

Chris



CHEMICAL MANUFACTURERS ASSOCIATION

FYI-0397-1289

March 18, 1997



FYI-97-001289

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

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 report is enclosed:

A 28-Day Repeated Dose Oral Toxicity Study of HBCD in Rats

Only volume 1 of 3 is enclosed with this letter. Volumes 2 and 3 contains 658 pages and only contain raw data.

This report does not include confidential information.

If you have any questions, please call me at 703-741-5637.

Sincerely,

Hasmukh Shah

Hasmukh Shah, Ph.D.
Director, CHF



8497000012

Enclosure

Contains No CBI

RECEIVED
OPPT/OSIC
97 MAR 31 PM 11:08



FINAL REPORT

VOLUME 1 OF 3
(Text and Summary Tables)

STUDY TITLE

**A 28-DAY REPEATED DOSE ORAL
TOXICITY STUDY OF HBCD IN RATS**

DATA REQUIREMENT

OECD Guideline, Section 407

STUDY DIRECTOR

Christopher P. Chengelis, Ph.D., D.A.B.T.

STUDY INITIATED ON

April 12, 1996

STUDY COMPLETION DATE

February 13, 1997

PERFORMING LABORATORY

WIL Research Laboratories, Inc.
1407 George Road
Ashland, Ohio 44805-9281

LABORATORY STUDY NUMBER

WIL-186004

SPONSOR PROJECT NUMBER

BFRIP 2.0-WIL HBCD

SPONSOR

Chemical Manufacturers Association
Brominated Flame Retardant Industry Panel
1300 Wilson Blvd.
Arlington, Virginia 22209

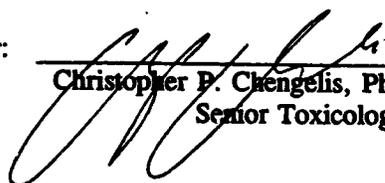
WIL-186004
CMA BFRIP

A 28-Day Repeated Dose Oral
Toxicity Study of HBCD in Rats

COMPLIANCE STATEMENT

This study, designated WIL-186004, was conducted in compliance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Regulations (40 CFR Part 792), October 16, 1989, the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [C (81) 30 (Final)], the Standard Operating Procedures of WIL Research Laboratories, Inc., and the protocol as approved by the sponsor (Chemical Manufacturers Association Brominated Flame Retardant Industry Panel). The protocol was designed and the study was conducted in accordance with the OECD Guidelines for testing of Chemicals, Health Effects Test Guidelines, Section 407, adopted May 12, 1981.

Study Director:



Christopher P. Chengelis, Ph.D., D.A.B.T.
Senior Toxicologist

13 Feb 97
Date

A 28-Day Repeated Dose Oral
Toxicity Study of HBCD in Rats

TABLE OF CONTENTS

VOLUME 1		Page
I.	Summary	14
II.	Objective	18
III.	Study Design	19
IV.	Experimental Procedures	20
A.	Introduction	20
B.	Test and Control Articles	20
1.	Test Article Identification	20
2.	Control Article Identification	21
3.	Preparation	21
4.	Administration	21
5.	Sampling and Analyses	22
C.	Animal Receipt and Acclimation/Pretest Period	23
D.	Animal Housing	23
E.	Diet, Drinking Water and Maintenance	24
F.	Environmental Conditions	24
G.	Assignment of Animals to Treatment Groups	24
H.	Clinical Observations and Survival	25
I.	Body Weights	25
J.	Food Consumption	26
K.	Functional Observational Battery	26
1.	Home Cage Observations	26
2.	Handling Observations	26
3.	Open Field Observations	27
4.	Sensory Observations	27
5.	Neuromuscular Observations	27
6.	Physiological Observations	27
L.	Locomotor Activity	27

VOLUME 1 (continued)		Page
M.	Clinical Pathology	28
	1. Hematology	28
	2. Serum Chemistry	29
N.	Pathology	29
	1. Macroscopic Examination	29
	2. Organ Weights	30
	3. Microscopic Examination	30
O.	Statistical Methods	31
P.	Data Retention	31
V.	Results	32
	A. Clinical Observations and Survival	32
	B. Body Weights	32
	C. Food Consumption	32
	D. Functional Observational Battery	33
	1. Home Cage Observations	33
	2. Handling Observations	33
	3. Open Field Observations	33
	4. Sensory Observations	34
	5. Neuromuscular Observations	34
	6. Physiological Observations	34
	E. Locomotor Activity	35
	F. Clinical Pathology	35
	1. Hematology	35
	2. Serum Chemistry	35
	G. Pathology	37
	1. Macroscopic Examination	37
	2. Organ Weights	37
	3. Microscopic Examination	38
VI.	Discussion and Conclusions	39

VOLUME 1 (continued)		<u>Page</u>
VII.	Key Personnel and Report Submission	40
VIII.	Pathologist Summary Statement	41
IX.	Quality Assurance Unit Statement	42
X.	References	45

A 28-Day Repeated Dose Oral
Toxicity Study of HBCD in Rats

INDEX OF TABLES

VOLUME 1 (continued)		Page
1.	Summary of Survival and Disposition	47
2.	Summary of Clinical Findings: Total Occurrence/No. of Animals (Prior to Scheduled Necropsy/Dispositions)	49
3.	Summary of Clinical Findings: Total Occurrence/No. of Animals (At Time of Dosing)	51
4.	Summary of Clinical Findings: Total Occurrence/No. of Animals (1-Hour Post-Dosing)	53
5.	Summary of Clinical Findings: Total Occurrence/No. of Animals (Recovery Period Observations)	55
6.	Body Weights (Grams) - Summary of Means	57
7.	Body Weight Gains (Grams) - Summary of Means	61
8.	Weekly Food Consumption (Grams/Animal/Day) - Summary of Means	55
9.	Functional Observational Battery Summary Incidence - Home Cage Observations (Week -1 Pretest Evaluation)	69
10.	Functional Observational Battery Summary Incidence - Home Cage Observations [%] (Week -1 Pretest Evaluation)	71
11.	Functional Observational Battery Summary Incidence - Home Cage Observations (Week 3 Evaluation)	73
12.	Functional Observational Battery Summary Incidence - Home Cage Observations [%] (Week 3 Evaluation)	75
13.	Functional Observational Battery Summary Incidence - Home Cage Observations (Week 5 Recovery Evaluation)	77
14.	Functional Observational Battery Summary Incidence - Home Cage Observations [%] (Week 5 Recovery Evaluation)	79

VOLUME 1 (continued)		<u>Page</u>
15.	Functional Observational Battery Summary Incidence - Handling Observations (Week -1 Pretest Evaluation)	81
16.	Functional Observational Battery Summary Incidence - Handling Observations [%] (Week -1 Pretest Evaluation)	87
17.	Functional Observational Battery Summary Incidence - Handling Observations (Week 3 Evaluation)	93
18.	Functional Observational Battery Summary Incidence - Handling Observations [%] (Week 3 Evaluation)	99
19.	Functional Observational Battery Summary Incidence - Handling Observations (Week 5 Recovery Evaluation)	105
20.	Functional Observational Battery Summary Incidence - Handling Observations [%] (Week 5 Recovery Evaluation)	111
21.	Functional Observational Battery Summary Incidence - Open Field Observations (Week -1 Pretest Evaluation)	117
22.	Functional Observational Battery Summary Incidence - Open Field Observations [%] (Week -1 Pretest Evaluation)	121
23.	Functional Observational Battery Summary Incidence - Open Field Observations (Week 3 Evaluation)	125
24.	Functional Observational Battery Summary Incidence - Open Field Observations [%] (Week 3 Evaluation)	129
25.	Functional Observational Battery Summary Incidence - Open Field Observations (Week 5 Recovery Evaluation)	133
26.	Functional Observational Battery Summary Incidence - Open Field Observations [%] (Week 5 Recovery Evaluation)	137
27.	Functional Observational Battery Summary Incidence - Sensory Observations (Week -1 Pretest Evaluation)	141
28.	Functional Observational Battery Summary Incidence - Sensory Observations [%] (Week -1 Pretest Evaluation)	145

