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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Science Review in Support of a Label Amendment and a Petition (Petition No. 0F06144) for an Exemption From the Requirements of Tolerances for EthylBloc™ (EPA Reg. No. 071297-1) Containing 0.14% 1-Methylcyclopropene (Chemical No. 224459). Review of Acute and Subchronic Toxicity Studies and Other Human Health Data/Information. DP Barcode D275229; Case No. 063215; Submission No. S597276; MRIDs 453803-01 to -08

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THRU: Freshteh Toghrol, Ph.D., Senior Scientist
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ACTION REQUESTED

AgroFresh, Inc. [formerly BioTechnologies for Horticulture, Inc (BTH, Inc.; a subsidiary of Rohm and Haas Company)] has submitted a new acute and subchronic toxicity studies and other human health data/information in support of: (i) a petition (Petition No. 0F06144) for an exemption from the requirements of tolerances for residues of 1-MCP on stored food commodities; and (ii) a label amendment to add indoor use on post-harvested fruits and vegetables. The new studies consist of a new acute inhalation study, a new battery of mutagenicity studies, a subchronic rat inhalation study, preliminary radiolabeled residue data, a dietary and worker risk assessment, and a new acute oral LD_{50} study.

1-MCP is the active ingredient in the end-use product, EthylBloc™ which contains 0.14% 1-MCP. EthylBloc™ is a powdered product that releases 1-MCP as a gas when mixed with water or a buffering agent. The end-use product is currently registered for non-food use on floral and nursery crops.

CONCLUSIONS AND RECOMMENDATIONS

1. The submitted acute toxicity, subchronic toxicity, and mutagenicity studies are acceptable and support registration, pending submission of an acceptable 90-day inhalation study. The registrant has indicated that the 90-day inhalation study is in progress.

2. BPPD concurs with the dietary and worker risk assessment submitted by the registrant (see below) for 1-MCP and CMP. When the product is used according to label directions, there is virtually no risk to consumers of treated food matrices or to workers handling the product formulations.

3. The petition for an exemption from the requirements of tolerances for 1-MCP on food, and the request for a label amendment to permit use of the product on food, are supported by the studies/data reviewed in this document and by previously submitted studies/data.

STUDY SUMMARIES

Acute Toxicity

Acute Oral Toxicity (OPPTS 870.1100; MRID 453803-08): Ten, young adult rats (5/sex) were each administered a single limit dose of 5000 mg/kg 1-methylcyclopropene alpha-cyclodextrin complex in corn oil by oral gavage. Following administration of the complex, rats were observed for clinical signs of toxicity and mortality for a period of 14 days. All rats survived and gained weight throughout the study period. Other than an observation of "scant feces"on Days 1 and 2 postdosing, no signs of toxicity were observed. Necropsies were negative. Based on the data, the rat acute oral LD_{50} for 1-MCP was >5000 mg/kg. Classification: Acceptable; Toxicity Category IV

Acute Inhalation Toxicity (OPPTS 870.1300; MRID 453803-01): Ten, young adult rats (5/sex) were each exposed to atmospheric concentrations of 2.5 mg/L (1126 ppm)1-MCP for 4-hours in whole body chambers. After exposure, the rats were observed for signs of clinical toxicity and mortality for the next 14 days. All rats survived and gained weight throughout the study period and there were no observations of clinical toxicity. Necropsies were negative. Based on the data, the rat acute inhalation LD_{50} for 1-MCP was >2.5 mg/L. Classification: Acceptable; Toxicity Category IV.

Subchronic Inhalation Toxicity

Two-Week Range-Finding Inhalation Toxicity - Female Rat (Non-guideline pilot study; MRID 453803-06): Groups of young adult female rats (7/group) were exposed by whole-body inhalation to atmospheres containing 1-MCP concentrations of 0, 100, 300, or 1000

ppm for a period of 6 hours/day, 5 days/weeks over the course of two consecutive weeks. Exposures were conducted on weekdays only and, in the second week, exposures were conducted on Monday through Thursday only. This resulted in a total of nine 6-hour exposures. Rats were observed daily for clinical signs of toxicity and for mortality. Body weights and food consumption were recorded on days 0, 3, 7, 10 of the study. Blood samples for hematology and clinical chemistries were collected at necropsy at the end of the study. Rats were subjected to necropsy on the day following the last exposure. Selected organs/tissues were collected, weighed, and examined microscopically. All rats survived and gained weight throughout the study, without any clinically observable signs of toxicity. In the 1000 ppm group but not at lower concentrations, red blood cell counts, hemoglobin, and hematocrits were decreased 8%, 6%, and 6%, respectively; and bilirubin was increased 40%. Absolute and relative spleen weights were increased, spleens were discolored, and microscopically, spleens exhibited increased cellularity primarily of red blood cell precursors in the red pulp at 1000 ppm. Similar histopathologic changes (but not increased spleen weights or grossly observable discoloration) of the spleen were detected in rats from the 300 ppm group. The NOAEL was 100 ppm and the LOAEL was 300 ppm based on splenic changes.

Because this was a range-finding study, presumably designed as a pilot for a subsequent longer term study, it was not intended to meet requirements for a subchronic toxicity study following EPA or OECD published protocol guidelines (e.g., length of exposure was too short, only one sex was used, histopathology was abbreviated). EPA OPPTS currently has no published protocol guidelines for so called "subacute" studies of 2 week's duration. Because of this, it does not satisfy the guideline requirement for a subchronic (i.e., 90-day) inhalation toxicity study (as set forth in EPA OPPTS 870.3465 or OECD 412 (*Repeated Dose Inhalation Toxicity: 28-day or 14-day Study*). However, this study does provide useful information.

Classification: Supplemental; however, the study demonstrates that effects on the spleen and blood chemistry occur at levels well above expected exposures to humans (or wildlife) when the product is used according to label directions. The registrant will be submitting a 90-day inhalation study.

Mutagenicity

Bacterial Reverse Mutation (Ames) Assay (OPPTS 870.5100; MRID 453803-02): Strains TA 98, TA100, TA102, TA1535, and TA1537 of *Salmonella typhimurium* were exposed for 24 hours to atmospheric concentrations of 1-methylcyclopropene (1-MCP) at nominal concentrations of 0, 100, 300, or 1000 ppm 1-MCP in an initial assay and 0, 100, 300, or 1000 ppm in a confirmatory assay; actual concentrations ranged from 78% to 126% of target concentrations. The test substance was generated via release from a 1-MCP/alpha-cyclodextrin complex (3.3% active ingredient). Atmospheric concentrations of 1-MCP were analyzed by gas chromatography at 1, 4, and 24 hr following the

initiation of the experiments. Following exposure, *S. typhimurium* cultures were incubated for a further 24 hours. Tester strains were exposed to 1-MCP in the presence and absence of a mammalian metabolic activation system (S-9). No increase in revertant frequencies was detected in either the initial or confirmatory assays in any of the 5 tester strains with or without S-9 metabolic activation. Positive control treatments yielded revertant frequencies within expected ranges. Based on the data, 1-methylcyclopropene is not a mutagen. Classification: Acceptable.

In vitro Mammalian Cell Gene Mutation Test (CHO/HGPRT)(OPPTS 870.5300; MRID 453803-03): Chinese hamster ovary (CHO) cells cultured *in vitro* were exposed for 24 hours to atmospheric concentrations of 1-methylcyclopropene (1-MCP) at nominal concentrations of 0, 100, 250, 500, or 1000 ppm. The test substance was generated via release from a 1-MCP/alpha-cyclodextrin complex (3.3% active ingredient). Concentrations in the air were analyzed by gas chromatography at 1 and 4 hours of treatment. The assay was performed in the presence and absence of a mammalian metabolic activation system (S-9). No cytotoxicity was reported at any test concentration in the absence of metabolic activation. There was no increase in induced mutant colonies over background at any concentrations in the initial trial. Increases in mutation frequency occurred in the confirmatory assay that were unrelated to dose, were not present in duplicate cultures, and did not exceed the mutant frequency required for a positive response. Some weak cytotoxicity (4-22%) was reported in the presence of metabolic activation in the initial trial and no cytotoxicity occurred in the confirmatory assay. No significant increase in the mutant frequency was induced at any concentration in the initial or confirmatory assay. There was no evidence of induced mutant colonies over background following 1-MCP vapor exposure of mammalian cells *in vitro*. Based on the data, 1-MCP is not a mutagen. Classification: Acceptable

In vitro Mammalian Chromosome Aberration Test with Human Lymphocytes OPPTS 870.5375; MRID 453803-04): In an initial study, cultured human lymphocytes were exposed for 4 hours to atmospheric concentrations of 1-methylcyclopropene (1-MCP) at nominal concentrations of 0, 100, 300, and 1,000 ppm and harvested approximately 22 hours after initiation of treatment; actual concentrations ranged from 64% to 116% of target concentrations. The test substance was generated via release from a 1-MCP/alpha-cyclodextrin complex (3.3% active ingredient). Replicate cultures of lymphocytes were exposed to 1-MCP with and without S-9 activation to determine the influence of a mammalian metabolic activation system upon aberration frequencies. At the end of the incubation period, cells were collected and scored for chromosome aberrations, polyploidy, and endoreduplication. In the initial assay, no increase in chromosome aberrations, polyploidy, or endoreduplication was observed at any exposure concentration. For lymphocytes not incubated with S-9 fraction, slight cytotoxicity may have been present at 100 and 1000 ppm (but not 300 ppm) as evidenced by reduced mitotic indices of 14% and 8%, respectively. The positive controls (with and without S-9) produced positive results in expected ranges. A confirmatory assay was conducted using

the same exposure concentrations and procedures. However, lymphocytes without an activation system were exposed to 1-MCP vapors for 19 instead of 4 hours as in the initial assay (lymphocytes with an activation system were exposed again for 4 hours in this subsequent confirmatory assay). Although not stated explicitly in the study report, the reason for increasing the exposure time in the confirmatory assay from 4 to 19 hours for lymphocyte cultures not having an S-9 metabolic activation system, apparently arose from the fact that the low and high (but not the mid) exposure concentrations had slightly reduced mitotic indices, suggesting possible cytotoxicity. Mitotic indices again were reduced for lymphocytes incubated without S-9 fraction at the low and high (but not mid) 1-MCP exposure concentrations, 45% and 5%, respectively. No increases were found at any exposure concentrations in chromosome aberrations, polyploidy, or endoreduplication. The positive control compounds(with and without S-9) yielded the expected increases, indicating that the test cells were responsive. Based on the data,1-MCP is not a claustogen. Classification: Acceptable.

In vivo Mammalian (Mouse) Micronucleus Test (OPPTS 870.5395; MRID 453803-05): Polychromatic erythrocytes from bone marrow, CD-1 mice were exposed (in groups of10/sex/exposure level) by whole body inhalation to atmospheric concentrations of 1-methylcyclopropene (1-MCP) at nominal concentrations of 0, 100, 300, or 1000 ppm for 6 hours. The high exposure group had an additional 4 animals/sex. Half of the controls and 1-MCP mice were sacrificed at 24 hours post-exposure and their micronuclei were evaluated. The second half were sacrificed 48 hours post-exposure. Two additional groups (5/sex) were exposed by intraperitoneal injection to: 1) water (10 ml/kg; vehicle for positive control) and 2) the positive control compound, mitomycin-C (2.0 mg/kg). These latter two groups were sacrificed 24 hours after injection and evaluated for the frequency of micronuclei. As an indication of cytotoxicity, the ratio of polychromatic to normochromatic erythrocytes also was assessed. Exposure to 1-MCP by inhalation did not increase the frequency of micronuclei in mice. No toxicity was evident in the ratio of poly- to normochromatic erythrocytes. The positive control increased the frequency of micronuclei, showing that the test subjects were appropriately sensitive to the induction of micronuclei. Based on the data, 1-MCP is not a mutagen. Classification: Acceptable

Dietary and Worker Risk Assessment (MRID 453803-07)

This report briefly summarizes the the toxicology of 1-MCP, its agricultural uses, and likely human exposures. Following this summary, the report compares estimated human doses to the acute rat lethality NOAEL and to the 2-week rat inhalation NOAEL. The factors by which the acute and 2-week NOAELs exceed the estimated human doses (both in workers and consumers) are calculated as the "margins of safety" (MOS) of 1-MCP. These MOS are on the order thousands for workers, to hundreds of thousands to millions for consumers, for 1-MCP-treated produce (i.e., the estimated human dose is less than the no-effect animal dose by factors of thousands to millions).

NOTE: Much of this information was previously submitted by the registrant and reviewed by the Agency (see various Memoranda listed in the Background section below) and deemed acceptable.

The toxicity of 1-MCP is summarized in the table below:

Study Type	Dose/concentration/effect
Acute Oral Toxicity	Rat LD_{50} >165 mg/kg; NOAEL >165 mg/kg
Acute Inhalation Toxicity	Rat LC_{50} >1000 ppm (> 2.2 mg/L)
Acute Dermal Toxicity	Rabbit LC_{50} > 2,000 mg/kg
Primary Eye Irritation	Slight (Rabbit)
Primary Dermal Irritation	Not an irritant (Rabbit)
Dermal Sensitization	Not a sensitizer (Guinea Pig)
Rat 2-Week Inhalation NOAEL	100 ppm (0.221 mg/L = 63 mg/kg-day)
Ames Mutagenicity	Negative
Mammalian Mutagenicity (CHO/HGPRT)	Negative
In vitro cytogenetics (human lymphocytes)	Negative
In vivo mouse micronucleus	Negative

For the dietary risk assessment, the study author assumed a theoretical worst-case scenario wherein all of the 1-MCP used in a treatment chamber was deposited in/on treated produce (apples in this scenario). Assuming:

(i) maximum application rate = 3.3% 1-MCP (0.068 g product/m^3; or 1 ppm v/v a.i./m^3);
(ii) 250 kg apples/m^3 (in treatment chamber); and
(iii) 100% 1-MCP absorbed in/on apples; then,

(0.068 g product/m^3) **X** (0.033 g a.i./g product) **X** (1 m^3/250 kg apples)

= 0.000009 g a.i./kg apples or 0.009 ppm a.i. (9 ppb a.i.).

NOTE: The concentration of 1-MCP is below the analytical method detection limit (using nonradiolabeled 1-MCP) in this worst case scenario .

Measured residues on apples (see Background section below for details) following a 24-hour treatment with ^{14}C-1-MCP were ≤ 7.7 ppb (mean = 3.8 ppb). These residues were below the theoretical maximum of 9 ppb.

Dietary Risk Assessment: Preliminary residue studies indicate that the mean radioactive residue remaining on apples following a 24-hr treatment with product will not exceed

approximately 4 ppb. Assuming a worst-case scenario wherein a 70 kg human consumes a diet of 1500 g of food/day and that ALL of the food contains 4 ppb of 1-MCP residues:

((0.000004 mg 1-MCP/g food) **X** (1500 g of food/day)) / 70 kg

= 8.6 x 10^{-5} mg/kg 1-MCP consumed.

Since the acute oral NOEL > 165 mg 1-MCP/kg:

(165 mg 1-MCP/kg) / 8.6 x 10^{-5} mg/kg 1-MCP = >1,900,000 Margin of Safety (MOS).

Using the same formula above, comparison of potential dietary exposure to the 2-week inhalation toxicity of 100 ppm (63 mg a.i/kg/day) gives an MOS >730,000.

Assuming a worst case scenario wherein ALL of the 1-MCP used in a treatment room was bound to the treated crop (i. e. 9 ppb 1-MCP), then:

Potential dietary exposure based on the acute oral NOEL gives an MOS >860,000 and if based on the 2-week inhalation NOEL gives an MOS >330,000.

Conclusion: Human dietary exposure is expected to be extremely low due to the gaseous nature of the active ingredient, its reversible binding to sites in/on the treated food matrix, its low use rate (≤1 ppm v/v), and its extremely low residues on food (≤9 ppb on a theoretical worst case scenario). Furthermore, numerous studies have demonstrated its low to no toxicity. If the product is used according to label directions, it is highly unlikely that any toxic effects will occur to humans or other nontarget organisms.

Worker Risk Assessment: Commercial applications of 1-MCP will be made in only sealed refrigerated and/or controlled-atmosphere chambers. The product will be applied by use of a proprietary packaging system for small (<500 m^3) chambers or with a proprietary generating system for larger (>500 m^3) chambers. In either chamber size, workers will be required to wear the appropriate PPE when working with the packaging or generating system and will leave the chamber immediately upon the initiation of treatment. The chamber will be posted with appropriate signage to deter entry into the chamber while treatment is in progress. Upon completion of treatment, the chambers will be vented prior to entry of workers. However, the study author determined that in the event of an inadvertent 30-minute exposure to workers to 1 ppm v/v 1-MCP, exposure and risk to 1-MCP would be low. The author showed calculations that demonstrated: there would be an MOS = 3150 for a 30-minute accidental exposure to 1-MCP.

3-Chloro-2-Methylpropene (CMP) Worker Risk Assessment: CMP is a starting material in the synthesis of 1-MCP and has been determined to be carcinogenic in laboratory animal studies. It is present in 1-MCP at ≤0.08%; therefore, in a treatment room, it will

theoretically be present at no more than 0.8 ppb. The study author presented calculations from Rohm & Haas Co. Health Hazard Review Committee demonstrating that a workplace exposure limit of 65 ppb CMP (i.e. a CMP concentration that workers may breathe all day during their working careers) has been established for CMP based on a 3000 MOS; the CMP analytical limit of detection is stated to be 0.4 ppb (MRID 453803-07, p. 11). Since it is unlikely that CMP will be present at more than 0.8 ppb in a treatment chamber, the MOS was calculated (by the study author) to be >243,000. Furthermore, when the product is used according to label directions, there is virtually no exposure of workers to CMP.

