



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

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

June 14, 2002

Memorandum

Subject: Secondary Review of Data Evaluation Records on Acute, Irritation, Sensitization and Developmental Toxicity Studies with 1-Methylcyclopropene (PC 224459). DP Barcode D283049; Submission S615722.

From: Roger Gardner, Toxicologist
Biochemical Pesticides Branch *Roger Gardner 6/17/02*
Biopesticides and Pollution Prevention Division (7511C)

Thru: Sheryl Reilly, Ph.D., Acting Chief
Biochemical Pesticides Branch *Sheryl K Reilly 6/17/02*
Biopesticides and Pollution Prevention Division (7511C)

To: Driss Brenmhend, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides and Pollution Prevention Division (7511C)

Action Requested

Secondary review of Data Evaluation Reports (DER, Attached) on four acute toxicity studies and one developmental toxicity study submitted by Agro Fresh, Inc. (MRIDs 454586-04 through -08) for 1-methylcyclopropene (MCP) and a formulated product, 1-methylcyclopropene-alpha-cyclodextrin complex (3.3% a.i.).

Recommendations and Conclusions

There were four acceptable acute toxicity study reports and one report on a developmental toxicity study submitted for consideration. Based on the study reviews prepared by the Chemical Hazard Evaluation Group at Oakridge National Laboratory, the 1-methylcyclopropene alpha-cyclodextrin product (containing 3.3% 1-methylcyclopropene) is placed into Toxicity Category IV for acute dermal toxicity as well as primary dermal and eye irritation. The formulated product was not a skin sensitizer. The acute oral toxicity study is waived because the formulation releases the gaseous active ingredient when moistened, and the acute inhalation toxicity study was waived because the

formulation releases the gaseous active ingredient when moistened. These results are sufficient to classify the product into Toxicity Category IV for acute inhalation toxicity.

The inhalation developmental toxicity study in rats showed that MCP caused no developmental effects at analytical concentrations as high as 1029 ppm (6.48 mg/L of air), and the maternal no-observed-adverse-effect level (NOAEL) was 0.67 mg/L. At the lowest-observed-adverse-effect level (LOAEL; 2.07 mg/L) exposed adult rats had increased incidences of darkened and/or enlarged spleens.

Acute Toxicity Studies

Four acute toxicity studies were submitted to support the registration of the 1-methylcyclopropene alpha-cyclodextrin (3.3% a.i.). All studies were acceptable, and the results are listed as follows:

Study	MRID No.	Results	Toxicity Category
Acute Oral-rat	---	Waived*	
Acute Dermal-rabbit	45458604	LD ₅₀ >5000 mg/kg	IV
Acute Inhalation-rat	---	Waived**	IV
Eye Irritation-rabbit	45458606	Slight irritant	IV
Skin Irritation-rabbit	45458605	Slight irritant	IV
Skin Sensitization-guinea pig (Modified Buehler)	45458607	Not a Sensitizer	N/A

* Waived because the formulation releases the gaseous active ingredient when moistened.

** Waived because there were no mortalities in the developmental toxicity study (MRID 45458608) after 14 consecutive daily exposures to air concentrations of 6.48 mg/L.

Developmental Toxicity Study

In an acceptable developmental toxicity study (MRID 45458608) 1-Methylcyclopropene gas was administered to groups of 22 timed-mated female rats via whole-body inhalation for 6 hours a day at target dose levels of 0, 100, 300, or 1000 ppm from gestation days (GD) 6 through 19, inclusive. Overall mean analytical concentrations were 0, 107, 329, or 1029 ppm (0, 0.67, 2.07, or 6.48 mg/L), respectively, however, individual daily measurements varied from 67.0-165.3, 240.4-462.5, and 798.7-1329.7 ppm, respectively. On GD 20, dams were sacrificed and necropsied, and all fetuses were examined externally, sexed, weighed, and euthanized. Approximately one-half of the fetuses in each litter were examined for visceral alterations, and the remainder were processed and examined for skeletal alterations.

There were no deaths or treatment-related clinical signs. At the 1000 ppm exposure level, mean body weight gain and food consumption were transiently decreased during GD 6-9 (56% and 23% less than controls, respectively; $p < 0.05$), with a compensatory increase in body weight gain during GD 9-12 (20% greater than controls; $p < 0.05$). Overall weight gain of the high-concentration group for the GD 6-19 dosing interval was also decreased, both without and with correction for gravid uterine weight (11 and 22% less than controls, respectively; $p < 0.05$ for corrected weight gain only). At the 300 and 1000 ppm exposure levels, darkened spleens were noted in 5/22 and 22/22 dams, respectively, and enlarged spleens were noted in 2/22 and 19/22 dams respectively; neither finding was observed in animals from the air control or low-concentration groups. In addition, histopathology conducted during the range-finding study detected treatment-related microscopic changes in the kidney at the 1000 ppm exposure level. These changes were characterized as multifocal basophilia of the cortical tubular epithelium with karyomegaly in some of the basophilic epithelial cells and accumulations of eosinophilic granular casts in the lumen of affected tubules. [Histopathology was not conducted in the current study.] There were no treatment-related effects on the intrauterine parameters of the treated groups compared to vehicle controls. **The maternal toxicity LOAEL for 1-methylcyclopropene in Crl:CD[®]BR rats dosed by whole-body inhalation exposure is 300 ppm (analytically verified 329 ppm [2.07 mg/L]), based on increased incidences of darkened and/or enlarged spleens. The maternal NOAEL is 100 ppm (analytically verified 107 ppm [0.67 mg/L]).**

There were no treatment-related increases in fetal deaths/resorptions or incidences of fetal structural alterations, and there was no evidence of altered growth or an effect on fetal sex ratios. **The developmental toxicity LOAEL for 1-methylcyclopropene in Crl:CD[®]BR rats dosed by whole-body inhalation exposure is not identified, and the developmental toxicity NOAEL is greater than or equal to 1000 ppm (analytically verified 1029 ppm [6.48 mg/L]).**

This inhalation developmental toxicity study in the rat is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental toxicity study in the rat (OPPTS 870.3700a; OECD 414).

DATA EVALUATION RECORD

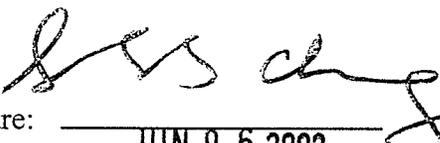
METHYLCYCLOPROPENE ALPHA-CYCLODEXTRIN COMPLEX (3.3% A.I.)

STUDY TYPE: ACUTE DERMAL TOXICITY - RAT (870.1200)
MRID 45458604

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 131

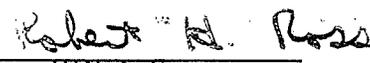
Primary Reviewer:
Susan Chang, M.S.

Signature: 
Date: JUN 0 6 2002

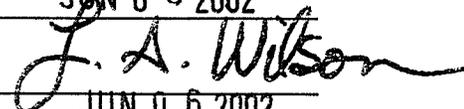
Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.),
Ph.D., D.A.B.T.

Signature: 
Date: JUN 0 6 2002

Robert H. Ross, M.S., Group Leader

Signature: 
Date: JUN 0 6 2002

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: 
Date: JUN 0 6 2002

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

DATA EVALUATION RECORD

Reviewed by:

Roger Gardner
6/13/02

Reviewer: Russell Jones or Paul Zubkoff, Ph.D.

STUDY TYPE: Acute Dermal Toxicity - Rats (OPPTS 870.1200)

MRID NO: 45458604

TEST MATERIAL: 1-Methylcyclopropene alpha-cyclodextrin complex
(3.3% a.i.)

