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CHEMICAL MANUFACTURERS ASSOCIATION

LANGLEY A. SPURLOCK, PH.D., CAE
VICE PRESIDENT
CHEMSTAR

November 26, 1996

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Dr. Lynn Goldman
Assistant Administrator
Office of Prevention, Pesticides and Toxic Substances TS-7101
Environmental Protection Agency
401 M Street, SW, Room 637, East Tower
Washington, DC 20460

Confidential No CBI

Dear Dr. Goldman:

The Chemical Manufacturers Association makes available to the public and appropriate government agencies final reports of environmental, health and safety research that it manages. In keeping with this policy, the following recently completed reports are enclosed:

- HEXABROMOCYCLODODECANE (HBCD): Chromosome Aberrations in Human Peripheral Blood Lymphocytes; 3194-55-6
- HEXABROMOCYCLODODECANE (HBCD): Maximization Test in Guinea Pigs;
- PENTABROMODIPHENYL OXIDE (PBDPO): Maximization Test in Guinea Pigs; 32534-81-9
- OCTABROMODIPHENYL OXIDE (OBDPO): Maximization Test in Guinea Pigs; 32536-52-0
- HEXABROMOCYCLODODECANE (HBCD): Closed Bottle Test; and,
- OCTABROMODIPHENYL OXIDE (OBDPO): Closed Bottle Test.

These reports do not include confidential information.

If you have any questions, please call Has Shah of my staff at 703-741-5637.

Sincerely,

Enclosure

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Final Report
MA Study No. M96AO62.1X64

FYI-0197-1281

MAXIMIZATION TEST IN GUINEA PIGS

Test Article

Octabromodiphenyl oxide

Author

Martin L. Wenk, Ph.D., D.A.B.T.

Study Completion Date

November 8, 1996

Performing Laboratory

Microbiological Associates, Inc. (MA)
9630 Medical Center Drive
Rockville, MD 20850

Laboratory Project Identification

M96AO62.1X64

Sponsor

Chemical Manufacturers Association
1300 Wilson Boulevard
Arlington, VA 22209

MICROBIOLOGICAL ASSOCIATES, INC.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study No. M96AO62.1X64 - Maximization Test in Guinea Pigs, was conducted in compliance with the Good Laboratory Practice Standards as published by the Organization for Economic Co-Operation and Development (OECD), OCDE/GD(92)32; and the United States Environmental Protection Agency (EPA) in 40 CFR Part 160 and 40 CFR 792 in all material aspects with the following exceptions:

The identity, strength, purity, and composition or other characteristics to define the test, control or reference substances have not been determined by the testing facility [(Section 105 (a)).

The stability of the test, control or reference substances under the test conditions has not been determined by the testing facility and is not included in the final report [(Sections 105 (a) and (b) and 185 (a) (5)).

Analyses to determine the uniformity, solubility or stability of the test, control or reference mixtures were not performed by the testing facility [(Section 113 (a)).

Signatures:

Martin L. Wenk, Ph.D., D.A.B.T. — Date: 11/18/96
Study Director

Sponsor: Hamukh Shah — Date: 11/19/96

Submitter: Hamukh Shah — Date: 11/19/96

QUALITY ASSURANCE STATEMENT

Study Title: MAXIMIZATION TEST IN GUINEA PIGS

Study Number: M96AC62.1X64

Study Director: Martin L. Wenk, Ph.D., D.A.B.T.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. FDA Good Laboratory Practice Regulations (21 CFR 58), the U.S. EPA GLPs (40 CFR 792 and 40 CFR 160), the UK GLP Compliance Programme, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

INSPECT ON 09 MAY 96, TO STUDY DIR 09 MAY 96, TO MGMT 09 MAY 96
PHASE: Protocol Review

INSPECT ON 13 JUN 96, TO STUDY DIR 13 JUN 96, TO MGMT 14 JUN 96
PHASE: Test and/or control material administration: intradermal injection

INSPECT ON 11 JUL 96, TO STUDY DIR 11 JUL 96, TO MGMT 16 JUL 96
PHASE: Scoring after topical application - challenge phase

INSPECT ON 17 JUL 96, TO STUDY DIR 17 JUL 96, TO MGMT 09 AUG 96
PHASE: Raw Data Audit

INSPECT ON 12 AUG 96, TO STUDY DIR 12 AUG 96, TO MGMT 14 AUG 96
PHASE: Draft Report

INSPECT ON 08 NOV 96, TO STUDY DIR 08 NOV 96, TO MGMT 08 NOV 96
PHASE: Draft to Final Report

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

Joan M. McGowan
QUALITY ASSURANCE

Nov 8, 1996
DATE

STUDY INFORMATION PAGE

Test article Identity: Octabromodiphenyl oxide

Vehicle Identities: Corn Oil and Petroleum Jelly

Study Initiation Date: May 31, 1996

Initiation of Dosing (Range-finding Test): June 13, 1996

Completion of In-Life Phase (Main Study): July 17, 1996

MA Study Number: M96AO62.1X64

Sponsor: Chemical Manufacturers Association
1300 Wilson Boulevard
Arlington, VA 22209

Sponsor Representative: Dr. Hasmukh C. Shah

Testing Facility: Microbiological Associates, Inc.
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Technical Staff: Cynthia Horner, B.S., LATG, RILAM - Toxicology Testing Facility Manager
Geof Hunt, LAT - Laboratory Supervisor
Thomas J. Downs, B.S. - Lead Technician
Harry Paulin, M.S. - Analytical Chemist
Anton Allen, D.V.M., Ph.D. - Director of Veterinary Medicine

Study Director: Martin L. Wenk 11/8/96
Martin L. Wenk, Ph.D., D.A.B.T. Date

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I. SUMMARY

The test article, octabromodiphenyl oxide, was administered in two stages (induction and challenge doses) to male guinea pigs. The induction doses were administered to the interscapular region as intradermal injections (phase I) and topical applications (phase II). The challenge dose was administered as topical applications to the flanks of each animal. Phase I of the induction doses consisted of three pairs of intradermal injections of 1)Freund's adjuvant and corn oil (50:50), 2)the test article at a concentration of 2.5% in corn oil, or 3)the test article at a concentration of 2.5% in the 50:50 corn oil/Freund's adjuvant solution which were administered to each treated animal (Group 2). The control animals (Group 1) received the same regimen but the test article was omitted. After a period of seven days, the treated animals (Group 2) received topical applications of neat test article (moistened in corn oil) on the previously treated interscapular sites.

Following a two week rest period, the challenge doses were administered. Each animal received a topical application of neat test article (moistened with corn oil) on the left flank and an empty Hilltop® Chamber on the right flank. Subsequent examination of the test sites indicated the test article produced no erythema or edema in any animal at 24, 48, 72, 96, or 120 hours after the challenge dose. Based on the percentage of animals responding (0%), the test article, octabromodiphenyl oxide was considered a non-sensitizer.

II. INTRODUCTION

The purpose of this study was to assess whether the test article caused delayed contact hypersensitivity in guinea pigs as measured by the Magnusson and Kligman method (Magnusson and Kligman, 1969) and Kligman and Basketter's paper "A critical commentary and updating of the guinea pig maximization test. Kligman A. and Basketter, D. Contact Dermatitis, 1995, 32, 129-134.". The experimental design complied with the EPA Health Effects Test Guidelines 40 CFR 798.4100 and the Organization for Economic Co-Operation and Development (OECD) Guidelines 406 for Testing of Chemicals, 1992.

III. MATERIALS AND METHODS

A. Test Article

Octabromodiphenyl oxide, a white powder, was received on May 21, 1996, assigned MA chemical number 96AO62, and stored at room temperature. The Sponsor was responsible for the determination and documentation of the identification, composition and potency of the test article.

B. Dose Preparation

For the topical applications of the Range-finding Test, the test article was mixed with petroleum jelly in a mortar/pestle to produce the appropriate concentrations. The intradermal injection preparations in corn oil were stirred on a Vortex stirrer and sonicated until a suspension was obtained. The high-dose of the topical application group received the test article undiluted or "neat".

For the Main Study, the test article was mixed with corn oil as described above. The dosage solutions which consisted of the test article (Group 2) or corn oil (Group 1) in Freund's complete adjuvant were prepared by mixing the appropriate components on a Vortex stirrer and sonicating until a suspension was obtained. Sodium lauryl sulfate which was used to irritate the interscapular test sites prior to phase II of the induction doses was diluted in petroleum jelly (in a mortar/pestle) to a concentration of 10%.

C. Test System

1. Animals

On June 4, 1996 male Dunkin Hartley albino (DHA) guinea pigs, approximately 4 weeks of age (Date of Birth: May 3, 1996), were obtained from Harlan Sprague Dawley, Madison, WI. The animals used for the Range-finding test were approximately five weeks of age at dosing.

For the Main Study, male Dunkin Hartley albino (DHA) guinea pigs, approximately 4 weeks of age (Date of Birth: May 17, 1996), were received on June 11, 1996 from Harlan Sprague Dawley, Madison, WI. The animals were approximately five weeks of age at dosing.

The supplier verified that the animals were free of any active infectious diseases prior to arrival at the Microbiological Associates facility.

Guinea pigs were chosen for the test system because they are specified as a species of choice for sensitization testing in EPA's Health Effects Test Guidelines and the OECD Guidelines.

2. Housing

All animals were housed in an American Association for Accreditation of Laboratory Animal Care (AAALAC)-accredited facility. Upon arrival the animals were checked for correct gender, examined to determine their health, and assigned study-unique quarantine/pretest identification numbers. Animals were housed two per cage and individually housed during the study in polycarbonate cages containing Sani-Chip Hardwood bedding (P.J. Murphy Forest Products, Montville, NJ) to absorb liquids.

The animals were transferred to clean cages weekly, and the racks were changed at least once every other week. The cage size (22 in. x 12.5 in. x 8 in.) conformed to the dimensions recommended in The Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication No. 86-23. All animals were housed in the same room from receipt until study completion. During the course of the study, the environmental conditions in the animal room were generally maintained with a temperature of 69-75°F and relative humidity of 30-70% on a 12 hour light/12 hour dark illumination cycle.

3. Husbandry

The animals were provided *ad libitum* access to drinking water (Washington Suburban Sanitary Commission Potomac Plant, Potomac, MD) via automatic watering devices and to Harlan TEKLAD Certified Guinea Pig #7006C feed (Harlan TEKLAD, Madison, WI) from arrival to termination. Annual water analyses and individual feed-lot analyses failed to reveal any contaminants which would have an effect on the results of the study. The results of these analyses are on file at MA. The animals were quarantined for approximately 1 week prior to test article administration, during which time they were observed daily for signs of illness or death. All animals assigned to the study appeared normal. No disease related observations were made during quarantine and all the animals were approved for use by the Clinical Veterinarian prior to being placed on test.