Dermal Risk to Workers: Since the active ingredient is a gas, and workers are unlikely to be present in treatment chambers when 1-MCP is being applied, there is virtually no dermal risk to workers from 1-MCP. The active ingredient has been shown to be nontoxic via oral, skin, and inhalation routes of exposure. Accidental, intermittent, inadvertent exposure to the product poses virtually no risk to workers.

BACKGROUND

EthylBloc™ (EPA Reg. No. 071297-1) is currently registered for non-food use on floral and nursery crops in enclosed, indoor areas. In support of this registration, the registrant has submitted acceptable product chemistry studies (OPPTS 810.1550-810.7950; Subdivision M Guidelines 151-10 to -17) and acute mammalian toxicity studies [OPPTS 870.1100 to 870.2600; Subdivision M 152-10 to -16 (see Memoranda from R. S Jones to D. Benmhend, dated 12/23/1998 and 3/1/1999)]; it was also initially concluded that the compound is not a mutagen. No data for non-target organisms and ecological effects were required because the product was not intended for use outdoors or in other non-enclosed areas.

More recently, the registrant requested an experimental use permit (EUP; EPA Reg. No. 71297-EUP-R) to permit the commercial indoor testing of EthylBloc™ on postharvest stored apples (EPA File No. 71297 -1). Under the EUP, the registrant intended to use a maximum of 52.9 lbs of EthylBloc™ (equivalent to 0.074 lbs of 1-MCP) on 10.8 million lbs of postharvested apples. This EUP was deemed acceptable by BPPD (see Memorandum from R. S. Jones to D Benmhend, dated 9/28/2000) provided it included a crop destruct requirement. The crop destruct requirement was included because there were no established tolerances or tolerance exemptions for residues of 1-MCP on apples or other food commodities.

A petition to establish a permanent exemption from the requirement of tolerances for residues of 1-MCP on food commodities was submitted to the Agency in April 2000 and is the subject of this review. It was noted in the EUP review of 9/28/2000, that since apples are also processed for juice, puree, applesauce, animal feed, etc, that residue data should be used to support the petition for a permanent tolerance exemption for 1-MCP and that these residue data should include data on all raw and processed apple commodities.

On 6/21/2000, a Notice of Filing of a Pesticide Petition to Establish a Tolerance for Certain Pesticide Chemicals in or on All Food Commodities appeared in the Federal Register Volume 65, Number 120; Docket Control No. PF-947), indicating that 1-MCP posed "no significant risk to humans or the environment." In a comment submitted to the Agency (see letter from M. Tichon, Valenti Biosciences Corporation, dated 7/20/2000), it was argued that "the literature indicates risks of adverse effects posed by toxicity of and exposure to 1-MCP are considerably greater than represented in the notice of filing" and that "these risks argue against the issuance of the proposed tolerance exemption. In a letter from S. Longacre to D. Benmhend (dated 12/19/2000), Agrofresh, Inc. submitted a response to these comments including information supporting the registrant's contention that the active ingredient would not cause adverse effects on humans and wildlife when the product is used according to label directions.

The information submitted by AgroFresh, Inc. to support the petition (Petition No. 0F06144) from the requirements of a tolerance for 1-MCP on all food commodities and a label amendment for EthylBloc™ (EPA Reg. No. 071297-1), containing 0.14% 1-MCP as its active ingredient, the comments submitted by Valenti BioSciences (dated 7/20/2000), and AgroFresh's response (dated 12/19/2000) to the Valenti BioSciences comments, were reviewed in a Memorandum from R. S. Jones to D. Benmhend (dated 2/21/2001). In the 2/21/2001 Memorandum, BPPD concurred with the AgroFresh conclusion that when EthylBloc™ (containing 0.14% 1-MCP as its active ingredient) is used according to label directions, exposure to humans and wildlife (by oral, dermal, inhalation, or eye pathways) is extremely low to non-existent. BPPD further concluded that the submitted data/evidence and scientific rationale submitted by AgroFresh indicated that there is a reasonable certainty of no harm with the use of EthylBloc™ (containing 0.14% 1-MCP as its active ingredient) on food commodities stored in closed, sealed treatment facilities and applied according to label directions. However, it is further noted that these determinations were not based upon any quantitative data (generated via guideline or non-guideline studies), but were based upon theoretical calculations.

Therefore, to alleviate concerns regarding 1-MCP residues on treated food, BPPD required the registrant to develop radioisotope techniques to determine whether any 1-MCP (and/or metabolites) remain on treated food after treatment. The registrant was also being required to re-conduct new acute oral toxicity and inhalation studies and the three study battery of genotoxicity/mutagenicity studies. The registrant subsequently submitted an acceptable preliminary analytical method using a Liquid Scintillation Counting (or LSC) method for determining ^{14}C-1-MCP residues in treated apples; the limit of quantitation was 1 ppb (see Memorandum from R. S. Jones to D. Benmhend, dated 5/03/2001). Accompanying this analytical method, were preliminary residue data obtained from three experiments using apples treated with ^{14}C-1-MCP. The submitted data, when corrected for mean analytical method recoveries, demonstrated that ^{14}C-1-MCP residues did not exceed 8.6 ppb in/on apples 24 hours following a 24-hr exposure to 1200 ppb ^{14}C-1-MCP (1.2x the maximum label application rate). Residues of ^{14}C-1-MCP were observed to be below the non-radiolabel analytical method detection limit of 10 ppb.

The current submission addresses the new toxicity studies submitted by the registrant to support the tolerance exemption petition and the label amendment to permit the use of the end-use product on food crop. The new toxicity studies were conducted using a new methodology that would assure that the test organisms were exposed to gaseous 1-MCP.

DATA EVALUATION RECORD

1-METHYLCYCLOPROPENE

STUDY TYPE: ACUTE ORAL TOXICITY (RAT)

Prepared for

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Primary Reviewer: Steven T. Cragg, PhD, DABT	Signature: Date:	Steven T. Cragg 3-July-2001
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EPA Reviewer: Biochemicals _____ Date 1/27/2002
Review Section__, ~~Toxicology~~ Branch ___ (75~~09~~C)
EPA Secondary Reviewer: _____ Date _____
Review Section__, ~~Toxicology~~ Biochemicals Branch ___ (75~~09~~C)

Data Evaluation Record

STUDY TYPE: Acute Oral Toxicity - Rat
OPPTS 870.1100 [§81-3]; OECD Guideline 401 [EEC Directive 92/69/EEC B.2]

DP BARCODE:	D275229	SUBMISSION CODE:	S597276
P.C. CODE:	071297-00001	TOX. CHEM. NO.:	
MRID No.:	453803-08		

TEST MATERIAL (PURITY): 1-Methylcyclopropene (95.80% active ingredient)

SYNONYMS: Cyclopropene, 1-methyl-; 1-MCP

CITATION: Parno, J.R., , Craig, L.P., Eberly, S.L., (2001). 1-Methylcyclopropene Alph-Cyclodextrin Complex (3.3 a.i.) Acute Oral Toxicity Study in Male and Female Rats. Rohm and Haas Company Toxicology Department Report No. 00R-199, February 8, 2001. MRID 453803-08. Unpublished.

SPONSOR: Rohm and Haas Company
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EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 453803-08), a single group of young adult rats (Crl:CD®BR) (5/sex) was administered single doses by gavage of 5000 mg/kg 1-methylcyclopropene alpha-cyclodexdrin complex. No control group was employed. After the single administration of the complex, rats were observed for clinical signs of toxicity and mortality for a period of 14 days.

Other than "scant feces" noted on days 1 and 2, no signs of toxicity or deaths occurred as a result of complex administration immediately after dosing or over the course of a 14-day observation period. Body weights were unaffected when compared to historical controls. No grossly observable lesions were evident at autopsy after the 14-day observation period.

LC50 (males)	› 5000 mg/kg
LC50 (females)	› 5000 mg/kg
LC50 (combined)	› 5000 mg/kg

1-Methylcyclopropene alpha-cyclodextrin complex falls into TOXICITY CATEGORY IV. At a dose of 5000 mg/kg, there were no mortalities, clinical signs of toxicity, necropsy findings, or body weight alterations. Thus, although the LD50 was not defined in this limit test, it exceeds 5,000 mg/kg, falling above the category IV cut-off of 5000 mg/kg for the LD50.

This was a limit test because no mortalities were seen at the exposure level tested. Presumably, additional exposure levels at lower concentrations would have been evaluated if mortality greater than 50% had occurred at the single concentration tested. In such a case, testing lower concentrations would have generated sufficient data to define the actual LD50. This level was sufficiently high to determine whether this compound falls into Toxicity Category IV (least toxic category). Thus, since no deaths occurred at the concentration tested, testing at lower concentrations would not have provided further information. Finally, the lack of a negative control is not considered a deficiency or limitation of this study

A negative control is not routinely used in acute toxicity testing unless a solvent other than water (or corn oil) is used to generate the test atmosphere and/or insufficient historical information is available concerning the solvent (see Protocol Guideline OPPTS 870.1100).

This acute oral toxicity study in rats is classified as acceptable. While only one group was tested (i.e., this was a limit test wherein only a lower limit for the LC50 could be established), the dose tested was sufficiently high to show a lack of toxicity. Moreover, a limit test complies with EPA policy to, where possible, reduce the number of animals used in toxicity testing.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A **Materials:**

1. Test Material: 1-Methylcyclopropene alpha-cyclodextrin complex mixed as a suspension in corn oil to a volume for dosing of 20 ml/kg (i.e., 5000 mg of the complex in 20 ml corn oil).

Description:	White solid (containing 3.4% active ingredient 1-methylcyclopropene)
Lot/Batch #:	Lot No. BAS 5-80; Sample No. 00-103
Purity	Mixture (confidential business information)
CAS #:	3100-04-7 (1-methylcyclopropene)
Test Article Analysis:	Analytical report (Rohm and Haas Report No. 00R-199) attached as appendix to the sponsor's LC50 report.

2. Vehicle and/or positive control:

Corn oil was used as the vehicle into which the complex was mixed as a suspension. Corn oil without the test material was not tested as a negative, vehicle control. Positive controls are not used routinely in acute toxicity tests.

3. Test animals:

Species:	Rat (males and females)
Strain:	Crl:CD®BR
Age and weight at dosing:	Males ~8 weeks (233-250 grams); females ~9 weeks (210-220 grams).
Source:	Charles River Laboratories, Raleigh, NC
Acclimation period:	Approximately one week.
Diet:	PMI Certified Rodent Diet 5002(C) (Purina Mills, Inc., Richmond, IN. Food was available *ad libitum*.
Water:	Automatic watering system *ad libitum* (reverse osmosis treated).
Housing:	Individual stainless steel wire mesh cages (dimensions 18 x 34 x 18 cm) suspended above absorbent-paper pan liners (changed 3X/wk).
Environmental Conditions:	
Temperature:	23°C monitored continuously
Humidity:	30-70% monitored continuously
Photoperiod:	12 hr light/12 hr dark.

B. STUDY DESIGN and METHODS:

1. In life dates - start: December 14, 2000 end: December 28, 2000

2. Animal assignment and treatment

Animals were assigned to the test group shown in Table 1. Following an overnight fast, rats were orally dosed (gavage) with 1-MCP-alpha-cyclodextrin complex suspended in corn oil to a dosing volume of 20 ml/kg (i.e., 5000 mg complex per 20 ml corn oil). Animals were observed for signs of toxicity at 0, 1, 2, and 4 hours after dosing, and daily thereafter for 14 days. Body weights were recorded on days 0 (prior to dosing), 7, and 14. After 14 day's observation, all animals were sacrificed and a necropsy was performed.

Table 1. Doses, No. Animals Treated, and Mortality Incidence

Dose (mg/kg)	No. animals treated	# dead/total (Males)	# dead/total (Females)	# dead/total (Combined)
5000 mg/kg	5/sex	0/5	0/5	0/10

3. Statistics
Because no mortalities occurred at the single exposure level tested, no LC50 was calculated.

II. **RESULTS AND DISCUSSION:**

A. Mortality is given in Table 1. No mortalities occurred as a result of a single oral administration of 1-MCP-alpha-cyclodextrin complex in corn oil.

The oral LD50 for males is › 5000 mg/kg
females is › 5000 mg/kg
combined is › 5000 mg/kg

These results place 1-Methylcyclopropene-alpha-cyclodextrin complex into Toxicity Category IV.

B. Clinical observations

No signs of toxicity were noted during or for 14 days after exposure to 1-methylcyclopropene-alpha-cyclodextrin complex. "Scant feces" was reported after dosing through day 2.

C. Body weight

1-Methylcyclopropene-alpha-cyclodextrin complex did not affect body weights

D. Necropsy

No adverse findings attributable to 1-methylcyclopropene-alpha-cyclodextrin complex were reported when animals were necropsied at end of the 14-day observation period.

E. Deficiencies

No deficiencies were noted.

DATA EVALUATION RECORD

1-METHYLCYCLOPROPENE

STUDY TYPE: ACUTE INHALATION TOXICITY - RAT

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
Crystal Station I
2800 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Tetrahedron, Inc.
1414 Key Highway
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Primary Reviewer: Steven T. Cragg, PhD, DABT	Signature: Date:	Steven T. Cragg 3-July-2001
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Quality Assurance: Waqi Alum, PhD.	Signature: Date:	W Alum July 3, 2001

EPA Reviewer: Biochemicals _____ Date 1/29/2002
Review Section__, ~~Toxicology~~ Biochemicals Branch ___ (7509C)
EPA Secondary Reviewer: _____ Date _____
Review Section__, ~~Toxicology~~ Biochemicals Branch ___ (7509C)

Data Evaluation Record

STUDY TYPE: Acute Inhalation Toxicity - Rat
OPPTS 870.1300 [§81-3]; OECD Guideline 403 [EEC Directive 92/69/EEC B.2]

DP BARCODE:	D275229	SUBMISSION CODE:	S597276
P.C. CODE:	071297-00001	TOX. CHEM. NO.:	
MRID No.:	453803-01		

TEST MATERIAL (PURITY): 1-Methylcyclopropene (95.80% active ingredient)

SYNONYMS: Cyclopropene, 1-methyl-; 1-MCP

CITATION: Ferguson, J.S., Craig, L.P., Bernacki, H.J., (2001). 1-Methylcyclopropene. Acute Inhalation Toxicity Study in Rats. Rohm and Haas Company Toxicology Department Report No. 00R-180, February 8, 2001. MRID 453803-01. Unpublished.

SPONSOR: Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, PA 19477-0904

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 453803-01), a single group of young adult rats (Crl:CD®BR) (5/sex) was exposed by inhalation for a period of 4 hours to a 1-methylcyclopropene (1-MCP) concentration of 2.5 mg/L (1126 ppm) in whole-body inhalation chambers under dynamic conditions. No negative (air-only) control group was employed. After exposures were completed, rats were observed for clinical signs of toxicity and mortality for a period of 14 days.

No signs of toxicity or deaths occurred during exposure or during the post-exposure 14-day observation period. Body weights were unaffected when compared to historical controls. No grossly observable lesions were evident at autopsy after the 14-day observation period.

LC50 (males)	› 2.5 mg/L (1126 ppm)
LC50 (females)	› 2.5 mg/L (1126 ppm)
LC50 (combined)	› 2.5 mg/L (1126 ppm)

1-Methylcyclopropene falls into TOXICITY CATEGORY IV. At an exposure level of 2.5 mg/L for 4 hours, there were no mortalities, clinical signs of toxicity, necropsy findings, or body weight alterations. Thus, although the LD50 was not defined in this limit test, it exceeds 2.5 mg/L, an exposure level that falls above the category IV cut-off of 2.0 mg/L for the LC50.

This was a limit test because no mortalities were seen at the exposure level tested. Presumably, additional exposure levels at lower concentrations would have been evaluated if mortality greater than 50% had occurred at the single concentration tested. In such a case, testing lower concentrations would have generated sufficient data to define the actual LC50. The report indicates that the single concentration tested was the highest that could be generated safely without the danger of explosion. This level also was sufficiently high to determine whether this compound falls into Toxicity Category IV (least toxic category). Thus, since no deaths occurred at the concentration tested, testing at lower concentrations would not have provided further information. Finally, the lack of a negative control is not considered a deficiency or

limitation of this study. A negative control is not routinely used in acute toxicity testing unless a solvent other than water is used to generate the test atmosphere and/or insufficient historical information is available concerning the solvent (see Protocol Guideline OPPTS 870.1300).

This acute inhalation study is classified as acceptable. While only one group was tested (i.e., this was a limit test wherein only a lower limit for the LC50 could be established), the concentration tested was sufficiently high to show a lack of toxicity. Moreover, a limit test complies with EPA policy to, where possible, reduce the number of animals used in toxicity testing.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A **Materials:**

1. Test Material: 1-Methylcyclopropene

Description:	Colorless gas
Lot/Batch #:	Lot No. RMJ6679A; Sample No. 00-118
Purity:	95.90%
CAS #:	3100-04-7
Test Article Analysis:	Analytical report (Rohm and Haas Report No. 00R-191) attached as appendix to the sponsor's LC50 report (see brief description below).

2. Vehicle and/or positive control:

No solvent or carrier vehicle was used as a vehicle to generate the test atmosphere since the test compound is a gas at room temperature. Air was used as a diluent to achieve the desired concentration. Positive control chemicals are not routinely used in acute LC50 tests.

3. Test animals:

Species:	Rat (males and females)
Strain:	Crl:CD®BR
Age and weight at dosing:	Males 8 weeks (255-275 grams); females 10 weeks (232-250 grams).
Source:	Charles River Laboratories, Kingston, NY
Acclimation period:	Approximately one week.
Diet:	PMI Certified Rodent Diet 5002(C) (Purina Mills, Inc., Richmond, IN. Food was available *ad libitum* except during exposure.
Water:	Automatic watering system *ad libitum* (reverse osmosis treated) except during exposure.
Housing:	Individual stainless steel wire mesh cages in inhalation chambers (dimensions 18 x 13 x 15 cm) or after exposures (dimensions 18 x 34 x 18 cm) suspended above absorbent-paper pan liners (changed 3X/wk).
Environmental Conditions:	
Temperature:	22±2°C (chambers); 22°C (non-exposure period).
Humidity:	30-70% (chambers); 35-51% (non-exposure period).
Air changes:	13/hr during exposures.
Photoperiod:	12 hr light/12 hr dark.