PROJECT NO: 00R-200

SPONSOR: Rohm and Haas Company, Toxicology Department, 727
Norristown Road, P.O. Box 904, Spring House, PA
19477-0904

TESTING FACILITY: Rohm and Haas Company, Toxicology Department, 727
Norristown Road, P.O. Box 904, Spring House, PA

TITLE OF REPORT: 1-Methylcyclopropene alpha-cyclodextrin complex
(3.3% a.i.) - Acute Dermal Toxicity Study in Male and
Female Rats

AUTHORS: J.R. Parno, L.P. Craig, and S.L. Eberly

STUDY COMPLETED: February 8, 2001

GOOD LABORATORY PRACTICE: GLP Compliant

CONCLUSION: The dermal LD₅₀ for males, females, and combined was
greater than 5000 mg/kg.

CLASSIFICATION: ACCEPTABLE -- TOXICITY CATEGORY IV

I. STUDY DESIGN

Test Material: 1-Methylcyclopropene alpha-cyclodextrin complex (3.3% 1-methylcyclopropene, a.i.), Lot No. BAS 5-80

Test Animals: Five male and 5 female Crl:CD®BR rats were received from Charles River Laboratories, Raleigh, NC and weighed 250-279 g (males) and 221-241 g (females) on the day of dosing. The animals (approximately 8-9 weeks old) were housed individually in suspended stainless steel cages with mesh fronts and bottoms. PMI Certified rodent Diet 5002 (C) and purified water were available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, approximately 23°C; relative humidity, 45-49%; and photoperiod, 12 hour light/dark cycle.

Methods: Rats were ear-tagged: Male – 00-02310, 00-02311, 00-02312, 00-02313, and 00-02314; Female – 00-02315, 00-02316, 00-02317, 00-02318, and 00-02319. The rats were quarantined for approximately one week. The test material (5000 mg/kg body weight) moistened with light white mineral oil (2:1 w/v) was applied topically to the shaved intact skin between flanks and shoulders in an area of approximately 10% of the body surface. The entire trunk was wrapped in a polyethylene sheet, covered with Elastoplast® and PEG® elastic bandages, and secured with adhesive tape. The coverings were removed after 24 hours and the excess test material was removed with a paper towel saturated with mineral oil. Cardboard collars were worn for 14 days to prevent preening of the application site. The test animals were observed for clinical signs of toxicity approximately 1, 2, and 4 hours after treatment and once daily thereafter for 14 days. The rats were weighed prior to treatment (day 0), and on days 7 and 14. The rats were euthanized on day 14 and necropsied.

II. RESULTS

Mortality: All rats survived the study.

Clinical Observations: Two females had scant feces on day 1 with recovery by day 2 and one male was passive on day 2 with recovery by day 3. Erythema, pocketing edema/edema, dark areas, desiccation, and/or scabs were noted on all animals throughout the study.

Body Weights: All rats gained weight during the study.

Gross Necropsy: No gross changes were noted.

III. DISCUSSION

The acute lethal dose (LD₅₀) is greater than 5000 mg/kg. This places 1-methylcyclopropene alpha-cyclodextrin complex (3.3% a.i.) in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE**.

DATA EVALUATION RECORD

METHYLCYCLOPROPENE ALPHA-CYCLODEXTRIN COMPLEX (3.3% A.I.)

STUDY TYPE: PRIMARY DERMAL IRRITATION - RABBIT (870.2500)
MRID 45458605

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 131

Primary Reviewer:
Susan Chang, M.S.

Signature: _____
Date: _____



JUN 0 6 2002

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.),
Ph.D., D.A.B.T.

Signature: _____
Date: _____



JUN 0 6 2002

Robert H. Ross, M.S., Group Leader

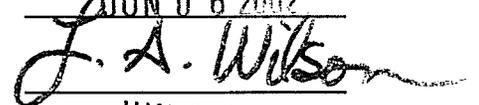
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Date: _____



JUN 0 6 2002

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: _____
Date: _____



JUN 0 6 2002

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DATA EVALUATION RECORD

Reviewed by:

Roy Gordon 6/13/02

Secondary Reviewer: Russell Jones or Paul Zubkoff, Ph.D.

STUDY TYPE: Primary Dermal Irritation - Rabbits (OPPTS 870.2500)

MRID NO: 45458605

TEST MATERIAL: 1-Methylcyclopropene alpha-cyclodextrin complex
(3.3% a.i.)

PROJECT NO: 00R-201

SPONSOR: Rohm and Haas Company, Toxicology Department, 727
Norristown Road, P.O. Box 904, Spring House, PA
19477-0904

TESTING FACILITY: Rohm and Haas Company, Toxicology Department, 727
Norristown Road, P.O. Box 904, Spring House, PA

TITLE OF REPORT: 1-Methylcyclopropene alpha-cyclodextrin complex
(3.3% a.i.) - Skin Irritation Study in Rabbits

AUTHORS: J.R. Parno, L.P. Craig, and S.L. Eberly

STUDY COMPLETED: February 8, 2001

GOOD LABORATORY PRACTICE: GLP Compliant

CONCLUSION: Very slight erythema was noted on three rabbits one hour after patch removal that cleared on two rabbits by day 2 but persisted on one rabbit through 72 hours. Another rabbit had very slight erythema 24 through 72 hours after patch removal. The primary irritation index was 0.4. 1-Methylcyclopropene alpha-cyclodextrin complex (3.3%) was slightly irritating.

CLASSIFICATION: ACCEPTABLE -- TOXICITY CATEGORY IV

I. STUDY DESIGN

Test Material: 1-Methylcyclopropene alpha-cyclodextrin complex (3.3% 1-methylcyclopropene, a.i.), Lot No. BAS 5-80

Test Animals: Six adult male New Zealand White rabbits were received from Covance Research Products, Inc., Denver, PA and weigh 2292-2843 g on the day of treatment. The animals, approximately 16-19 weeks old, were housed individually in suspended stainless steel cages. PMI Certified High Fiber Rabbit Diet 5325 (125 g/day) and purified water (*ad libitum*) were available. The environmental conditions of the animal room were as follows: temperature, 18-19°C; relative humidity, 57-59%; and photoperiod, 12 hour light/dark cycle.

Methods: Rabbits were ear-tagged: 00-27251, 00-27252, 00-27253, 00-27254, 00-27255, and 00-27278. The rabbits were quarantined for approximately two weeks. The fur around the entire trunk between the flank and shoulders was shaved approximately 24 hours prior to treatment. The rabbits received a single 0.5 g of test material moistened with 1.0 mL of light white mineral oil (1:2 w/v) applied onto a 1.0-inch square gauze-lined adhesive bandage which was placed on the shaved intact skin of each rabbit. The entire trunk was wrapped with a semi-occluded dressing. The covering was removed 4 hours later and the site wiped with paper towels saturated with tap water. The animals were observed for mortality and clinical signs of toxicity daily. Dermal examination was recorded at 1, 24, 48, and 72 hours after removal of the patch.

II. RESULTS

Mortality: All rabbits survived the study.

Dermal responses: Very slight erythema was noted on three rabbits one hour after patch removal that cleared on two rabbits by day 2 but persisted on one rabbit through 72 hours (Table 1). Another rabbit had very slight erythema 24 through 72 hours after patch removal. The primary irritation index was 0.4.

Irritation Scores:

TABLE 1. Summary of individual rabbit's dermal irritation scores with time

Animal No.		Hours			
		1	24	48	72
M	00-27251	0/0 ^a	37255	37255	37255
	00-27252	37255	0	0	0
	00-27253	0	0	0	0
	00-27254	37255	0	0	0
	00-27255	37255	37255	37255	37255
	00-27278	0	0	0	0

Data taken from Table 1, p. 12, MRID 45458605.