D. Test Procedure

1. Range-Finding Test

a. Randomization

Four animals to be used in the range-finding test were randomized 7 days prior to dosing. The animals were chosen from the stock population using a computer-generated random number table and assigned to one of two test groups as indicated in the table presented below. Each animal was given a unique identification number which was displayed on an ear tag and on a cage card which was placed on the front of each cage. The cage card also listed the study number, test article identification, dose level and displayed color coded indicators of those parameters. Raw data records and specimens were also identified by the unique animal identification number. A summary of the design of the Range-finding test is presented on the next page:

Dose Level(s)	Route of Administration	Dose	No. of Animals
Neat[A], 25%, 12.5%, 6.25% (in petroleum jelly)	Topical Application	Hilltop® Chamber with test material	2
5%, 2.5%, 1% and 0.5% (in corn oil)	Intradermal Injection	0.1 ml	2
TOTAL			4

[A] The neat test article was moistened with corn oil for the topical application.

b. Treatment

Approximately twenty-four hours prior to dosing, the fur of the interscapular area of the two animals to be treated by intradermal injection was clipped to expose an area roughly 4 by 6 cm. For the animals to be treated topically, an area large enough to accept two Hilltop® Chambers was shaved on each flank prior to application. Dosing for the Range-finding test was performed on June 13, 1996.

(1) Intradermal Injection

Two animals each received four intradermal injections of the test article at concentrations of 0.5%, 1.0%, 2.5% and 5.0%, at separate sites on the shaved interscapular region. Each injection was administered just inside the periphery of the shaved area at a volume of 0.1 ml.

(2) Topical Applications

The two animals which were treated topically on the shaved flanks with four concentrations (neat-moistened with corn oil, 25%, 12.5%, and 6.25%) of the test article using 2 Hilltop® Chambers per flank. Each Hilltop® Chamber was loaded with 0.5 gms of one of the four concentrations. The Hilltop® Chambers were placed on separate locations and secured in place with 2" hypoallergenic cloth tape and adhesive tape. The Hilltop® Chambers were left in place for 48 hours.

c. Types and Frequency of Observations and Measurements

The animals which were administered intradermal injections of the test article were evaluated for necrosis, ulcerations, erythema, and edema 48 hours after dosing. The animals which were

administered topical applications of the test article were evaluated for dermal lesions (erythema and edema) at the time of unwrapping according to Draize (see section III.D.2.c).

d. Dose Selection for the Main Study

The highest concentration which caused mild to moderate irritation (for each route of administration) was selected to be used for the corresponding route of administration in the induction phase of the main study. The highest concentration of the topical application route of administration that did not cause erythema was selected to be used in the challenge phase of the main study. No ulcerations or necrosis was noted at any intradermal injection site during the Range-finding test. None of the concentration levels administered by dermal injection produced mild to moderate irritation, but the 2.5% concentration level caused very slight irritation. Therefore, the 2.5% dose level was used for the intradermal injections (phase I) of the induction dose of the main study and the test article was administered neat in the topical application (phase II) of the induction dose of the main study. The highest topical application concentration level (neat) was also used for the challenge phase of the main study.

e. Sacrifice

After final evaluation of the treatment sites, the animals were sacrificed by lethal injection of Beuthanasia®-D Special euthanasia solution. No necropsies were performed.

2. Main Study

a. Randomization

The animals used in the main study were randomized two days prior to dosing. The weight ranges of guinea pigs (252.7 - 351.6 grams) did not exceed ± 20% of the mean weight. Using a stratified body weight program (Innovative Programming Associates, Inc., Princeton, NJ)., each animal was randomly assigned to a test group and given an identification number which was displayed on a cage card on the front of each cage and on an ear tag. The cage card also listed the study number, test article identification, dose level and displayed color coded indicators of those parameters. Raw data records and specimens were labeled with the animal identification number. A summary of the experimental design is presented on the following page:

Group	Treatment Regimen	Dose	No. of Animals
1	Induction Dose: Intradermal injections: One pair each of Freund's Adjuvant, Vehicle and Vehicle plus Freund's Adjuvant. Topical Application: No treatment because neat material applied to the treated animals.	0.1 ml	10
	Challenge Dose: Topical Application: Separate and single application of neat test article and empty Hilltop® Chamber.	0.5 g	
2	Induction Dose: Intradermal injections: One pair each of Freund's Adjuvant, Test Material and Test Material plus Freund's Adjuvant. Topical Application: Single application of neat test article.	0.1 ml 0.5 g	20
	Challenge Dose: Topical Application: Separate and single application of the neat test article and an empty Hilltop® Chamber.	0.5 g	

b.

Treatment

(1) Induction Doses

Phase I (intradermal injections) induction dosing was performed on June 19, 1996. Approximately twenty-four hours prior to dosing, the fur of the interscapular region of each animal was clipped to expose an area roughly 4 by 6 cm.

(a) Intradermal Injections

During phase 1, three pairs of intradermal injections were given to each animal at injection sites 1, 2 and 3 as shown in Diagram 1. Each injection (0.1 ml) was administered just inside the periphery of the shaved area.

The first pair of injections (site 1) consisted of a 50:50 solution of corn oil and Freund's complete adjuvant. The second pair of injections (site 2) consisted of corn oil (Group 1) or the test article at a concentration of 2.5% in corn oil (Group 2). The third pair of injections (site 3) consisted of corn oil (Group 1) or the test article (Group 2) at a concentration level of 2.5% in Freund's adjuvant.

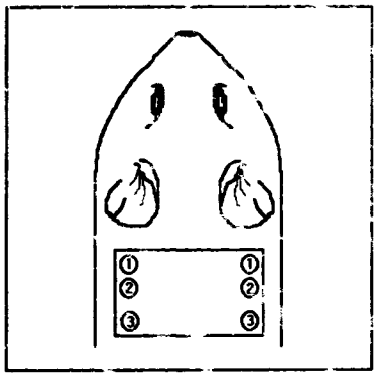

Diagram 1
Intradermal Injection Sites

On Day 7, the interscapular area which received the intradermal injections was shaved again (approximately 4 x 6 cm) with an electric clipper. Since the test article did not cause irritation when applied topically in the Range-finding test, the test area was treated with 0.5 gms of 10% sodium lauryl sulphate in petroleum jelly, in order to create a local irritation.

(b) Topical Application

On Day 8, a topical application (phase II) of the neat test material (moistened with corn oil) was applied to the shaved interscapular area using a Hilltop® Chamber which was loaded with 0.5 gms of the test article and placed in the interscapular area of each Group 2 animal. Since the test article was administered neat, the Group 1 animals were not treated during Phase II. The chambers were secured in place with 2" hypoallergenic cloth tape and adhesive tape. The chambers were left in place for 48 hours.

(2) Challenge Dose

After the completion of the induction phase, all animals were given a two week rest period, after which, a

challenge dose (24-hour exposure) was given to both groups.

Approximately twenty-four hours prior to the challenge dose, the fur of both flanks was clipped to expose an area approximately 5 by 5 cm. For each animal, one empty Hilltop® chamber was applied to the right flank and a second chamber was loaded with 0.5 gms of neat test article (moistened with corn oil) and applied to the left flank. The chambers were secured as before and removed 24 hours later.

Approximately 24 hours after removing the chambers the challenge area was cleaned using a gauze moistened with the vehicle. The animals were scored according to Draize (see section III.D.2.c)

c. **Types and Frequency of Observations and Measurements**

All animals were observed daily for mortality/morbundity. Body weights for all surviving animals were recorded on Test Days 1 (prior to dosing) and following the last day of observation (Day 29).

Dermal lesions were scored approximately 24, 48, 72, 96 and 120 hours after the challenge dose.

○ Erythema and eschar 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 eschar formation (injuries in depth)	4
Total possible erythema score	4

○ Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slightly edema (edges of area well defined by definite raising)	2
Moderate edema (raised approx. 1.0 mm)	3
Severe edema (raised more than 1.0 mm extending beyond the area of exposure	4
Total possible erythema score	4
Total possible primary irritation	8

d. Terminal Office

After the last examination, the animals were sacrificed by lethal injection of Beuthanasia®-D Special euthanasia solution. No necropsies were performed.

E. Data Analyses

1. Calculations

a. Body Weights

Means and standard deviations were calculated for the body weights of each group of the Main Study on Days 1 and 29. An Analysis of Variance Test (ANOVA) was performed on body weights comparing the treated animals (Group 2) to the control animals (Group 1).

b. Grading of Test Material

The grading of the test material was based on the number of guinea pigs showing a response (defined as a score of 1 or greater) at 48 hours post-challenge dose.

Percentage of Animals Responding (Based on 20 animals)	No. of Animals Responding	Grade	Classification
5	1	I	Weak
10 - 30	2 - 6	II	Mild
35 - 65	7 - 13	III	Moderate
70 - 80	14 - 16	IV	Strong
85 - 100	17 - 20	V	Extreme

F. Archives

Upon completion of the final report, all raw data and reports will be maintained by the Quality Assurance Unit of Microbiological Associates, Incorporated, Rockville, Maryland in accordance with the relevant Good Laboratory Practices Regulations. Unused dosage preparations and all residual test article was discarded following administration to the test system. The retention of any required reserve sample of test article will be the responsibility of the Sponsor.

Written permission from the Sponsor will be obtained to discard or transfer original records during the required retention period. If a transfer of original records occurs, true copies (i.e. photocopies, microfilm, or other accurate reproductions of the original records) will be retained for at least the minimum retention period specified by the relevant regulations in accordance with MA Standard Operating Procedures (SOP) No. OPQP2601.

G. Deviations from the Protocol

For the topical application of the challenge phase, cloth bandages were used rather than Coban elastic bandages.

The temperature and/or humidity were sporadically out of the protocol-prescribed range. The duration of these excursions was from 2 to 6 hours. The temperature exceeded the range by 1°F and the humidity exceeded the range by 4 percentage points.

The animals were transferred to clean cages twice a week rather than three times as prescribed by the protocol.

These deviations from the protocol did not have any negative effects on the results of the study.