B. STUDY DESIGN and METHODS:

1. In life dates - start: December 15, 2000 end: December 29, 2000

2. Exposure conditions

During exposures, rats were housed in stainless steel wire mesh cages (with dimensions of 18 x 13 x 15 cm) within a 240 liter Plexiglas® and stainless steel inhalation chamber. Assuming rats averaged no more than 250 grams in weight, or 250 ml total volume, 1 rat would displace no more than 250 ml total volume. 10 rats would displace 2,500 ml or 2.5 liters, which is slightly more than 1% of the chamber volume (well within the guideline of 5%).

3. Animal assignment and treatment

Animals were assigned to the test group noted in Table 1. Rats were exposed to 1-MCP by whole body inhalation exposure for 4 hours. They were observed for signs of toxicity during exposure, immediately upon removal from the chambers after exposure, 4 hours post-exposure, and daily thereafter for 14 days. After 14 day's observation, all animals were sacrificed and a necropsy was performed. Chamber airflow was monitored continuously. The airflow rate was 51 liters/minute, resulting in 13 air changes per hour, exceeding the guidance standard of 10 changes per hour.

Table 1. Concentrations, exposure conditions, mortality/animals treated.

Nominal Conc.	Analytical Conc.* (± S. D.)	MMAD (µm)	GSD (µm)	# dead/total (Males)	# dead/total (Females)	# dead/total (Combined)
1,000 ppm or 2.21 mg/L	1126 (± 108) ppm or 2.49 (± 0.238) mg/L	N/A*	N/A*	0/5	0/5	0/10

* The analytical or actual concentration is given along with the standard deviation (S.D.); N =8.
** Not Applicable. MMAD (mass median aerodynamic diameter) and GSD (geometric standard deviation) are not relevant since 1-MCP is a gas.

4. Generation of the test atmosphere and description of the chamber

Chamber atmospheres were generated by connecting a 1-MCP filled Tedlar Sampling bag through a tube and, using a Gilmont Flowmeter, metering 1-MCP from the bag into a 240 liter Plexiglas® and stainless steel chamber containing the test subjects. 1-MCP was drawn into the top of the chamber via negative pressure and mixed with air inside the chamber to achieve the desired concentration. Chamber atmospheres were sampled twice hourly during the exposure period. Chamber airflow was monitored continuously. The airflow rate was 51 liters/minute, resulting in 13 air changes per hour. The time to t_{99} (equilibration time to reach 99% of target concentration) was 22 minutes. Test subjects remained in the chamber exposed to the target concentration for 4 hours after t_{99} was reached, ensuring 4 hours of exposure to the target concentration.

Test atmosphere concentration

The test atmosphere was sampled twice hourly (at approximately 30 minute intervals) over the 4-hour course of exposure. Samples (0.5 ml) were taken from the chamber with a gas tight

syringe and injected directly into a gas chromatograph. This chromatograph is described in the appended analytical report as a "Hewlett Packard Series 6890 HPLC sytem." HPLC usually stands for high pressure liquid chromatography, not gas chromatography. This may be an error. The toxicity report refers to gas chromatographic analysis. And other parts of the analytical report appended to the back of the toxicology report describe column conditions and that leave no doubt that gas chromatography was employed. A flame ionization detector was used to detect 1-MCP. Results of the analysis are given in Table 1 above.

Particle size determination

Particle size determination is not relevant for this compound, which is a gas. Consequently, mass median aerodynamic diameters and the geometric standard deviations of the diameters, in micrometers, is not reported in Table 1.

5. Statistics

Because no mortalities occurred at the single exposure level tested, no LC50 was calculated.

II. Results and Discussion:

A. Mortality is given in Table 1. No mortalities occurred as a result of exposure to 1-MCP.

The LC50 for males is	› 2.5 mg/L
females is	› 2.5 mg/L
combined is	› 2.5 mg/L

These results place 1-Methylcyclopropene into Toxicity Category IV.

B. Clinical observations

No signs of toxicity were noted during or for 14 days after exposure to 1-methylcyclopropane.

C. Body weight

1-Methylcyclopropene did not affect body weights

D. Necropsy

No adverse findings attributable to 1-methylcyclopropene were reported when animals were necropsied at the end of the 14-day observation period.

E. Deficiencies

1. To generate the test atmosphere within the chamber, 1-MCP was not mixed with air to the desired concentration in a plenum prior to introduction of 1-MCP into the inhalation chamber. Rather, pure 1-MCP was introduced directly into the chamber and then allowed to mix with air to achieve the desired concentration. This might be acceptable if the report had indicated that chamber air samples were taken from breathing zone of the test subjects. Repeated 1-MCP measurements were consistent within the chamber but whether samples were taken from the breathing zone was not indicated. Better yet would have been sampling from different locations throughout the chamber showing consistent measurements. Since repeated measurements were taken (twice hourly during the exposure period), which showed consistent concentrations, and since a gas such as 1-MCP would

be expected to quickly mix and equilibrate with diluent air, this method of atmosphere generation is not considered serious enough to compromise the integrity of the study.

2. Isobutylene was used as the standard for 1-MCP. Standard concentrations of 1-MCP were not made and used to generate a calibration curve for the GC. It was assumed that the GC detector would have the same sensitivity for isobutylene as for 1-MCP and that the area under the curve for isobutylene would directly correspond to the concentration of 1-MCP (after adjusting for molecular weight differences). But justification for this assumption was not provided in the report. Since it is generally recognized that the areas under the curve are consistent for stucturally similar chemicals on the same GC column, this assumption is not considered serious enough to compromise the integrity of the study.

3. Two GC peaks were ascribed to 1-MCP. The first major peak eluted after 3.3 minutes while a second, smaller peak eluted after 3.4 minutes. The area under the curve of both peaks was used to determine the concentration of 1-MCP. The second peak may have been a contaminant or a thermal degradation product. In the second case, adding the two peaks together is justified. If the former case, adding the two areas may still be justified since assessing the toxicity of the formulation (with the contaminant included) is the desired goal. The area of the second peak was 4.8% of the combined areas. Consequently, even if the contribution of the second peak is not included, this would not change the toxicity category of this compound.

DATA EVALUATION RECORD

1-METHYLCYCLOPROPENE

STUDY TYPE: TWO-WEEK RANGE-FINDING INHALATION TOXICITY (RAT)

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
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Arlington, VA 22202

Prepared by

Tetrahedron, Inc.
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Primary Reviewer: Steven T. Cragg, PhD, DABT	Signature: Steven T. Cragg Date: 3-July-2001
Secondary Reviewer Nasin Begin, PhD	Signature: Nasrin Begum Date: 7/3/01
Quality Assurance: Waqi Alum, PhD.	Signature: W. Alum Date: July 3, 2001

EPA Reviewer: Biochemicals, Date 1/27/2002
Review Section__, ~~Toxicology~~ Branch ___ (75~~0~~C)
EPA Secondary Reviewer: _____, Date _____
Review Section__, ~~Toxicology~~ Biochemicals Branch ___ (75~~0~~C)

Data Evaluation Record

STUDY TYPE: Range-Finding Inhalation Toxicity - Female Rat
no guidelines (this is a pilot study)

DP BARCODE:	D275229	SUBMISSION CODE:	S597276
P.C. CODE:	071297-00001	TOX. CHEM. NO.:	
MRID No.:	453803-06		

TEST MATERIAL (PURITY): 1-Methylcyclopropene (95.80% active ingredient)

SYNONYMS: Cyclopropene, 1-methyl-; 1-MCP

CITATION: Ferguson, J.S., Craig, L.P., Lampe, K.R., (2001). 1-Methylcyclopropene Two-Week Inhalation Range-Finding Study in Female Rats. Rohm and Haas Company Toxicology Department Report No. 00R-183A, March 22, 2001. MRID 453803-06. Unpublished.

SPONSOR: Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, PA 19477-0904

EXECUTIVE SUMMARY: In a 2-week range-finding inhalation toxicity study (MRID 453803-06), groups of young adult female rats (Crl:CD®BR) (7/group) were exposed by whole-body inhalation to atmospheres containing 1-methylcyclopropene (1-MCP) for a period of 6 hours/day, 5 days/weeks over the course of two consecutive weeks. Male rats were not employed in this study. Exposures were conducted on weekdays (no exposures were conducted on weekends) and, in the second week, exposures were conducted on Monday through Thursday only. This resulted in a total of nine 6-hour exposures. Rats were exposed to 1-methylcyclopropene (1-MCP) concentrations of 0, 100, 300, or 1000 ppm in whole-body inhalation chambers under dynamic conditions. Rats were observed daily for clinical signs of toxicity and for mortality. Body weights and food consumption were recorded on days 0, 3, 7, 10 of the study. Blood samples for hematology and clinical chemistries were collected at necropsy at the end of the study. Rats were subjected to necropsy on the day following the last exposure. Selected organs/tissues were collected, weighed, and examined microscopically.

All rats survived exposure to 1-MCP with no clinically observable signs of toxicity. No effects on body weights or food consumption were found at any exposure level. In the 1000 ppm group but not at lower concentrations, red blood cell counts, hemoglobin, and hematocrits were decreased 8%, 6%, and 6%, respectively. Also at this exposure level but not below, bilirubin was increased 40%. Absolute and relative spleen weights were increased, spleens were discolored, and microscopically, spleens exhibited increased cellularity primarily of red blood cell precursors in the red pulp at 1000 ppm. Similar histopathologic changes (but not increased spleen weights or grossly observable discoloration) of the spleen were detected in rats from the 300 ppm group. The NOAEL for this study was 100 ppm and the LOAEL was 300 ppm based on splenic changes.

Because this was a range-finding study, presumably designed as a pilot for a subsequent longer term study, it was not intended to meet requirements for a subchronic toxicity study following EPA or OECD published protocol guidelines (e.g., length of exposure was too short, only one sex was used, histopathology was abbreviated). EPA OPPTS currently has no published protocol guidelines for so called

"subacute" studies of 2 week's duration. Because of this, it does not satisfy the guideline requirement for a subchronic (i.e., 90-day) inhalation toxicity study (as set forth in EPA OPPTS 870.3465). OECD has protocol 412 ("Repeated Dose Inhalation Toxicity: 28-day or 14-day Study"). However, this study does not satisfy OECD test protocol 412 (e.g., only one sex was used, no "recovery" group was employed, etc.). However, this study does provide useful supplementary information.

COMPLIANCE: Flagging statements (confidentiality and GLPs) were provided. The GLP statement included in the report and signed by the study director indicates that the study was conducted according to published FIFRA GLPs but had not received a Quality Assurance audit because it was a range-finding study "designed to estimate toxicity or to provide information needed for dose selection for subsequent studies."

I. MATERIALS AND METHODS

A **Materials:**

1. Test Material: 1-Methylcyclopropene

Description:	Colorless gas
Lot/Batch #:	Lot No. RMJ6679A; Sample No. 00-105
Purity:	96.42%
CAS #:	3100-04-7
Test Article Analysis:	Analytical report (Rohm and Haas Report No. 00R-183A) attached as appendix to the sponsor's LC50 report (see brief description below).

2. Vehicle and/or positive control:

No solvent or carrier vehicle was used as a vehicle to generate the test atmosphere since the test compound is a gas at room temperature. Air was used as a diluent to achieve the desired concentration.

3. Test animals:

Species:	Rat (females only)
Strain:	Crl:CD®BR
Age and weight at dosing:	Age not specified (120-125 grams).
Source:	Not specified.
Acclimation period:	Not specified.
Diet:	Not specified.
Water:	Not specified.
Housing:	Not specified.

Environmental Conditions (not specified for general housing outside inhalation chambers):

Environmental Conditions inside inhalation chambers:

Temperature:	20.5±1.3°C
Humidity:	68.3±11.1%
Chamber air flow rate:	79.0±00 liters/minute
Chamber volume:	240 liters
Air changes:	19.75/hr during exposures.
Photoperiod:	12 hr light/12 hr dark.

B. STUDY DESIGN and METHODS:

1. In life dates - start: Not specified end: Not specified Report date: 22 March 2001

2. Exposure conditions

During exposures, rats were housed in stainless steel wire mesh cages (with dimensions of 18 x 13 x 15 cm) within a 240 liter Plexiglas® and stainless steel inhalation chamber. Assuming rats averaged no more than 250 grams in weight, or 250 ml total volume, 1 rat would displace no more than 250 ml total volume. 10 rats would displace 2,500 ml or 2.5 liters, which is slightly more than 1% of the chamber volume (within the guideline of 5%).

3. Animal assignment and treatment

Animals were assigned to the test group noted in Table 1. Rats were exposed to 1-MCP by whole body exposure for 6 hr/day, 5 d/wk for the first week and 4 d/wk for the second week for a total of 9 exposures (no exposures occurred on weekends).

Table 1. Concentrations, exposure conditions, females/group

Group	Nominal Conc.	Actual Conc. (± S.D.)	No. Females/Group
1 (air-only)	0 ppm	0 ppm	7
2	100 ppm	104.9 ppm (± 8.6)	7
3	300 ppm	306 ppm (± 13.6)	7
4	100 ppm	999.8 ppm (± 51.6)	7

4. Generation of the test atmosphere and description of the chamber

Chamber atmospheres were generated by connecting a 1-MCP filled Tedlar Sampling bag through a tube and, using a Gilmont Flowmeter, metering 1-MCP into a 240 liter Plexiglas® and stainless steel chamber containing the test subjects. 1-MCP was drawn into the top of the chamber via negative pressure and mixed with air inside the chamber to achieve the desired concentration. Chamber atmospheres were sampled twice hourly. Chamber airflow was monitored continuously. The airflow rate was ~50 liters/minute, resulting in ~12 air changes per hour. The time to t_{99} (equilibration time to reach 99% of target concentration) less than 10% of total exposure time. Test subjects remained in the chamber exposed to the target concentration for 6 hours after t_{99} was reached, ensuring 6 hours of exposure to the target concentration.

Test atmosphere concentration

The test atmosphere was sampled twice hourly over the 6-hour course of daily exposures. Samples (0.5 ml) were taken from the chamber with a gas tight syringe and injected directly into a gas chromatograph. A flame ionization detector was used to detect 1-MCP. Results of the analysis are given in Table 1 above.

Particle size determination

Particle size determination is not relevant for this gas. Consequently, mass median aerodynamic diameters and geometric standard deviations are not reported in Table 1.

5. Statistics

Statistics were not described.

C. METHODS:

1. Observations

Animals were inspected daily for signs of toxicity and mortality at the conclusion of each exposure day.

2. Body weight

Animals were weighed prior to exposure on days 0, 3, 7, and 10 and reported as group average body weights and group average body weight gains.

3. Food consumption

Food consumption was recorded on days 0, 3, 7, and 10 and reported as daily group averages for days 0-3, 4-7, and 7-10.

4. Ophthalmic examination

No ophthalmic examinations were conducted.

5. Blood collection Blood was collected for hematology and clinical chemistries from the abdominal aorta in anesthetized subjects immediately preceding sacrifice and autopsy. Whether subjects were fasted overnight was not reported. The CHECKED (X) parameters were examined (see tables below).

A. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular hemoglobin (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines.

Blood clotting measurements were not reported.

B. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	----------	X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALP)	X	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatinine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (ALT, aka SGPT)*		
X	Serum aspartate aminotransferase (AST, aka SGOT)*		
X	Gammaglutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines.

6. Urinalysis

No urinalyses were performed.

7. Sacrifice and Pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In this range-finding study, only a few of the required tissues were collected and examined histologically. The organs marked (XX) were, in addition, weighed.

The following tissues were collected and examined histologically: bone marrow from femur (not analyzed), bone from sternum (with marrow), both kidneys, larynx, liver, lungs, nasal cavity, spleen, trachea. Kidneys, liver, lungs, and spleen were weighed.

II. RESULTS

A. Observations:

1. Toxicity - No observable signs of toxicity occurred as a result of exposure to 1-MCP

2. Mortality - No mortality occurred as a result of exposure to 1-MCP.

B. Body weight and weight gain

1-Methylcyclopropene did not affect body weights or relative body weight gains.

C. Food consumption

Food consumption was unaffected by exposure to 1-MCP.

D. Ophthalmic examination

Ophthalmic examinations were not conducted.

E. Blood work

1. Hematology

At 1000 ppm, red blood cell counts, hemoblobin, and hematocrits were decreased compared to controls by 8%, 6%, and 6%, respectively, compared to controls. These decreases were statistically significant at the 0.05 level. Rats exposed to lower concentrations of the test material were unaffected. No other hematological parameters were affected at any exposure level.

2. Clinical Chemistry

At 1000 ppm, bilirubin was increased 40% (statistically significant at $p < 0.05$). No other clinical chemistry parameters were different from controls at this or other exposure levels.

F. Urinalysis

Urinalyses were not conducted.

G. Sacrifice and Pathology

1. Organ weights

Organ weight changes were observed only in subjects exposed to the highest levels of 1-MCP exposure (1000 ppm). At this level, a 30 - 31% increase in spleen weights was found, which the authors considered treatment-related as this finding correlated with gross and microscopic pathology of this organ. Statistically significant increases were also found for kidney and lung weights. The authors did not consider these latter changes related to 1-MCP exposure as the increases were slight and pathologic examination did not reveal damage to these organs.

2. Gross pathology

In the 1000 ppm group, 4 of 7 subjects had discolored spleens and a single subject had an enlarged spleen. No other pathologic changes were grossly observable.