^aErythema/Edema

Description of rating method:

Evaluation of Skin Reaction	<u>Score</u>
Erythema formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight escharformation (injuries in depth)	4
<u>Edema Formation</u>	
No edema	
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised by more than 1 mm extending beyond the area of exposure)	4

III. DISCUSSION

Very slight erythema was noted on three rabbits one hour after patch removal that cleared on two rabbits by day 2 but persisted on one rabbit through 72 hours. Another rabbit had very slight erythema 24 through 72 hours after patch removal. The primary irritation index was 0.4. 1-Methylcyclopropene alpha-cyclodextrin complex (3.3%) was slightly irritating and is in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE**.

DATA EVALUATION RECORD

METHYLCYCLOPROPENE ALPHA-CYCLODEXTRIN COMPLEX (3.3% A.I.)

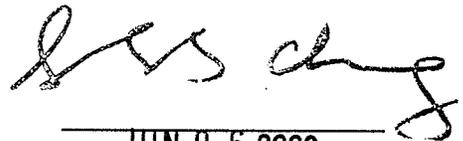
STUDY TYPE: PRIMARY EYE IRRITATION - RABBIT (870.2400)
MRID 45458606

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 131

Primary Reviewer:
Susan Chang, M.S.

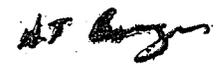
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Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.),
Ph.D., D.A.B.T.

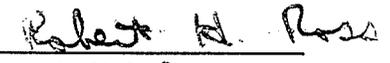
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Robert H. Ross, M.S., Group Leader

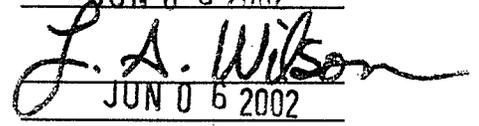
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Quality Assurance:
Lee Ann Wilson, M.A.

Signature: _____
Date: _____



JUN 0 6 2002

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DATA EVALUATION RECORD

Reviewed by:

Roger Gardner 6/13/02

Secondary Reviewer: Russell Jones or Paul Zubkoff, Ph.D.

STUDY TYPE: Acute Eye Irritation - Rabbits (OPPTS 870.2400)

MRID NO: 45458606

TEST MATERIAL: 1-Methylcyclopropene alpha-cyclodextrin complex (3.3% a.i.)

PROJECT NO: 00R-202

SPONSOR: Rohm and Haas Company, Toxicology Department, 727 Norristown Road, P.O. Box 904, Spring House, PA 19477-0904

TESTING FACILITY: Rohm and Haas Company, Toxicology Department, 727 Norristown Road, P.O. Box 904, Spring House, PA

TITLE OF REPORT: 1-Methylcyclopropene alpha-cyclodextrin complex (3.3% a.i.) - Eye Irritation Study in Rabbits

AUTHORS: J.R. Parno, L.P. Craig, and S.L. Eberly

STUDY COMPLETED: February 8, 2001

GOOD LABORATORY PRACTICE: GLP Compliant

CONCLUSION: No corneal opacity, iritis, or positive conjunctival irritation was noted on any rabbit. The maximum average score was 5.0 at one hour after test material instillation. 1-Methylcyclopropene alpha-cyclodextrin complex (3.3% a.i.) was minimally irritating.

CLASSIFICATION: ACCEPTABLE -- TOXICITY CATEGORY IV

I. STUDY DESIGN

Test Material: 1-Methylcyclopropene alpha-cyclodextrin complex (3.3% 1-methylcyclopropene, a.i.), Lot No. BAS 5-80

Test Animals: Six adult male New Zealand White rabbits were received from Covance Research Products, Inc., Denver, PA and weigh 2729-3201 g on the day of treatment. The animals, approximately 20 weeks old, were housed individually in suspended stainless steel cages. PMI Certified High Fiber Rabbit Diet 5325 (125 g/day) and purified water (*ad libitum*) were available. The environmental conditions of the animal room were as follows: temperature, 18-19°C; relative humidity, 57-58%; and photoperiod, 12 hour light/dark cycle.

Methods: Rabbits were ear-tagged: 00-27279, 00-27280, 00-27281, 00-27282, 00-27283, and 00-27285. The rabbits were quarantined for approximately two weeks. The test material (0.1 g/eye/animal) as received was applied in the conjunctival sac of one eye, and the eye held closed for about one second. The contralateral eye served as control. The eyes were examined and scored 1, 24, 48 and 72 hours after test material instillation. All treated and control eyes were washed with 0.9% physiological saline for approximately 60 seconds after the 24- hour reading.

II. RESULTS

Mortality: No animals died during the study.

Ocular Lesions: No corneal opacity was noted on any rabbit. In addition, iritis or positive conjunctival irritation was not noted on any rabbit (Table 1). The maximum average score was 5.0 at one hour after test material instillation.

TABLE 1. Summary of Eye Irritation Scores with Time: Conjunctiva and Iris				
Score Conditions	1 hour	24 hours	48 hours	72 hours
Conjunctiva				
Erythema	0 to 1	0 to 1	0 to 1	0
Chemosis	1	0	0	0
Discharge	0 to 2	0	0	0
Iris	0	0	0	0

Irritation score is based on Draize Method

Scale for Scoring Ocular Lesions

Grades for Ocular Lesions

Cornea Opacity: degree of density (area most dense taken for reading)	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible.	1
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas; no details of iris visible; size of pupil barely discernible	3
Opaque cornea, iris not discernible through the opacity	4
Area of corneal involved:	
None	0
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than tree quarters	3
Greater than three quarters, up to whole area	4
Iris: Normal or not sufficiently abnormal to rate a score of 1	
Marked deepened rugae, congestion, swelling, moderate circumcorneal hyperemia or injection, any of these or combination thereof, iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these).	2
Conjunctive redness: (refers to palpebral and bulbar conjunctiva, excluding cornea and iris)	
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color; individual vessels not easily discernible	2
Diffuse beefy red	3
Chemosis: lids and/or nictating membranes	
No swelling	0
Any swelling above normal (includes nictitating membrane).	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids more than half closed	4
Discharge No discharge	
Any amount different from normal (does not include small amounts observed in inne canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

TABLE 2. Summary of Total ^a and Primary Eye Irritation Scores with Time					
	Animal #	1 h	24 h	48 h	72 h
M	00-27279	8	2	0	0
	00-27280	4	0	0	0
	00-27281	2	2	0	0
	00-27282	8	2	2	0
	00-27283	4	0	0	0
	00-27285	4	2	0	0
^b Total		5	1.3	0.3	0

^aFormula: Total Irritation Score = I + II + III, where,

I = Corneal Score= [Density (A) x Area (B)] x 5

II= Iris Score= Severity x 5

III= Conjunctival Score= [Erythema (A) + Chemosis (B) + Discharge (C)] x 2

^bPrimary Irritation = Sum of Total Irritation Scores ÷ 6

III. DISCUSSION

No corneal opacity, iritis, or positive conjunctival irritation was noted on any rabbit. The maximum average score was 5.0 at one hour after test material instillation. Although approximately 50% of the test material fell from the eye, enough remained in contact with the treated eye, that the amount was sufficient to cover the entire cornea and surrounding surface.

1-Methylcyclopropene alpha-cyclodextrin complex (3.3% a.i.) was minimally irritating and is in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE**.