IV. RESULTS

A. Dermal Scores (Range-Finding Test)

The individual scores for ulceration, necrosis, erythema and edema for the Range-Finding Test were reviewed by the Study Director. The animals which received the intradermal injections exhibited no ulcerations or necrosis. It was noticed that irritation occurred in the 2.5% concentration level but no irritation was observed at the 5.0% level. This was an unusual situation and the Study Director selected the 2.5% concentration level to be used for the intradermal injection of the induction phase of the main study and neat test article was used for the topical application in the induction and challenge phase.

B. Dermal Scores (Main Study)

Individual scores for erythema and edema for the Main Study animals are presented in Table 1 (scores at 24, 48, 72, 96, and 120 hours post challenge dose application) .

Examination of the flank test sites at 24, 48, 72, 96, and 120 hours post challenge dose unwrapping failed to reveal any irritation at any test site in any animal from either group.

C. Body Weights

Individual and group mean body weights are presented in Table 2. Mean body weights are represented graphically in Figure 1.

Statistical analysis failed to show any significant ($p \leq 0.05$) difference in the Day 29 body weights of the treated animals when compared to the control animals.

V. DISCUSSION

Due to the lack of a response to the test article after the challenge dose, octabromodiphenyl oxide is considered a non-sensitizer when administered to guinea pigs via intradermal injections and topical applications and requires no grading classification (Table 3).

VI. REFERENCES

Draize, J.H., Woodward, G., Calvery, H.O. *Methods for the Study of Irritation and Toxicity Substances Applied Topically to the Skin and Mucous Membranes*, Journal of Pharmacology and Experimental Therapeutics. 83:377-390, 1944.

Magnusson, B., Kligman, A.M. *The Identification of Contact Allergens by Animal Assay. The Guinea Pig Maximization Test*, The Journal of Investigative Dermatology. 52: 268-276, 1969

Kligman, A.M., Basketter, D.A. *A Critical Commentary and Updating of the Guinea Pig Maximization Test*. Contact Dermatitis. 32: 129-134, 1995.

MICROBIOLOGICAL ASSOCIATES INC.

TABLES AND FIGURES

MAXIMIZATION TEST IN GUINEA PIGS

TABLE 1
DERMAL SCORES (CHALLENGE DOSE)
CONTROL SITE

Treatment Group	Animal Number	24 Hours[1]		48 Hours		72 Hours		96 Hours		120 Hours	
		Ery.	Edema	Ery.	Edema	Ery.	Edema	Ery.	Edema	Ery.	Edema
1 (Challenge Only)	31	0	0	0	0	0	0	0	0	0	0
	32	0	0	0	0	0	0	0	0	0	0
	33	0	0	0	0	0	0	0	0	0	0
	34	0	0	0	0	0	0	0	0	0	0
	35	0	0	0	0	0	0	0	0	0	0
	36	0	0	0	0	0	0	0	0	0	0
	37	0	0	0	0	0	0	0	0	0	0
	38	0	0	0	0	0	0	0	0	0	0
	39	0	0	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0	0	0
2 (Treated)	41	0	0	0	0	0	0	0	0	0	0
	42	0	0	0	0	0	0	0	0	0	0
	43	0	0	0	0	0	0	0	0	0	0
	44	0	0	0	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0	0	0	0
	46	0	0	0	0	0	0	0	0	0	0
	47	0	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0	0
	49	0	0	0	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0	0	0	0
	51	0	0	0	0	0	0	0	0	0	0
	52	0	0	0	0	0	0	0	0	0	0
	53	0	0	0	0	0	0	0	0	0	0
	54	0	0	0	0	0	0	0	0	0	0
	55	0	0	0	0	0	0	0	0	0	0
	56	0	0	0	0	0	0	0	0	0	0
	57	0	0	0	0	0	0	0	0	0	0
	58	0	0	0	0	0	0	0	0	0	0
	59	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0

Ery. = Erythema [1]Hours after the challenge dose application.

MAXIMIZATION TEST IN GUINEA PIGS

TABLE 1 (cont.)
DERMAL SCORES (CHALLENGE DOSE)
TEST ARTICLE SITE

Treatment Group	Animal Number	24 Hours[1]		48 Hours		72 Hours		96 Hours		120 Hours	
		Ery.	Edema	Ery.	Edema	Ery.	Edema	Ery.	Edema	Ery.	Edema
1 (Challenge Only)	31	0	0	0	0	0	0	0	0	0	0
	32	0	0	0	0	0	0	0	0	0	0
	33	0	0	0	0	0	0	0	0	0	0
	34	0	0	0	0	0	0	0	0	0	0
	35	0	0	0	0	0	0	0	0	0	0
	36	0	0	0	0	0	0	0	0	0	0
	37	0	0	0	0	0	0	0	0	0	0
	38	0	0	0	0	0	0	0	0	0	0
	39	0	0	0	0	0	0	0	0	0	0
	40	0	0	0	0	-	0	0	0	0	0
2 (Treated)	41	0	0	0	0	0	0	0	0	0	0
	42	0	0	0	0	0	0	0	0	0	0
	43	0	0	0	0	0	0	0	0	0	0
	44	0	0	0	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0	0	0	0
	46	0	0	0	0	0	0	0	0	0	0
	47	0	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0	0
	49	0	0	0	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	0	0	0	0
	51	0	0	0	0	0	0	0	0	0	0
	52	0	0	0	0	0	0	0	0	0	0
	53	0	0	0	0	0	0	0	0	0	0
	54	0	0	0	0	0	0	0	0	0	0
	55	0	0	0	0	0	0	0	0	0	0
	56	0	0	0	0	0	0	0	0	0	0
	57	0	0	0	0	0	0	0	0	0	0
	58	0	0	0	0	0	0	0	0	0	0
	59	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0

Ery. = Erythema

[1]Hours after the challenge dose application.

MAXIMIZATION TEST IN GUINEA PIGS

TABLE 2

BODY WEIGHTS (Grams)

Group No.	Animal No.	Day 1	Day 29
1	31	341.7	477.1
	32	330.0	528.8
	33	360.5	571.7
	34	315.9	510.6
	35	360.9	599.3
	36	348.0	555.7
	37	304.0	527.2
	38	355.6	616.6
	39	361.1	633.7
	40	325.4	573.5
	Mean	340.3	559.4
	S.D.	20.6	49.5
	(N)	(10)	(10)
2	41	337.8	498.3
	42	319.1	527.8
	43	354.3	481.6
	44	362.8	629.4
	45	388.5	588.4
	46	333.4	578.3
	47	329.0	548.2
	48	318.8	589.4
	49	324.1	542.4
	50	308.6	481.6
	51	320.1	522.4
	52	350.8	510.0
	53	344.0	523.9
	54	340.2	500.7
	55	319.8	522.1
	56	307.4	524.8
	57	350.0	551.5
	58	333.8	518.7
	59	351.0	500.8
	60	306.7	461.4
	Mean	335.0	530.1
	S.D.	20.8	41.6
	(N)	(20)	(20)

MAXIMIZATION TEST IN GUINEA PIGS

TABLE 3

TEST MATERIAL GRADE

Percentage of Animals Responding	No. of Animals Responding (Based on a Group of 20)	Grade	Classification
0%	0	NA*	Non-irritant

NOTE: The grading of the test material is based on the number of guinea pigs showing a response (defined as a score of 1 or greater) 48 hours post-chamber removal.

* No animals responded to the test article as defined above. A minimum of one animal responding is necessary for a grade of I and a classification of "weak".

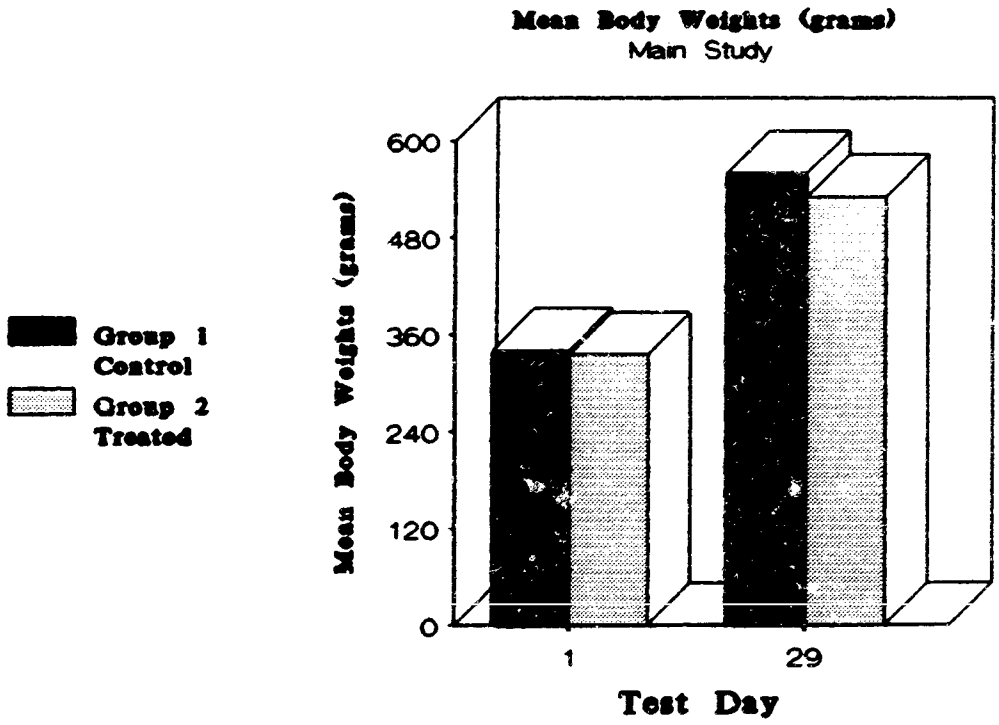
Mean Body Weights (grams)
Main Study
Group 1
Control
Group 2
Treated
Mean Body Weights (grams)
600
480
360
240
120
0
1
29
Test Day

FIGURE 1

VIII. APPENDIX

APPENDIX A
PROTOCOL AND AMENDMENTS

QA APPROVED

PROTOCOL AMENDMENT
#1

DATE: July 31, 1996

SPONSOR: Chemical Manufacturers Association

STUDY NO.: M96AO62.1X64

STUDY TITLE: Maximization Test in Guinea Pigs

Revisions:

1. Page 2, Section 4.3.1, "Storage Conditions to Maintain Stability"; Add data for sections 4.3.1.1 to 4.3.1.4. Room temperature, ambient, ambient and none, respectively.

   Reason: Omitted from protocol.

2. Page 3, Section 6.5, "Source of Animals"; Revise to Harlan-Sprague Dawley, Madison, WI.

   Reason: Error.