3. Microscopic pathology

a. Non-neoplastic

At exposure levels of 300 and 1000 ppm, spleens exhibited an increase in extramedulary hematopoiesis that the study investigators considered related to treatment. This finding was characterized "by increased cellularity, primarily of the erythrocytic precursors in the red pulp of the spleen at 306 and 1000 ppm." When sternbral bone marrow was examined, no clear difference in cellularity was apparent at any dose level.

b. Neoplastic - not relevant for subacute studies.

III. DISCUSSION

A. In this two-week range-finding inhalation study conducted with female rats at 0, 100, 300, or 1000 ppm, 1-MCP showed an adverse affect on erythrocytes as evidenced by decreased circulating red blood cells and increased extramedullary hematopoiesis in the spleen, found at the two higher exposure concentrations. A NOAEL of 100 ppm and a LOAEL of 300 ppm was established in this study based on these hematological effects.

B. Deficiencies

1. This study does not meet requirements for a subchronic study under Subdivision F Guidelines. The major reasons are insufficient exposure time (2 weeks vs 90 days), only one sex was employed, and limited histopathology. Documentation also is lacking for animal husbandry (e.g., housing, temperature and humidity during non-exposure periods), and other study parameters. However, it should be noted that this study does not appear intended to have met Subdivision F requirements for subchronic studies. The report indicates that it is a "range-finding" study intended to provide preliminary toxicological information and/or to serve as a basis for selecting exposure levels for a subsequent, longer-term study. As such, this study provides useful supplementary information.

2. To generate the test atmosphere within the chamber, 1-MCP was not mixed with air to the desired concentration in a plenum prior to introduction of 1-MCP into the inhalation chamber. Rather, pure 1-MCP was introduced directly into the chamber and then allowed to mix with air to achieve the desired concentration. This might be acceptable if the report had indicated that chamber air samples were taken from the breathing zone of the test subjects. Repeated 1-MCP measurements were consistent within the chamber but whether samples were taken from the breathing zone was not indicated. Better yet would have been sampling from different locations throughout the chamber showing consistent measurements. Since repeated measurements were taken (twice hourly during the exposure period), which showed consistent concentrations, and since a gas such as 1-MCP would be expected to quickly mix and equilibrate with diluent air, this method of atmosphere generation is not considered serious enough to compromise the integrity of the study.

3. Isobutylene was used as the standard for 1-MCP. Standard concentrations of 1-MCP were not made and used to generate a calibration curve for the GC. It was assumed that the GC detector would have the same sensitivity for isobutylene as for 1-MCP and that the area under the curve for isobutylene would directly correspond to the concentration of 1-MCP (after adjusting for molecular weight differences). But justification for this assumption was not provided in the report. Since it is generally recognized that the areas under the curve are consistent for stucturally similar chemicals on the same GC column, this assumption is not considered serious enough to compromise the integrity of the study.

PROPERTY OF ROHM AND HAAS

1-Methylcyclopropene
Two-Week Inhalation Range-Finding Study in Female Rats
Protocol No. 00P-183A Report No. 00R-183A

Table 4
Body weight (g)
Mean Values: F e m a l e s

Group	Test Substance	Dose PPM		Observation Periods (days) Pretest phase 0	Dosing phase 0	3	7	10
1	Air Control	0	Mean	125.4	149.2	164.0	182.4	192.5
			SD	7.4	9.5	8.9	12.0	15.1
			N	7	7	7	7	7
2	1-MCP	105	Mean	125.8	148.5	162.0	181.4	189.7
			SD	6.0	6.9	7.7	9.1	9.7
			N	7	7	7	7	7
3	1-MCP	306	Mean	125.1	149.5	160.9	181.0	192.4
			SD	5.2	5.7	6.9	10.2	11.3
			N	7	7	7	7	7
4	1-MCP	1000	Mean	121.3	150.7	159.9	178.4	189.7
			SD	14.5	4.3	5.6	8.6	9.5
			N	7	7	7	7	7

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1-Methylcyclopropene
Two-Week Inhalation Range-Finding Study in Female Rats
Protocol No. 00P-183A Report No. 00R-183A

Table 5

Body weight (g)
Mean Cumulative Change: F e m a l e s

Group	Dose PPM		Observation Periods (weeks) Dosing phase 0[a]	1[b]	1[c]
1	0	Mean	14.8	33.2	43.3
		SD	3.6	6.6	9.6
		N	7	7	7
2	105	Mean	13.5	32.9	41.2
		SD	6.7	8.1	8.6
		N	7	7	7
3	306	Mean	11.5	31.5	43.0
		SD	3.1	5.8	8.9
		N	7	7	7
4	1000	Mean	9.2	27.7	39.0
		SD	4.4	10.3	9.1
		N	7	7	7

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1-Methylcyclopropene
Two-Week Inhalation Range-Finding Study in Female Rats
Protocol No. 00P-183A Report No. 00R-183A

Table 6

Summary of Average Daily Feed Consumption (Grams)
F e m a l e s

Group	Test Substance	Dose PPM		Dosing phase Observation Periods (Days) 3	7	10
1	Air Control	0	Mean	16.4	18.0	18.1
			SD	1.8	2.0	2.2
			N	7	7	7
2	1-MCP	105	Mean	16.3	19.0	18.8
			SD	1.3	0.9	1.0
			N	7	7	7
3	1-MCP	306	Mean	16.5	19.2	19.4
			SD	2.4	1.7	2.0
			N	7	7	7
4	1-MCP	1000	Mean	15.3	19.9	18.7
			SD	1.4	1.2	0.8
			N	7	7	7

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PROPERTY OF ROHM AND HAAS COMPANY

1-Methylcyclopropene
Two-Week Inhalation Range-Finding Study in Female Rats
Protocol No. 00P-183A Report No. 00R-183A

Table 7

Hematology
Day 11
Mean Values: Female

	Group	Dose PPM		WBC 10E3/CMM	RBC 10E6/CMM	HGB G/DL	HCT %	MCV CM	MCH PG	MCHC %	PLT 10E3/CMM
1	Air Control	0	Mean	7.1	5.91	13.5	38.8	65.7	22.9	34.8	1240
			SD	2.8	0.35	0.5	1.6	1.6	0.7	0.6	122
			N	7	7	7	7	7	7	7	7
2	1-MCP	105	Mean	8.7	5.60	13.2	37.5	67.0	23.5	35.1	1254
			SD	0.8	0.41	0.6	2.1	1.9	0.9	0.6	149
			N	7	7	7	7	7	7	7	7
3	1-MCP	306	Mean	8.3	5.61	13.2	37.6	67.1	23.6	35.1	1320
			SD	2.9	0.26	0.5	1.4	1.8	0.6	0.4	158
			N	7	7	7	7	7	7	7	7
4	1-MCP	1000	Mean	8.5	5.43*	12.7*	36.4*	67.1	23.4	34.9	1324
			SD	2.6	0.32	0.6	1.9	1.2	0.5	0.3	174
			N	7	7	7	7	7	7	7	7

WBC=White Blood Cell Count RBC=Red Blood Cell Count HGB=Hemoglobin HCT=Hematocrit
MCV=Mean Cell Volume MCH=Mean Cell Hemoglobin PLT=Platelet MET=Methemoglobin G/DL=Gram/Deciliter
10E3/CMM=1,000 Cells/Cubic Millimeter MCHC=Mean Cell Hemoglobin Concentration %=Percent CM=Cubic Microns
10E6/CMM=1,000,000 Cells/Cubic Millimeter PG=Picogram PT=Prothrombin Time SEC=Seconds

* Indicates a Statistically Significant Difference from Control (p<0.05).

PROPERTY OF ROHM AND HAAS COMPANY

1-Methylcyclopropene
Two-Week Inhalation Range-Finding Study in Female Rats
Protocol No. 00P-183A Report No. 00R-183A

Table 8

White Blood Cell Differentials
Day 11
Mean Values: Male

	Group	Dose PPM		MONO %	LMPH %	SEGS %	EOS %
1	Air Control	0	Mean	1	83	15	1
			SD	1	6	6	1
			N	7	7	7	7
2	1-MCP	0	Mean	0	84	15	1
			SD	1	4	4	1
			N	7	7	7	7
3	1-MCP	0	Mean	1	81	16	2
			SD	1	7	6	1
			N	7	7	7	7
4	1-MCP	0	Mean	1	82	17	0
			SD	1	6	5	1
			N	7	7	7	7

MONO=Monocyte LMPH=Lymphocyte SEGS=Segmented Neutrophils ATYP=Atypical Lymphocytes
NSGS=Non-Segmented Neutrophils EOS=Eosinophils BASO=Basophils NRBC=Nucleated Red Blood Cells

* Indicates a Statistically Significant Difference from Control (p<0.05).

PROPERTY OF ROHM AND HAAS COMPANY

1-Methylcyclopropene
Two-Week Inhalation Range-Finding Study in Female Rats
Protocol No. 00P-183A Report No. 00R-183A

Table 9

Clinical Chemistry
Day 11
Mean Values: Female

	Group	Dose PPM		AST U/L	ALT U/L	ALP U/L	GGT U/L	CA MG/DL	PHOS MG/DL	TRIG MG/DL	ALB G/DL	TP G/DL	BUN MG/DL
1	Air Control	0	Mean	102	44	220	0	10.6	9.0	54	3.9	5.5	16.2
			SD	22	5	26	0	0.2	0.8	13	0.2	0.2	1.8
			N	7	7	7	7	7	7	7	7	7	7
2	1-MCP	105	Mean	86	47	202	0	10.5	8.7	50	3.9	5.5	15.6
			SD	20	7	33	0	0.3	0.6	20	0.2	0.3	1.4
			N	7	7	7	7	7	7	7	7	7	7
3	1-MCP	306	Mean	84	47	203	0	10.8	9.6	68	3.9	5.6	15.9
			SD	11	10	30	0	0.4	0.5	35	0.2	0.3	1.7
			N	7	7	7	7	7	7	7	7	7	7
4	1-MCP	1000	Mean	97	47	239	0	10.6	9.1	49	4.0	5.6	15.3
			SD	35	9	41	0	0.2	0.7	27	0.2	0.2	1.8
			N	7	7	7	7	7	7	7	7	7	7

* Indicates a Statistically Significant Difference from Control (p<0.05).

PROPERTY OF ROHM AND HAAS COMPANY

1-Methylcyclopropene
Two-Week Inhalation Range-Finding Study in Female Rats
Protocol No. 00P-183A Report No. 00R-183A

Table 9

Clinical Chemistry
Day 11
Mean Values: Female

	Group	Dose PPM		CREA MG/DL	BILI MG/DL	GLU MG/DL	CHOL MG/DL	NA MMOL/L	K MMOL/L	CL MMOL/L	AG RATIO	GLOB G/DL
1	Air Control	0	Mean	0.26	0.10	179	74	141	4.5	106	2.5	1.6
			SD	0.06	0.02	33	15	1	0.3	2	0.4	0.2
			N	7	7	7	7	7	7	7	7	7
2	1-MCP	105	Mean	0.25	0.10	179	69	141	4.1	106	2.5	1.6
			SD	0.03	0.03	12	7	1	0.0	1	0.3	0.1
			N	7	7	7	7	7	7	7	7	7
3	1-MCP	306	Mean	0.22	0.10	167	75	141	4.4	104	2.4	1.7
			SD	0.04	0.02	23	8	1	0.5	2	0.2	0.2
			N	7	7	7	7	7	7	7	7	7
4	1-MCP	1000	Mean	0.28	0.14*	179	74	141	4.4	105	2.5	1.6
			SD	0.08	0.04	21	10	2	0.4	2	0.2	0.1
			N	7	7	7	7	7	7	7	7	7

* Indicates a Statistically Significant Difference from Control (p<0.05).

DATA EVALUATION RECORD

1-METHYLCYCLOPROPENE

STUDY TYPE: BACTERIAL REVERSE MUTATION ASSAY
WITH *SALMONELLA TYPHIMURIUM*
(AMES TEST)

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
Crystal Station I
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Prepared by

Tetrahedron, Inc.
1414 Key Highway
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Primary Reviewer: Steven T. Cragg, PhD, DABT	Signature: Steven T. Cragg Date: 3-July-2001
Secondary Reviewer Nasin Begin, PhD	Signature: Nasrin Begum Date: 7/3/01
Quality Assurance: Waqi Alum, PhD.	Signature: [illegible] Date: July 3, 2001

EPA Reviewer: Biochemicals _____, Date 1/29/2002
Review Section__, ~~Toxicology~~ Branch ___ (75~~0~~C)
EPA Secondary Reviewer: _____, Date _____
Review Section__, ~~Toxicology~~ Biochemicals Branch ___ (75~~0~~C)

Data Evaluation Record

STUDY TYPE: Bacterial Reverse Mutation Assay with Salmonella typhimurium (i.e., Ames Test) OPPTS 870.5100 [§84-2]; OECD Guideline 471 [EEC Directive 92/69/EEC B.2]

DP BARCODE:	D275229	SUBMISSION CODE:	S597276
P.C. CODE:	071297-00001	TOX. CHEM. NO.:	
MRID No.:	453803-02		

TEST MATERIAL (PURITY): 1-Methylcyclopropene (95.80% active ingredient)

SYNONYMS: Cyclopropene, 1-methyl-; 1-MCP

CITATION: O'Neill, P.J., Frederick, C.B., (2001). 1-Methylcyclopropene Vapor Released from 1-Methylcyclopropene Alpha-Cyclodextrin Complex (3.3% a.i.): *Salmonella typhimurium.* Gene Mutation Assay. Rohm and Haas Company Toxicology Department Report No. 00RC-193A, February 27, 2001. MRID 453803-02. Unpublished.

SPONSOR: Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, PA 19477-0904

EXECUTIVE SUMMARY: In a reverse mutation assay in bacteria, (MRID 453803-02), strains TA 98, TA100, TA102, TA1535, and TA1537 of *S. Typhimurium* were exposed for 24 hours to atmospheres containing 1-methylcyclopropene (1-MCP) released from a 1-MCP/alpha-cyclodextrin complex (a.i. 3.3%) at nominal concentrations of 0, 100, 300, or 1000 ppm 1-MCP in an initial assay and 0, 100, 300, or 1000 ppm in a confirmatory assay. Concentrations were analyzed by gas chromatography at three time points (1, 4, and 24 hr) over a 24-hour exposure period. After exposure to 1-MCP, cultures were incubated for a further 24 hours. Tester strains were exposed to 1-MCP in the presence and absence of a mammalian metabolic activation system. The activation system consisted of the post-mitochondrial centrifugation (S-9) fraction of liver from rats treated with the P-450 enzyme inducer, Aroclor 1254.

1-MCP was not tested at higher concentrations than 1000 ppm because, according to the authors, higher concentrations might result in an explosion hazard. No cytotoxicity was reported at any test concentration. No increase in revertant frequencies was detected in either the initial or confirmatory assays in any of the 5 tester strains with or without S-9 metabolic activation. An exception was for Strain TA1535 at 1000 ppm only in the confirmatory test (with activation), where revertant frequencies were exactly two times background. Because the background was unusually low by historical standards, a repeat confirmatory assay was performed with only TA1535. This repeat confirmatory test was negative. Chemical analysis of exposure atmospheres indicated that actual concentrations ranged from 78% to 126% of target concentrations. The strains reacted appropriately to positive control chemicals, yielding revertant frequencies within expected ranges. The results of this study indicate that 1-methylcyclopropene is not mutagenic in this test system.

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guidelines 84-2 for *in vitro* mutagenicity (bacterial reverse mutation) data and was conducted according the EPA OPPTS 870.5100 protocol for this assay.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A **Materials:**

1. Test Material: 1-Methylcyclopropene

Description:	Colorless gas released from *alpha*-cyclodextrin matrix (3.3% a.i.) (white solid with business confidential composition included as Confidential Attachment).
Lot/Batch #:	Lot No. BAS 5-80.
Purity:	3.3% within matrix; varying percentages when allowed to vaporize from matrix in Tedlar exposure bags upon addition of water.
CAS #:	3100-04-7 (a.i.).
Solvent used:	None (cells were exposed directly to 1-MCP vapor).
Other comments:	1-MCP is released from the alpha-cyclodextrin matrix when mixed with warm water.

2. Control Materials:

Negative:
alpha-Cyclodextrin powder without 1-methylcyclopropene (with water added to simulate releasing conditions)

Positive:

Nonactivation:			
1-Nitrofluorene	50	µg/plate	TA98
Sodium azide	2	µg/plate	TA100, TA 1535
9-Aminoacridine	100	µg/plate	TA1537
Mitomycin-C	2	µg/plate	TA102
Activation			
2-Anthramine	5	µg/plate	TA98, TA100, TA1535
2-Anthramine	6	µg/plate	TA1537
2-Anthramine	12	µg/plate	TA102

3. Activation: S9 derived from

_X_ Aroclor 1254	_X_ Induced	_X_ Rat	_X_ Liver
____ Phenobarbital	____ Non-induced	____ Mouse	____ Lung
____ None		____ Hamster	____ Other
____ Other			____ Other

S-9 mix was purchased from Molecular Toxicology, Inc. [Moltox], Lot No. 955 and 1111, and consisted of the following:

Component	Final Concentration
Nicotinamide-adenine dinucleotide phosphate (NADP)	4 mM
Glucose-6-phosphate	5 mM
Magnesium chloride ($MgCl_2$)	8 mM
Potassium chloride (KCl)	33 mM
Sodium phosphate buffer, pH 7.4	100 mM
Liver homogenate (S-9) from Aroclor 1254 treated rats	10 µl/ml

4. Test Cells: *S. typhimurium* strains (obtained from B. Ames, U California, Berkeley)

___ TA97 __X__TA98 __X__TA100 __X__TA102 __X__TA1535
_X_ TA1537 ____TA1538

Properly maintained? Y
Periodically checked for Mycoplasma contamination? Y
Periodically checked for karyotype stability? Y
Periodically "cleansed" against high spontaneous background? Y

5. Test compound atmospheric concentrations used

"Initial Definitive" assay:
Nonactivated conditions: 0, 100, 300, 1000 ppm (nominal)
Activated conditions: 0, 100, 300, 1000 ppm (nominal)
"Confirmatory" assay:
Nonactivated conditions: 0, 10, 50, 500, 1000 ppm (nominal)
Activated conditions: 0, 10, 50, 500, 1000 ppm (nominal)
"Repeat Confirmatory" assay (with strain TA 1535 only):
Nonactivated conditions: 0, 10, 50, 500, 1000 ppm (nominal)
Activated conditions: 0, 10, 50, 500, 1000 ppm (nominal)

All strains (triplicate plates) were used for each treatment condition.