VOLUME 1 (continued)		Page
29.	Functional Observational Battery Summary Incidence - Sensory Observations (Week 3 Evaluation)	149
30.	Functional Observational Battery Summary Incidence - Sensory Observations [%] (Week 3 Evaluation)	153
31.	Functional Observational Battery Summary Incidence - Sensory Observations (Week 5 Recovery Evaluation)	157
32.	Functional Observational Battery Summary Incidence - Sensory Observations [%] (Week 5 Recovery Evaluation)	161
33.	Functional Observational Battery Summary Incidence - Neuromuscular Observations (Week -1 Pretest Evaluation)	165
34.	Functional Observational Battery Summary Incidence - Neuromuscular Observations [%] (Week -1 Pretest Evaluation)	167
35.	Functional Observational Battery Summary Incidence - Neuromuscular Observations (Week 3 Evaluation)	169
36.	Functional Observational Battery Summary Incidence - Neuromuscular Observations [%] (Week 3 Evaluation)	171
37.	Functional Observational Battery Summary Incidence - Neuromuscular Observations (Week 5 Recovery Evaluation)	173
38.	Functional Observational Battery Summary Incidence - Neuromuscular Observations [%] (Week 5 Recovery Evaluation)	175
39.	Functional Observational Battery Summary Incidence - Physiological Observations (Week -1 Pretest Evaluation)	177
40.	Functional Observational Battery Summary Incidence - Physiological Observations (Week 3 Evaluation)	179
41.	Functional Observational Battery Summary Incidence - Physiological Observations (Week 5 Recovery Evaluation)	181
42.	Group Mean Motor Activity Counts (Week -1 Pretest Evaluation)	183
43.	Group Mean Motor Activity Counts (Week 3 Evaluation)	187

	<u>Page</u>
VOLUME 1 (continued)	
44. Group Mean Motor Activity Counts (Week 5 Recovery Evaluation)	191
45. Hematology Values - Summary of Means	195
46. Leukocyte Differential Count (%) - Summary of Means	203
47. Leukocyte Counts - Summary of Means	207
48. Serum Chemistry Values - Summary of Means	211
49. Gross Necropsy Observations Incidence Summary (Week 4 Primary Necropsy)	223
50. Gross Necropsy Observations Incidence Summary (Week 6 Recovery Necropsy)	224
51. Organ Weights (Grams) - Summary of Means (Week 4 Primary Necropsy)	225
52. Organ Weights (Grams) - Summary of Means (Week 6 Recovery Necropsy)	229
53. Organ Weights Relative to Final Body Weights (Grams/100 Grams) - Summary of Means (Week 4 Primary Necropsy)	233
54. Organ Weights Relative to Final Body Weights (Grams/100 Grams) - Summary of Means (Week 6 Recovery Necropsy)	237
55. Histomorphological Diagnosis - Summary Incidence (Week 4 Primary Necropsy)	241
56. Histomorphological Diagnosis - Summary Incidence (Week 6 Recovery Necropsy)	255
VOLUME 2	
57. Individual Survival and Disposition	269
58. Individual Clinical Observations (Prior to Scheduled Necropsy/Dispositions)	273
59. Individual Clinical Observations (At Time of Dosing)	279

VOLUME 2 (continued)		Page
60.	Individual Clinical Observations (1-Hour Post-Dosing)	283
61.	Individual Clinical Observations (Recovery Period Observations)	287
62.	Individual Body Weights (Grams)	288
63.	Individual Body Weight Gains (Grams)	296
64.	Individual Food Consumption (Grams/Animal/Day)	304
65.	Individual Functional Observational Battery Data - Home Cage Observations (Week -1 Pretest Evaluation)	312
66.	Individual Functional Observational Battery Data - Home Cage Observations (Week 3 Evaluation)	320
67.	Individual Functional Observational Battery Data - Home Cage Observations (Week 5 Recovery Evaluation)	328
68.	Individual Functional Observational Battery Data - Handling Observations (Week -1 Pretest Evaluation)	332
69.	Individual Functional Observational Battery Data - Handling Observations (Week 3 Evaluation)	356
70.	Individual Functional Observational Battery Data - Handling Observations (Week 5 Recovery Evaluation)	380
71.	Individual Functional Observational Battery Data - Open Field Observations (Week -1 Pretest Evaluation)	392
72.	Individual Functional Observational Battery Data - Open Field Observations (Week 3 Evaluation)	408
73.	Individual Functional Observational Battery Data - Open Field Observations (Week 5 Recovery Evaluation)	416
74.	Individual Functional Observational Battery Data - Sensory Observations (Week -1 Pretest Evaluation)	424
75.	Individual Functional Observational Battery Data - Sensory Observations (Week 3 Evaluation)	440

	VOLUME 2 (continued)	<u>Page</u>
76.	Individual Functional Observational Battery Data - Sensory Observations (Week 5 Recovery Evaluation)	448
77.	Individual Functional Observational Battery Data - Neuromuscular Observations (Week -1 Pretest Evaluation)	456
78.	Individual Functional Observational Battery Data - Neuromuscular Observations (Week 3 Evaluation)	472
79.	Individual Functional Observational Battery Data - Neuromuscular Observations (Week 5 Recovery Evaluation)	488
80.	Individual Functional Observational Battery Data - Physiological Observations (Week -1 Pretest Evaluation)	496
81.	Individual Functional Observational Battery Data - Physiological Observations (Week 3 Evaluation)	504
82.	Individual Functional Observational Battery Data - Physiological Observations (Week 5 Recovery Evaluation)	512
83.	Individual Motor Activity Counts (Week -1 Pretest Evaluation)	516
84.	Individual Motor Activity Counts (Week 3 Evaluation)	532
85.	Individual Motor Activity Counts (Week 5 Recovery Evaluation)	548
	VOLUME 3	
86.	Individual Hematology Values (Week 4 Primary Necropsy)	557
87.	Individual Hematology Values (Week 6 Recovery Necropsy)	565
88.	Individual Leukocyte Differential Count (%) (Week 4 Primary Necropsy)	569
89.	Individual Leukocyte Differential Count (%) (Week 6 Recovery Necropsy)	577
90.	Individual Leukocyte Counts (Week 4 Primary Necropsy)	581
91.	Individual Leukocyte Counts (Week 6 Recovery Necropsy)	589

	VOLUME 3 (continued)	<u>Page</u>
92.	Individual Serum Chemistry Values (Week 4 Primary Necropsy)	593
93.	Individual Serum Chemistry Values (Week 6 Recovery Necropsy)	609
94.	Individual Gross and Microscopic Description of Organs (Week 4 Primary Necropsy)	617
95.	Individual Gross and Microscopic Description of Organs (Week 6 Recovery Necropsy)	669
96.	Individual Organ Weights and Final Body Weights (Grams) (Week 4 Primary Necropsy)	697
97.	Individual Organ Weights and Final Body Weights (Grams) (Week 6 Recovery Necropsy)	705
98.	Individual Organ Weights Relative to Final Body Weights (Grams/100 Grams) (Week 4 Primary Necropsy)	709
99.	Individual Organ Weights Relative to Final Body Weights (Grams/100 Grams) (Week 6 Recovery Necropsy)	717

A 28-Day Repeated Dose Oral
Toxicity Study of UHED in Rats

INDEX OF APPENDICES

	<u>Page</u>
VOLUME 3 (continued)	
A. Analyses of Dosing Preparations (Sponsor-Generated Data)	721
B. Gravimetric Analysis of Dosing Formulations (WIL Research Laboratories, Inc.)	729
C. Pretest Clinical Observations	763
D. Scoring Criteria for Functional Observational Battery	766
E. Summaries of Validation Studies (WIL-99026, WIL-99032, WIL-99034 and WIL-99035)	782
F. Clinical Pathology Methods, Procedures and References	813
G. WIL Functional Observational Battery Historical Control Data	818
H. WIL Motor Activity Historical Control Data	873
I. Clinical Pathology Historical Control Data (WIL Research Laboratories, Inc.)	880
J. Study Protocol	903

A 28-Day Repeated Dose Oral
Toxicity Study of HBCD in Rats

I. SUMMARY

The test article, hexabromocyclododecane (HBCD), Lot #3571, in the vehicle, corn oil, was administered orally by gavage to three groups of Sprague-Dawley Crl: CD BF rats for a period of 28 consecutive days. Dose levels were 125 (low), 350 (mid), or 1000 (high) mg/kg/day, administered in a dosage volume of 5 ml/kg. The test groups consisted of 6 males and 6 females in the 125 and 350 mg/kg/day groups and 12 males and 12 females in the 1000 mg/kg/day group. A concurrent control group comprised of 12 males and 12 females received the vehicle, corn oil, for 28 consecutive days at a dosage volume of 5 ml/kg. At the end of the dosing period, 6 animals/sex/group were euthanized and necropsied. The remaining 6 animals/sex in the control and 1000 mg/kg/day groups remained on-test untreated for a 14 day recovery period. At the end of the recovery period, all animals were euthanized and necropsied.