Chemical:	Task 131						
	Average Scores			Unwashed			
	Hours	Days					
	1	24	48	72	4	7	10
I. Cornea							
A. Opacity	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B. Area	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Score	0.0	0.0	0.0	0.0	0.0	0.0	0.0
II. Iris							
A. Values	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Score	0.0	0.0	0.0	0.0	0.0	0.0	0.0
III. Conjunctivae							
A. Hyperemia	0.8	0.7	0.2	0.0	0.0	0.0	0.0
B. Chemosis	1.0	0.0	0.0	0.0	0.0	0.0	0.0
C. Discharge	0.7	0.0	0.0	0.0	0.0	0.0	0.0
Score	5.0	1.3	0.3	0.0	0.0	0.0	0.0
Total Score	5.0	1.3	0.3	0.0	0.0	0.0	0.0

The chemical is classified Mild Irritant

Chemical:	SK 9913-3P			
	Rabbit Number 1			
	Hours			
	1	24	48	72
I. Cornea				
A. Opacity	0	0	0	0
B. Area	0	0	0	0
(A*B)*5	0	0	0	0
II. Iris				
A. Values	0	0	0	0
(A*5)	0	0	0	0
III. Conjunctivae				
A. Hyperemia	1	1	0	0
B. Chemosis	1	0	0	0
C. Discharge	2	0	0	0
(A+B+C)*2	8	2	0	0
Total Score	8	2	0	0

	Rabbit Number 2			
	Hours			
	1	24	48	72
I. Cornea				
A. Opacity	0	0	0	0
B. Area	0	0	0	0
(A*B)*5	0	0	0	0
II. Iris				
A. Values	0	0	0	0
(A*5)	0	0	0	0
III. Conjunctivae				
A. Hyperemia	1	0	0	0
B. Chemosis	1	0	0	0
C. Discharge	0	0	0	0
(A+B+C)*2	4	0	0	0
Total Score	4	0	0	0

	Rabbit Number 3			
	Hours			
	1	24	48	72
I. Cornea				
A. Opacity	0	0	0	0
B. Area	0	0	0	0
(A*B)*5	0	0	0	0
II. Iris				
A. Values	0	0	0	0
(A*5)	0	0	0	0
III. Conjunctivae				
A. Hyperemia	0	1	0	0
B. Chemosis	1	0	0	0
C. Discharge	0	0	0	0
(A+B+C)*2	2	2	0	0
Total Score	2	2	0	0

	Rabbit Number 4			
	Hours			
	1	24	48	72
I. Cornea				
A. Opacity	0	0	0	0
B. Area	0	0	0	0
(A*B)*5	0	0	0	0
II. Iris				
A. Values	0	0	0	0
(A*5)	0	0	0	0
III. Conjunctivae				
A. Hyperemia	1	1	1	0
B. Chemosis	1	0	0	0
C. Discharge	2	0	0	0
(A+B+C)*2	8	2	2	0
Total Score	8	2	2	0

	Rabbit Number 5			
	Hours			
	1	24	48	72
I. Cornea				
A. Opacity	0	0	0	0
B. Area	0	0	0	0
(A*B)*5	0	0	0	0
II. Iris				
A. Values	0	0	0	0
(A*5)	0	0	0	0
III. Conjunctivae				
A. Hyperemia	1	0	0	0
B. Chemosis	1	0	0	0
C. Discharge	0	0	0	0
(A+B+C)*2	4	0	0	0
Total Score	4	0	0	0

	Rabbit Number 6			
	Hours			
	1	24	48	72
I. Cornea				
A. Opacity	0	0	0	0
B. Area	0	0	0	0
(A*B)*5	0	0	0	0
II. Iris				
A. Values	0	0	0	0
(A*5)	0	0	0	0
III. Conjunctivae				
A. Hyperemia	1	1	0	0
B. Chemosis	1	0	0	0
C. Discharge	0	0	0	0
(A+B+C)*2	4	2	0	0
Total Score	4	2	0	0

DATA EVALUATION RECORD

METHYLCYCLOPROPENE ALPHA-CYCLODEXTRIN COMPLEX (3.3% A.I.)

STUDY TYPE: SKIN SENSITIZATION - GUINEA PIG (870.2600)
MRID 45458607

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 131

Primary Reviewer:
Susan Chang, M.S.


Signature: _____
Date: JUN 06 2002

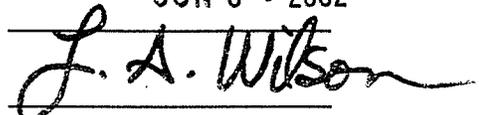
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H. Tim Borges, M.T.(A.S.C.P.),
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Date: JUN 06 2002

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Date: JUN 06 2002

Quality Assurance:
Lee Ann Wilson, M.A.


Signature: _____
Date: JUN 06 2002

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DATA EVALUATION RECORD

Reviewed by:

Roger Gordon . 6/13/02

Secondary Reviewer: Russell Jones or Paul Zubkoff, Ph.D.

STUDY TYPE: Skin Sensitization - Guinea Pigs (OPPTS 870.2600)

MRID NO: 45458607

TEST MATERIAL: 1-Methylcyclopropene alpha-cyclodextrin complex
(3.3% a.i.)

PROJECT NO: 00R-203

SPONSOR: Rohm and Haas Company, Toxicology Department, 727
Norristown Road, P.O. Box 904, Spring House, PA
19477-0904

TESTING FACILITY: Rohm and Haas Company, Toxicology Department, 727
Norristown Road, P.O. Box 904, Spring House, PA

TITLE OF REPORT: 1-Methylcyclopropene alpha-cyclodextrin complex
(3.3% a.i.) - Dermal Sensitization Study in Guinea Pigs

AUTHORS: J.R. Parno, D.M. Anderson, and T.L. Danberry

STUDY COMPLETED: February 7, 2001

GOOD LABORATORY PRACTICE: GLP Compliant

CONCLUSION: Scattered mild redness was noted on 0/20 and 1/20 test animals 24 and 48 hours, respectively, after challenge. The vehicle control animals had no reaction after challenge. The results of the positive control study were appropriate. 1-Methylcyclopropene alpha-cyclodextrin complex (3.3%) was not a dermal sensitizer.

CLASSIFICATION: ACCEPTABLE

I. STUDY DESIGN

Test Material: 1-Methylcyclopropene alpha-cyclodextrin complex (3.3% 1-methylcyclopropene, a.i.), Lot No. BAS 5-80

Test Animals: Fifty-seven outbred female Hartley guinea pigs received from Charles River Kinston Facility, Kingston, NY. Forty-five animals were assigned to groups and weighed 393-492 g at study commencement. The animals (approximately 8 weeks old) were housed individually in suspended stainless steel cages with wire mesh fronts and bottoms. PMI certified Guinea Pig Diet No. 5026 and purified water were available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 21.7-22.2°C; relative humidity, 48-52%; photoperiod, and 12 hour light/dark cycle.

Methods: Guinea pigs were identified by cage cards and grouped: Treated – 00-31586, 00-31577, 00-31587, 00-31554, 00-31548, 00-31563, 00-31588, 00-31570, 00-31572, 00-31544, 00-31600, 00-31551, 00-31559, 00-31549, 00-31592, 00-31589, 00-31564, 00-31574, 00-31594, and 00-31576; Irritation Control (vehicle control) – 00-31547, 00-31571, 00-31579, 00-31558, 00-31573, 00-31546, 00-31557, 00-31545, 00-31580, and 00-31581; Positive Control – 00-31591, 00-31582, 00-31568, 00-31552, 00-31555, 00-31599, 00-31560, 00-31583, 00-31585, and 00-31562; Irritation Control for Positive Control – 00-31569, 00-31550, 00-31593, 00-31566, and 00-31553. The guinea pigs were quarantined for approximately two weeks. The animals were induced and challenged according to the maximization test. The midline over the shoulder region of 45 female guinea pigs was shaved. Three pairs of intradermal injections (0.1 mL/site) were made into a 2 cm x 4 cm clipped area of skin of the guinea pigs on day 1. The injectables were Freund's complete adjuvant (diluted with equal volume of mineral oil), 10% w/w test material in mineral oil, and 10% w/w test material in a 1:1 emulsion of Freund's complete adjuvant with mineral oil. On day 7, the same scapular region was clipped and pretreated with 10% w/w sodium lauryl sulfate. On day 8, 0.4 g of test material moistened with 0.5 mL of mineral oil, absorbed onto an approximate 2 cm x 4 cm filter paper, was applied to the intradermal injection area under occlusion for 48 hours. The vehicle control animals were treated similarly to the test animals with the exception that the test material was omitted from the intradermal injections and topical application. On day 22, the sides of the test animals and the control animals were clipped approximately 6 hours prior to challenge. The animals were topically challenged with 0.2 g of test material moistened with mineral oil (right side) and mineral oil alone (left side) at naive sites for six hours. The positive control animals were treated similarly to the tested animals with the exception that 5% w/v suspension of hexylcinnamaldehyde (HCA, intradermal injection) and undiluted hexylcinnamaldehyde (topical induction) were used. The irritation control animals for the positive controls were treated similarly to the positive control animals with hexylcinnamaldehyde omitted. The positive controls and the irritation controls for the positive control animals were treated with 0.3 mL of mineral oil alone, 15% w/v, 20% w/v, and 25% w/v of HCA in mineral oil at challenge. The sites were evaluated 24 and 48 hours post exposure.