B. TEST PERFORMANCE

1. Type of Salmonella assay
_X_ Standard plate test
____ Pre-incubation test
____ 'Prival" modification (i.e., azo-reduction method)
____ Spot test
____ Other (describe)

2. Protocol:

To achieve the desired atmospheric concentration, 1-MCP was released from a quantity of alpha-cyclodextrin matrix selected to achieve a predetermined concentration inside a 12 liter Tedlar air-tight bag. 1-MCP was released when the *alpha*-cyclodextrin matrix containing it was dissolved with warm water inside the bag. Minimal agar dishes (in triplicate) overlaid with the five bacterial strains (with and without metabolic activation systems) were placed inside the bags prior to release of 1-MCP. Once water was mixed with the 1-MCP-containing *alpha*-cyclodextrin matrix, bags were sealed and placed into incubators at 37°C for 24 hours. The atmospheres inside the bags were sampled at 1, 4 and 24 hours by gas chromatography (with a flame ionization detector). After 24 hours, plates were removed from the bags, lids were added, and plates were returned to the incubator for an additional 24 hours. Colonies were counted at the end of at least 48 hours incubation. To achieve a positive response, revertant counts in strains exposed to the test material had to exceed negative control counts by a factor of two. Toxicity was defined as the elimination of a uniform background lawn.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay

No results of a preliminary cytotoxicity assay were reported. Although the criteria for judging cytotoxicity (i.e., elimination or diminution of uniform background lawn) was stated in the "methods" section describing the definitive and confirmatory assays, the issue of cytotoxicity was not addressed in the results section of the report. Nevertheless, the fact that revertant colonies for 1-MCP-exposed plates were approximately the same as for negative controls indicates a lack of toxicity, even at the highest exposure level tested.

B. Definitive assay (0, 100, 300, and 1000 ppm)

Revertant counts did not exceed the negative control for any of the five tester strains at any concentration, with or without metabolic activation. Positive control revertant counts were within expected ranges. See Table 1 for mean summary revertant counts.

C. Confirmatory assay (0, 10, 50, 500, 1000 ppm)

For four of the five tester strains, revertant counts did not exceed the negative control at any concentration, with or without metabolic activation. For tester strain TA 1535, revertant counts exceeded negative control counts by a factor of exactly 2 at 1000 ppm only. Because the negative control was considered low by historical standards, it was suspected that this result was spurious. As a result, the test was repeated using only this strain (see below). Positive control revertant counts were within expected ranges for all strains. See Table 2 for mean summary revertant counts.

D. Repeat Confirmatory assay with TA 1535 only (0, 10, 50, 500, 1000 ppm)

When retested with strain TA 1535 only, 1-MPC did not increase revertant counts above background levels at any concentration, with or without metabolic activation. Positive control revertant counts were within expected ranges. See Table 2 for mean summary revertant counts.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This study is acceptable. Five strains of *Salmonella typhimurium* were used with and without metabolic activation over a wide exposure range with the highest practicable concentration at the upper end of the range. Positive controls were appropriate for the strain and activation system status producing revertant frequencies within expected ranges. Toxicity does not seem to have been a problem in this study although it was not addressed directly in the report. The results of this study indicate that 1-methylcyclopropene does not cause reverse mutations in *Salmonella typhimurium* under the conditions tested.

B. <u>Deficiencies</u>

1. Although it is not a major discrepancy, the report did not describe how positive controls were handled (presumably they weren't put into bags).

2. Toxicity should have been addressed more directly (e.g., as in a discussion of range finding studies where background lawns in the petri dishes were examined and observations noted). From the summary tables reporting revertant numbers, it is apparent that revertants were approximately similar to the negative control values. Thus, no toxicity could have been occurring or the numbers

would have been less at the higher exposure levels. The fact that toxicity is not addressed directly in the report should not impact the integrity of this study or the conclusions drawn.

3. Isobutylene was used as the standard for 1-MCP. Standard concentrations of 1-MCP were not made and used to generate a calibration curve for the GC. It was assumed that the GC detector would have the same sensitivity for isobutylene as for 1-MCP and that the area under the curve for isobutylene would directly correspond to the concentration of 1-MCP (after adjusting for molecular weight differences). But justification for this assumption was not provided in the report. Since it is generally recognized that the areas under the curve are consistent for stucturally similar chemicals on the same GC column, this assumption is not considered serious enough to compromise the integrity of the study.

Table 1
Definitive Assay Revert Count Summaries

		Mean revertant counts (+/- standard deviation)				
	S-9	Strain TA98	Strain TA100	Strain TA102	Strain TA1535	Strain TA1537
Solvent Controls						
Cyclodextrin	+	30 (+/- 8)	111 (+/- 14)	281 (+/- 15)	28 (+/- 10)	17 (+/- 5)
Cyclodextrin	-	24 (+/- 5)	123 (+/- 10)	249 (+/- 15)	21 (+/- 4)	8 (+/- 2)
Positive Controls						
2-Anthramine	+	1470* (+/- 70)	1182* (+/- 48)	1250* (+/- 58)	315* (+/- 13)	423* (+/- 44)
2-Nitrofluorene	-	683* (+/- 31)				
Sodium azide	-		498* (+/- 15)		585* (+/- 50)	
Mitomycin-C	-			1035* (+/- 25)		
9-Aminoacridine	-					721* (+/- 162)
Exposure Levels						
1000 ppm	+	28 (+/- 9)	137 (+/- 11)	259 (+/- 17)	22 (+/- 2)	16 (+/- 3)
300 ppm	+	37 (+/- 3)	109 (+/- 18)	266 (+/- 14)	26 (+/- 8)	16 (+/- 2)
100 ppm	+	38 (+/- 7)	110 (+/- 13)	287 (+/- 34)	19 (+/- 4)	17 (+/- 3)
1000 ppm	-	20 (+/- 2)	115 (+/- 11)	252 (+/- 10)	23 (+/- 5)	11 (+/- 3)
300 ppm	-	21 (+/- 0)	127 (+/- 15)	245 (+/- 22)	27 (+/- 9)	10 (+/- 1)
100 ppm	-	23 (+/- 6)	134 (+/- 13)	233 (+/- 20)	23 (+/- 6)	9 (+/- 4)

* Positive response (more than 2 times background).

Table 2
Confirmatory Assay Revert Count Summaries

		Mean revertant counts (+/- standard deviation)					Repeat
	S-9	Strain TA98	Strain TA100	Strain TA102	Strain TA1535	Strain TA1537	Strain TA1535
Solvent Controls							
Cyclodextrin	+	32 (+/- 6)	143 (+/- 14)	211 (+/- 14)	14 (+/- 3)	14 (+/- 4)	18 (+/- 4)
Cyclodextrin	-	27 (+/- 7)	128 (+/- 18)	196 (+/- 5)	10 (+/- 2)	11 (+/- 3)	16 (+/- 5)
Positive Controls							
2-Anthramine	+	1178* (+/- 54)	1082* (+/- 68)	1388* (+/- 46)	286* (+/- 67)	387* (+/- 35)	210* (+/- 23)
2-Nitrofluorene	-	645* (+/- 34)					
Sodium azide	-		474* (+/- 16)		414* (+/- 24)		550* (+/- 12)
Mitomycin-C	-			936* (+/- 30)			
9-Aminoacridine	-					484* (+/- 163)	
Exposure Levels							
1000 ppm	+	32 (+/- 4)	178 (+/- 46)	236 (+/- 19)	**28* (+/- 6)**	12 (+/- 4)	22 (+/- 5)
500 ppm	+	34 (+/- 6)	151 (+/- 15)	261 (+/- 5)	19 (+/- 9)	11 (+/- 3)	25 (+/- 10)
50 ppm	+	43 (+/- 3)	199 (+/- 17)	233 (+/- 10)	14 (+/- 3)	14 (+/- 2)	26 (+/- 4)
10 ppm	+	38 (+/- 5)	191 (+/- 7)	249 (+/- 3)	19 (+/- 6)	10 (+/- 4)	22 (+/- 2)
1000 ppm	-	24 (+/- 3)	176 (+/- 10)	207 (+/- 4)	19 (+/- 4)	11 (+/- 3)	14 (+/- 12)
500 ppm	-	25 (+/- 8)	158 (+/- 16)	202 (+/- 15)	12 (+/- 3)	9 (+/- 2)	20 (+/- 2)
50 ppm	-	27 (+/- 2)	153 (+/- 22)	199 (+/- 13)	13 (+/- 2)	8 (+/-1)	16 (+/- 2)
10 ppm	-	30 (+/- 3)	152 (+/- 8)	222 (+/- 9)	12 (+/- 6)	6 (+/-3)	25 (+/- 3)

* Positive response (more than 2 times background).

DATA EVALUATION RECORD

1-METHYLCYCLOPROPENE

STUDY TYPE: IN VITRO MAMMALIAN CHROMOSOME ABERRATION TEST WITH HUMAN LYMPHOCYTES

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
Crystal Station I
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Arlington, VA 22202

Prepared by

Tetrahedron, Inc.
1414 Key Highway
Baltimore, MD 21230

Primary Reviewer: Steven T. Cragg, PhD, DABT	Signature: Steven T. Cragg Date: 3-July-2001
Secondary Reviewer Nasin Begin, PhD	Signature: Nasrin Begum Date: 7/3/01
Quality Assurance: Waqi Alum, PhD.	Signature: W Alum Date: July 3, 2001

EPA Reviewer: Biochemicals _____, Date 1/24/2002
Review Section__, ~~Toxicology~~ Branch ___ (75[illegible]C)
EPA Secondary Reviewer: _____, Date _____
Review Section__, ~~Toxicology~~ Branch ___ (75[illegible]C)
Biochemicals

Data Evaluation Record

STUDY TYPE: *In vitro* Mammalian Chromosome Aberration Test with Human Lymphocytes OPPTS 870.5375 [§84-2]; OECD Guideline 473 [EEC Directive 92/69/EEC B.2]

DP BARCODE: D275229
P.C. CODE: 071297-00001
MRID No.: 453803-04
SUBMISSION CODE: S597276
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): 1-Methylcyclopropene (95.80% active ingredient)

SYNONYMS: Cyclopropene, 1-methyl-; 1-MCP

CITATION: Murli, H.M., (2001). 1-Methylcyclopropene Vapor Released from 1-Methylcyclopropene Alpha-Cyclodextrin Complex (3.3% a.i.): Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes. Rohm and Haas Company Sponsored Study. Report No. 00RC-194, March 14, 2001. Conducted at Covance Laborartories, Covance Study No. 21917-0-449OECD, MRID 453803-04. Unpublished.

SPONSOR: Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, PA 19477-0904

EXECUTIVE SUMMARY: In an initial *in vitro* mammalian chromosome aberration test (MRID 453803-04), cultured human lymphocytes were exposed for approximately 4 hours to atmospheres containing 1-methylcyclopropene (1-MCP) released from a 1-MCP/alpha-cyclodextrin complex (a.i. 3.3%) at nominal concentrations of 0, 100, 300, and 1,000 ppm and harvested approximately 22 hours after initiation of treatment. Replicate cultures of lymphocytes were exposed to 1-MCP with and without an Aroclor-induced rat liver S-9 fraction to determine the influence of a mammalian metabolic activation system upon aberration frequencies. At the end of the incubation period, cells were collected, stained, and mounted on slides where one hundred cells, if possible, per replicate were scored for chromosome aberrations, polyploidy, and endoreduplication. 1-MCP was not tested at higher concentrations than 1,000 ppm because, according to the authors, higher concentrations might result in an explosion hazard. Results of this initial assay indicated no increase in chromosome aberrations, polyploidy, or endoreduplication at any exposure concentration. For lymphocytes not incubated with S-9 fraction, slight cytotoxicity may have been present at 100 and 1000 ppm (but not 300 ppm) as evidenced by reduced mitotic indices of 14% and 8%, respectively. The positive control compounds, mitomycin C (without S-9) and cyclophosphamide (with S-9), produced positive results in expected ranges.

Subsequent to the initial assay, a confirmatory assay was conducted using the same exposure concentrations and procedures. However, lymphocytes without an activation system were exposed to 1-MCP vapors for 19 instead of 4 hours as in the initial assay (lymphocytes with an activation system were exposed again for 4 hours in this subsequent confirmatory assay). Although not stated explicitly in the report, the reason for increasing the exposure time in the confirmatory assay from 4 to 19 hours for lymphocyte cultures not having an S-9 metabolic activation system, apparently arose from the fact that the low and high (but not the mid) exposure concentrations had slightly reduced mitotic indices, suggesting possible cytotoxicity. Mitotic indices again were reduced for lymphocytes incubated without S-9 fraction at the low and high (but not mid) 1-MCP exposure concentrations, 45% and 5%, respectively. No increases

were found at any exposure concentrations in chromosome aberrations, polyploidy, or endoreduplication. The positive control compounds, mitomycin C (without S-9) and cyclophosphamide (with S-9), yielded the expected increases, indicating that the test cells were responsive.

Chemical analysis of exposure atmospheres indicated that actual concentrations ranged from 64% to 116% of target concentrations. The cultured cells reacted appropriately to positive control chemicals, yielding revertant frequencies within expected ranges. The results of this study indicate that 1-methylcyclopropene is not claustogenic in this test system.

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guidelines 84-2 for *in vitro* mammalian chromosome aberration data (OPPTS 870.5375).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided

I. MATERIALS AND METHODS

A **Materials:**

1. Test Material: 1-Methylcyclopropene

Description:	Colorless gas released from *alpha*-cyclodextrin matrix (3.3% a.i.)
Lot/Batch #:	Lot No. BAS 5-80 (white solid with business confidential composition).
Purity:	3.3% within matrix; varying percentages when allowed to vaporize from matrix in Tedlar exposure bags upon addition of water.
CAS #:	3100-04-7 (a.i.).
Test Article Analysis:	Analytical report attached to this report as "APPENDIX II - ANALYSIS OF 1-METHYLCYCLOPROPENE VAPOR CONCENTRATIONS" (pages 54-66).

2. Control Materials:

Negative:
alpha-Cyclodextrin powder without 1-methylcyclopropene (with water added to simulate releasing conditions)

Positive:

Nonactivation:		
Mytomycin C	0.750, 1.00, & 1.50	µg/ml (initial test - ~3 hr)
Mytomycin C	0.150, 0.200, & 0.300	µg/ml (confirmatory test - 19 hr)
Activation		
Cyclophosphamide	20, 30, & 50	µg/ml

3. Activation: S9 derived from

X Aroclor 1254	X Induced	X Rat	X Liver
___ Phenobarbital	___ Non-induced	___ Mouse	___ Lung
___ None		___ Hamster	___ Other
___ Other			___ Other

S-9 mix was purchased from Molecular Toxicology, Inc. [Moltox], Lot No. 1049 and consisted of the following:

Component	Final Concentration
Nicotinamide-adenine dinucleotide phosphate (NADP)	1.5 mg/ml (1.8 mM)
Isocitric acid	2.7 mg/ml (10.5 mM)
Liver homogenate (S-9) from Aroclor 1254 treated rats	15.0 µg/ml (1.5%)

4. Test Organisms: Human peripheral lymphocytes obtained from health volunteers (i.e., adult non-smokers with no history of radiotherapy, chemotherapy, or drug usage and no current viral infections). To induce division, cells were stimulated with phytohemagglutinin. At predetermined intervals after exposure to the test compound, cells were treated with colchicine to arrest them in metaphase for counting.
Properly maintained? Y

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

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

5. Test compound atmospheric concentrations used

Initial assay (~4 hr exposure [+/- S9] to 1-MCP; harvest at ~22 hr):
Nonactivated conditions: 0, 100, 300, 1000 ppm (nominal)
Activated conditions: 0, 100, 300, 1000 ppm (nominal)
Confirmatory assay (~19 hr exposure [-S9], ~4 hr exposure [+S9] to 1-MCP; harvest at ~22 hr):
Nonactivated conditions: 0, 100, 300, 1000 ppm (nominal)
Activated conditions: 0, 100, 300, 1000 ppm (nominal)

Duplicate cultures were used for each treatment condition.

B. TEST PERFORMANCE

1. Preliminary cytotoxicity assay:

Part of the cytogenetic assay. Mitotic index was used to evaluate cytotoxicity.

2. Cytogenetic assay protocol:

Atmosphere generation: To achieve the desired atmospheric concentration, 1-MCP was released from a quantity of alpha-cyclodextrin matrix selected to achieve a predetermined concentration inside a 12 liter Tedlar air-tight bag. 1-MCP was released when the *alpha*-cyclodextrin matrix containing it was dissolved with warm water inside the bag. Minimal agar dishes (in duplicate) overlaid with the human lymphocytes (with and without metabolic activation systems) were placed inside the bags prior to release of 1-MCP. Once water was mixed with the 1-MCP-containing *alpha*-cyclodextrin matrix, bags were sealed and placed into incubators at 37°C for 24 hours. The atmospheres inside the bags were sampled at 1, 4 and 24 hours by gas chromatography (with a flame ionization detector). Cell cultures were exposed to nominal concentrations of 0, 100, 300, and 1000 ppm.