Animals were observed twice daily for mortality and moribundity. Clinical signs were recorded daily. Body weight and food consumption were measured weekly. Functional observational battery and motor activity evaluations were performed during weeks -1 (pretest), 3, and 5 (recovery). Samples for hematology and serum chemistry evaluations were collected at the primary (28 day) and recovery (42 day) necropsies. Complete necropsies were performed on all rats. The brain, liver, kidney, heart, spleen, testes and epididymis or ovaries, adrenal glands, and thymus from all animals were weighed at each necropsy. Approximately 40 tissues were collected and preserved at each necropsy from all animals. The following tissues were examined microscopically from the control and high dose animals: liver, kidney, heart, spleen, testes (males), prostate (males), seminal vesicles (males), epididymis (males), ovaries (females), adrenal glands, thymus, bone with marrow (sternebra), brain, stomach, cecum, duodenum, ileum, jejunum, lymph node, peripheral nerve (sciatic), spinal cord, lung, trachea, uterus (females), urinary

bladder, and all gross lesions.) The lungs, liver, kidneys, stomach, gross lesions and target organs were examined in all dose levels.

Survival was not affected by administration of the test article. All animals survived to the scheduled necropsy. Clinical signs observed during the study were nonspecific, low in incidence, non-dose-related and not considered related to test article.

Body weights, weight gain and food consumption of treated animals were compared statistically by sex and treatment day to their respective control groups ($p < 0.05$ or $p < 0.01$). Body weight, weight gain and food consumption was not affected by treatment. No statistically significant differences in body weight between control and treated animals were detected with the exception of an increase in mean female body weight in the 350 mg/kg/day during week 2 of treatment. Mean female body weight at that time point was 196 g vs 179 in the control group. No statistically significant differences in body weight gain between control and treated animals were detected with the exception of a decrease in mean male body weight gain in the 1000 mg/kg/day recovery group during week 1 of recovery. Mean male body weight gain at that time point was 21 g vs 31 in the control group; mean male body weight was not statistically different from the control mean. No statistically significant differences in food consumption between control and treated animals were detected with the exception of an increase in mean female food consumption in the 350 mg/kg/day during weeks -1, 1, and 2 of treatment. Mean female food consumption at those time points were 18, 17 and 17 g vs 16, 15 and 15 g in the control group, respectively.

Functional observation battery and motor activity results from treated animals were compared statistically by sex and treatment day to their respective control groups ($p < 0.05$). These parameters were not affected by treatment with the test article. No statistically significant differences were observed between treated and control animals at any time point.

No statistically significant differences between treated and control animals were found for hematology parameters with the exception of an increase in the mean

activated partial thromboplastin time in the 1000 mg/kg/day males on week 4 and a decrease in the mean prothrombin time in the 1000 mg/kg/day females on week 4. These statistical differences were not of toxicological significance.

No toxicologically significant effects on serum chemistry values related to test article administration were observed at the 28 day primary and 42 day recovery necropsies. Scattered instances of statistically significant differences between treated and control animals were detected for some serum chemistry parameters at the 28 day primary necropsy. These scattered statistical differences were not considered toxicologically significant because the statistical differences occurred: in the absence of a dose response, in the absence of the accompanying clinical chemistry changes expected, in the opposite direction from what occurs in a toxic state, in a direction which is without physiologic significance, or due to potential interference with the laboratory method. No statistically significant differences in serum chemistry parameters were detected between groups at the 42 day recovery necropsy.

No gross lesions which could be attributed to the test article were detected at either necropsy. Gross lesions were nonspecific, low in incidence, non-dose-related and considered incidental.

No microscopic lesion which could be attributed to the test article were detected on histopathologic exam. Microscopic changes were nonspecific, low in incidence, non-dose-related and considered incidental.

No statistical significant differences in organ weight or organ to body weight ratios were detected between control and treated animals with one exception. Absolute liver weights were statistically significantly increased with respect to control means at the 28 day necropsy in males in the high dose and females in the mid and high dose. Liver to body weight ratios in mid and high dose males and low, mid and high dose females were statistically significantly increased at the 28 day necropsy. At the recovery necropsy, male absolute and liver to body weight ratio were statistically comparable to the control mean whereas female absolute liver weights and liver to body weight ratio were statistically significantly increased with respect to control mean. The difference in absolute liver weight between control and treated

WIL-186004
CMA BFRIP

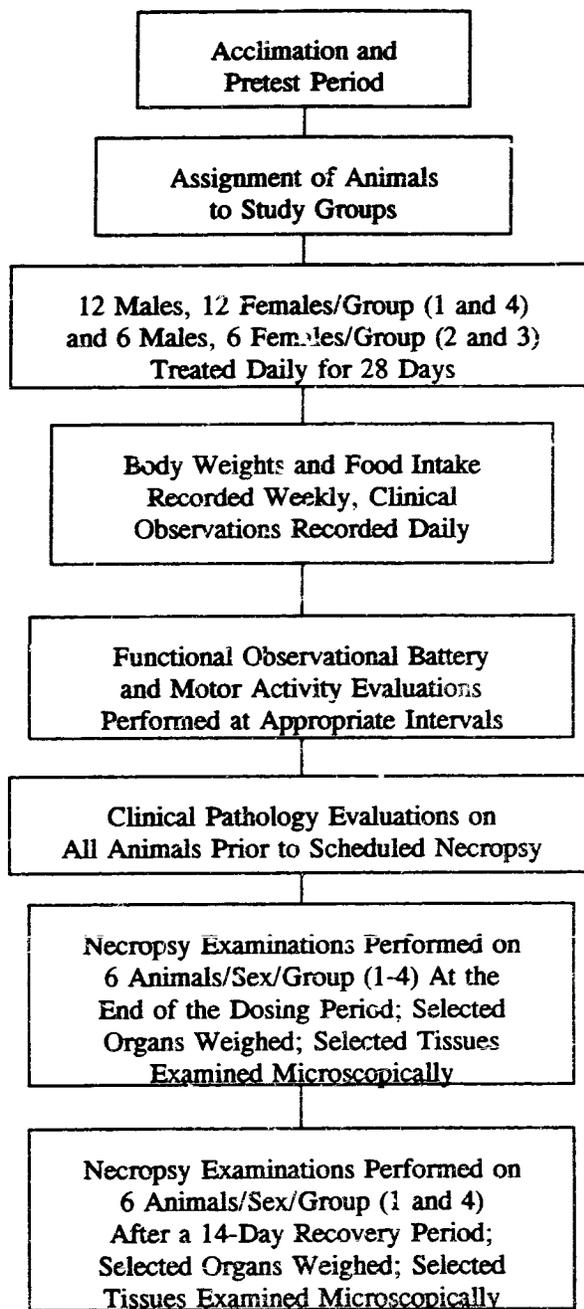
females was less pronounced at the end of the recovery period, indicating the increase in liver weight was reversible in females as well as males. In the absence of test article related histopathologic and serum chemistry changes, increases in liver weight are considered an adaptive, rather than a toxic response, are not uncommon in the rat, and are most likely the result of microsomal induction.