II. RESULTS

Mortality: No deaths were observed in any of the group.

Body Weights: All guinea pigs gained weight during the study.

Skin Effects: The reaction after inductions was not reported. Scattered mild redness was noted on 0/20 and 1/20 animals 24 and 48 hours, respectively, after challenge. The vehicle control animals had no reaction after challenge. The results of the positive control study were appropriate.

TABLE 1. Summary of Individual Erythema Challenge Scores with Time ^a						
Time	24 hours			48 hours		
Erythema Score	0	1	2	0	1	2
Treated	20	0	0	19	1	0
Naive Control	10	0	0	10	0	0

^aNumber of animals affected

Scale for Scoring Skin Reaction

No reaction	0
Scattered mild redness	1
Moderate and diffuse redness	2
Intense redness and/or swelling	3

III. DISCUSSION

Scattered mild redness was noted on 0/20 and 1/20 test animals 24 and 48 hours, respectively, after challenge. The vehicle control animals had no reaction after challenge. The results of the positive control study were appropriate. 1-Methylcyclopropene alpha-cyclodextrin complex (3.3%) was not a dermal sensitizer. The packet is classified **ACCEPTABLE**.

DATA EVALUATION RECORD

ETHYLBLOC/224459
[1-METHYLCYCLOPROPENE]

STUDY TYPE: PRENATAL DEVELOPMENTAL TOXICITY STUDY - RAT;
[OPPTS 870.3700a (§83-3a)]; OECD 414
MRID 45458608

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
BPPD Work Assignment #130

Primary Reviewer:
Donna L. Fefee, D.V.M.

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JUN 05 2002

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This review may have been altered subsequent to the contractors' signatures above.

ETHYLBLOC/224459

EPA Reviewer: Unassigned
Biopesticides and Pollution Prevention Division (7511C)
EPA Secondary Reviewer: Roger Gardner, Ph.D.
Biopesticides and Pollution Prevention Division (7511C)

Signature: Roger Gardner
Date: 6/13/02
Signature: _____
Date: _____

DATA EVALUATION RECORD
TXR#:

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700a [§83-3a];
OECD 414.

PC CODE: 224459

DP BARCODE: D283049/071297-1
SUBMISSION NO.: S615722

TEST MATERIAL (PURITY): 1-Methylcyclopropene (Ethylbloc; 96.42% a.i.)

SYNONYMS: none reported

CITATION: Wood, S., L. Craig, H. Bernacki, Jr., et al. (2001) 1-Methylcyclopropene: inhalation (whole-body) developmental toxicity study in rats. Rohm and Haas Company, Toxicology Department, 727 Norristown Road, P.O. Box 904, Spring House, PA 19477-0904. Laboratory report number 00R-181, April 18, 2001. MRID 45458608. Unpublished.

SPONSOR: Rohm and Haas Company (Applicant is Agro Fresh, Inc.).

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45458608) 1-Methylcyclopropene gas (96.42% a.i., lot # RMJ6675B) was administered to 22 timed-mated female CrI:CD®BR rats/dose via whole-body inhalation for 6 hours a day at target dose levels of 0, 100, 300, or 1000 ppm from gestation days (GD) 6 through 19, inclusive. Overall mean analytical concentrations were 0, 107, 329, or 1029 ppm (0, 0.67, 2.07, or 6.48 mg/L), respectively, however, individual daily measurements varied from 67.0-165.3, 240.4-462.5, and 798.7-1329.7 ppm, respectively. On GD 20, dams were sacrificed and necropsied, and all fetuses were examined externally, sexed, weighed, and euthanized. Approximately one-half of the fetuses in each litter were examined for visceral alterations, and the remainder were processed and examined for skeletal alterations.

There were no deaths or treatment-related clinical signs. At the 1000 ppm exposure level, mean body weight gain and food consumption were transiently decreased during GD 6-9 (56% and 23% less than controls, respectively; $p < 0.05$), with a compensatory increase in body weight gain during GD 9-12 (20% greater than controls; $p < 0.05$). Overall weight gain of the high-concentration group for the GD 6-19 dosing interval was also decreased, both without and with correction for gravid uterine weight (11 and 22% less than controls, respectively; $p < 0.05$ for corrected weight gain only). At the 300 and 1000 ppm exposure levels, darkened spleens were noted in 5/22 and 22/22 dams, respectively, and enlarged spleens were noted in 2/22 and 19/22 dams respectively; neither finding was observed in animals from the air control or low-

concentration groups. In addition, histopathology conducted during the range-finding study detected treatment-related microscopic changes in the kidney at the 1000 ppm exposure level. These changes were characterized as multifocal basophilia of the cortical tubular epithelium with karyomegaly in some of the basophilic epithelial cells and accumulations of eosinophilic granular casts in the lumen of affected tubules. [Histopathology was not conducted in the current study.] There were no treatment-related effects on the intrauterine parameters of the treated groups compared to vehicle controls. **The maternal toxicity LOAEL for 1-methylcyclopropene in Crl:CD®BR rats dosed by whole-body inhalation exposure is 300 ppm (analytically verified 329 ppm [2.07 mg/L]), based on increased incidences of darkened and/or enlarged spleens. The maternal NOAEL is 100 ppm (analytically verified 107 ppm [0.67 mg/L]).**

There were no treatment-related increases in fetal deaths/resorptions or incidences of fetal structural alterations, and there was no evidence of altered growth or an effect on fetal sex ratios. **The developmental toxicity LOAEL for 1-methylcyclopropene in Crl:CD®BR rats dosed by whole-body inhalation exposure is not identified, and the developmental toxicity NOAEL is greater than or equal to 1000 ppm (analytically verified 1029 ppm [6.48 mg/L]).**

This inhalation developmental toxicity study in the rat is classified **Acceptable/Guideline** and satisfies the guideline requirement for a developmental toxicity study in the rat (OPPTS 870.3700a; OECD 414). However, it must be noted that significant variation in the analytical exposure concentrations at all dose levels make the actual dosages to the animals uncertain.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

- Test material:** 1-Methylcyclopropene

Description:	A colorless gas
Lot #:	RMJ6675B
Purity:	96.42 % a.i.
Compound Stability:	Stable for several months in the liquid state, at -78 °C
CAS #of TGAI:	Not available
Structure:	Not available

- Vehicle and/or positive control:** The control group was exposed to conditioned laboratory air, only. There was no positive control used in the study.