Initial assay: Peripheral venous blood (0.6 ml) collected from healthy human volunteers was mixed in 15 ml heparinized tubes with 9.6 ml of culture medium consisting of RPMI 1640 supplemented with 15% fetal bovine serum (FBS), penicillin, (100 units/ml), streptomycin (100 ul/ml), L-glutamine (2 mM), and 2% phytohemagglutinin M (PHA-M). This mixture was incubated for two days prior to exposure to facilitate cell division. For the non-activation portion

(-S9) of the assay, the cell cultures were transferred to petri dishes and exposed to the test atmospheres described above for approximately 4 hours. After exposure, cells were washed with buffered saline, refed with RPMI 1640 medium, transferred to 15 ml centrifuge tubes and incubated for the remaining ~18 hr culture period until harvest. Approximately 2 hours prior to harvest, colchicine (Colcemid®) was added to arrest cells in metaphase. For the activation portion of the assay, cell cultures were treated identically to the non-activation samples except that during exposure to 1-MCP, cell medium contained S-9 fraction but not fetal bovine serum (FBS). Cells were harvested after ~22 hours total incubation by centrifuging the cells, discarding the supernatant, swelling the cells with 75 mM KCl hypotonic solution, and then fixing the cells in methanol:acetic acid (3:1 v/v). Cells were then mounted on slides and stained with 5% Geimsa. Slides were coded and subsequently read for mitotic index and chromosomal aberrations.

Confirmatory assay: The confirmatory assay was conducted identically to the initial assay except that cells without S-9 activation systems were exposed to the various concentrations of 1-MCP for 19 instead of 4 hours. This was done to further explore a finding in the initial assay consisting of a slight reduction in mitotic indices at the low and high exposure levels, suggesting cytotoxicity. Cells with S-9 activation were, as in the initial assay, exposed for ~4 hours.

Protocol specifics:

a. Cell Treatment

Cells were exposed to 1-MCP for ~ 4 hours in the initial assay (+/- S9) and, in the confirmatory assay, ~4 hours (+S9) and ~19 hours (-S9).

b. Spindle inhibition

Inhibitor used/concentration: chochicine (Colcemid®)/0.1 µl/ml

Administration time: approximately 2 hours prior to harvest

c. Cell harvest

Cells were harvested after 22 hours total incubation for both the initial and confirmatory assays.

d. Details of slide preparation

See protocol description above.

e. Metaphase analysis

No. cells examined per dose:	100 per culture (in duplicate)
Scored for structural:	Yes
Scored for numerical:	No
Coded prior to analysis:	Yes

f. Evaluation criteria

% cells with structural aberrations
% cells with more than one structural aberration

evidence for dose-response
Doses with <50% reduction in mitotic index not read (none in this study)

g. Statistical analysis

Fisher's exact test was used to compare exposed cells to negative controls. Cochran-Armitage was used for linear trend.

II. REPORTED RESULTS

A. Initial assay (0, 100, 300, and 1000 ppm) (4hr exposure to 1-MCP; 22 hr total incubation)

No increase in chromosomal aberrations was found at any exposure concentration. For cells not incubated with an S-9 activation system, mitotic indices in the low and high exposure groups were slightly reduced (14% and 8%, respectively). The reductions in mitotic indices were slight and no dose-response for toxicity was evident. To further explore possible cytotoxicity, in the confirmatory assay, cells incubated without S-9 were exposed to 1-MCP for 19 hours (+S-9 cultures were, again, exposed for 4 hours). See the attached tables for individual culture and average results.

B. Confirmatory assay (0, 10, 50, 500, 1000 ppm) (4 hr [+S9] & 19 hr [-S9] exposure to 1-MCP; 22 hr total incubation)

No increase in chromosomal aberrations was found at any exposure concentration. For cells not incubated with an S-9 activation system, mitotic indices in the low and high exposure groups again were reduced (45% and 5%, respectively). While the low dose mitotic index was reduced more than in the initial assay, a dose-response for cytotoxicity still was not found as the high dose had a higher mitotic index than the low dose and the mid dose was comparable to controls. See the attached tables for individual culture and average results.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This study is acceptable. Cultured human lymphocytes were exposed to 1-MCP with and without metabolic activation over a wide exposure range with the highest practicable concentration at the upper end of the range. Positive controls were appropriate for activation system status producing aberration frequencies in the expected ranges. Toxicity does not seem to have been a problem in this study. The results of this study indicate that 1-methylcyclopropene does not cause chromosomal aberrations in cultured human lymphocytes under the conditions tested.

B. Deficiencies

1. Isobutylene was used as the standard for 1-MCP. Standard concentrations of 1-MCP were not made and used to generate a calibration curve for the GC. It was assumed that the GC detector would have the same sensitivity for isobutylene as for 1-MCP and that the area under the curve for isobutylene would directly correspond to the concentration of 1-MCP (after adjusting for molecular weight differences). But justification for this assumption was not provided in the report. Since it is generally recognized that the areas under the curve are consistent for stucturally similar chemicals on the same GC column, this assumption is not considered serious enough to compromise the integrity of the study.

Pages 45-48 Access to FIFRA Registration Data is restricted under FIFRA section 10(g)

DATA EVALUATION RECORD

1-METHYLCYCLOPROPENE

STUDY TYPE: IN VIVO MAMMALIAN ERYTHROCYTE MICRONUCLEUS TEST (MOUSE)

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
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2800 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Tetrahedron, Inc.
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Primary Reviewer: Steven T. Cragg, PhD, DABT	Signature: Steven T. Cragg Date: 3-July-2001
Secondary Reviewer Nasin Begin, PhD	Signature: Nasrin Begum Date: 7/3/01
Quality Assurance: Waqi Alum, PhD.	Signature: W Alum Date: July 3, 2001

EPA Reviewer: Biochemicals _____, Date 1/24/2002
Review Section__, ~~Toxicology~~ Branch ___ (75~~00~~C)
EPA Secondary Reviewer: _____, Date _____
Review Section__, ~~Toxicology~~ Biochemicals Branch ___ (75~~00~~C)

Data Evaluation Record

STUDY TYPE: *In vivo* Mammalian (Mouse) Micronucleus Test
OPPTS 870.5395 [§84-2]; OECD Guideline 474 [EEC Directive 92/69/EEC B.2]

DP BARCODE:	D275229	SUBMISSION CODE:	S597276
P.C. CODE:	071297-00001	TOX. CHEM. NO.:	
MRID No.:	453803-05		

TEST MATERIAL (PURITY): 1-Methylcyclopropene (95.80% active ingredient)

SYNONYMS: Cyclopropene, 1-methyl-; 1-MCP

CITATION: Sames, J.L., Frederick, C.B., (2001). 1-Methylcyclopropene: Micronucleus Assay in CD-1 Mouse Bone Marrow Cells. Rohm and Haas Company Report No. 00RC-194, February 5, 2001. MRID 453803-05. Unpublished.

SPONSOR: Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, PA 19477-0904

EXECUTIVE SUMMARY: In an *in vivo* micronucleus assay with polychromatic erythrocytes from bone marrow, CD-1 mice were exposed (in groups of10/sex/exposure level) by whole body inhalation to 1-methylcyclopropene (1-MCP) at concentrations of 0 (air-control), 100, 300, or 1000 ppm for a single 6 hour period. The high exposure group had an additional 4 animals/sex for a total of 14/sex). Half of the air-controls and 1-MCP subjects were sacrificed 24 hours after exposure and processed for evaluation of micronuclei. The second half were sacrificed 48 hours post-exposure. Two additional groups (5/sex) were exposed by intraperitoneal injection to: 1) water, at a dose of 10 ml/kg (vehicle for positive control) and 2) the positive control compound, mitomycin-C, administered in water at a dose of 2.0 mg/kg. These latter two groups were sacrificed 24 hours after injection and evaluated for the frequency of micronuclei. For each animal, at least 2000 polychromatic erythrocytes were examined for the presence or absence of micronuclei. As an indication of cytotoxicity, the ratio of polychromatic to normochromatic erythrocytes also was assessed.

Results indicated that 1-MCP did not increase the frequency of micronuclei in mice under the conditions of exposure. No toxicity was evident in the ratio of poly- to normochromatic erythrocytes. The positive control agent, mytomycin-C, increased the frequency of micronuclei, showing that the test subjects were appropriately sensitive to the induction of micronuclei.

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guidelines 84-2 for the Mammalian Erythrocyte Micronuclei Test (OPPTS 870.5395).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

Study Design

Group	Agent	Target Conc (ppm)	Actual Conc ± S.D. (ppm)	No./sex (24-hr sac.)	No./sex (48-hr sac.)
1	Air-only	0	0	5	5
2	1-MCP	100	107 ± 29	5	5
3	1-MCP	300	304 ± 60	5	5
4	1-MCP	1000	956 ± 138	7	7
5	Water (i.p.)	10 ml/kg	10 ml/kg	5	5
6	Mitomycin-C (i.p.)	2 mg/kg in water @ 10ml/kg	2 mg/kg in water @ 10ml/kg	5	0

A MATERIALS

1. Test Material: 1-Methylcyclopropene

Description: Colorless gas
Lot/Batch #: Lot No. RMJ6606B; Sample No. TD00-102
Purity: 96.4%
CAS #: 3100-04-7
Test Article Analysis: Analytical report (Rohm and Haas Report No. 00R-232) attached as appendix to this report.

2. Control Materials:

Negative:
Air-only control (whole body inhalation)
Water control (negative control as vehicle for mitomycin-C) injected i.p.

Positive:
Mytomycin-C (in distilled water) at a dose of 2 mg/kg injected i.p.

3. Test compound administration:

Volume of test substance administered: Not applicable for test substance. Administered as gas in atmosphere. For mitomycin-C positive control agent, administered in water at a volume of 10 ml/kg.

Route of administration: For 1-MCP test substance, whole body *inhalation. For mitomycin-C positive control*, intraperitoneal (i.p.) injection.

Dose (exposure concentrations) used: See table above.

4. Test animals:

a. Species: Mouse Strain: CD-1 Age: 8 weeks
Weight: 22-28 grams (sex not specified)

b. No. animals used per dose: 5 males and 5 females (except for 1-MCP mice exposed to 1000 ppm; for this group, 7 males and 7 females were employed).

c. Properly maintained? Yes

B. TEST PERFORMANCE

1. Treatment and Sampling Times:

1-MCP-exposed mice: Mice were exposed to atmospheres containing 1-MCP in Plexiglas® and stainless steel whole-body inhalation chambers for 6 hours with an internal volume of 240 L. Half of the mice were sacrificed 24 hours post-exposure and bone marrow was collected for evaluation. The remainder were sacrificed 48 hours post-exposure.

2. Generation of the test atmosphere, description of the chamber, and analysis of test atmosphere:

Chamber atmospheres were generated by connecting a 1-MCP filled Tedlar Sampling bag through a tube and, using a Gilmont Flowmeter, metering 1-MCP into a 240 liter Plexiglas® and stainless steel chamber containing the test subjects. 1-MCP was drawn into the top of the chamber via negative pressure and mixed with air inside the chamber to achieve the desired concentration. Chamber atmospheres were sampled twice hourly. Chamber airflow was monitored continuously. The airflow rate ranged between 41 and 43 liters/minute for the 4 chambers, resulting in <10 air changes per hour. Animal volumes comprised less than 1% of chamber volumes (i.e., 28 grams [maximum weight of mouse] = 28 ml x 14 mice [maximum] = 392 ml = 0.392 L; divided by 240 L = 0.2%). Chamber temperatures ranged from 68-70°F and humidity between 70-75%.

The test atmosphere was sampled twice hourly (at approximately 30 minute intervals) over the 6-hour course of exposure. Samples (0.5 ml) were taken from the chamber with a gas tight syringe and injected directly into a gas chromatograph. This chromatograph is described in the appended analytical report as a "Hewlett Packard Series 6890 HPLC sytem." HPLC usually stands for high pressure liquid chromatography, not gas chromatography. This may be an error. The toxicity report refers to gas chromatographic analysis. And other parts analytical report appended to the back of the toxicology report describe column conditions and that leave no doubt that gas chromatography was employed. A flame ionization detector was used to detect 1-MCP. Results of the analysis are given in Table 1 above.

3. Tissues and Cells Examined:

Animals from the 1-MCP and air control groups were sacrificed by cervical dislocation at 24 hours or 48 hours post-exposure. Positive control animals and water controls were sacrificed 24 hours after dosing. Bone marrow was collected from both femurs by flushing and mixing into 15 ml centrifuge tubes containing 1 ml fetal bovine serum. Slides were prepared as described below

Polychromatic erythrocytes collected from bone marrow (femur)

To determine polychromatic/normochromatic cell ratio (for cytotoxicity determination)

No. of polychromatic erythrocytes (PCE 1) examined per animal:	< 1,000
No. of normochromatic erythrocytes (PCE) examined per animal:	< 1,000

To determine frequency of micronuclei among polychromatic cells (PCE 2)

No. of polychromatic erythrocytes (PCE 1) examined per animal:	< 2,000

4. Details of slide preparation

Slides were prepared from at least five animals per group (3 slides per animal). After removing marrow from both femurs and flushing into 15 ml centrifuge tubes, cells were centrifuged. After centrifuging at 120 g for 5 minutes, supernatant was removed and cells were resuspended in remaining solution, smeared onto clean microscope slides, allowed to dry for >1 hour, fixed in methanol for 15 minutes, dried again, and stained with Acridine Orange.

5. Statistical methods

Males and females, time of sacrifice, and 1-MCP exposure level were analyzed separately. Ratios of micronuclei-bearing PCEs to total PCEs read, expressed as percentages, were transformed by taking their arcsine square root (to convert to a normal statistical distribution). Statistics were performed upon the converted values. A three-way Analysis of Variance (ANOVA) was used to determine whether statistically significant differences were present between sexes, among groups, and time of sacrifice. If the ANOVA F statistic was significant for 1-MCP exposure relative to control, contrasts were performed to determine linear dose-response trend, and quadratic dose-response. Pair-wise comparisons between each 1-MCP group and air controls were performed using Dunnett's t-test. These statistical tests were performed using the Statistical Analysis System (SAS) program, version 6.09 enhanced.

6. Evaluation criteria

A positive response was recorded if a statistically significant increase occurred in micronuclei-bearing PCEs from animals exposed to the test article compared to concurrent air-only controls, or if a significant dose-response was found. If concurrent controls showed unusually low responses, historical controls could be substituted. To validate a negative response, the positive control compound should fall within normal limits for the laboratory to show that the test system was responsive to this type of effect (i.e., be at least 2-fold higher than water controls).

II. REPORTED RESULTS

1-Methylcyclopropene (1-MCP) did not increase the frequency of micronuclei in polychromatic erythrocytes (PCEs) from the bone marrow of mice (at least 5/sex/group) exposed at concentrations of 0, 100, 300, or 1000 ppm (0.22, 0.66, or 2.2 mg/l). This was true for both post-exposure sacrifice time points (24 and 48 hours). The ratio of poly- to normochromatic erythrocytes was not affected by 1-MCP exposure, indicating the absence of toxicity. Mitomycin-C caused a greater than 2-fold increase in the frequency of micronuclei, indicating that the test system was sensitive to the induction of this toxicity endpoint (the increase in mitomycin-C treated mice was actually on the order of 50-fold greater than any of the negative controls).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This study is acceptable. Sufficient numbers of test animals sacrificed at standard post-exposure time points were exposed to three concentrations of 1-MCP (100, 300, and 1000

ppm). Sufficient numbers of cells were collected and read. Results were compared using valid statistical procedures to a concurrent control group exposed only to air. No increase in micronuclei occurred at any exposure level, for either sex, at either sacrifice time point. No toxicity was evident as measured by the ratio of polychromatic to normochromatic erythrocytes. Statistically significant increases in micronucleated cells occurred in mice treated with the positive control agent, mitomycin-C, indicating that the test system was appropriately responsive to this genetic toxicity endpoint. Under the described experimental conditions, 1-methylcyclopropene does not induce micronuclei in mice.

B. Deficiencies

1. To generate the test atmosphere within the chamber, 1-MCP was not mixed with air to the desired concentration in a plenum prior to introduction of 1-MCP into the inhalation chamber. Rather, pure 1-MCP was introduced directly into the chamber and then allowed to mix with air to achieve the desired concentration. This might be acceptable if the report had indicated that chamber air samples were taken from breathing zone of the test subjects. Repeated 1-MCP measurements were consistent within the chamber but whether samples were taken from the breathing zone was not indicated. Better yet would have been sampling from different locations throughout the chamber showing consistent measurements. Since repeated measurements were taken (twice hourly during the exposure period), which showed consistent concentrations, and since a gas such as 1-MCP would be expected to quickly mix and equilibrate with diluent air, this method of atmosphere generation is not considered serious enough to compromise the integrity of the study.

2. Isobutylene was used as the standard for 1-MCP. Standard concentrations of 1-MCP were not made and used to generate a calibration curve for the GC. It was assumed that the GC detector would have the same sensitivity for isobutylene as for 1-MCP and that the area under the curve for isobutylene would directly correspond to the concentration of 1-MCP (after adjusting for molecular weight differences). But justification for this assumption was not provided in the report. Since it is generally recognized that the areas under the curve are consistent for stucturally similar chemicals on the same GC column, this assumption is not considered serious enough to compromise the integrity of the study.

3. Two GC peaks were ascribed to 1-MCP. The first major peak eluted after 3.3 minutes while a second, smaller peak eluted after 3.4 minutes. The area under the curve of both peaks was used to determine the concentration of 1-MCP. The second peak may have been a contaminant or a thermal degradation product. In the second case, adding the two peaks together is justified. If the former case, adding the two areas may still be justified since the assessing the toxicity of the formulation (with the contaminant included) is the desired goal. The area of the second peak was 4.8% of the combined areas. Consequently, even if the contribution of the second peak is not included, this would not change the toxicity category of this compound.