In conclusion, no systemic toxicity was observed at any dose level. Based on the results of this study, the NOAEL (No Observed Adverse Effect Level) of HBCD administered orally to male and female rats for 28 consecutive days was 1000 mg/kg/day.

II. OBJECTIVE

The objective of the study was to evaluate the possible toxic effects of Hexabromocyclododecane (HBCD) when administered orally (gavage) to rats for 28 days. This included evaluation of potential neurotoxicity by functional observational battery (FOB) and motor activity (MA) assessment. Reversibility, persistence or delayed occurrence of toxic effects was evaluated by a 14-day post-treatment recovery period. The selected route of administration was oral by gavage as this route is an acceptable and standard method for administering test article per OECD Guideline 407. The animal model, the Sprague-Dawley CrI:CD®BR rat, is recognized as appropriate for subchronic toxicity studies and was selected based on the availability of historical control data.

III. STUDY DESIGN



IV. EXPERIMENTAL PROCEDURES

A. INTRODUCTION

The experimental start date, initiating with test article administration, was May 17, 1996 (week 0). The primary necropsy was on June 14, 1996. The recovery necropsy was on June 28, 1996. The experimental termination date, concluding with the last pathology examination, was October 8, 1996.

B. TEST AND CONTROL ARTICLES

1. TEST ARTICLE IDENTIFICATION

Hexabromocyclododecane (HBCD) was received from Chemical Manufacturers Association via Wildlife International Limited, Easton, Maryland, as follows:

<u>Label Identification</u>	<u>No. of Containers Received</u>	<u>Description</u>	<u>Date Received</u>
#3577 HBCD Approximately 300 g (Composite* of #3462, 3519, and 3551) Prep. date: 2/19/96 Exp. date: 12/16/96 [WIL Log No. 3014A]	One Bottle Gross weight 646.3 g	White powder	4/17/96
HBCD Test substance no. 3577 Composite* of no's 3462, 3519, 3551 Prep. date: 2/19/96 Expires: 12/16/96 [WIL Log No. 3014B]	One Bottle Gross weight 430.1 g	White powder	6/7/96

* The test article was a composite sample of equal portions of commercial HBCD manufactured by CMA BFRIP members.

The stability and purity of the bulk test article were the responsibility of the sponsor. For purposes of dose calculations, the test article was considered to be 100% HBCD. The bulk test article was stored in a sealed container in a dry, well-ventilated area at room temperature (68°F ± 4°F) and was

considered to be stable under these conditions. On April 19, 1996, a 1.013 g sample was taken from the first shipment of test article and stored in the archives at WIL Research Laboratories, Inc. An approximate one gram reserve sample was to be taken from the second shipment of test article in accordance with WIL standard operating procedures. However, after the remaining test article had been returned to the sponsor, it was discovered that a retention sample was not collected from this shipment. This deviation from the protocol had no impact on the scientific validity of the study as both shipments were of the same lot number.

2. CONTROL ARTICLE IDENTIFICATION

The vehicle control article utilized in preparation of the test mixtures and for administration to the control group was Mazola® corn oil manufactured by Best Foods, CPC International, Inc., Englewood Cliffs, New Jersey.

3. PREPARATION

A sufficient amount of corn oil to dose the control group animals was placed in a labeled storage container. The vehicle control article was stirred continuously throughout the sampling and dosing procedures using a magnetic stir bar and stir plate.

The appropriate amount of test article, HBCD, for each group was weighed into a tared, precalibrated storage container. A stir bar was added and the container was tared a second time. An appropriate volume of vehicle was added and the weight of the formulation was recorded. The formulations were stirred continuously throughout the sampling and dosing procedures using magnetic stir bars and plates.

All dosing formulations were prepared daily and stored at room temperature. Test article concentration and homogeneity of the dosing formulations were verified using gravimetric determination.

4. ADMINISTRATION

The test mixtures were administered orally by gastric intubation via a 16-gauge stainless steel gavage cannula (Popper and Sons, Inc., Hyde Park, New

York 11040) as a single daily dose for 28 consecutive days through the day prior to the scheduled primary necropsy. A dose volume of 5 ml/kg was used for all dosage levels. The concurrent control group animals received the vehicle, corn oil, on a comparable regimen at a dose volume of 5 ml/kg. Individual dosages were adjusted based on the most recent body weights, to provide the correct mg/kg/day dose. On May 28, 1996 (study day 11), all males and the first female in the 350 mg/kg/day group were inadvertently dosed using the dose volumes for all males and the first female in the 125 mg/kg/day group, and on June 7, 1996 (study day 21), the first four males in the 1000 mg/kg/day group were inadvertently dosed using the dose volumes for the first four males in the 350 mg/kg/day group. All animals that were underdosed received a correcting dose amount. In the 350 mg/kg/day group, three males (nos. 50285, 50286 and 50315) were overdosed by 0.1 ml and one female (no. 50322) was overdosed by 0.03 ml. One male (no. 50283) in the 1000 mg/kg/day group was overdosed by 0.2 ml. This deviation from the protocol had no impact on the outcome of the study as all animals received the correct dosing solution and only a limited number of animals received a slightly higher than intended dosage. The following table presents the study group assignment.

<u>Group Number</u>	<u>Test Article</u>	<u>Dosage Level (mg/kg/day)</u>	<u>Concentration (mg/ml)</u>	<u>Dosage Volume (ml/kg)</u>	<u>Number of Animals</u>	
					<u>Males</u>	<u>Females</u>
1	Vehicle	0	0	5	12	12
2	HBCD	125	25	5	6	6
3	HBCD	350	70	5	6	6
4	HBCD	1000	200	5	12	12

5. SAMPLING AND ANALYSES

Prior to the initiation of dosing, sample dosing suspensions were prepared for Groups 2 and 4, and duplicate 1 ml aliquots were withdrawn from the middle stratum of each suspension immediately after preparation. The suspensions were stirred overnight using magnetic stir bars and plates, and duplicate 1 ml aliquots were withdrawn from the middle stratum of each

suspension approximately 24 hours following preparation. The samples were frozen as soon as possible after collection and were shipped on dry ice to Albemarle Corporation Technical Center, Baton Rouge, Louisiana, on May 9, 1996, for stability analysis. Duplicate one-gram samples of the test article were collected and frozen on the first and last days of test article administration, and were shipped on dry ice to Albemarle Corporation Technical Center on May 20, 1996 and June 17, 1996, respectively, for stability analysis. The results of these analyses are presented in Appendix A.

Gravimetric assays of the dosing formulations for verification of test article concentration and homogeneity were performed at WIL Research Laboratories, Inc. Concentration determinations were performed daily prior to dose administration. Homogeneity determinations were performed on study days 0, 13 and 27. The results of these analyses are presented in Appendix B.

C. ANIMAL RECEIPT AND ACCLIMATION/PRETEST PERIOD

Forty male and forty female Sprague-Dawley CrI:CD®BR rats were received on May 2, 1996, from Charles River Laboratories, Inc., Portage, Michigan. The animals were 28 days old upon receipt. Each animal was examined by a qualified technician on the day of receipt. Animals were uniquely identified by Monel metal eartags displaying the permanent identification number. All animals were housed for a 15-day acclimation and pretest period; observations were made twice daily for mortality and general changes in appearance or behavior.

Pretest data collection began on May 6, 1996. Individual body weights were recorded and detailed physical examinations were performed on the first and last days of pretest week -1 (May 10 and 16, 1996, respectively). Individual food consumption was measured for the interval week -1 to week 0. For computer entry, pretest data were assigned to computer protocol number WIL-186004P. Pretest clinical observations are presented in Appendix C.

D. ANIMAL HOUSING

Upon arrival, all animals were housed three per cage by sex for a minimum of three days. Thereafter, all animals were housed individually in clean, wire-mesh

cages suspended above cage-board. All animals were maintained by the animal husbandry staff in accordance with the "Guide for the Care and Use of Laboratory Animals"¹. The animal facilities at WIL Research Laboratories, Inc., are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

E. DIET, DRINKING WATER AND MAINTENANCE

The basal ration used in this study was Purina® Certified Rodent Chow® #5002. The diet utilized at WIL Research Laboratories, Inc., is a certified feed with appropriate analyses performed by the manufacturer and provided to WIL Research Laboratories, Inc. Municipal water supplying the facility is sampled and analyzed for contaminants according to Standard Operating Procedures. The results of these analyses are maintained at WIL Research Laboratories, Inc. Contaminants were not present in animal feed or water at levels sufficient to interfere with the objectives of this study. The basal diet and drinking water delivered by an automatic watering system were provided *ad libitum* throughout the study period except during the period of fasting prior to blood collection when food but not water was withheld.