Martin L. Ward — Study Director — 7/31/96 — Date

Hamukh Shah — Sponsor's Approval — 8/5/96 — Date

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MA Study Number: M96AO62.1X64

4.0 TEST ARTICLE:

4.1 Name or Code No.: Octabromodiphenyl oxide

4.2 MA Test Article Number: 96AO62

4.3 Stability and Handling of Test Article:

4.3.1 Storage Conditions to Maintain Stability:

4.3.1.1 Temperature: Room temperature

4.3.1.2 Humidity: Ambient

4.3.1.3 Light: Ambient

4.3.1.4 Special Requirements: None

4.3.2 Special Handling Procedures: N/A MLW 7/31/96

4.4 Vehicle: For injection, sterile physiological saline (0.9% w/v) will be used for aqueous-soluble test articles. Aqueous-insoluble materials will be given as a solution in light mineral oil, corn oil, polyethylene glycol 400, DMSO or propylene glycol (in that order of preference). If solutions are not possible, 2% carboxymethylcellulose, corn oil, or mineral oil will be used to form an injectable suspension for a 23 gauge needle. For topical applications of solids, the test article will be applied as a neat paste formed by wetting with the appropriate vehicle or a w/w concentration in petroleum jelly. Acetone but not DMSO may be added as a solvent used for topical applications. If a liquid, the test article will be applied neat or solubilized or suspended with the appropriate vehicle (solution first choice) as determined for injections.

4.5 Test Article Analysis: The Sponsor will be responsible for the determination and documentation of the composition and stability of the test article.

THIS PAGE REVISED Study M96AO62.1X64
Martin L. Wenk, Ph.D., D.A.B.T. July 31, 1996
Study Director Date

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MA Study Number: M96AO62.1X64

4.6 Test Article Retention Sample:

The retention of any required reserve sample of the test article will be the responsibility of MA.

5.0 TESTING SCHEDULE:

5.1 Proposed Initiation of Dosing: June 4, 1996

5.2 Proposed Completion of Observations: July 12, 1996

5.3 Proposed Submission Date of Draft Report to Sponsor: August 12, 1996

5.4 Proposed Submission Date of Final Report: Two weeks following receipt of Sponsor comments.

6.0 TEST SYSTEM:

6.1 Species: Guinea Pigs

6.2 Strain: Hartley (DHA)

6.3 Number per Sex: Thirty-four Males

6.4 Body Weight: The test animals will weigh at least 300 g at the start of the study.

6.5 Source of Animals: Harlan Sprague Dawley, Madison, WI.

5.6 Justification for Selection of Test System: Guinea pigs are recommended for sensitization testing in the EPA Health Effects Test Guidelines and the OECD Guidelines.

6.7 Procedure for Unique Identification of Test System: Upon arrival, each animal will be given a study-unique pretest number. During the test animal selection process, each test animal will be assigned a test animal number unique to it within

THIS PAGE REVISED Study M96AO62.1X64

Martin L. Wenk, Ph.D., D.A.B.T. July 31, 1996
Study Director Date

MICROBIOLOGICAL ASSOCIATES, INC.

MICROBIOLOGICAL ASSOCIATES, INC.

MA Study Number: M96AO62.1X64

MAXIMIZATION TEST IN GUINEA PIGS

1.0 PURPOSE OF THE STUDY:

The purpose of this study is to assess whether a test article causes delayed contact hypersensitivity in guinea pigs as measured by the Magnusson and Kligman method (Magnusson and Kligman, 1969) and Kligman and Basketter's recent paper "A critical commentary and updating of the guinea pig maximization test. Kligman A. and Basketter, D. Contact Dermatitis, 1995, 32, 129-134." The experimental design complies with the EPA Health Effects Test Guidelines 40 CFR 798.4100 and the Organization for Economic Co-Operation and Development (OECD) Guidelines 406 for Testing of Chemicals, 1992.

2.0 SPONSOR:

2.1 Name: **Chemical Manufacturers Association**

2.2 Address: 1300 Wilson Boulevard
Arlington, VA 22209

2.3 Representative: Dr. Hasmukh C. Shah

3.0 TESTING FACILITY:

3.1 Name: Microbiological Associates Inc. (MA)

3.2 Address: 9630 Medical Center Drive
Rockville, Maryland 20850

3.3 Study Director: Martin L. Wenk, Ph.D, D.A.P.T.

3.4 Animal Facility Manager: Cynthia Horner, B.S., LATG, RILAM

3.5 Laboratory Supervisor: Geof Hunt, LAT

3.6 Lead Technician: Thomas J. Downs, B.S.

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MA Study Number: M96AO62.1X64

4.0 TEST ARTICLE:

4.1 Name or Code No.: Octabromodiphenyl oxide

4.2 MA Test Article Number: 96AO62

4.3 Stability and Handling of Test Article:

4.3.1 Storage Conditions to Maintain Stability:

4.3.1.1 Temperature:

4.3.1.2 Humidity:

4.3.1.3 Light:

4.3.1.4 Special Requirements:

4.3.2 Special Handling Procedures:

4.4 Vehicle: For injection, sterile physiological saline (0.9% w/v) will be used for aqueous-soluble test articles. Aqueous-insoluble materials will be given as a solution in light mineral oil, corn oil, polyethylene glycol 400, DMSO or propylene glycol (in that order of preference). If solutions are not possible, 2% carboxymethylcellulose, corn oil, or mineral oil will be used to form an injectable suspension for a 23 gauge needle. For topical applications of solids, the test article will be applied as a neat paste formed by wetting with the appropriate vehicle or a w/w concentration in petroleum jelly. Acetone but not DMSO may be added as a solvent used for topical applications. If a liquid, the test article will be applied neat or solubilized or suspended with the appropriate vehicle (solution first choice) as determined for injections.

4.5 Test Article Analysis: The Sponsor will be responsible for the determination and documentation of the composition and stability of the test article.

4.6 Test Article Retention Sample:

The retention of any required reserve sample of the test article will be the responsibility of MA.

5.0 TESTING SCHEDULE:

5.1 Proposed Initiation of Dosing: June 4, 1996

5.2 Proposed Completion of Observations: July 12, 1996

5.3 Proposed Submission Date of Draft Report to Sponsor: August 12, 1996

5.4 Proposed Submission Date of Final Report: Two weeks following receipt of Sponsor comments.

6.0 TEST SYSTEM:

6.1 Species: Guinea Pigs

6.2 Strain: Hartley (DHA)

6.3 Number per Sex: Thirty-four Males

6.4 Body Weight: The test animals will weigh at least 300 g at the start of the study.

6.5 Source of Animals: Hazleton Research Products, Denver, PA.

6.6 Justification for Selection of Test System: Guinea pigs are recommended for sensitization testing in the EPA Health Effects Test Guidelines and the OECD Guidelines.

6.7 Procedure for Unique Identification of Test System: Upon arrival, each animal will be given a study-unique pretest number. During the test animal selection process, each test animal will be assigned a test animal number unique to it within

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the population making up the study. This number will appear as an ear tag and will also appear on a cage card visible on the front of each cage. The cage card will additionally contain the study number, test article, dose level and study type. Raw data records and specimens will also be identified by the unique test animal number.

6.8 Housing: Animals will be housed in polycarbonate cages with Sani-Chip heat-treated hardwood bedding (P.J. Murphy Forest Products Corporation, Montville, NY). Animals will be housed in an environmentally controlled room at a temperature of 69-75°F and at a relative humidity of 30-70%, with a 12 hr light/12 hr dark cycle. Animals will be housed two per cage during quarantine and individually following test animal selection. The cage size will conform to the upper weight range as recommended in the Guide for the Care and Use of Laboratory Animals, DHEW (NIH) No. 86-23. All animals will be transferred to clean cages with fresh bedding three times a week.

6.9 Acclimation Period: All animals will be acclimated for at least one week. During this time, animals will be observed daily for signs of illness or death and all unusual observations will be reported to the Study Director or Clinical Veterinarian. Animals will be examined and approved for use by the Clinical Veterinarian (RAQA #11). Any sickly animal will be eliminated prior to the test animal selection process.

6.10 Food: Harlan TEKLAD Guinea Pig 7006C (Harlan TEKLAD, Madison, WI) will be provided ad libitum from arrival until termination. The lot number or milling date will be recorded in the raw data. There are no contaminants in the feed which are expected to influence the study.

6.11 Water: Tap water from an automatic watering system will be provided ad libitum from arrival until termination. The water source is Washington Suburban Sanitary Commission, Potomac Plant; no additional treatment. The water meets USEPA drinking water standards and is monitored at least annually for levels of organophosphorus pesticides, metals, and coliform bacteria and other contaminants.

6.12 Animal Welfare Compliance Statements: Daily observations and the ability to euthanize animals for humane reasons will minimize unavoidable discomfort and

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pain to study animals. The Clinical Veterinarian will be consulted if animal health or welfare issues arise during the course of the study.

7.0 EXPERIMENTAL DESIGN:

7.1 Procedure Used to Assign Animals to Treatment Groups: Following the quarantine period, a sufficient number of animals for the range-finder will be chosen from the stock using a computer generated random number table for the Range-Finding Test(s). Following the Range-Finding Test(s), thirty animals from the remaining stock will be randomized into two experimental groups on the basis of body weight using a computer-generated randomization program.

7.2 Range-Finding Test(s): Four animals will be used for the initial range-finding test (2 animals/route of administration):

Dose Level(s)	Route of Administration	Dose	No.of Animals
If a liquid: Neat, 75%, 40%, 10% If a solid: Neat as a paste, 25%, 12.5% and 6.25% in petroleum jelly	Topical Application	Load patch or Hilltop® Chamber with test material[1]	2
As a solution (preferably) or an injectable suspension: 5%, 2.5%, 1% and 0.5%	Intradermal Injections	0.1 ml	2

[1] Exact amount to be documented in the raw data.

7.2.1 Test Procedure: An approximate 4 by 6 cm area of the interscapular region of each of two animals will be clipped free of fur approximately 24 hours prior to the intradermal injections. An area large enough to accept two 2 x 4 cm patches will be shaved from each flank of two other animals prior to the topical application.

7.2.1.1 Intradermal Injections: Twenty-four hours later two animals will receive 4 intradermal injections of four different concentrations, each one at a separate interscapular site. Each injection will be 0.1 ml and will be given just inside the periphery of the shaved area. The location and

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concentration of the injection will be documented in the raw data for each animal.