3. Test animals:

Species:	Rat
Strain:	CrI:CD®BR
Age/weight at study initiation:	11-14 weeks of age; 198.4-266.0 g on GD 3
Source:	Charles River Laboratories, Kingston, NY
Housing:	Individually in suspended, stainless steel cages measuring approximately 18 x 18 x 34 cm
Diet:	PMI Certified Rodent Diet 5002 (meal) <i>ad libitum</i> except during exposure
Water:	Purified by reverse osmosis and available <i>ad libitum</i>
Environmental conditions:	Temperature: Approximately 22 °C Humidity: 33-58% Air changes: Not reported Photoperiod: 12 hrs dark/12 hrs light
Acclimation period:	None

B. PROCEDURES AND STUDY DESIGN:

- In life dates:** Start: November 17, 2000; End: December 7, 2000
- Mating:** The females were mated with sexually mature males of the same strain by the supplier (Charles River Laboratories). The day of conception was designated as gestation day (GD) 0, and the females were shipped to arrive at the testing facility on GD 1 or 2; however, the method used to confirm mating was not reported.
- Animal assignment:** Animals were assigned to the dose groups given in Table 1 by means of a randomization procedure based on stratified GD 3 body weights so that group mean body weights and standard deviations were homogenous.

Test group	Nominal conc. (ppm)	Analytical conc. in ppm (mg/L) ^a	Chamber temp. (°C.)	Relative humidity (%)	# Assigned
Control	0	0 (0)	20.4-23.6	62.3-89.3	22
Low (LCT)	100	107 (0.67)	20.2-23.2	69.7-90.8	22
Mid (MCT)	300	329 (2.07)	19.4-23.1	71.0-92.3	22
High (HCT)	1000	1029 (6.48)	20.9-24.3	71.3-87.0	22

Data taken from text table, p. 13 and pp. 26-29, MRID 45458608.

^a Calculated by reviewer using the conversion (ppm x M.W)/24,450 = mg/L and a Molecular Weight of 154.

- Concentration selection rationale:** The exposure concentrations were selected based on the results from a range-finding study in which 10 pregnant rats per group were administered 1-methylcyclopropene gas via whole-body inhalation for 6 hours a day at target levels of 0, 300, or 1000 ppm from gestation days (GD) 6 through 19, inclusive. The results of the range-finding study are summarized in the Appendix. In addition, the target high-concentration level of 1000 ppm is considered to be the maximum safe air concentration, as higher concentrations can result in explosion.
- Generation of the test atmosphere/chamber description:** A 240-Liter Plexiglas® and stainless steel dynamic exposure chamber was used. For each exposure level, a vial of the

liquid test material was attached to a Tedlar Gas Sampling bag by approximately 6 inches of Tygon® tubing, then placed in a water bath at a temperature of 25-35°C. The liquid test material began to boil, vaporized into a gas, and was transferred through connecting tubing into the sampling bag. When the transfer of the gas was complete, the sampling bag was closed, and the vial was disconnected. For the 1000 ppm exposure level, the procedure was repeated with a second vial of the test material, using the same gas sampling bag. The bag was then connected to the appropriate chamber, and the gas was metered using a Gilmont Flowmeter to obtain the desired concentration. The gas was drawn into the top of the chamber by maintaining the chamber pressure at negative 1.2 to 3.8 inches of water relative to the laboratory and was mixed with conditioned laboratory air inside the chamber. The airflow rates through the chambers were maintained at 51-52 L/min for the test material exposures and at 79 L/min for the air control group, which was intended to maintain oxygen concentration at greater than 19%, temperature at 22±2°C., and relative humidity at 30-70%. Exposure conditions (daily averages) are given in Table 1, above.

Time to equilibrium (T_{99}) was 22 minutes.

Test atmosphere concentrations were determined via gas chromatography. During each exposure, 6 samples were collected from all test material exposure groups at approximately 1-hour intervals by drawing 0.5 mL of chamber air into a gas-tight syringe, and a single sample was collected from the air control group using the same method. The samples were immediately injected into a gas chromatograph and analyzed.

Results: Absence of the test material was confirmed in the samples taken from the chamber of the air control group. Mean daily concentrations of the test atmosphere of the 100 ppm group ranged from 90.8-132 ppm, with individual samples varying from 67.0-165.3 ppm and 26.5% of the samples differing from target by greater than 15%. Mean daily concentrations of the test atmosphere of the 300 ppm group ranged from 285-381 ppm, with individual samples varying from 240.4-462.5 ppm and 34.3% of the samples differing from target by greater than 15%. Mean daily concentrations of the test atmosphere of the 1000 ppm group ranged from 941-1119 ppm, with individual samples varying from 798.7-1329.7 ppm and 13.7% of the samples differing from target by greater than 15%.

The analytical data indicated that there was significant variation in the actual exposure concentrations to the study animals, both between and within exposures.

- 6. Test article administration:** All exposures were once daily on GD 6-19 by the whole-body inhalation route. The timing of the 6-hour exposure period began after T_{99} was reached, and the animals of the treated groups remained in the exposure chamber for at least as long as the T_{99} after the generator was turned off.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** The animals were checked for clinical signs daily, including removal of the animals from the cage for examination. During GD 6-20, an additional observation was conducted to check for mortality or moribundity. Body weights were recorded on GD 3, 6, 9, 12, 15, 18, and 20, and food consumption data were recorded for the following intervals: GD 3-6, 6-9, 9-12, 12-15, 15-18, and 18-20. Dams were sacrificed on GD 20 by CO₂ gas asphyxiation and subjected to gross necropsy. The uterus was excised and weighed, and numbers of corpora lutea and numbers and positions of implantation sites, live and dead fetuses, and early and late resorptions were recorded. Uteri of grossly non-gravid females were stained with 10% ammonium sulfide to detect very early resorptions. In addition, maternal spleens were retained in 10% buffered formalin for possible future histopathological examination.
2. **Fetal evaluations:** All live fetuses were weighed, sexed, and examined externally. Alternating live fetuses from each litter were subjected to visceral examination by the Staples' Technique, and the heads from these fetuses were fixed in Bouin's fixative, then examined using the technique of Barrows and Taylor. The remaining fetuses were processed for staining with alizarin red S and subjected to skeletal examination. Fetuses with external abnormalities were subjected to visceral examination of the head and body and skeletal examination of the body only.

D. DATA ANALYSIS:

1. **Statistical analyses:** Maternal body weight and food consumption data, gravid uterine weights, and fetal body weights were analyzed using a one-way ANOVA, followed by Dunnett's test when significant. Incidence rates of pregnancy, adult gross necropsy findings, maternal death, and litters with total resorptions were analyzed using a 2xN Chi-square test, followed by Fisher's exact test with a Bonferroni correction for multiple comparisons when significant. Numbers of corpora lutea, implantations, live and dead fetuses, early and late resorptions, and fetal alterations were analyzed using a 2xN Kruskal-Wallis non-parametric ANOVA, followed by a 2x2 Kruskal-Wallis non-parametric ANOVA with a Bonferroni correction for multiple comparisons when significant. The litter, i.e. the proportion of affected fetuses per litter or the litter mean, was considered the unit of statistical analysis. A minimum significance level of $p < 0.05$ was used for all tests.
2. **Indices:** The following indices were calculated from cesarean section records of animals in the study: % preimplantation loss and % postimplantation loss. The formulas used were not provided in the study report.
3. **Historical control data:** With the following exception, historical control data were not provided to allow comparison with concurrent controls. Preimplantation loss data from 4 developmental toxicity studies conducted in 1991-1994 were included in the study report. Three of these studies were oral (gavage) studies using unspecified vehicles, and the remaining study was an inhalation study. It is unknown whether these studies used the same strain of rat used in the current study.