F. ENVIRONMENTAL CONDITIONS

All animals were housed throughout the acclimation period and during the study in an environmentally-controlled room. Controls were set to maintain temperature at 72° ± 4°F (22° ± 2°C), relative humidity at approximately 30-70% and a minimum of 10 air exchanges per hour. Room temperature and relative humidity were recorded twice daily. Actual recorded ranges for temperature and humidity were 71.9° to 73.0°F (22.2° to 22.8°C) and 33.7% to 67.3%, respectively, during the study period. Light timers were set to provide a 12-hour light/12-hour dark photoperiod.

G. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS

On May 16, 1996 (the day prior to test article administration), all available rats were weighed and examined in detail for physical abnormalities. Animals judged suitable for testing, as determined by the study director, were selected for use in

the computerized randomization procedure. The individual body weights were entered into the WIL Toxicology Data Management System (WTDMS™). A printout containing the animal numbers, corresponding body weights and individual group assignments was generated based on computer-generated random number permutations. The animals then were arranged according to the printout. The control and 1000 mg/kg/day groups each consisted of 12 males and 12 females. The 125 and 350 mg/kg/day groups each consisted of six males and six females. After randomization into study groups, the animals were then randomized into two study replicates to allow for the reasonable conduct of the FOB and locomotor activity assessments. Each dose group and sex were equally represented within each study replicate. Animal no. 50292 was replaced by animal no. 50289 on study day -1 as animal no. 50292 died shortly after being handled for pretest clinical observations and weighing. The selected animals were approximately six weeks old at the initiation of dosing; body weight values ranged from 159 to 222 grams for the males and from 121 to 161 grams for the females. Several animals weighed less than the protocol-specified minimum weight (175 g for males, 125 g for females) at the initiation of dosing. This deviation had no impact on the outcome of the study as all animals were within the protocol-specified age range (4-8 weeks) at the initiation of dosing.

H. CLINICAL OBSERVATIONS AND SURVIVAL

The animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity. Clinical observations were performed on all animals at the time of dosing and approximately one to two hours following dosing (designated as one hour post-dosing for reporting purposes). During the 14-day recovery period, clinical observations were performed daily on the remaining six animals/sex in the control and high dose groups. Each animal received a detailed physical examination prior to the scheduled necropsy.

I. BODY WEIGHTS

Individual body weights were measured and recorded weekly beginning one week prior to test article administration (week -1). Mean body weight changes

were calculated for each study week. A final body weight was recorded for each animal on the day of scheduled necropsy.

J. FOOD CONSUMPTION

Individual food consumption was measured weekly beginning one week prior to test article administration (week -1). Food intake was calculated as g/animal/day for the corresponding body weight intervals.

K. FUNCTIONAL OBSERVATIONAL BATTERY

Functional observational battery (FOB) evaluations were performed on six animals/sex/group during the pretest week, the fourth week of test article administration and the last week of the recovery period (weeks -1, 3 and 5, respectively). The same six animals/sex in the control and 1000 mg/kg/day groups were used for each evaluation interval. Testing was performed by the same technicians without knowledge of the animal group assignment. The FOB was performed in a sound-proofed room equipped with a white noise generator set to operate at 70 ± 10 db, with the following exception. Home cage observations were performed in the animal room. All animals were observed for the following parameters as described below (refer to Appendix D for a detailed description of the scoring criteria used for each observation):

1. HOME CAGE OBSERVATIONS

Posture
Convulsions/Tremors
Feces consistency

Biting
Palpebral (eyelid) closure

2. HANDLING OBSERVATIONS

Ease of removal from cage
Lacrimation/Chromodacryorrhea
Piloerection
Palpebral closure
Red/Crusty deposits
Eye prominence

Ease of handling animal in hand
Salivation
Fur appearance
Respiratory rate/character
Mucous membranes/Eye/Skin color
Muscle tone

3. OPEN FIELD OBSERVATIONS (evaluated over a 2 minute observation period)

Mobility	Gait
Rearing	Arousal
Convulsions/Tremors	Urination/Defecation
Grooming	Gait score
Bizarre/Stereotypic behavior	Backing
Time to first step (seconds)	

4. SENSORY OBSERVATIONS

Approach response	Touch response
Startle response	Tail pinch response
Pupil response	Eyeblink response
Forelimb extension	Hindlimb extension
Air righting reflex	Olfactory orientation

5. NEUROMUSCULAR OBSERVATIONS

Hindlimb extensor strength	Grip strength-hind and forelimb
Hindlimb foot splay	Rotarod performance

6. PHYSIOLOGICAL OBSERVATIONS

Catalepsy	Body weight
Body temperature	

L. LOCOMOTOR ACTIVITY

Locomotor activity observations were performed on six animals/sex/group during the pretest week, the fourth week of test article administration and the last week of the recovery period, (weeks -1, 3 and 5, respectively). The same six animals/sex from the control and 1000 mg/kg/day groups were used for each evaluation interval. Locomotor Activity, recorded after the completion of the FOB, was measured automatically using the Digiscan 'Micro' Animal Activity System (Omnitech Electronics, Inc., Columbus, OH 43228). This is a personal computer-controlled system consisting of individual test chambers which utilize a series of infrared photobeams surrounding a clear plastic, rectangular cage to quantify an animal's motor activity. The testing of treatment groups was done according to replicate sequence. The activity system was operated in the Stagger Start mode of

operation. Data were collected in one-minute epochs and the test session was 41 minutes in duration for each animal. Animal placement into the activity cage in the Stagger Start mode of operation initiated the data collection process. The first epoch was often incomplete due to the placement of the animal in the activity cage. For this reason, the first minute of data was deleted. The remaining 40 minutes of data collection (4, 10 minute subsessions) were compiled for tabular presentation.

Locomotor Activity was divided into two categories: total and ambulatory activity. Total motor activity was defined as a combination of fine motor skills (i.e. grooming; interruption of one or two adjacent photobeams) and ambulatory motor activity (interruption of three or more consecutive photobeams). Refer to Appendix E for additional details on the motor activity methodology.

M. CLINICAL PATHOLOGY

Blood samples for clinical pathology evaluations were taken from the vena cava of all animals just prior to the scheduled necropsy. The animals were fasted overnight prior to necropsy and the collection of blood samples. Clinical pathology methods, procedures and references are presented in Appendix F.

1. HEMATOLOGY

The following hematologic evaluations were conducted on blood samples collected from all animals at the scheduled necropsies:

Total Leukocyte Count (White Cell)	Differential Leukocyte Count
Erythrocyte Count (Red Cells)	-Neutrophil
Hemoglobin	-Lymphocyte
Hematocrit	-Monocyte
Mean Corpuscular Volume (MCV)	-Eosinophil
Mean Corpuscular Hemoglobin (MCH)	-Basophil
Mean Corpuscular Hemoglobin Concentration (MCHC)	Prothrombin Time (Pro Time)
Platelet Count	Activated Partial Thromboplastin Time (APTT)
Platelet Estimate*	
RBC Morphology*	

* = Presented only on individual tables

() = Designates tabular abbreviation

2. SERUM CHEMISTRY

The following serum chemistry evaluations were conducted on blood samples collected from all animals at the scheduled necropsies.