Necrosis and ulcerations will be evaluated 48 hours after injection. The highest concentration that causes mild to moderate skin irritation will be used for the intradermal induction phase of the Main Study. However, if the material produces necrosis or ulcerations at all doses tested, then additional Range-Finding Tests will be conducted until a suitable dose level is determined. If additional Range-Finding Tests are necessary, they will be performed at an additional cost and with the Sponsor's approval.

7.2.1.2 Topical Applications: The other two animals will receive 4 topical applications of the four different concentrations using two 2 x 4 cm pieces of filter paper or two suitable size Hilltop® Chambers on each flank of each animal. If a patch is used, it will be loaded with the test material as described in Section 7.2. A plastic cover (e.g. dam with occluded surface) will be placed over the patch to form an occlusive barrier. If a Hilltop® Chamber is used, it will be loaded with the test material as described in Section 7.2. The patch or Hilltop® Chamber will be secured in place with a Coban elastic bandage and adhesive tape. This will be left in place for 48 hours.

Animals will be scored at the time of unwrapping according to the Section 7.4.3. The highest concentration that causes mild to moderate irritation will be used for the induction phase of the Main Study. The highest concentration causing no erythema will be used for the challenge phase of the Main Study. However, if the material produces levels of irritation greater than necessary to select induction and challenge doses then additional Range-Finding Tests will be conducted until suitable dose levels are determined. If additional Range-Finding Tests are necessary, they will be

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performed at an additional cost and with the Sponsor's approval.

7.3 Main Study: Thirty animals will be used for the main study:

Group	Treatment Regimen	Dose	No. of Animals
1 (Challenge Only)	Induction Dose: Intradermal Injections: One pair each of Freund's Adjuvant, Vehicle and Vehicle plus Freund's Adjuvant).	0.1 ml	10
	Topical Application: Single application of the vehicle if vehicle employed OR no treatment if neat material applied.	load patch	
	Challenge Dose: Topical Application: Separate and single application of test article and vehicle (if used).	load patch	
2 (Treated)	Induction Dose: Intradermal Injections: One pair each of Freund's Adjuvant, Test Material and Test Material plus Freund's Adjuvant).	0.1 ml	20
	Topical Application: Single application of test article.	load patch	
	Challenge Dose: Topical Application: Separate and single application of the test article and vehicle (if used).	load patch	

7.3.1 Test Procedure: An approximate 4 by 6 cm area of the interscapular region of each animal will be clipped free of fur approximately 24 hours prior to each induction dose. An approximate 5 by 5 cm area of each flank of each animal will be clipped free of fur approximately 24 hours prior to the challenge dose.

7.3.1.1 Induction Doses: Induction doses shall be in two phases: intradermal injections and a topical application. During phase 1, three pairs of intradermal injections will be given to each animal at the injection sites (1, 2 and 3) as shown in Figure 1.

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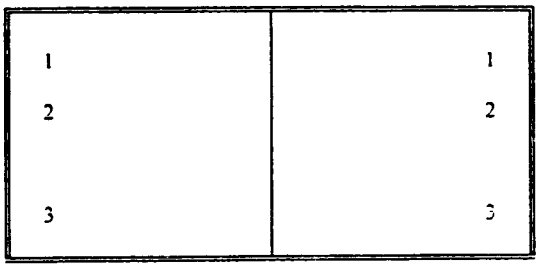
Cranial
Clipped interscapular region



Caudal

Figure 1 - Location of intradermal injection sites.

For the **Challenge Only Group** (Group 1), the first pair of injections (1) will be adjuvant alone. The second pair of injections (2) will be the vehicle, selected in the range-finder for intradermal injections, alone. The third pair (3) of injections will be the chosen vehicle in Freund's complete adjuvant. Similar injections will be given to each **Treated Group** (Group 2) with the exception the test material in the selected vehicle and at the concentration determined in the range-finder will be substituted for the vehicle.

Freund's complete adjuvant will be prepared for Groups 1 and 2 as an emulsified 50:50 mixture with the vehicle selected for the intradermal injections of the test article. It will then be injected alone or in combination with the test article at the concentration determined by the range-finder or the test article vehicle.

Each injection will be 0.1 ml and will be given just inside

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the periphery of the shaved interscapular area that will bear the 2 x 4 cm patch during the topical phase of induction (see below).

On Day 7, the interscapular area which received the intradermal injections will be shaved again (approximately 4 x 6 cm) with an electric clipper. If the test article is not a skin irritant, the test area will be treated with 0.5 ml of 10% sodium lauryl sulphate in vaseline, in order to create a local irritation.

On Day 8, a topical application (phase 2) of the test material or vehicle (if appropriate) will be applied to the shaved interscapular area, used for the intradermal injections, using a 2 x 4 cm piece of filter paper or suitable size Hilltop® Chambers. The concentration of the topically applied test material will be at doses set by the Range-Finding Test (mild to moderate irritation).

If a patch is used, it will be loaded with the test material. A plastic cover (e.g. dam with occluded surface) will be placed over the patch to form an occlusive barrier. If a Hilltop® Chamber is used, it will be loaded with the test material. The patch or Hilltop® Chamber will be secured in place with a Coban elastic bandage and adhesive tape. The exact amount of test material applied will be recorded in the study data. This will be left in place for 48 hours.

7.3.1.2 Challenge Dose: After the completion of the induction phase, all animals will be given a two week rest period. Following the rest period, a final challenge dose (24-hour exposure) will be given to both groups.

An approximate 5 by 5 cm area of each flank of each animal will be clipped free of fur approximately 24 hours prior to the challenge dose.

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A topical application of the test material or vehicle (if appropriate) will be applied to the shaved flank using a 2 x 2 cm piece of filter paper or a Hilltop® Chamber. The concentration of the topically applied test material will be at doses set by the Range-Finding Test (non-irritating). If a patch is used, it will be loaded (e.g. saturated) with the test material or vehicle (if appropriate) and the exact amount will be recorded in the study data. A plastic cover (e.g. dam with occluded surface) will be placed over the patch to form an occlusive barrier. If a Hilltop® Chamber is used, it will be loaded (e.g. saturated) with the test material or vehicle (if appropriate). The patch or Hilltop® Chamber will be secured in place with a Coban elastic bandage and adhesive tape. Both the **Challenge Only Group** (Group 1) and the **Treated Group** (Group 2) will be treated as follows:

- Single topical dose of the test material given on the **left** flank.
- Single topical dose of the vehicle (if appropriate) given on the **right** flank.

The patch or Hilltop Chamber® will be removed 24 hours later. Approximately 21 hours after removing the patch the challenge area is cleaned and shaved if necessary. The animals will be scored as described in Sections 7.4.2 and 7.4.3. At the time dermal lesions are scored after the challenge exposure, consideration shall be given to the need to re-challenge all animals. A re-challenge may be needed in order to distinguish a hyperirritability syndrome from true sensitization (Kligman and Basketter, 1995). This can be especially important in differentiating the etiology of mild skin responses. The need for a re-challenge shall be performed after a three (3) week rest period and at an additional cost to the sponsor. The re-challenge shall be

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carried out as described in Section 7.3.1.2 and the type and frequency of observations and measurements shall be the same as that described in Section 7.4 for the initial challenge.

7.4 Type of Frequency of Observations and Measurements:

7.4.1 Body Weights: Body weights will be recorded on Test Day 1 and on the last day of observation for all animals.

7.4.2 Evaluation of Dermal Irritation: Dermal lesions will be scored approximately 24, 48, 72, 96 and 120 hours after the challenge dose application (at unwrapping 24, 48, 72 and 96 hours after unwrapping).

7.4.3 Scale for Scoring Dermal Lesions: Draize, J.H., Woodward, G., Calvery, H.O. *Methods for the Study of Irritation and Toxicity Substances Applied Topically to the Skin and Mucous Membranes*, Journal of Pharmacology and Experimental Therapeutics. 83:377-390, 1944.

o Erythema and eschar 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 eschar formation (injuries in depth)	4
Total possible erythema score	4
o Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slightly edema (edges of area well defined by definite raising)	2
Moderate edema (raised approx. 1.0 mm)	3
Severe edema (raised more than 1.0 mm extending beyond the area of exposure	4
Total possible erythema score	4
Total possible primary irritation	8

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7.4.4 Mortality/Unusual Reactions: Animals will be observed once daily during the course of the study. Any unusual reactions will be recorded if observed.

7.4.5 Method of Euthanasia: After the last examination in both the Range-Finding Test and Main Study, the animals will be euthanized by lethal injection of Beuthanasia®-D Special euthanasia solution. No necropsies will be performed.

8.0 RECORDS AND SAMPLE ARCHIVES:

8.1 Records: Upon completion of the final report, all raw data and reports will be maintained by the Quality Assurance Unit of Microbiological Associates, Rockville, MD in accordance with the relevant Good Laboratory Practice Regulations.

8.2 Test Article: Test article will be stored under appropriate conditions with access restricted to authorized personnel. Unless arrangements are made to the contrary, unused dosage preparations will be disposed of following administration to the test system and all residual test article will be returned to the Sponsor following finalization of the report.

9.0 DATA AND REPORTING:

9.1 Grading of Test Material: The grading of the test material is based on the number of guinea pigs showing a responses (defined as a score of 1 or greater) at 48 hour post-challenge dose.

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Percentage of Animals Responding	No. of Animals (Based on a Group of 20)	Grade	Classification
0 - 8	1	I	Weak
9 - 28	2 - 6	II	Mild
29 - 64	7 - 13	III	Moderate
65 - 80	14 - 16	IV	Strong
81 - 100	17 - 20	V	Extreme

9.2 Treatment of Results: Data will be summarized in tabular form, showing for each individual animal the skin reaction results of the challenge exposure at 24, 48, 72, and 96 hours after exposure.

9.3 Study Report Requirements: Results presented in the final report will include, but not be limited to, the following:

- Summary
- Materials and method
- Individual irritation scores
- Results of the Range-Finding Test
- Initial and final individual body weights of the animals with a mean and standard deviation.
- Interpretation of the test is based on the Grading and Classification as defined by the Magnusson and Kligman method and as discussed by Kligman and Basketter,1995.

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MA Study Number: M96AO62.1X64

9.4 Interpretation of the Results: The test results will provide an estimate of the overall sensitization potential of the test material, i.e. essentially a non-sensitizer, a weak sensitizer, a moderate sensitizer, or a potent sensitizer.