II. RESULTS:**A. MATERNAL TOXICITY:**

- Mortality and clinical observations:** There were no deaths or abortions during the study, and no treatment-related clinical signs were noted.
- Body weight** - Selected body weight data are summarized in Table 2. Mean absolute body weights and the terminal body weight corrected for gravid uterine weight of the air control and treated groups were similar. The mean body weight gain of the high-concentration group was transiently decreased to 56% less than controls during GD 6-9, followed by a compensatory 20% increase compared to controls during GD 9-12. Weight gain of the high-concentration group for the entire dosing interval was 11% less than controls and 22% less than controls when corrected for gravid uterine weights. These differences are considered treatment-related. There were no remarkable differences between the body weights and body weight gains of the low- and mid-concentration groups compared with the air control group.

Gestation day	Target chamber concentration in ppm (# of Dams)			
	Control (22)	100 (20)	300 (22)	1000 (22)
Absolute body weights				
3	231.9±3.22	232.4±2.48	231.2±2.35	231.3±2.85
6	251.5±3.14	251.5±2.55	250.0±2.05	250.3±2.82
9	268.4±3.15	265.8±3.02	265.6±2.58	257.6±3.80
15	305.2±3.57	301.8±3.53	303.2±3.44	297.1±4.71
20	370.8±4.69	368.7±4.40	368.4±5.61	356.3±5.81
Adjusted GD 20 body weight ^b	295.1±3.9	290.2±4.2	295.7±3.5	284.1±4.2
Body weight gains				
Days 3-6 (pretreatment)	19.6±1.00	19.1±1.02	18.9±0.87	19.0±0.94
Days 6-9	16.9±1.08	14.3±1.18	15.6±1.27	7.4±1.59 * (44) ^c
Days 9-12	17.2±1.03	17.9±0.79	18.0±1.00	20.7±0.88 * (120)
Days 6-20 (treatment)	119.3±3.14	117.2±3.44	118.4±4.64	106.0±4.03 (89)
/Adjusted GD 6-20 BW gain ^d	43.6±2.14	38.7±3.10	45.8±2.77	33.8±2.97 * (78)

Data taken from Tables 5, 6, 7 and Appendix 6, pages 32, 33, 34, and 101-104, respectively, MRID 45458608.

^a Data expressed as Mean ± Standard Error.

^b Adjusted GD 20 body weight = GD 20 weight minus gravid uterine weight.

^c Numbers in parentheses equal percent of control, calculated by reviewer.

^d Adjusted GD 6-20 BW gain = GD 6-20 BW gain minus gravid uterine weight.

Significantly different from control: * p < 0.05

- Food consumption:** At the highest concentration level, the GD 6-9 group mean food consumption was decreased 23% compared to that of controls (p<0.05). There were no other treatment-related effects on food consumption.

4. **Gross pathology:** Treatment-related abnormal gross necropsy findings were noted at 300 and 1000 ppm and included the following: darkened spleen in 5/22 and 22/22 dams, respectively (vs. 0/22 controls; $p < 0.05$ for high-dose animals only); and enlarged spleen in 2/22 and 19/22 dams, respectively (vs. 0/22 controls; $p < 0.05$ for high-concentration animals only).

5. **Cesarean section data:** Data collected at cesarean section are summarized in Table 3. There were no treatment-related effects on the numbers of viable litters, mean numbers of resorptions, implantations, corpora lutea, and live or dead fetuses, fetal weights, or fetal sex ratios of the treated groups compared to controls. The statistically significant increase in the mean preimplantation loss of the 1000 ppm group as compared to controls was not considered treatment-related, as dosing began after implantation, there were no remarkable differences in numbers of implantations, and the values at all exposure levels were quite low.

TABLE 3. Cesarean section observations ^a				
Observation	Target chamber concentration (ppm)			
	0	100	300	1000
# Animals Assigned (Mated)	22	22	22	22
# Animals Pregnant	22	20	22	22
Pregnancy Rate (%)	100	90.9	100	100
# Nonpregnant	0	2	0	0
Maternal Wastage	0	0	0	0
# Died	0	0	0	0
# Abortions or early deliveries	0	0	0	0
Total # Corpora Lutea	313	282	284	311 ^b
Corpora Lutea/Dam	14.2±0.34	14.1±0.43	12.9±0.68	14.8±0.63 ^b
Total # Implantations	289	260	265	273 ^c
Implantations/Dam	13.1±0.30	13.0±0.36	12.0±0.68	12.4±0.50 ^c
Total # Litters	22	20	22	22
Total # Live Fetuses	275	252	256	263
Live Fetuses/Dam	12.5±0.40	12.6±0.42	11.6±0.70	12.0±0.49
Total # Dead Fetuses	0	0	0	0
Total # Resorptions	14	8	9	10
Early	14	8	9	9
Late	0	0	0	1
Resorptions/Dam Total	0.6±0.21	0.4±0.17	0.4±0.13	0.5±0.11
Early	0.6±0.21	0.4±0.17	0.4±0.13	0.4±0.11
Late	0±0	0±0	0±0	0.0±0.05
Litters with Total Resorptions	0	0	0	0
Mean Fetal Weight (g)	3.8±0.05	3.9±0.05	3.9±0.05	3.7±0.04
Males	3.9±0.05	4.0±0.04	4.0±0.05	3.8±0.05
Females	3.7±0.06	3.8±0.06	3.8±0.05	3.6±0.04
Sex Ratio (Mean % Male)	50.5±2.98	46.9±3.77	52.4±3.18	48.8±3.75
Preimplantation Loss (Mean %)	7.4±1.34	7.3±1.85	7.2±2.61	15.6±3.0 ^b
No. per animal	1.1±0.21	1.1±0.30	0.9±0.30	2.5±0.55 *
Postimplantation Loss (Mean %)	5.0±1.70	3.2±1.39	3.9±1.25	3.7±0.94

Data taken from Tables 3, 10, and Appendix 9, pp. 30, 37-39, and 118-121, respectively, MRID 45458608.

^aData are given as Mean ± Standard Error, where appropriate.

^bData are from 21/22 pregnant females due to exclusion of data from one animal that had an erroneous value recorded for corpora lutea.

^c Includes implantations from the animal that had an erroneous value recorded for corpora lutea.

Significantly different from control: * p <0.05

B. DEVELOPMENTAL TOXICITY:

The total numbers of fetuses (litters) in the air control, low-, mid-, and high-concentration groups were 275 (22), 252 (20), 256 (22), and 263 (22), respectively. Fetal morphological data are given in Tables 4a, 4b, and 4c. There were no treatment-related effects on the incidences of fetal alterations.

1. **External examination:** There were no external malformations noted. Hematomas were observed on 1 fetus from the air control group and 2 fetuses from one litter from the high-concentration group and were the only external variations recorded.
2. **Visceral examination:** The only visceral malformation was a dilated pulmonary trunk in one fetus from the air control group. A malpositioned right carotid branch was observed in one fetus from each of the 300 and 1000 ppm groups. There were no other visceral malformations or variations recorded.
3. **Skeletal examination:** There were no skeletal malformations noted. The incidence rates of total skeletal variations, types of skeletal variations, and individual skeletal variations were similar among the treated and control groups.

TABLE 4a. External examinations [fetal (litter) incidences]				
Observations	Target chamber concentration (ppm)			
	0	100	300	1000
Number examined	275 (22)	252 (20)	256 (22)	263 (22)
Variation				
Hematoma	1 (1)	0 (0)	0 (0)	2 (1)

Data taken from Table 11, p. 40, MRID 45458608.

TABLE 4b. Visceral examinations [Fetal (Litter) incidences]				
Observations	Target chamber concentration (ppm)			
	0	100	300	1000
Number examined	144 (22)	133 (20)	133 (22)	137 (22)
Number affected	1 (1)	0 (0)	1 (1)	1 (1)
Malformations				
Dilated pulmonary trunk	1 (1)	0 (0)	0 (0)	0 (0)
Variations				
Malpositioned right carotid branch	0 (0)	0 (0)	1 (1)	1 (1)

Data taken from Table 12, p. 41, MRID 45458608.