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Rohm and Haas Report No. 00R-232
1-Methylcyclopropene: Micronucleus Assay
in CD-1 Mouse Bone Marrow Cells

Table II

Mean Summary Data
Male Animals

Group	Day	Dose PPM		NCE	PCE1	MNP	PCE2	PCE TOTAL	PNR RATIO	MNC %
1 Air Control	1	0	Mean	386	704	3	1385	2089	1.90	0.13
			SD	70	79	2	126	52	0.53	0.07
			N	5	5	5	5	5	5	5
2 1 MCP	1	100	Mean	469	640	4	1456	2096	1.71	0.20
			SD	208	226	3	233	34	1.05	0.13
			N	5	5	5	5	5	5	5
3 1 MCP	1	300	Mean	425	651	3	1431	2082	1.55	0.15
			SD	44	34	1	60	33	0.25	0.05
			N	5	5	5	5	5	5	5
4 1-MCP	1	1000	Mean	435	649	4	1459	2107	1.60	0.21
			SD	87	120	2	114	41	0.64	0.07
			N	7	7	7	7	7	7	7
5 Water Control	1	0	Mean	517	552	2	1349	1901	1.09	0.09
			SD	62	65	1	347	406	0.23	0.05
			N	5	5	5	5	5	5	5
6 Air Control	2	0	Mean	496	618	3	1488	2106	1.26	0.15
			SD	26	61	2	93	48	0.19	0.08
			N	5	5	5	5	5	5	5
7 1-MCP	2	100	Mean	465	671	3	1384	2055	1.50	0.16
			SD	85	112	1	112	12	0.41	0.04
			N	5	5	5	5	5	5	5
8 1-MCP	2	300	Mean	395	702	3	1414	2116	1.85	0.15
			SD	73	107	2	99	51	0.51	0.08
			N	5	5	5	5	5	5	5
9 1 MCP	2	1000	Mean	486	716	4	1402	2119	1.57	0.18
			SD	143	300	2	288	60	0.60	0.08
			N	7	7	7	7	7	7	7
10 Water Control	2	0	Mean	390	693	2	1373	2066	1.96	0.12
			SD	105	143	1	160	45	0.88	0.04
			N	5	5	5	5	5	5	5
11 Mitomycin C	1	2	Mean	535	596	112	1508	2104	1.37	5.31 #
			SD	210	168	13	203	55	0.86	0.57
			N	5	5	5	5	5	5	5

Rohm and Haas Report No. 00R-232
1-Methylcyclopropene: Micronucleus Assay
in CD-1 Mouse Bone Marrow Cells

Table II

Mean Summary Data
Female Animals

Group	Day	Dose PPM		NCE	PCE1	MNP	PCE2	PCE TOTAL	PNR RATIO	MNC %
1 Air Control	1	0	Mean	435	673	3	1403	2077	1.55	0.14
			SD	27	49	2	62	34	0.12	0.08
			N	5	5	5	5	5	5	5
2 1-MCP	1	100	Mean	425	656	3	1450	2105	1.59	0.16
			SD	69	50	2	21	39	0.37	0.10
			N	5	5	5	5	5	5	5
3 1-MCP	1	300	Mean	398	688	4	1408	2096	1.75	0.17
			SD	37	52	3	53	26	0.30	0.13
			N	5	5	5	5	5	5	5
4 1-MCP	1	1000	Mean	433	633	4	1469	2102	1.57	0.18
			SD	97	73	2	78	35	0.56	0.08
			N	7	7	7	7	7	7	7
5 Water Control	1	0	Mean	425	651	3	1471	2121	1.63	0.13
			SD	84	112	1	83	54	0.61	0.06
			N	5	5	5	5	5	5	5
6 Air Control	2	0	Mean	396	740	4	1431	2171	2.00	0.20
			SD	95	108	3	102	34	0.68	0.13
			N	5	5	5	5	5	5	5
7 1-MCP	2	100	Mean	416	698	4	1417	2115	1.71	0.17
			SD	51	69	1	66	55	0.35	0.04
			N	5	5	5	5	5	5	5
8 1-MCP	2	300	Mean	389	691	3	1418	2109	1.92	0.16
			SD	98	89	1	113	29	0.74	0.05
			N	5	5	5	5	5	5	5
9 1-MCP	2	1000	Mean	458	624	3	1451	2075	1.49	0.16
			SD	113	128	1	127	40	0.61	0.06
			N	7	7	7	7	7	7	7
10 Water Control	2	0	Mean	445	711	2	1413	2124	1.96	0.10
			SD	187	164	1	155	55	1.09	0.06
			N	5	5	5	5	5	5	5
11 Mitomycin-C	1	2	Mean	579	515	114	1556	2071	1.03	5.51 #
			SD	193	150	16	156	31	0.53	0.81
			N	5	5	5	5	5	5	5

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DATA EVALUATION RECORD

1-METHYLCYCLOPROPENE

STUDY TYPE: IN VITRO MAMMALIAN CELL GENE MUTATION TEST
(CHO/HGPRT LOCUS TEST)

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
Crystal Station I
2800 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Tetrahedron, Inc.
1414 Key Highway
Baltimore, MD 21230

Primary Reviewer: Steven T. Cragg, PhD, DABT	Signature: Steven T. Cragg Date: 3-July-2001
Secondary Reviewer Nasin Begin, PhD	Signature: Nasin Begum Date: 7/3/01
Quality Assurance: Waqi Alum, PhD.	Signature: [illegible] Date: July 3, 2001

EPA Reviewer: Biochemicals _____, Date 1/29/2002
Review Section__, ~~Toxicology~~ Branch ___ (7509C)
EPA Secondary Reviewer: _____, Date _____
Review Section__, ~~Toxicology~~ Biochemicals Branch ___ (7509C)

Data Evaluation Record

STUDY TYPE: *In vitro* Mammalian Cell Gene Mutation Test (CHO/HGPRT)
OPPTS 870.5300 [§84-2]; *OECD Guideline 476 [EEC Directive 92/69/EEC B.2]*

DP BARCODE:	D275229	SUBMISSION CODE:	S597276
P.C. CODE:	071297-00001	TOX. CHEM. NO.:	
MRID No.:	453803-03		

TEST MATERIAL (PURITY): 1-Methylcyclopropene (95.80% active ingredient)

SYNONYMS: Cyclopropene, 1-methyl-; 1-MCP

CITATION: Cifone, M.A., (2001). 1-Methylcyclopropene Vapor Released from 1-Methylcyclopropene Alpha-Cyclodextrin Complex (3.3% a.i.): CHO HGPRT Forward Mutation Assay with a Confirmatory Assay and Duplicate Cultures. Rohm and Haas Company Toxicology Department Report No. 00RC-195, March 27, 2001. MRID 453803-03. Unpublished.

SPONSOR: Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, PA 19477-0904

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus, Chinese hamster ovary (CHO) cells cultured *in vitro* were exposed to atmospheres containing 1-methylcyclopropene (1-MCP) vapor released from a 1-MCP/alpha-cyclodextrin complex (a.i. 3.3%) at nominal concentrations of 0, 100, 250, 500, or 1000 ppm. Concentrations in the air were analyzed by gas chromatography at 1 and 4 hours of treatment. The assay was performed in the presence and absence of a mammalian metabolic activation system consisting of the post-mitochondrial centrifugation (S9) fraction of liver from rats treated with the P-450 enzyme inducer, Aroclor 1254.

1-MCP was not tested at higher concentrations than 1,000 ppm because, according to the authors, higher concentrations might result in an explosion hazard. No cytotoxicity was reported at any test concentration in the absence of metabolic activation. There was no increase in induced mutant colonies over background at any concentrations in the initial trial. Increases in mutation frequency occurred in the confirmatory assay that were unrelated to dose, were not present in duplicate cultures, and did not exceed 15 x 10^6, the mutant frequency required for a positive response. Some weak cytotoxicity (4-22%) was reported in the presence of metabolic activation in the initial trial and no cytotoxicity occurred in the confirmatory assay. No significant increase in the mutant frequency was induced at any concentration in the initial or confirmatory assay. There was no evidence of induced mutant colonies over background following 1-MCP vapor exposure of mammalian cells *in vitro*.

This study is classified as acceptable. It satisfies the requirement for FIFRA Test Guidelines 84-2 for *in vitro* mammalian cell gene mutation test data and was conducted according the EPA OPPTS 870.5300 protocol for this assay.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A **Materials:**

1. Test Material: 1-Methylcyclopropene

Description:	Colorless gas released from *alpha*-cyclodextrin matrix (3.3% a.i.) (white solid with business confidential composition included as Confidential Attachment).
Lot/Batch #:	Lot No. BAS 5-80.
Purity:	3.3% within matrix; varying percentages when allowed to vaporize from matrix in Tedlar exposure bags upon addition of water.
CAS #:	3100-04-7 (a.i.).
Solvent used:	None (cells were exposed directly to 1-MCP vapor).
Other comments:	1-MCP is released from the matrix when mixed with warm water.

2. Control Materials:

Negative: *alpha*-Cyclodextrin powder without 1-methylcyclopropene (with water added to simulate releasing conditions)

Negative reference: Nothing added. Cultures prepared with or without S9 activation and incubated under standard conditions

Positive:

Nonactivation:

5-Bromo-2'-deoxyuridine (BrdU) 50 µg/plate

Activation

Methylcholanthrene 5 µg/plate

3. Activation: S9 derived from

_X_ Aroclor 1254	_X_ Induced	_X_ Rat	_X_ Liver
____ Phenobarbital	____ Non-induced	____ Mouse	____ Lung
____ None		____ Hamster	____ Other
____ Other			____ Other

S-9 mix was purchased from Molecular Toxicology, Inc. [Moltox], Lot No. 955 and 1111, and consisted of the following:

Component	Final Concentration
Nicotinamide-adenine dinucleotide phosphate (NADP)	0.8 mM
Glucose-6-phosphate	1.0 mM
Calcium chloride	2.0 mM
Potassium chloride (Kcl)	6.0 mM
Magnesium chloride ($MgCl_2$)	2.0 mM
Phosphate	10.0 mM
Liver homogenate (S-9) from Aroclor 1254 treated rats	10 µl/ml

4. Test Cells: mammalian cells in culture

___ mouse lymphoma L5178Y cells

_X_ Chinese hamster ovary (CHO) cells; clone K1-BH_4

___ V79 cells (Chinese hamster lung fibroblasts)

___ other

Properly maintained? Y
Periodically checked for Mycoplasma contamination? Y
Periodically checked for karyotype stability? Y
Periodically "cleansed" against high spontaneous background? Y

Media

a) Culture medium:
Ham's Nutrient Mixture F12
Supplements:
L-glutamine
gentamicin
Fungizone
fetal bovine serum (8% v/v)

b) Cleansing medium:
Culture medium (THMG medium) with reduced serum content (5%) supplemented with 1.0 x 10-4 M glycine and 3.2 x 10-6 M methotrexate

c) Recovery medium:
THMG medium without methotrexate and with fetal bovine serum increased to 8%.

d) Mutant selection medium:
hypoxanthine-free F12 culture medium containing 4 µg/ml 6-thioguanine and 5% fetal bovine serum

5. Locus examined

hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
selection agent: 6-thioguanine (6-TG)

6. Test compound concentrations used

Nonactivated:
0, 100, 250, 500, 1000 ppm (nominal) in the culture atmosphere

Activated
0, 100, 250, 500, 1000 ppm (nominal) in the culture atmosphere

B. TEST PERFORMANCE

1. Cell treatment:
   a. Cells (4 x 10^6 seeded per dish in duplicate) exposed to test compound, negative or positive controls for 4 hours nonactivated or activated.
   b. After washing, cells cultured for an expression period of 7 days before cell selection.
   c. After expression, 2 x 10^5 cells/dish (12 dishes/group) were cultured for 7 days in selection medium to determine number of mutants and 200 cells per dish (3 dishes/group) were cultured for 7 days without selecting agent to determine cloning efficiency.

2. Test atmosphere generation and exposure of cultures:

To achieve the desired atmospheric concentration, 1-MCP was released from a quantity of alpha-cyclodextrin matrix selected to achieve a predetermined concentration inside a 12 liter Tedlar air-tight bag. 1-MCP was released when the *alpha*-cyclodextrin matrix containing it was dissolved

with warm water inside the bag. Petri dishes containing the CHO cells (with and without metabolic activation systems) were placed inside the bags prior to release of 1-MCP. Once water was mixed with the 1-MCP-containing *alpha*-cyclodextrin matrix, bags were sealed and placed into incubators at 37°C for 4 hours. The atmospheres inside the bags were sampled after 1 and at 4 hours by gas chromatography (with a flame ionization detector). After 4 hours, plates were removed from the bags, lids were added, and plates were returned to the incubator for additional incubation

3. Statistical methods

Statistical tables developed by Kastenbaum and Bowman (1970), for Poisson-distributed data

4. Evaluation criteria

Controls should have absolute cloning efficiencies between 50% and 115%. Background mutant frequencies generally should be from 0 to 10×10^{-6}. Positive control mutant frequencies should be statistically elevated over controls at the $p > 0.01$ significance level. The high concentration should not reduce survival more than 10 to 25%.

For a response to be considered positive;

cultures exposed to the test material should exhibit a positive dose-response with statistically significant increases in revertant frequency compared to concurrent controls. Mutant frequencies must be greater than 15×10^{-6}. Results should be comparable between initial and confirmatory assays and among replicates.

For a response to be considered negative;

cultures exposed to the test material (that have at least 10% survival) should not exhibit a statistical increase over controls at the 0.05 significance level. A repeat assay does not confirm an earlier result.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay

A cytotoxicity assay revealed little if any toxicity at concentrations up to 1000 ppm.

B. Initial assay (0, 100, 250, 500, 1000 ppm)

Revertant counts did not exceed the concurrent negative control at any concentration, with or without metabolic activation. Positive control revertant counts were within expected ranges. See attached tables for results.

C. Confirmatory assay (0, 100, 250, 500, 1000 ppm)

Revertant counts did not exceed the concurrent negative control at any concentration, with or without metabolic activation. Positive control revertant counts were within expected ranges. See attached tables for results.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This study is acceptable. 1-MCP did not induce forward mutations in Chinese Hamster Overy cells at the HGPRT locus, with or without metabolic activation over a wide exposure range with the highest practicable concentration at the upper end of the range. Positive controls were appropriate for the activation system status producing revertant

frequencies in the expected ranges. Toxicity did not interfere with the assay. The results of this study indicate that 1-methylcyclopropene vapor does not cause reverse mutations in cultured CHO cells at the HGPRT locus under the conditions tested.

B. Deficiencies

During chemical analysis of the exposure atmosphere, isobutylene was used as the standard for 1-MCP. Standard concentrations of 1-MCP were not made and used to generate a calibration curve for the GC. It was assumed that the GC detector would have the same sensitivity for isobutylene as for 1-MCP and that the area under the curve for isobutylene would directly correspond to the concentration of 1-MCP (after adjusting for molecular weight differences). But justification for this assumption was not provided in the report. Since it is generally recognized that the areas under the curve are consistent for stucturally similar chemicals on the same GC column, this assumption is not considered serious enough to compromise the integrity of the study.

References

Kastenbaum, M.A., Bowman, K.O., 1970. Tables for determining the statistical significance of mutation frequencies. Mutation Research 86:193-214.