10.0 STANDARD OPERATING PROCEDURES AND PROTOCOL ALTERATION:

All procedures not specified in this protocol will be performed in accordance with the Microbiological Associates Inc. Standard Operating Procedures Manual and in compliance with the appropriate EPA Good Laboratory Practice Standards. This study will be conducted in compliance with the appropriate EPA or OECD guidelines.

This study will be performed in compliance with the provisions of the Good Laboratory Practice Regulations for EPA TSCA Regulations, 40 CFR 792 and OECD ISBN 922-84-12367-9.

Alterations of the study protocol may be made after the study has been initiated. In the event that the sponsor verbally authorizes a change in protocol, such change will be honored by Microbiological Associates Inc. A protocol amendment will then be issued by Microbiological Associates Inc. which will include the change and the justification, to be dated and signed by both study director and sponsor.

Will this study be submitted to an Agency? YES

Please list the agency(ies). EU, EPA

Please state the appropriate guidelines to be followed. EU, EPA, OECD

11.0 SIGNATURES:

Hamill Stal — Sponsor Approval — 5-29-96 — Date

Walter L Ward — Study Director — 5/31/96 — Date

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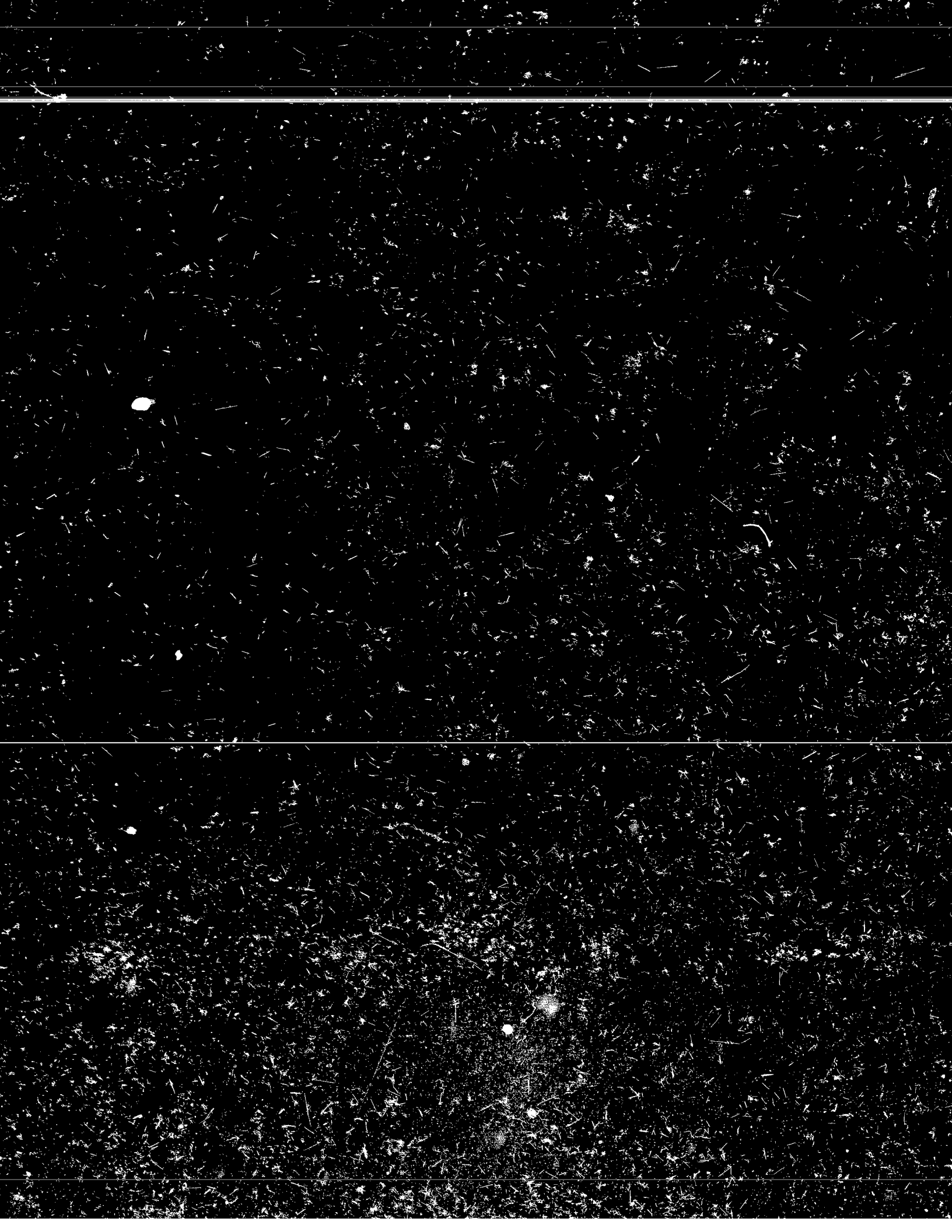

FYI - 0197 - 1285

OCTABROMODIPHENYL OXIDE (OBDPO): CLOSED BOTTLE TEST

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 439E-101

AUTHORS:
Edward C. Schaefer
Doug Haberlien

STUDY INITIATION DATE: June 11, 1996

STUDY COMPLETION DATE: November 11, 1996

Submitted to

Chemical Manufactures Association's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

WILDLIFE INTERNATIONAL LTD.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Chemical Manufacturer's Association Brominated Flame Retardant Industry Panel

TITLE: Octabromodiphenyl Oxide (OBDPO) Closed Bottle Test

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 439E-101

STUDY COMPLETION: November 11, 1996

This study was conducted to conform with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989; and OECD, ISBN 92-84-12367-9 Paris 1982, with the following exceptions:

The reference substance, obtained from J.T. Baker (Phillipsburg, NJ), was not characterized in compliance with Good Laboratory Practice Standards.

Characterization and stability of the test substance was not conducted in compliance with Good Laboratory Practice Standards.

Verification of the test concentrations, stability and homogeneity of the test and reference substances in the test medium were not determined.

STUDY DIRECTOR:

Edward C. Schaefer
Study Director

11-11-96
DATE

SPONSOR APPROVAL

Sponsor

DATE

Applicant/Submitter

DATE

QUALITY ASSURANCE
OCTABROMODIPHENYL OXIDE (OBDPO): CLOSED BOTTLE TEST
PROJECT NO.: 439E-101

This study was examined for conformance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Part 792, 17 August 1989; and OECD, ISBN 92-84-12367-9, Paris 1982. The dates of all audits and inspections and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Test substance preparation	June 17, 1996	June 18, 1996	June 19, 1996
Test initiation	June 18, 1996	June 25, 1996	June 27, 1996
DO measurements	July 9, 1996	July 9, 1996	July 10, 1996
Data and Draft Report	August 5 and 6, 1996	August 6, 1996	August 16, 1996
Final Report	November 11, 1996	November 11, 1996	November 11, 1996

Lisa T. Drottar
Quality Assurance Representative

11-11-96
DATE

REPORT APPROVAL

SPONSOR: Chemical Manufacturer's Association Brominated Flame Retardant Industry Panel

TITLE: Octabromodiphenyl Oxide (OBDPO) Closed Bottle Test

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 439E-101

STUDY DIRECTOR:

Edward C. Schaefer
Study Director

11/11/96
DATE

MANAGEMENT:

James P. Swigert, Ph.D.
Manager, Analytical Chemistry

11/11/96
DATE

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SUMMARY

SPONSOR:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel
CONTACT:	Hasmukh Shah, Ph.D.
LOCATION OF STUDY, RAW DATA AND A COPY OF FINAL THE REPORT:	Wildlife International Ltd. Easton, MD 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	439E-101
STUDY:	Octabromodiphenyl Oxide (OBDPO): Closed Bottle Test
TEST CONCENTRATION:	15 mg/L
TEST DATES:	Experimental Start - June 18, 1996 Experimental Termination - July 16, 1996
LENGTH OF EXPERIMENTAL PHASE:	28 Days

TEST SUBSTANCE:	PERCENT BIODEGRADATION:
Octabromodiphenyl Oxide (OBDPO)	0.0

INTRODUCTION

Tests of ready biodegradability, by definition, provide limited opportunity for acclimation and biodegradation to occur. A positive result in a test of ready biodegradability is an indication that the test substance will undergo rapid and ultimate biodegradation in the environment. A negative result in a test of ready biodegradability does not necessarily mean that the test substance will not be biodegraded under relevant environmental conditions but that additional testing may be needed.

OBJECTIVE

The objective of the study was to measure dissolved oxygen uptake over a 28 day period and express it as a percentage of the theoretical oxygen demand (ThOD) or chemical oxygen demand (COD).

EXPERIMENTAL DESIGN

The test contained an inoculum control group, a reference group, and a treatment group. The blank control, reference, and treatment groups contained ten replicate test chambers. The inoculum control was used to measure the dissolved oxygen consumption of the inoculum and was not dosed with a carbon source. The reference chambers were dosed with sodium benzoate, a substance known to be biodegradable, at a concentration of 2 mg/L. The treatment group test chambers were used to evaluate the test substance at 15 mg/L. Measurements of oxygen consumption were performed on two test chambers from the control, reference and treatment groups on days 0, 7, 14, 21, and 28.

MATERIALS AND METHODS

This study was conducted according to the procedures outlined in the protocol, Octabromodiphenyl Oxide (OBDPO): Closed Bottle Test, (Appendix I). The protocol was based on the procedures specified in the OECD Guideline for Testing of Chemicals, Guideline 301D (1) and Council of the European Communities, Guideline C.4-E, *Closed Bottle* (2); and TSCA Title 40 of the Federal Code of Regulations, Part 796, Section 3200: *Ready Biodegradability: Closed Bottle Test* (3).

Test Substance

The test substance used in this study was a composite of the following three samples of octabromodiphenyl oxide (OBDPO):

Manufacturer:	Ameribrom
Sample ID:	Octabromodiphenyl Oxide FR 1208
Date:	14-12-95
Ref. No.:	4118
Batch No.:	951107
Expiration Date:	Not Given
Date Received:	March 7, 1996
Wildlife ID:	3601

Manufacturer:	Great Lakes Chemical
Sample ID:	Octabromodiphenyl Oxide
Lot. No.:	5525DI21A
Prepared:	March 7, 1996
Expiration Date:	March 7, 1997
Date Received:	March 12, 1996
Wildlife ID:	3603

Manufacturer:	Albemarle
Sample ID:	SAYTEX 111
Lot. No.:	20405-1B
Expiration Date:	December 16, 1996
Date Received:	December 20, 1995
Wildlife ID:	3517

The composite OBDPO sample was prepared on April 10, 1996 and was assigned Wildlife International Ltd. identification number 3637. The composite was prepared by combining equal parts

of the three manufacturers' products and mixing for approximately two hours. Subsamples of the composite sample were collected from the left and right sides of the top, middle, and bottom of the container. The subsamples were shipped to the Albemarle Corporation and the Great Lakes Chemical Corporation for analysis to determine the homogeneity of the mixture. An additional sample of the composite was collected from the mixture and shipped to Albemarle Corporation for characterization of the test substance. After the initial mixing, the composite was mixed for an additional 2 hours. Subsamples of the remixed sample were collected from the left and right sides of the top, middle and bottom of the container. These subsamples were shipped to the Albemarle Corporation and the Great Lakes Chemical Corporation.