Glucose	Gamma Glutamyltransferase (Glutamyl Transfer)
Blood Urea Nitrogen	Serum Aspartate Aminotransferase (Aspartat Transfer)
Blood Creatinine	Serum Alanine Aminotransferase (Alanine Transfer)
Total Protein	Serum Alkaline Phosphatase (Alkaline Phos'tse)
Albumin	Sodium
Albumin/Globulin Ratio (A/G Ratio)	Potassium
Globulin	Total Cholesterol (Cholesterol)
Calcium	
Phosphorus	
Chloride	
Total Bilirubin (Total Bili)	

() = Designates tabular abbreviation

N. PATHOLOGY

1. MACROSCOPIC EXAMINATION

A complete necropsy was conducted on all animals that were euthanized at the end of the dosing period or following a two week recovery period. All animals were euthanized by carbon dioxide asphyxiation and exsanguinated. The necropsy included, but was not limited to, examination of the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities including viscera. At the time of necropsy, the following tissues and organs were collected and preserved in 10% neutral buffered formalin:

Adrenals (2)*	Lymph node (mesenteric)*
Aorta	Mammary gland (females only)
Bone with marrow (sternbrae)*	Ovaries with oviducts (2)*
Bone marrow smear (from femur) ^a	Pancreas
Brain (forebrain, midbrain, hindbrain)*	Peripheral nerve (sciatic)*
Eyes with optic nerve (2)	Pituitary
Gastrointestinal tract	Prostate*
Esophagus	Salivary glands [submaxillary (2)]
Stomach*	Seminal vesicles (2)*
Duodenum*	Skeletal muscle (vasus medialis)
Jejunum*	Skin
Ileum*	Spinal cord (cervical, midthoracic, lumbar)*
Cecum*	Spleen*
Colon	Testes with epididymides (2) ^{ab}
Rectum	Thymus*
Heart*	Thyroids [with parathyroids if present (2)]
Kidneys (2)*	Trachea*
Liver (sections of two lobes)*	Urinary bladder*
Lungs [including bronchi, fixed by inflation with fixative (2)]*	Uterus with vagina*
Lymph node (mediastinal)*	All gross lesions*

^a = Bone marrow smears were obtained at the scheduled necropsy but were not placed in 10% neutral buffered formalin.

^b = The testes and epididymides were preserved in Bouin's solution.

2. ORGAN WEIGHTS

The following organs from animals euthanized at the week 4 primary and week 6 recovery necropsies were weighed:

Adrenals	Liver
Brain	Ovaries
Epididymides	Spleen
Heart	Testes
Kidneys	Thymus

Paired organs were weighed together. Organ to final body weight ratios were calculated.

3. MICROSCOPIC EXAMINATION

After fixation, specified tissues (designated with an asterisk in Section IV.N.1.) were sent to Colorado Histo-Prep, Inc., Fort Collins, Colorado, for

trimming and staining (hematoxylin-eosin). All tissues from the control and high dose group animals designated with an asterisk in Section IV.N.1 and the lungs, liver, kidneys, stomach and gross lesions from the low and mid dose group animals were examined microscopically. Microscopic examination of these tissues was conducted by Carney B. Jackson, D.V.M., B.S. An. Sci., D.A.C.V.P., D.A.C.V.P.M., Assistant Director of Pathology and Veterinary Medicine, WIL Research Laboratories, Inc.

O. STATISTICAL METHODS

All analyses were conducted using two-tailed tests for minimum significance levels of 1% and 5% comparing the treatment groups to the vehicle control group by sex. All means were presented with standard deviations (S.D.) and the numbers of sampling units (n) used to calculate the means. All statistical tests were performed by a Digital® MicroVAX® 3400 computer with appropriate programming. Analysis of body weight, body weight change, food consumption, clinical pathology values, continuous functional observational battery (FOB) data and absolute and relative organ weight data were subjected to a one-way analysis of variance, followed by Dunnett's test². Discontinuous (ordinal or descriptive) FOB data were analyzed using Fisher's exact test². Statistical tests for locomotor activity data were performed using a personal computer installed with SAS/STAT statistical software³. Clinical laboratory values for cell types that occur at a low incidence (i.e., monocytes, eosinophils and basophils) were not subjected to statistical analysis.

P. DATA RETENTION

The sponsor will have title to all documentation records, raw data, specimens or other work product generated during the performance of the study. All work product including raw paper data and specimens will be retained in the archives at WIL Research Laboratories, Inc., as per protocol.

Raw data in magnetic form, a retention sample of the test article and the original final report will be retained in the archives at WIL Research Laboratories, Inc., in compliance with regulatory requirements.

V. **RESULTS**

A. **CLINICAL OBSERVATIONS AND SURVIVAL**

Summary Data: Tables 1, 2, 3, 4, 5

Individual Data: Tables 57, 58, 59, 60, 61

All animals survived to the scheduled necropsies.

No clinical signs which could be attributed to the test article were observed at the clinical examinations performed at the time of dosing, one hour following dosing, during the recovery period or prior to the scheduled necropsies. Clinical findings in the treated groups occurred similarly in the control group and/or in a limited number of animals. No relationship to the test article was evident.

B. **BODY WEIGHTS**

Summary Data: Tables 6, 7

Individual Data: Tables 62, 63

Mean body weights in the treated groups were statistically comparable to the control group means throughout the dosing and recovery periods, with the following exception. Mean body weight in the 350 mg/kg/day group females was significantly ($p < 0.05$) increased on week 2. Mean body weight gains in all treated groups were statistically comparable to the control group values throughout the dosing period. During the recovery period (weeks 4-5 and 5-6), mean body weight gain in the 1000 mg/kg/day group males was reduced (statistically significant at $p < 0.01$ for week 4-5) in comparison to the male control group mean. However, these decreases were not attributed to treatment as no test article-related effects on body weight were noted during the dosing period. In the 1000 mg/kg/day group females, mean body weight gains were statistically comparable to the control group values during the recovery period.

C. **FOOD CONSUMPTION**

Summary Data: Table 8

Individual Data: Table 64

Food consumption, evaluated as g/animal/day, was not affected by test article administration at any dose level. The only statistically significant ($p < 0.05$ or

$p < 0.01$) differences from the control group were slight increases in food consumption in the 125 and/or 350 mg/kg/day group females during the first two weeks of test article administration. However, the values were comparable to their respective pretest values and decreases, rather than increases, in food consumption are generally considered to be toxicologically significant; no relationship to treatment was evident. All other food consumption values in the treated groups were similar to the control group values.

D. FUNCTIONAL OBSERVATIONAL BATTERY

1. HOME CAGE OBSERVATIONS

Summary Data: Tables 9, 10, 11, 12, 13, 14

Individual Data: Tables 65, 66, 67

Historical Control Data: Appendix G

No remarkable differences were apparent between the control and treated groups when the home cage observations were evaluated during study weeks -1 (pretest period), 3 and 5 (recovery period).

2. HANDLING OBSERVATIONS

Summary Data: Tables 15, 16, 17, 18, 19, 20

Individual Data: Tables 68, 69, 70

Historical Control Data: Appendix G

No remarkable differences were apparent between the control and treated groups when the handling observations were evaluated during study weeks -1 (pretest period), 3 and 5 (recovery period).

3. OPEN FIELD OBSERVATIONS

Summary Data: Tables 21, 22, 23, 24, 25, 26

Individual Data: Tables 71, 72, 73

Historical Control Data: Appendix G

No remarkable differences were apparent between the control and treated groups when the open field observations were evaluated during study weeks -1 (pretest period), 3 and 5 (recovery period).

4. SENSORY OBSERVATIONS

Summary Data: Tables 27, 28, 29, 30, 31, 32

Individual Data: Tables 74, 75, 76

Historical Control Data: Appendix G

No remarkable differences were apparent between the control and treated groups when the sensorimotor observations were evaluated during study weeks -1 (pretest period), 3 and 5 (recovery period).

5. NEUROMUSCULAR OBSERVATIONS

Summary Data: Tables 33, 34, 35, 36, 37, 38

Individual Data: Tables 77, 78, 79

Historical Control Data: Appendix G

No test article-related differences were apparent between the control and treated groups when the neuromuscular responses were evaluated during study weeks -1 (pretest period), 3 and 5 (recovery period). The only statistically significant ($p < 0.01$) differences from the control group were decreased hindlimb grip strengths in the 125 and 1000 mg/kg/day group females at the week 3 evaluation. However, similar values were observed for these groups during the pretest evaluation and the 1000 mg/kg/day group female mean at the week 5 recovery evaluation was comparable to the control group value; therefore, the decreases could not be attributed to the test article.