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Table 1
Initial Mutation Assay Without Metabolic Activation

Sample Name: 1-Methylcyclopropene alpha-cyclodextrin complex (3.3% a.i.), (Sample Number TD No. 00-103, Lot No. BAS 5-80)
Sponsor: Rohm and Haas Company **Test Date:** January 31, 2001 **Selective Agent:** 4 ug/mL 6-thioguanine **Cells seeded for analysis:** 200/dish for C.E., 2 x 10^4/dish for mutants
Assay Number: 21917-0-435 OECD **Expression Time:** 7 days

Nonactivation Test Condition	Survival to Treatment: Mean Colony Number ± SD			Survival to Treatment: % Neg. Control	Relative Population Growth (% of Control)	Mean Colonies Dish Number 1	2	3	4	5	6	7	8	9	10	11	12	Total Mutant Colonies	Absolute C.E. ± SD (%)			Mutant Frequency in 10E-6 Units[a]
Negative Control[b]	191.0	±	16.5	100.8	113.2	1	0	0	0	0	0	0	0	0	0	0	0	1	79.4	±	12.1	0.5
Negative Control[b]	166.7	±	5.0	93.2	87.1	0	1	1	1	1	0	1	0	1	2	0	1	9	101.4	±	8.5	3.7
Negative Reference[c]	190.7	±	10.7	106.6	56.0	2	0	0	0	0	0	0	0	0	0	0	0	2	99.5	±	9.3	0.8
Negative Reference[c]	203.3	±	3.1	113.6	56.1	0	0	1	1	0	0	0	1	0	1	0	0	4	86.5	±	2.8	1.9
Positive Control[d]	144.7	±	18.6	80.9	25.2	27	15	17	17	16	21	25	20	15	16	12	18	219	84.7	±	3.0	107.8**
Positive Control[d]	163.7	±	9.0	91.5	41.1	21	16	19	19	18	12	19	17	21	25	15	18	220	98.2	±	6.9	93.4**
Test Article[e]																						
100	188.0	±	2.6	105.1	39.5	0	1	0	1	2	0	2	0	0	C	0	0	6	92.7	±	1.6	2.9
100	187.7	±	23.7	104.9	63.2	0	0	2	0	0	0	0	0	0	0	1	0	3	86.0	±	10.0	1.5
250	170.3	±	6.1	95.2	83.0	0	0	0	1	0	0	0	1	1	0	0	0	3	103.7	±	11.4	1.2
250	185.3	±	9.2	103.6	67.5	1	0	0	0	0	C	0	0	0	0	0	0	1	91.5	±	7.1	0.5
500	185.7	±	43.7	103.8	75.9	0	0	1	1	0	0	1	0	1	1	0	1	6	118.0	±	6.0	2.1
500	181.7	±	22.5	101.6	93.5	0	0	0	1	0	0	0	1	1	0	1	0	4	83.2	±	9.5	2.0
1000	170.7	±	10.2	95.4	58.9	0	1	0	1	0	0	1	1	0	1	0	0	5	91.4	±	5.0	2.3

[a]Mutant Frequency = Total mutant colonies / (No. of dishes x 2 x 10E5 x absolute CE)
[b]Negative Control = Cultures exposed to alpha-cyclodextrin powder, 300 mg, with 20 mL of water under the same conditions as test article treated cultures.
[c]Negative Reference Control = Cultures containing cells and RPMI 1640 culture medium, incubated under standard culturing conditions.
[d]Positive Control = 50 µg/mL 5-Bromo-2'-deoxyuridine
[e]ppm
** Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq 15 \times 10E\text{-}6$
CE = Cloning efficiency
C = Contaminated

Table 2
Confirmatory Mutation Assay Without Metabolic Activation

Sample Name: 1-Methylcyclopropene alpha-cyclodextrin complex (3.3% a.i.), (Sample Number TD No. 00-103, Lot No. BAS 5-80)

Sponsor: Rohm and Haas Company **Test Date:** February 09, 2001 **Selective Agent:** 4 ug/mL 6-thioguanine **Cells seeded for analysis:** 200/dish for C.E.,
Assay Number: 21917-0-435 OECD **Expression Time:** 7 days 2 x 10^5/dish for mutants

Nonactivation Test Condition	Survival to Treatment: Mean Colony Number ± SD			Survival to Treatment: % Neg Control	Relative Population Growth (% of Control)	Mean Colonies Dish Number 1	2	3	4	5	6	7	8	9	10	11	12	Total Mutant Colonies	Absolute C.E. ± SD (%)			Mutant Frequency in 10E-6 Units[a]
Negative Control[b]	201.3	±	9.1	96.5	68.2	0	2	0	1	1	0	1	3	2	0	1	1	12	108.4	±	5.0	4.6
Negative Control[b]	216.0	±	4.4	103.5	104.0	0	0	1	0	0	1	0	0	1	0	0	1	4	89.7	±	3.2	1.9
Negative Reference[c]	258.0	±	15.4	123.6	138.8	1	0	0	1	1	1	0	0	1	1	3	2	11	103.2	±	9.9	4.4
Negative Reference[c]	235.0	±	24.6	112.6	154.1	2	1	3	0	1	1	1	0	1	0	2	3	15	106.4	±	15.5	5.9
Positive Control[d]	185.7	±	12.3	89.0	126.6	10	12	12	13	13	18	13	19	15	6	14	10	155	98.5	±	6.1	65.6***
Positive Control[d]	151.0	±	15.9	72.4	100.1	11	21	19	11	13	16	13	14	18	22	15	11	190	99.2	±	4.5	79.8***
Test Article[e]																						
100	209.3	±	13.1	100.3	88.0	0	4	0	4	1	1	1	1	3	1	1	3	20	76.7	±	12.4	10.9**
100	247.3	±	18.0	118.5	117.5	0	0	1	1	1	0	0	0	0	0	0	1	4	111.0	±	6.3	1.5
250	229.0	±	21.7	109.7	96.4	1	0	1	1	2	1	2	2	0	1	1	1	13	90.7	±	2.8	6.0
250	202.7	±	47.1	97.1	103.0	1	1	1	1	0	1	2	4	1	1	0	1	14	100.2	±	4.2	5.8
500	238.0	±	3.5	114.0	161.4	0	1	0	0	1	2	2	0	1	0	1	0	8	109.2	±	5.5	3.1
500	238.3	±	5.1	114.2	69.0	1	0	1	0	1	1	1	0	0	2	1	0	8	110.0	±	3.0	3.0
1000	228.0	±	61.5	109.2	67.7	0	0	1	1	2	2	0	0	1	1	3	0	11	108.8	±	3.9	4.2
1000	227.0	±	10.4	108.8	131.1	2	0	1	1	3	1	1	1	3	2	2	0	17	100.5	±	6.4	7.0*

[a]Mutant Frequency = Total mutant colonies / (No. of dishes x 2 x 10E5 x absolute CE)
[b]Negative Control = Cultures exposed to alpha-cyclodextrin powder, 300 mg, with 20 mL of water under the same conditions as test article treated cultures.
[c]Negative Reference Control = Cultures containing cells and RPMI 1640 culture medium, incubated under standard culturing conditions.
[d]Positive Control = 50 µg/mL 5-Bromo-2'-deoxyuridine
[e]ppm
* Significant increase: Kastenbaum Bowman test $p \leq 0.05$ but mutant frequency $\leq 15 \times 10E-6$
** Significant increase: Kastenbaum Bowman test $p \leq 0.01$ but mutant frequency $\leq 15 \times 10E-6$
*** Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq 15 \times 10E-6$
CE = Cloning efficiency

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Table 3
Initial Mutation Assay With Metabolic Activation

Sample Name: 1-Methylcyclopropene alpha-cyclodextrin complex (3.3% a.i.), (Sample Number TD No. 00-103, Lot No. BAS 5-80)

Sponsor: Rohm and Haas Company **Test Date:** January 31, 2001 **Selective Agent:** 4 ug/mL 6-thioguanine **Cells seeded for analysis:** 200/dish for C.E.,
Assay Number: 21917-0-435 OECD **Expression Time:** 7 days 2 x 10^5/dish for mutants

Nonactivation Test Condition	Survival to Treatment: Mean Colony Number ± SD			Survival to Treatment: % Neg. Control	Relative Population Growth (% of Control)	Mean Colonies Dish Number 1	2	3	4	5	6	7	8	9	10	11	12	Total Mutant Colonies	Absolute C.E. ± SD (%)			Mutant Frequency in 10E-6 Units[a]
Negative Control[b]	183.7	±	13.6	95.5	100.2	1	2	1	2	0	1	0	2	0	1	1	0	11	82.7	±	14.1	5.5
Negative Control[b]	201.0	±	13.5	104.5	99.7	0	2	1	1	1	1	0	0	3	0	0	0	9	113.7	±	6.1	3.3
Negative Reference[c]	202.7	±	24.1	105.4	61.1	0	0	1	1	0	3	0	1	1	1	1	0	9	120.9	±	10.0	3.1
Negative Reference[c]	148.3	±	24.7	77.1	59.0	0	0	0	1	0	0	0	1	1	0	0	1	4	102.7	±	6.5	1.6
Positive Control[d]	135.0	±	19.0	70.2	43.0	20	19	C	19	19	21	21	21	20	19	25	25	231	108.5	±	4.3	96.8**
Positive Control[d]	123.7	±	12.5	64.3	55.4	12	28	19	28	20	24	17	27	22	21	21	16	255	88.2	±	22.1	120.5**
Test Article[e]																						
100	184.7	±	8.1	96.0	96.6	0	2	0	1	0	0	0	1	0	3	0	0	7	64.2	±	15.8	4.5
100	208.7	±	10.6	108.5	68.8	2	2	0	1	1	0	1	0	0	1	2	1	11	84.5	±	7.4	5.4
250	177.0	±	5.6	92.0	79.6	0	1	0	1	2	0	0	1	1	C	1	0	7	98.2	±	6.3	3.2
250	184.3	±	4.2	95.8	76.3	1	0	0	0	0	0	1	0	1	0	0	0	3	87.9	±	3.7	1.4
500	170.7	±	1.2	88.7	105.1	0	0	0	0	2	4	1	2	0	1	2	2	12	115.4	±	9.5	4.3
500	151.7	±	14.6	78.8	87.9	1	1	0	3	1	0	0	1	2	2	1	0	12	96.5	±	4.8	5.2
1000	171.0	±	5.6	88.9	97.9	1	0	0	0	2	1	0	0	1	0	0	0	5	102.7	±	5.5	2.0
1000	184.3	±	10.1	95.8	80.5	1	2	1	1	1	1	1	0	1	0	0	1	10	95.0	±	9.7	4.4

[a]Mutant Frequency = Total mutant colonies / (No. of dishes x 2 x 10E5 x absolute CE)
[b]Negative Control = Cultures exposed to alpha-cyclodextrin powder, 300 mg, with 20 mL of water under the same conditions as test article treated cultures.
[c]Negative Reference Control = Cultures containing cells and RPMI 1640 culture medium, incubated under standard culturing conditions.
[d]Positive Control = 5 µg/mL 3-methylcholanthrene
[e]ppm
** Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq 15 \times 10E\text{-}6$
CE = Cloning efficiency
C = Contaminated

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Table 4
Confirmatory Mutation Assay With Metabolic Activation

Sample Name: 1-Methylcyclopropene alpha-cyclodextrin complex (3.3% a.i.) (Sample Number TD No. 00-103, Lot No. BAS 5-80)

Sponsor: Rohm and Haas Company **Test Date:** February 28, 2001 **Selective Agent:** 4 ug/mL 6-thioguanine **Cells seeded for analysis:** 200/dish for C.E., 2 x 10^5/dish for mutants
Assay Number: 21917-0435 OECD **Expression Time:** 7 days

Nonactivation Test Condition	Survival to Treatment: Mean Colony Number ± SD			Survival to Treatment: % Neg. Control	Relative Population Growth (% of Control)	Mean Colonies Dish Number 1	2	3	4	5	6	7	8	9	10	11	12	Total Mutant Colonies	Absolute C.E. ± SD (%)			Mutant Frequency in 10E-6 Units[a]
Negative Control[b]	193.7	±	5.9	101.1	94.7	0	1	1	0	0	1	2	2	1	1	0	2	11	78.0	±	2.2	5.9
Negative Control[b]	189.3	±	6.8	98.9	105.3	1	2	0	0	3	0	1	0	2	0	2	0	11	88.7	±	5.1	5.2
Negative Reference[c]	215.7	±	11.0	112.6	94.1	1	2	0	1	2	1	1	1	1	2	3	2	17	82.7	±	4.6	8.6
Negative Reference[c]	228.3	±	1.5	119.2	104.6	3	0	0	1	1	1	1	1	3	1	0	1	13	91.7	±	6.0	5.9
Positive Control[d]	192.0	±	15.0	100.3	81.6	20	34	30	26	34	25	19	39	26	21	24	30	328	81.5	±	7.0	167.7**
Positive Control[d]	226.0	±	2.6	118.0	70.0	28	35	37	35	39	48	47	41	41	54	31	29	465	91.2	±	1.8	212.5**
Test Article																						
100	208.7	±	4.5	109.0	120.2	0	2	1	2	1	0	0	3	1	2	0	1	12	83.5	±	2.6	6.0
100	199.7	±	13.3	104.3	120.1	0	2	0	1	1	0	0	0	0	0	0	1	5	80.7	±	6.3	2.6
250	166.0	±	17.5	86.7	94.7	0	4	0	2	0	0	1	1	0	0	1	1	10	88.8	±	7.7	4.7
250	181.7	±	9.0	94.9	105.0	0	1	1	1	0	0	0	0	3	0	0	1	7	61.5	±	1.8	4.7
500	229.0	±	12.1	119.6	113.4	2	0	1	0	3	1	2	0	0	1	1	2	13	88.7	±	7.3	6.1
500	232.3	±	7.1	121.3	103.8	0	0	0	1	2	0	1	1	1	0	2	0	8	95.0	±	10.5	3.5
1000	241.7	±	5.0	126.2	106.8	0	0	0	1	0	0	C	1	0	1	1	1	5	83.5	±	5.7	2.7
1000	204.7	±	4.0	106.9	118.4	1	0	0	0	1	0	0	1	0	0	1	0	4	91.7	±	6.7	1.8

[a]Mutant Frequency = Total mutant colonies / (No. of dishes x 2 x 10E5 x absolute CE)
[b]Negative Control = Cultures exposed to alpha-cyclodextrin powder, 300 mg, with 20 mL of water under the same conditions as test article treated cultures.
[c]Negative Reference Control = Cultures containing cells and RPMI 1640 culture medium, incubated under standard culturing conditions.
[d]Positive Control = 5 µg/mL 3-methylcholanthrene
[e]ppm
** Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq 15 \times 10E\text{-}6$.
CE = Cloning efficiency
C = contaminated

PERIODICAL REPORT ANALYSIS COMPANY PL...

DATA EVALUATION RECORD

1-METHYLCYCLOPROPENE

STUDY TYPE: DIETARY AND WORKER RISK ASSESSMENTS

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticides Programs
U.S. Environmental Protection Agency
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Prepared by

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Primary Reviewer: Steven T. Cragg, PhD, DABT	Signature: Steven T. Cragg Date: 3-July-2001
Secondary Reviewer Nasin Begin, PhD	Signature: Nasrin Begum Date: 7/3/01
Quality Assurance: Waqi Alum, PhD.	Signature: WAlam Date: July 3, 2001

EPA Reviewer: Biochemical _____, Date 1/29/2002
Review Section __, ~~Toxicology~~ Branch ___ (75~~0~~9C)
EPA Secondary Reviewer: _____, Date _____
Review Section __, ~~Toxicology~~ Branch ___ (75~~0~~9C)
Biochemicals

Data Evaluation Record

STUDY TYPE: Dietary and Worker Risk Assessment

DP BARCODE:	D275384, D275385	SUBMISSION CODE:	S598245
P.C. CODE:	069592-1	TOX. CHEM. NO.:	
MRID No.:	453853-07		

TEST MATERIAL (PURITY): 1-Methylcyclopropene (95.80% active ingredient)

SYNONYMS: Cyclopropene, 1-methyl-; 1-MCP

CITATION: Longacre, S.L., Hazelton, G.A., (2001). 1-Methylcyclopropene (1-MCP): Dietary and Worker Risk Assessment. Rohm and Haas Company Toxicology Department Report No. AGREG-01-01, April 5, 2001. MRID 453803-07. Unpublished.

SPONSOR: Rohm and Haas Company
Toxicology Department
727 Norristown Road
Spring House, PA 19477-0904

EXECUTIVE SUMMARY: This report briefly summarizes the agricultural uses, likely human exposures, and the toxicology of 1-methylcyclopropene (1-MCP). Following this summary, the report compares estimated human doses to the acute rat lethality NOAEL and to the 2-week rat inhalation NOAEL. The factors by which the acute and 2-week NOAELs exceed the estimated human doses (both in workers and consumers) are calculated as the "margins of safety" (MOS) of 1-MCP. These MOS are on the order thousands for workers, to hundreds of thousands to millions for consumers, for 1-MCP-treated produce (i.e., the estimated human dose is less than the no-effect animal dose by factors of thousands to millions). The toxicity of 1-MCP is summarized below.

Study Type	Dose/concentration/effect
Rat Oral Toxicity (LD50)	>165 mg/kg (since no deaths occurred, this dose represents the effective acute oral lethality NOAEL)
Rat Inhalation Toxicity (LC50)	>1000 ppm (> 2.2 mg/L)
Rabbit Dermal Toxicity (LD50)	> 2,000 mg/kg
Rabbit Eye Irritation	Slight
Rabbit Dermal Irriation	No irritation
Guinea Pig Dermal Sensitization	Not a sensitizer
Rat 2-Week Inhalation NOAEL	100 ppm (0.221 mg/L = 63 mg/kg-day)
Ames Mutagenicity	Negative
Mammalian Mutagenicity (CHO/HGPRT)	Negative
In vitro cytogenetics (human lymphocytes)	Negative
In vivo mouse micronucleus	Negative

COMPLIANCE: The report indicates that protocol or GLP guidelines are not applicable for this type of report (it did not generate original data but compared previously submitted toxicology data with estimated exposures). Consequently, no OPPTS Protocol Guideline is cited as having been followed and a signed and dated GLP compliance page indicates that GLPs do not apply to this report. A page also is provided indicating that this report does not contain business confidential information.

A. RESULTS

	Assume all 1-MCP on produce*	Measured amount 1-MCP on produce**
1-MCP on produce	9 ppb	4 ppb
% affected produce assumed in human diet*	100%	100%
Consumer MOS (acute oral rat lethality NOAEL)	1,900,000	730,000
Consumer MOS (2-wk rat inhalation NOAEL)	860,000	330,000
Worker MOS (2-wk rat inhalation study)	3,150	N/A

* 100% of an amount of 1-MCP was considered to transfer quantitatively (i.e., completely) to apples confined in an airtight space. simulating fumigation treatment. Human diet was assumed to include 100% 1-MCP treated produce.
** A known amount of radiolabeled 1-MCP was placed into a known volume of space containing apples and the amount transferred to the apples was measured.

B. DISCUSSION

The exposure assumptions used in this risk assessment were reasonable and tended, where uncertainty was present, to err conservatively on the side of protecting health. For example, to calculate consumer dose, it was conservatively assumed that 100% of the human diet was treated with 1-MCP whereas it is probable that only a fraction of the diet would be so treated. Assumptions about respiration rates, body weights of rats and humans were typical. Spot-checks of the calculations did not reveal mathematical mistakes.

It would have been desirable to calculate a margin of safety (MOS) based on NOAELs from a subchronic or chronic animal study. A smaller margin of safety may have resulted since longer term studies tend to show effects at lower doses (i.e., would have a lower NOAEL). However, longer term studies do not seem to have been undertaken to date. Thus, calculating MOS based on longer term studies was not possible. While mutagenicity results suggest a lack of carcinogenic potential, genotoxicity studies cannot rule out noncarcinogenic effects occurring from longterm exposure that might occur at lower levels than seen in the 2-week pilot rat (9 exposures; females only) inhalation study. Consequently, the margins of safety calculated in this report are useful but should not be considered definitive. The fact that the reported MOS are quite large, even though based on short term studies, is reassuring.

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Chemical: Cyclopropene,1-methyl-

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