The theoretical oxygen demand (ThOD) value used to calculate the percent degradation of the test substance was 0.38 mg O_2/mg.

Stock Solution Preparation

A suspension of the test substance was prepared at a nominal concentration of 100 mg/L in Nanopure water. In addition, a stock solution of the reference substance, sodium benzoate, was prepared at a nominal concentration of 100 mg/L in Nanopure water.

Test Medium

The test medium was a modified biochemical oxygen demand (BOD) test dilution water and was prepared using Nanopure water as described in Protocol Appendix I. The mineral medium was aerated for approximately 20 minutes and then allowed to stand overnight at test temperature. Prior to use, the dilution water was aerated for approximately 10 minutes to achieve a dissolved oxygen (DO) concentration of $\geq$8.5 mg O_2/L.

Test Apparatus and Conditions

The test chambers were 300 mL BOD bottles. The test was conducted at 20±3°C. Test chambers were identified by project number, test substance ID, and test concentration, and bottle number.

Test Inoculum

Secondary clarifier supernatant was collected from Prospect Bay Wastewater Treatment Facility, Grasonville, Maryland on June 18, 1996. The supernatant was filtered through glass wool and then aerated until used. A standard plate count was performed on the inoculum. Plates were incubated at 20 ± 3 °C for approximately 48 hours.

Preparation of Test Chambers

Aerated mineral medium was added to the BOD bottles so that they are about one-half full. Adequate volumes of test substance and reference substance stock solution necessary to achieve a concentrations of 15 and 2 mg/L, respectively, were added to the appropriate bottles. Approximately 0.5 mL of inoculum was added to all bottles. Sufficient mineral medium was then added to each bottle so that all bottles were completely full. The bottles were stoppered and capped.

Sample Analysis

The dissolved oxygen concentration was determined using a Yellow Springs Instruments Dissolved Oxygen Meter. Dissolved oxygen measurements were performed on two test chambers from the control, reference and treatment groups on days 0, 7, 14, 21, and 28. Day zero samples were analyzed immediately after all bottles had been prepared.

Calculations

The average oxygen uptake exhibited by the control, reference, and treatment groups was calculated for each sampling interval. The biochemical oxygen demand (BOD) was calculated for each sampling interval using the following equation:

$$BOD = \frac{mgO_2/L\ uptake\ test\ substance - mgO_2/L\ uptake\ blank}{mg\ test\ substance/L\ in\ vessel}$$

The percent degradation was calculated using the following equation:

$$\% \ degradation = \frac{BOD \ (mg \ O_2/mg \ test \ substance)}{ThOD \ (mg \ O_2/mg \ test \ substance)} \ X \ 100$$

RESULTS AND DISCUSSION

Observations and Measurements

The temperature range recorded during the test was 18 to 20°C and was within the protocol specified range throughout the test. The result of the standard plate count performed on the inoculum was 3.7×10^4 CFU/mL.

The average oxygen uptake exhibited by the control, reference, and treatment groups at each sampling interval is presented in Table 1. The oxygen depletion of the inoculum control was ≤ 1.5 mg O_2/L. The calculated biochemical oxygen demand (BOD) and percent degradation results are also presented in Table 1. Degradation of the test substance was not observed over the 28 day test period.

The viability of the inoculum and validity of the test was supported by the results of the reference substance, sodium benzoate, degrading approximately 88%. An average percent biodegradation of greater than 60% was achieved by day 7, thereby fulfilling the criteria for a valid test(1).

CONCLUSION

Degradation of the test substance, Octabromodiphenyl oxide at 15 mg/L, was not observed over the 28-day test period.

REFERENCES

1 **Organisation for Economic Cooperation and Development.** 1992. *Ready Biodegradability: Closed Bottle Test.* OECD Guideline 301D.

2 **Council of the European Communities.** Directive 67/548/EEC. Annex V. Guideline C.4-E, *Closed Bottle.*

3. **TSCA Title 40 of the Federal Code of Regulations**. 1994. Part 796 Section 3200: *Ready Biodegradability. Closed Bottle Test.*

Table 1

Average Oxygen Uptake, Biochemical Oxygen Demand (BOD) and Percent Degradation Results

Test Substance	Conc. (mg/L)	Day	DO (mg/L)	DO Uptake	Average Uptake	BOD (mg/mg)	Percent Degradation
Control	NA	0	9.0	NA	NA	NA	NA
Control	NA	0	9.0	NA			
Control	NA	7	8.8	0.2	0.2	NA	NA
Control	NA	7	8.8	0.2			
Control	NA	14	8.7	0.3	0.3	NA	NA
Control	NA	14	8.7	0.3			
Control	NA	21	8.3	0.7	0.7	NA	NA
Control	NA	21	8.3	0.7			
Control	NA	28	8.2	0.8	0.8	NA	NA
Control	NA	28	8.3	0.7			
Benzoate	2	0	9.0	NA	NA	NA	NA
Benzoate	2	0	9.0	NA			
Benzoate	2	7	6.1	2.9	2.9	1.4	82
Benzoate	2	7	6.1	2.9			
Benzoate	2	14	5.8	3.2	3.2	1.5	88
Benzoate	2	14	5.8	3.2			
Benzoate	2	21	5.6	3.4	3.5	1.4	82
Benzoate	2	21	5.5	3.5			
Benzoate	2	28	5.2	3.8	3.8	1.5	88
Benzoate	2	28	5.3	3.7			
OBDPO	15	0	9.0	NA	NA	NA	NA
OBDPO	15	0	9.1	NA			
OBDPO	15	7	8.8	0.2	0.2	0.0	0
OBDPO	15	7	8.9	0.1			
OBDPO	15	14	8.4	0.6	0.6	0.0	0
OBDPO	15	14	8.4	0.6			
OBDPO	15	21	8.4	0.6	0.7	0.0	0
OBDPO	15	21	8.2	0.8			
OBDPO	15	28	8.4	0.6	0.7	0.0	0
OBDPO	15	28	8.2	0.8			

APPENDIX I

Study Protocol and Protocol Deviations

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PROTOCOL

OCTABROMODIPHENYL OXIDE (OBDPO): CLOSED BOTTLE TEST

Organisation for Economic Cooperation and Development
OECD Guideline 301D

and

Council of the European Communities Directive 67/548/EEC
Annex V, Guideline C.4-E

and

TSCA Title 40 of the Federal Code of Regulations
Part 796, Section 3200

Submitted To

Chemical Manufacturers Association's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

WILDLIFE INTERNATIONAL LTD.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

April 17, 1996

PROTOCOL NO.: 439/041796/CBT/SUB439

APPENDIX I

WILDLIFE INTERNATIONAL LTD.

OCTABROMODIPHENYL OXIDE (OBDPO): CLOSED BOTTLE TEST

SPONSOR:	Chemical Manufacturers Association's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209
SPONSOR'S REPRESENTATIVE:	Dr. Hasmukh Shah
TESTING FACILITY:	Wildlife International Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Edward C. Schaefer
LABORATORY MANAGEMENT:	James P. Swigert Ph.D. Manager of Aquatic Toxicology

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date:	6-12-96	Experimental Termination Date:	7-10-96
Project No.:	439E-101	Study Room:	Biodegradation
Test Concentrations:	15 mg/L	Int/Date:	EMS 6-5-96
Test Substance No.:	3637	Receipt Date:	4-11-96

PROTOCOL APPROVAL

E. C. Schaefer Jr. — STUDY DIRECTOR — 6-11-96 — DATE

James P. Swigert — LABORATORY MANAGEMENT — 6/11/96 — DATE

Hasmukh Shah — SPONSOR'S REPRESENTATIVE — May 28, 1996 — DATE

INTRODUCTION

Tests of ready biodegradability, by definition, provide limited opportunity for acclimation and prolonged biodegradation to occur. A positive result in a test of ready biodegradability is an indication that the test substance will undergo rapid and ultimate biodegradation in the environment. A negative result in a test of ready biodegradability does not necessarily mean that the test substance will not be biodegraded under relevant environmental conditions but that additional testing may be needed.

OBJECTIVE

The objective of the study will be to measure dissolved oxygen uptake over a 28 day period and express it as a percentage of the theoretical oxygen demand (ThOD) or chemical oxygen demand (COD).

EXPERIMENTAL DESIGN

The test will contain an inoculum control group, a reference group, and a treatment group. The blank control, reference, and treatment groups will contain ten replicate test chambers. The inoculum control is used to measure the dissolved oxygen consumption of the inoculum and will not be dosed with a carbon source. The reference chambers will be dosed with sodium benzoate, a substance known to be biodegradable, at a concentration of 2 mg/L. The treatment group test chambers within the treatment group will be used to evaluate the test substance at 15 mg/L. Measurements of oxygen consumption will be performed on two test chambers from the control, reference and treatment groups on days 0, 7, 14, 21, and 28.

MATERIALS AND METHODS

Test methods are based on the procedures specified in the OECD Guideline for Testing of Chemicals, Guideline 301D (1); Council of the European Communities, Guideline C.4-E, *Closed Bottle* (2); and TSCA Title 40 of the Federal Code of Regulations, Part 796, Section 3200: *Ready Biodegradability: Closed Bottle Test* (3).

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Test Substance

The test substance will be a composite sample of octabromodiphenyl oxide (OBDPO) produced by three manufacturers (Albemarle Corporation, Ameribrom LTD, and Great Lakes Chemical Corporation). The composite OBDPO sample will be composed of equal parts of each of the three manufacturers' product. Prior to use in the study, equal weights of test substance from each manufacturer will be placed in an appropriate plastic container and mixed on a laboratory shaker for a minimum of two hours to form a composite OBDPO sample for use as the test substance. Subsamples of the composite sample will be collected from the left and right sides of the top, middle and bottom of the container. The subsamples will be analyzed by Albemarle Corporation to determine the homogeneity of the mixture. An additional sample of the composite will be collected indiscriminately from the mixture and analyzed by Albemarle Corporation to characterize the test substance. If additional mixing or other procedures are necessary based on the results of the analysis, details of the procedure will be amended to the protocol. The mean OBDPO percent of the homogeneity samples will be used for the study.