TABLE 4c. Skeletal examinations [fetal (litter) incidences]				
Observations	Target chamber concentration (ppm)			
	0	100	300	1000
Number examined	131 (22)	118 (20)	123 (22)	127 (22)
Number affected	46 (18)	35 (15)	30 (15)	47 (19)
Variations				
Reduced ossification of parietal(s)	5 (3)	3 (2)	2 (1)	4 (3)
Reduced ossification of interparietal	6 (3)	7 (4)	8 (3)	5 (3)
Dumbbell shaped thoracic vertebral centrum(-a)	17 (10)	9 (6)	11 (8)	18 (10)
Cleaved thoracic vertebral centrum	2 (2)	4 (4)	3 (3)	4 (4)
Unossified thoracic vertebral centrum (-a)	0 (0)	0 (0)	0 (0)	3 (3)
Reduced ossification of thoracic vertebral centrum(-a)	0 (0)	0 (0)	0 (0)	3 (2)

Data taken from Table 13, pp. 42-47, MRID 45458608

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The study author concluded that the NOEL for maternal toxicity was 100 ppm (analytically verified 107 ppm), and that there was no evidence of developmental toxicity in exposure levels up to and including 1000 ppm (analytically verified 1029 ppm).

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** At exposure concentrations of 300 and 1000 ppm, maternal toxicity was evident as darkened and/or enlarged spleens, which are considered to be adverse effects of treatment in the absence of histopathology data indicating otherwise. Grossly abnormal spleens were also observed in animals exposed to these concentrations in the range-finding study. At 1000 ppm, there was also decreased body weight gain and food consumption during GD 6-9, with a compensatory increase in body weight gain during GD 9-12, and this group had decreased weight gain for the entire dosing interval both with and without correction for gravid uterine weights. In addition, histopathology conducted during the range-finding study detected treatment-related microscopic changes in the kidney at the 1000 ppm exposure level.

Therefore, the maternal toxicity LOAEL for 1-methylcyclopropene in CrI:CD®BR rats exposed by whole-body inhalation is 300 ppm (analytically verified 329 ppm [2.07 mg/L]), based on increased incidences of darkened and/or enlarged spleens. The maternal NOAEL is 100 ppm (analytically verified 109 ppm [0.67 mg/L]).

2. **Developmental toxicity:**

- a. **Deaths/resorptions:** Maternal treatment did not result in an increase in fetal deaths or resorptions.

- b. **Altered growth:** There were no treatment-related effects on fetal weights or fetal ossification rates.
- c. **Developmental variations:** Exposure to the test material did not result in an increased incidence of fetal developmental variations.
- d. **Developmental malformations:** Exposure to the test material did not result in an increased incidence of fetal developmental malformations. In fact, there was a very low incidence of malformations for the study overall, with only a single documented malformation: dilated pulmonary trunk in a fetus from the control group.

Therefore, the developmental toxicity LOAEL for 1-methylcyclopropene in Crl:CD®BR rats exposed by whole-body inhalation is not identified, and the developmental toxicity NOAEL is greater than or equal to 1000 ppm (analytically verified 1029 ppm [6.48 mg/L]).

- C. **STUDY DEFICIENCIES:** In general this was a well-conducted study; however, there was one major deficiency. There was significant variation in the analytical exposure concentrations, with fully 25% of the individual samples varying from target by more than $\pm 15\%$. This variation occurred at all concentration levels, both within and between days, and made the actual exposure concentrations to the animals uncertain. However, the high-concentration group was certainly exposed to concentrations greater than the 2:0 mg/L required for a limit test, as the analytical concentrations for this group varied from 798.7-1329.7 ppm (equivalent to 5.03-8.38 mg/L). Since there was no developmental toxicity evident at this exposure level, the study is considered acceptable.

In addition, the following minor deficiencies were noted:

- On four occasions, there were minor excursions outside the specified exposure temperature range of $22 \pm 2^\circ\text{C}$.
- Relative humidity inside the exposure chambers ranged from 62.3-92.3%, rather than the 40-60% specified in the guidelines (or even the 30-70% specified in the protocol), and no explanation for the discrepancy was provided.

These deficiencies do not affect study classification.

APPENDIX: Prenatal Developmental Toxicity Study - Rat; Range-finding**TEST MATERIAL (PURITY):** 1-Methylcyclopropene (Ethylbloc; 96.42% a.i.)**CITATION:** Wood, S., L. Craig, H. Bernacki, Jr., et al. (2001) 1-Methylcyclopropene: inhalation (whole-body) developmental toxicity study in rats. Rohm and Haas Company, Toxicology Department, 727 Norristown Road, P.O. Box 904, Spring House, PA 19477-0904. Laboratory report number 00R-181, April 18, 2001. MRID 45458608. Unpublished.

In a range-finding developmental toxicity study (included in the main study, MRID 45458608) 1-Methylcyclopropene gas (96.42% a.i., lot # RMJ6675B) was administered to 10 timed-mated female CrI:CD[®]BR rats/group via whole-body inhalation for 6 hours a day at target concentration levels of 0, 300, or 1000 ppm (mean actual concentrations of 0, 329, or 1074 ppm) from gestation days (GD) 6 through 19, inclusive. On GD 20, dams were necropsied and subjected to cesarean section. Gravid uterine weights and numbers and locations of fetuses, early and late resorptions, total implantations and corpora lutea were recorded, and live fetuses were weighed, sexed, and examined externally. In addition, representative samples of liver, kidneys, trachea, lung, larynx, and the nasal cavity were collected from 5/10 dams of each group, processed, and examined histologically.

There were no deaths or clinical signs of toxicity. At 1000 ppm, mean body weight gain was decreased for GD 6-9 (54% of controls; $p < 0.05$) and increased during GD 9-12 (128% of controls; $p < 0.05$), and this group's net body weight gain corrected for gravid uterine weight was also decreased (87% of controls; n.s.). Corresponding differences in food consumption by high-concentration animals were also noted during the GD 6-9 and 9-12 intervals (87 and 108% of controls, respectively; $p < 0.05$ for GD 6-9 only). Abnormal maternal gross necropsy observations included the following: enlarged spleen in 8/10 and 1/10 dams treated at 1000 and 300 ppm respectively; darkened spleen in 9/10 and 1/10 dams treated at 1000 and 300 ppm, respectively; and pale kidneys in 1/10 dams treated at 1000 ppm. None of these findings were observed in control animals. At 1000 ppm, treatment-related microscopic changes were noted in 5/5 of the kidneys examined. These changes were characterized as multifocal basophilia of the cortical tubular epithelium with karyomegaly or karyorrhexis in some of the basophilic epithelial cells and accumulations of eosinophilic granular casts in the lumen of affected tubules. There were no treatment-related effects on intrauterine parameters, fetal sex ratios or fetal weights of the treated groups compared to controls. There were no treatment-related increases in fetal deaths or resorptions or incidences of fetal external malformations or variations.

Based on the results of this study, the target concentrations selected for the main study were 0, 100, 300, and 1000 ppm. The highest dose level was expected to result in minimal maternal toxicity; however, according to the MRID 45458608, this concentration is considered the maximum safe air concentration, as higher concentrations have the potential to cause explosion.

DATA FOR ENTRY INTO ISIS

Developmental Study - rats (870.3700a)

PC code	MRID	Study	Species	Duration	Route	Admin	Conc. range ppm	Concentrations ppm	NOAEL ppm	LOAEL ppm	Target organ	Comments
224459	45458608	developmental	rats	GD 6-19	inhal.	whole-body	100-1000	0, 100, 300, 1000	100	300	spleen, kidney, body weight	Maternal
224459	45458608	developmental	rats	GD 6-19	inhal.	whole-body	100-1000	0, 100, 300, 1000	≥ 1000	unknown	none	Developmental