genetic material.
- Slides were not coded prior to scoring.
- Cells were not exposed to the test material without S9 activation for one full cell cycle (≈12-14 hours for human lymphocytes). However, there was no suggestive evidence of a clastogenic effect at either dose without S9 activation. It is, therefore, unlikely that the outcome of the study would be altered by extending the treatment time in the nonactivated phase of testing.

Structural chromosome aberrations

Summary of results

Table 3: mutagenicity data

fixation interval: 24 h after start of the treatment

Test group	number of cells analysed	conc. per ml	S9 mix	per cent aberrant cells incl. gaps	excl. gaps	exchanges
Solvent-control	200	0.0 µg	-	0.50	0.50	0.00
Positive-control EMS	200	0.72 mg	-	12.00	9.00	2.50
Test-article	200	3.0 µg	-	1.00	0.00	0.00
Test-article	200	5.0 µg	-	2.00	1.00	0.00
Solvent-control	200	0.0 µg	+	2.00	1.00	0.00
Positive-control CPA	200	19.8 µg	+	9.50	9.00	4.00
Test-article	200	3.0 µg	+	3.00	1.00	0.50
Test-article	200	5.0 µg	+	1.50	1.00	0.00

Table 4: mutagenicity data

fixation interval: 48 h after start of the treatment

test group	number of Muta cells analysed	conc. per ml	S9 mix	per cent aberrant cells incl. gaps	excl. gaps	exchanges
Solvent-control	200	0.0 µg	-	1.50	1.00	0.00
Test-article	200	3.0 µg	-	1.50	1.50	0.00
Test-article	200	5.0 µg	-	5.00	0.50	0.00
Solvent-control	200	0.0 µg	+	4.00	1.00	0.00
Test-article	200	3.0 µg	+	2.00	1.00	0.50
Test-article	200	5.0 µg	+	2.00	1.50	0.00