Information on the characterization of test, control or reference substances is required by Good Laboratory Practice Standards (GLP), 40 CFR Part 792.31. The Sponsor is responsible for providing Wildlife International Ltd. written verification that the OBDPO composite sample has been characterized according to GLP's prior to its use in the study. If written verification of GLP test substance characterization is not provided to Wildlife International Ltd., it will be noted in the compliance statement of the final report. The attached form IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR (Appendix I) will be used to provide information necessary for GLP compliance.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

Stock Solution Preparation

A suspension of the test substance will be prepared at a nominal concentration of 100 mg/L in high quality water (e.g. Nanopure). In addition, a stock solution of the reference substance,

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sodium benzoate, will be prepared at a nominal concentration of 100 mg/L in high quality water. The stock solutions may be stored in a refrigerator for a maximum of three days.

Test Medium

The test medium is a modified biochemical oxygen demand (BOD) test dilution water and will be prepared using high quality water as described in Appendix I. The mineral medium will be aerated for approximately 20 minutes and then allowed to stand overnight at test temperature. The dissolved oxygen (DO) concentration of the mineral medium will be measured. The mineral medium will be reaerated if the measured DO is less than 8.5 mgO_2/L.

Test Apparatus and Conditions

The test chambers will be 300 mL BOD bottles. The test will be conducted at 20±3°C. Test chambers will be identified by project number, WIL test substance ID, and test concentration, and bottle number.

Test Inoculum

Secondary clarifier supernatant will be collected from Prospect Bay Wastewater Treatment Facility, Grasonville, Maryland. The supernatant will be filtered through glass wool and then aerated until used. A standard plate count will be performed on the inoculum. Plates will be incubated at 20 ± 3°C for approximately 48 hours.

Preparation of Test Chambers

Aerated mineral medium will added to the BOD bottles so that they are about one-half full. Adequate volumes of test and reference substance stock solutions necessary to achieve a concentration of 15 and 2 mg/L, respectively, will be added to the appropriate bottles. Approximately 0.5 mL of inoculum will be added to all bottles. Sufficient mineral medium will then be added to each bottle so that all bottles are completely full. The bottles will be stoppered and capped.

Sample Analysis

The dissolved oxygen concentration will be determined using a Yellow Springs Instruments Dissolved Oxygen Meter or equivalent. Dissolved oxygen measurements will be performed on two

APPENDIX I

test chambers from the control, reference and treatment groups on days 0, 7, 14, 21, and 28. Day zero samples will be analyzed immediately after all bottles have been prepared.

Calculations

The average oxygen uptake exhibited by the control, reference, and treatment groups will be calculated at each sampling interval. The biochemical oxygen demand (BOD) will be calculated after for each sampling interval using the following equation:

$$BOD = \frac{mgO_2/L \text{ uptake test substance} - mgO_2/L \text{ uptake blank}}{mg \text{ test substance/L in vessel}}$$

The percent degradation will be calculated using the following equation:

$$\% \text{ degradation} = \frac{\text{BOD (mg } O_2\text{/mg test substance)}}{\text{ThOD (mg } O2\text{/mg test substance)}} \times 100$$

Results

No bias is expected in this study and statistical methods will not be used in the analysis of the data. Interpretation of the results will be based on the following:

1. Oxygen depletion in the inoculum blank is ≤ 1.5 mgO_2/L.
2. The reference substance must achieve 60% degradation by day 14.

RECORDS TO BE MAINTAINED

Records to be maintained will include, but not limited to, the following:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance as provided by Sponsor.
3. Study initiation and termination dates.
4. Experimental initiation and termination dates.
5. Stock solution concentration calculations and solution preparation.
6. Inoculum source and pretreatment data.

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7. Results of DO analysis.
8. Temperature range recorded during test period.
9. Copy of final report.

FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International Ltd. The report is to include, but is not limited to, the following, when applicable:

1. Name and address of facility performing the study.
2. Dates on which the study was initiated and completed.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures stated in the approved protocol,including any changes in the original protocol.
5. Identification and characterization of the test substance as provided by Sponsor including name, CAS number, percent active, percent carbon, theoretical ThOD, and other characteristics, if provided by the Sponsor.
6. A description of the transformations and calculations performed on the data.
7. A description of the methods used and reference to any standard method employed.
8. A description of the test system.
9. A description of the preparation of the test solutions, the testing concentrations, the route of administration, and the duration of the test.
10. A description of all circumstances that may of affected the quality or integrity of the data.
11. The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.
12. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
13. The location where the raw data and final report are to be stored.
14. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and findings reported to the Study Director and Management.

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CHANGES TO THE FINAL REPORT

If it is necessary to make corrections or additions to the final report after if has been accepted, such changes shall be made in the form of an amendment issued by the Study Director. The amendment shall clearly identify the part of the study that is being amended and the reasons for the alteration. Amendments shall be signed and dated by the Study Director and Laboratory QA.

CHANGING OF PROTOCOL

Changes in the protocol will be made in the form of a written amendment that is signed by the Study Director, Laboratory Management, and the Sponsor. The amendment will become part of the original protocol.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 792)and OECD (ISBN 92-84-12367-9). Each study conducted by Wildlife International Ltd. is routinely examined by the Wildlife International Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Raw data for all work performed at Wildlife International Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International Ltd. site or at an alternative location to be specified in the final report.

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REFERENCES

1 **Organisation for Economic Cooperation and Development.** 1992. *Ready Biodegradability: Closed Bottle Test.* OECD Guideline 301D.

2 **Council of the European Communities.** Directive 67/548/EEC. Annex V. Guideline C.4-E, *Closed Bottle.*

3 **TSCA Title 40 of the Federal Code of Regulations.** 1994. Part 796, Section 3200: *Ready Biodegradability: Closed Bottle Test.*

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APPENDIX I. Test Medium

The following stock solutions used to prepare the test medium may be purchased or prepared as described below:

(a) Potassium dihydrogen orthophosphate, KH_2PO_4 ..... 8.50 g
Dipotassium hydrogen orthophosphate, K_2HPO_4 ..... 21.75 g
Disodium hydrogen orthophosphate dihydrate,
$Na_2HPO_4 \cdot 2H_2O$ ..... 33.40 g
Ammonium chloride, NH_4Cl ..... 0.50 g

Dissolve in high quality water and make up to 1 liter.

(b) Calcium chloride solution, $CaCl_2$ ..... 27.50 g

Dissolve in high quality water and make up to 1 liter.

(c) Magnesium sulfate, $MgSO_4 \cdot 7H_2O$ ..... 22.50 g

Dissolve in high quality water and make up to 1 liter.

(d) Ferric chloride, $FeCl_3 \cdot 6H_2O$ ..... 0.25 g

The test medium will contain the following standard reagent solutions per liter of high quality water (e.g. Nanopure):

1 mL of phosphate buffer solution, APHA, pH 7.2
1 mL of calcium chloride solution, APHA, 2.75%
1 mL of magnesium sulfate solution, APHA, 2.25%
1 mL of ferric chloride solution, APHA, 0.025%

The constituents of the test medium are not known to contain any contaminants that are reasonably expected to be present and are known to be capable of interfering with the study.

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APPENDIX II
IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): _____

Test Substance Sample Code or Batch Number: _____

Test Substance Purity (% Active Ingredient): _____ Expiration Date: _____

Solubility: Water: _____ Theoretical Oxygen Demand: _____

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance and reference standard been determined prior to its use in this study in accordance with GLP Standards? Yes ____ No ____

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International Ltd.

_____

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? Yes ____ No __

Other pertinent stability information: _____

_____

IV. Test Concentrations:

☐ Adjust test concentration to 100% a.i. based upon the purity (%) given above.

☐ Do not adjust test concentration to 100% a.i. Test the material AS IS.

V. Toxicity Information:

Mammalian: Rat LD50 _ ___ Mouse LD50 ____

Aquatic: Invertebrate Toxicity (EC/LC50) Fish Toxicity (LC50)

_____ _____

_____ _____

Other Toxicity Information (including findings of chronic and subchronic tests):

_____

VI. Classification of the Compound:

_____ Insecticide _____ Herbicide _____ Fungicide
_____ Microbial Agent _____ Economic Poison

Other: _ _____

APPENDIX I

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: Octabromodiphenyl Oxide (OBDPO): Closed Bottle Test

PROTOCOL NO.: 439/041796/CBT/SUB439 **DEVIATION NO.:** 1

SPONSOR: Chemical Manufacturers Association **PROJECT NO.:** 439E-101

DATE OF DEFACTO DEVIATION: April 10, 1996

DEVIATION: Page 4, Test Substance:

Subsamples of the composite mixture were sent to both the Albemarle Corporation and the Great Lakes Chemical Corporation.

REASON:

The samples were sent to the Great Lakes Chemical Corporation for preliminary homogeneity testing.

IMPACT:

This deviation had no impact on the integrity of the study.

STUDY DIRECTOR — DATE 8-8-96

LABORATORY MANAGEMENT — DATE 8/8/96

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APPENDIX I

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: Octabromodiphenyl Oxide (OBDPO): Closed Bottle Test

PROTOCOL NO.: 439/041796/CBT/SUB439 **DEVIATION NO.:** 2

SPONSOR: Chemical Manufacturers Association **PROJECT NO.:** 439E-101

DATE OF DEFACTO DEVIATION: April 29, 1996

DEVIATION: Page 4, Test Substance:

After the initial mixing, the composite was mixed for an additional 2 hours. Subsamples of the composite mixture were sent to the Albemarle Corporation and to the Great Lakes Chemical Corporation.

REASON:

The preliminary homogeneity testing indicated that additional mixing was necessary.

IMPACT:

This deviation had no impact on the integrity of the study.

STUDY DIRECTOR — DATE: 8-8-96

LABORATORY MANAGEMENT — DATE: 8/8/96

SAD3

APPENDIX II

Personnel Involved in the Study

The following key Wildlife International Ltd. personnel were involved in the conduct or management of this study:

(1) James P. Swigert, Manager, Aquatic Toxicology
(2) Edward C. Schaefer, Study Director, Biodegradation
(3) Doug Haberlein, Biologist, Biodegradation
(4) Joel I. Stenzel, Senior Chemist, Analytical Chemistry