6. PHYSIOLOGICAL OBSERVATIONS

Summary Data: Tables 39, 40, 41

Individual Data: Tables 80, 81, 82

Historical Control Data: Appendix G

No remarkable differences were apparent between the control and treated groups when the physiological parameters were evaluated during study weeks -1 (pretest period), 3 and 5 (recovery period).

E. LOCOMOTOR ACTIVITY

Summary Data: Tables 42, 43, 44

Individual Data: Tables 83, 84, 85

Historical Control Data: Appendix H

No remarkable differences in mean ambulatory and total motor activity values in the 125, 350 and 1000 mg/kg/day group males and females were observed during study weeks -1 (pretest period), 3 and 5 (recovery period).

F. CLINICAL PATHOLOGY

1. HEMATOLOGY

Summary Data: Tables 45, 46, 47

Individual Data: Tables 86, 87, 88, 89, 90, 91

No test article-related effects on hematology parameters were observed at the week 4 primary or week 6 recovery evaluations. A slight increase in mean activated partial thromboplastin time (APTT) in the 1000 mg/kg/day group males was statistically significant ($p < 0.05$) when compared to the control group value at the week 4 evaluation. However, APTT increases of this magnitude are not considered to be toxicologically significant. A statistically significant ($p < 0.01$) decrease in mean prothrombin time occurred in the 1000 mg/kg/day group females at the week 4 evaluation. However, increases, rather than decreases, in prothrombin time are generally considered to be toxicologically significant. No other remarkable differences in hematology parameters were observed.

2. SERUM CHEMISTRY

Summary Data: Table 48

Individual Data: Table 92, 93

Historical Control Data: Appendix I

No adverse effects related to test article administration on serum chemistry parameters were observed at any dose level at the 28 day primary necropsy or the 42 day recovery necropsy.

Scattered instances of statistically significant differences between treated and control animals were detected for some serum chemistry parameters at the 28 day primary necropsy. These scattered statistical differences were not considered an adverse effect of the test article because the statistical differences occurred: in the absence of a dose response; in the absence of the accompanying clinical chemistry changes expected (i.e. the increase in total plasma protein in mid and high dose males and high dose females was not accompanied by an increase in hematocrit and total red blood cell count numbers); in the opposite direction from what occurs in a toxic state (i.e. the increase in globulin and albumin/globulin ratio in mid dose males and the increase in albumin in high dose females where a decrease in these parameters is indicative of toxicity); in a direction which is without physiologic significance (i.e. the decrease BUN and creatinine in mid dose females, the decrease in serum alanine aminotransferase and aspartate aminotransferase in low, mid and high dose males and mid and high dose males, respectively); due to interference with the laboratory method (i.e. the increase in serum chloride in mid and high dose males and in low, mid and high dose females - free bromine in the test article can artificially elevate chloride by reacting in the laboratory method as free chloride anions). Additionally, although detected as statistically different from concurrent control values, the absolute change in the measured parameters were small and within the range of historical control values for this species, strain, sex and laboratory (i.e. total plasma protein and the albumin/globulin ratio in mid dose males, cholesterol in mid and high dose females, serum glucose in high dose females, serum calcium in high dose females).

No statistically significant differences in serum chemistry parameters were detected between groups at the 42 day recovery necropsy.

G. PATHOLOGY

1. MACROSCOPIC EXAMINATION

Summary Data: Tables 49, 50

Individual Data: Tables 94, 95

No test article-related gross lesions were observed at the week 4 primary necropsy or at the week 6 recovery necropsy. Gross lesions in the control and treated groups occurred with similar frequency or were observed in single animals.

2. ORGAN WEIGHTS

Summary Data: Tables 51, 52, 53, 54

Individual Data: Tables 96, 97, 98, 99

No statistical significant differences in organ weight or organ to body weight ratios were detected between control and treated animals with one exception. Absolute liver weights were statistically significantly increased with respect to control means at the 28 day primary necropsy in males in the high dose and females in the mid and high dose. Liver to body weight ratios in mid and high dose males and low, mid and high dose females were statistically significantly increased at the 28 day necropsy. At the recovery necropsy, male absolute and liver to body weight ratio were statistically comparable to the control mean at the recovery necropsy whereas female absolute liver weights and liver to body weight ratio were statistically significantly increased with respect to control mean. The difference in absolute liver weights between control and treated females was less pronounced at the end of the recovery period, indicating the increase in liver weight was reversible in females as well as males. In the absence of test article related histopathologic and serum chemistry changes, increases in liver weight are considered an adaptive, rather than a toxic response, are not uncommon in the rat, and are most likely the result of microsomal induction.

3. MICROSCOPIC EXAMINATION

Summary Data: Tables 55, 56

Individual Data: Tables 94, 95

No test article-related histopathological lesions were observed at the week 4 primary or week 6 recovery necropsies. Lesions noted in the treated groups occurred in a limited number of animals, in a manner which was not dose-related, or with similar frequency in the control group.

VI. DISCUSSION AND CONCLUSIONS

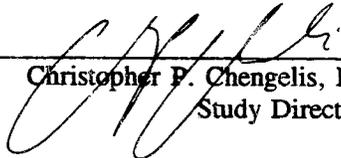
All animals survived to scheduled necropsy. No clinical signs related to test article administration were observed.

No test article-related effects on body weight, body weight gain, food consumption, functional observation battery or locomotor activity were found in either sex at any dose during either the 28 day dosing interval or during the 14 day recovery period.

No test article-related adverse effects on serum chemistry or hematology parameters were detected during the study. No test article-related gross or microscopic lesions were found at any dose level in either sex at either necropsy.

Absolute and relative organ weights were unaffected by test article administration with the exception of liver weight. Absolute liver weight was statistically increased at the 28 day necropsy in males of the high dose and in females at the mid and high dose; relative liver weights were increased in the mid and high dose males and in low, mid and high dose females. At the day 42 recovery necropsy, male absolute and relative liver weight were statistically comparable to the control mean. Female absolute and relative liver weight was statistically increased relative to the control mean at the recovery necropsy. This difference was less pronounced than at the 28 day necropsy, which indicates the increase in female liver was reversible as was the male liver weight. Increases in liver weight in the absence of test article-related changes in serum chemistry and histopathology are likely the result of microsomal induction, which is an adaptive response in the rat and not evidence of toxicity.

In conclusion, no evidence of systemic toxicity was observed at any dose level or time point. Based on the results of this study the NOAEL (No Observed Adverse Effect Level) of HBCD administered orally to male and female rats for 28 consecutive days was 1000 mg/kg/day.



Christopher P. Chengelis, Ph.D., D.A.B.T.
Study Director

13 Feb 97
Date

VII. KEY PERSONNEL AND REPORT SUBMISSION
Study Supervisors:

Kerin Clevidence, B.S.	Group Supervisor of Gross Pathology and Developmental Toxicology Laboratory
Sally A. Keets, A.S.	Manager of Vivarium
Gary R. Kiplinger, B.S.	Manager of General Toxicology
Ken D. Miller, B.A., M.T.(ASCP)	Senior Groups Supervisor - Clinical Pathology
Daniel W. Sved, Ph.D.	Director of Metabolism and Analytical Chemistry

Report Prepared By:



J. Paul DuBois, B.S.
Report Writer II

13 Feb 97
Date

Reviewed By:



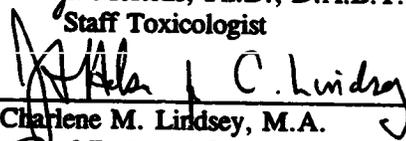
Carney B. Jackson, D.V.M., B.S. An. Sci.,
D.A.C.V.P., D.A.C.V.P.M.
Assistant Director of Pathology and Veterinary Medicine
Study Pathologist

13 Feb 97
Date



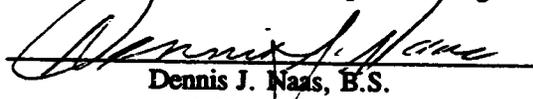
Jozef J.W.M. Mertens, Ph.D., D.A.B.T.
Staff Toxicologist

2/13/97
Date



Charlene M. Lindsey, M.A.
Manager of Technical Report Writing

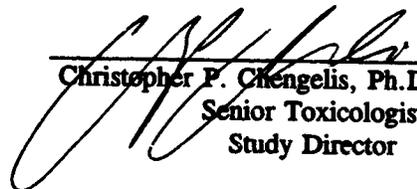
2/13/97
Date



Dennis J. Naas, B.S.
Assistant Director of Toxicology

2/13/97
Date

Approved and Submitted By:



Christopher P. Chengelis, Ph.D., D.A.B.T.
Senior Toxicologist
Study Director

13 Feb 97
Date

VIII. PATHOLOGIST SUMMARY STATEMENT

Study animals were pre-designated for euthanization either at the end of the dosing period (week 4) or following a two-week recovery period (week 6). All animals were euthanized by carbon dioxide asphyxiation and exsanguinated. A complete macroscopic examination was performed on each study animal that included, but was not limited to, the external surfaces, all orifices and cranial, thoracic, abdominal and pelvic cavities including viscera. No test article-related external or internal findings were noted at the week 4 primary or the week 6 recovery necropsies.

Specified tissues (refer to section IV.N.1.) were preserved in 10% neutral buffered formalin and prepared for microscopic evaluation by Colorado Histo-Prep, Inc., Fort Collins, Colorado. Microscopic examination of the tissues was performed by Carney B. Jackson, D.V.M., B.S. An. Sci., D.A.C.V.P., D.A.C.V.P.M., Assistant Director of Pathology and Veterinary Medicine, WIL Research Laboratories, Inc.

Findings (lesions) in the test article-treated groups occurred either in a limited number of animals, in a manner which was not dose-related or with similar frequency in the control group.

In conclusion, under the conditions of this study, no test article-related macroscopic or microscopic findings (lesions) were apparent at any dose level at the week 4 primary and week 6 recovery necropsies.

Carney B. Jackson

Carney B. Jackson, D.V.M., B.S. An. Sci.,
D.A.C.V.P., D.A.C.V.P.M.

Assistant Director of Pathology and Veterinary Medicine
Study Pathologist

13 Feb 97

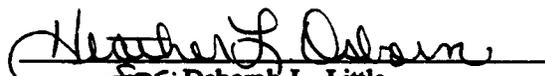
Date

IX. QUALITY ASSURANCE UNIT STATEMENT

<u>Date(s) of Inspection(s)</u>	<u>Phase Inspected</u>	<u>Date(s) Findings Reported to Study Director</u>	<u>Date(s) Findings Reported to Management</u>
5/17/96	Test Material Preparation and Analysis	5/17/96	6/20/96
5/28/96	Animal Care & Equipment	5/28/96	6/20/96
6/11/96	Functional Observational Battery	6/11/96	7/30/96
6/14/96	Blood Collection & Analysis	6/14/96	7/30/96
6/14/96	Necropsy	6/14/96	7/30/96
9/24/96	Study Records Vol. I-1	9/26/96	10/24/96
9/23, 24, 26, 27/96	Study Records Vols. I-2 & I-3	9/27/96	10/24/96
9/24/96	Study Records Vol. I-4	9/26/96	10/24/96
9/25/96	Study Records Vol. Rx-1	9/26/96	10/24/96
9/25/96	Study Records Vol. N-1	9/26/96	10/24/96
9/26/96	Study Records Vols. C-1, 2, 3	9/26/96	10/24/96
10/10/96	Study Records Vol. H/P-1	10/10/96	11/26/96
10/14/96	Draft Report (Appendix B only)	10/14/96	11/26/96
9/26, 10/3, 4, 6, 7, 14, 20, 21/96	Draft Report (w/o Appendix B)	10/21/96	11/26/96

The study was conducted and inspected in accordance with the United States EPA Good Laboratory Practice Regulations, the OECD Principles of Good Laboratory Practice, the Standard Operating Procedures of WIL Research Laboratories, Inc., and the protocol. Quality Assurance findings, derived from the inspections during the conduct of the study from the inspections of the raw data and the draft report, are documented and have been reported to the study director. A status report is submitted to management monthly.

The raw data, retention sample(s), if applicable, and the final report in the Archives at WIL Research Laboratories, Inc., or another location specified by the sponsor.


for: Deborah L. Little
Manager of Quality Assurance

2/13/97
Date

WIL-186004
CMA BFRIP

Colorado Histo-Prep, Inc.

Post Office Box 270733 • Fort Collins, CO 80527 • 970-493-2660

CH-P Study # 1948

Client's Study #WIL-186004

QUALITY ASSURANCE STATEMENT

This study has been conducted in accordance with the EPA Health Effects Test Guidelines (40 CFR Part 792) and the OECD GLP's, C(81) 30 (Final) Annex 2, including appropriate SOP's and designated recording requirements.

The study was inspected by a member of the Quality Assurance Unit and reported to the Project Director and Management as recorded below.

Comparisons were made between the raw data and the final report, and they were found to be in agreement. All records were reviewed to assure that any problems found during the course of a Quality Assurance Inspection were corrected and properly documented.

<u>PHASE REVIEWED</u>	<u>REVIEWED BY</u>	<u>DATE REVIEWED</u>	<u>PROJECT DIRECTOR</u>	<u>MANAGEMENT</u>
Tissue receipt in file	Aimee Kramer	07-12-96	10-18-96	10-18-96
Protocol reviewed	Aimee Kramer	07-11-96	10-18-96	10-18-96
Tissue list compared to protocol	Aimee Kramer	07-11-96	10-18-96	10-18-96
Animal numbers, sex, necropsy dates reviewed	Aimee Kramer	07-12-96	10-18-96	10-18-96
Trimming	Aimee Kramer	07-15-96	07-15-96	07-15-96
Embedding	Aimee Kramer	07-19-96	07-19-96	07-19-96
Microtomy	Aimee Kramer	07-25-96	07-25-96	07-25-96
Slides compared to blocks	Aimee Kramer	07-25-96	10-18-96	10-18-96
Slides compared to blocks	Aimee Kramer	07-30-96	10-18-96	10-18-96
Blocks compared to inventory	Aimee Kramer	07-30-96	10-18-96	10-18-96
Wet tissue compared to inventory	Aimee Kramer	10-18-96	10-18-96	10-18-96
Handwritten forms checked for writeovers	Aimee Kramer	07-29-96	10-18-96	10-18-96
Computer forms compared to CH-P animal forms	Aimee Kramer	07-30-96	10-18-96	10-18-96
Necropsy sheets compared to animal forms	Aimee Kramer	07-29-96	10-18-96	10-18-96
Slides returned	Aimee Kramer	07-26-96		
Slides returned	Aimee Kramer	07-30-96		
Blocks returned	Aimee Kramer	10-18-96		
Wet tissue finalized for shipment	Aimee Kramer	10-18-96		

WIL-186004
CMA BFRIP

Colorado Histo-Prep, Inc.

Post Office Box 270733 • Fort Collins, CO 80527 • 970-493-2660

The raw histology data consisting of paper records related to this study are archived at Colorado Histo-Prep, Inc., located at 319 Lincoln Court, Fort Collins, CO 80524. Raw data of blocks, slides, and wet tissues are temporarily stored at Colorado Histo-Prep, Inc. until forwarded to client.

All raw Quality Assurance data are archived at Colorado Histo-Prep, Inc., Fort Collins, CO 80524. Storage is in accordance with the EPA and OECD guidelines.


Aimee R. Kramer
Quality Assurance Mgr.

10/18/96
Date

WIL-186004
CMA BFRIP

X. REFERENCES

1. National Research Council (1996) Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences. National Academy Press, Washington, D.C.
2. BMDP (1979) Biomedical Computer Programs. (Dixon, W.J. and Brown, M.B., eds.) University of California Press, Berkeley, CA, pp. 612, 780, 781.
3. SAS (1991) SAS/STAT User's Guide, Version 6, 4th Edition. SAS Institute, Cary, North Carolina, 1028 pages.