Report Title: OECD Acute Inhalation Toxicity Evaluation

Test Type: Acute Respiratory Toxicity

Conducting Laboratory and Location: International Research and Development Corporation, Mattawau, Michigan

Test Substance(s): G0539.04 Dust aerosol at 4.4, 2.0 or 4.9 mg/L. The 2.0 and 4.9 aerosols were micronized to reduce particle size.

Species: Rat

# of Animals: 30 male and female rats (5 in each group)

Test Conditions: Rats were exposed for 4 hours to 4.4, 4.9 , or 2.0 mg/L of a dust aerosol of G0539.04. The 4.4 mg/L dosed group received neat material. The 2.0 and 4.9 mg/L dosed groups received micronized material as the neat material has a large particle size. Animals were observed for 14 days.

Results: The LC50 for inhalation exposure was estimated to be > 4.9 mg/L.

Study #: 191-1456
Report Date: 2/21/90
QA Statement/GLP compliance: Yes

Accession #: 35755

3

000215

#10

International Research
and Development Corporation
MATTAWAN MICHIGAN U.S.A. 49071 TELEPHONE (616) 668-3336

SPONSOR: The Procter & Gamble Company

TEST ARTICLE: G0539.04

SUBJECT: OECD Acute Inhalation Toxicity Evaluation

DATE OF SUBMISSION: February 21, 1990

191-1456

"credence through research"

TABLE OF CONTENTS

		Page
I.	Quality Assurance Statement	1
II.	Synopsis	2
III.	Objective	3
IV.	Test Material	4
V.	Experimental Design	5
VI.	Material and Methods	6
	A. Animals	6
	B. Test Material Administration	6
	1. Animal Exposure	6
	2. Generation of Exposure Atmosphere	7
	3. Analysis of Exposure Atmosphere	8
	a. Nominal Exposure Concentration	8
	b. Actual Exposure Concentration	8
	c. Aerosol Particle Size	9
	C. General Observations	9
	1. Appearance, Behavior and Mortality	9
	2. Body Weights	9
	D. Pathology	9
	1. Necropsy	9
VII.	Results	10
	A. Nominal Exposure Concentrations	10
	B. Actual Exposure Concentrations	10
	C. Aerosol Particle Size	10
	D. General Observations	11
	1. Appearance and Behavior	11
	2. Body Weights	11
	E. Pathology	12
	1. Necropsy and Macroscopic Observations	12

TABLE OF CONTENTS
(continued)

	Page
IX. Signatures	13
X. Conclusion	14
FIGURE NO.	
1-2. Schematic Diagram of Generation and Exposure System	15-16
3. Graphical Representation of Aerosol Particle Size for Group I	17
4. Graphical Representation of Aerosol Particle Size for Group II	18
5. Graphical Representation of Aerosol Particle Size for Group III	19
TABLE NO.	
1. Individual Sample Data	20
2-3. Summary of Clinical Findings	21-28
APPENDIX A	
Individual Clinical Findings	29-45
APPENDIX B	
Individual Body Weights	46-48
APPENDIX C	
Individual Post-Mortem Observations	49-51
APPENDIX D	
Protocol	52-63

International Research and Development Corporation

I. QUALITY ASSURANCE STATEMENT

Study Title: OECD Acute Inhalation Toxicity Evaluation
Test Article: G0539.04

This report has been reviewed by the International Research and Development Corporation Quality Assurance Department in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations of June 20, 1979 and as modified by the final rule effective October 5, 1987.

An inspection of the protocol for this study was conducted on May 8, 1989. A randomly sampled phase of the conduct of the study was inspected on May 19, 1989. Findings resulting from inspections, from a data audit, and from a review of the report were reported to management and the Study Director on November 5, 1989.

Approved By: Margery J. Wirth, B.S. — 2/20/90
Margery J. Wirth, B.S.
Director, Quality Assurance
Date

II. SYNOPSIS

Three groups of Sprague-Dawley derived albino rats (5 males and 5 females in each group) were exposed for 4 hours to a dust aerosol atmosphere of the G0539.04 test material at actual concentrations of 4.4, 4.9 or 2.0 mg/L. The equivalent aerodynamic diameters of the test material aerosols were 10 microns for the 4.4 mg/L exposure, 4.4 microns for the 4.9 mg/L exposure and 4.2 microns for the 2.0 mg/L exposure. One male and three females from the 4.9 mg/L group died either during the exposure or within 24 hours post-exposure. A high incidence of labored breathing and gasping was noted in the two groups exposed to the smaller particle size. Body weight gain was depressed except for females exposed to 2.0 mg/L. Macroscopic abnormalities observed at necropsy, white material in the trachea, gas filled intestines, red mottled lungs and dark kidneys, were only observed in those animals which died on study. No exposure related abnormalities were noted in those animals surviving 14 days. The LC_{50} was estimated to be equal-to-or-greater-than 4.9 mg/L.

III. OBJECTIVE

The objective of this study was to evaluate the acute toxicity of the experimental compound when administered via the inhalation route according to guidelines of the Organization of Economic Coordination and Development issued June, 1981.

IV. TEST MATERIAL

The test material was received from The Procter and Gamble Company, Cincinnati, Ohio.

Date received: 4-27-89

Amount received: 2067.8 g in two containers one of which was broken when received.

Label identification: G0539.04
BYO 874

IRDC number assigned: 10020

Date received: 5-19-89

Amount received: 2035.08 g

Label identification: G0539.04
BYO 874

IRDC number assigned: 10020B

V. EXPERIMENTAL DESIGN

Three groups of 5 male and 5 female rats each were used for this study. Each group was exposed for 4 hours to a dust aerosol atmosphere of the test material. One group was exposed to the test material as received. Since the particle size was outside the respirable range, two additional exposures were conducted with air-micronized test material.

The animals were observed for pharmacotoxic signs immediately after the exposure and then daily during a 14-day post-exposure observation period. Body weights were recorded just before exposure and on days 7 and 14 post-exposure. At the end of the post-exposure observation period, the animals were sacrificed and subjected to gross necropsy in which major organs in the abdominal and thoracic cavities were observed for macroscopic abnormalities.

The following table summarizes the experimental design:

Group Number	Desired Exposure Conditions	Number of Animals	
		Males	Females
I	5 mg/L neat material	5	5
II	5 mg/L micronized material	5	5
III	2.5 mg/L micronized material	5	5

This study was initiated on May 5, 1989 with the exposure of Group I and terminated on July 24, 1989 with the necropsy of Group III.

VI. MATERIAL AND METHODS

A. ANIMALS

Sprague-Dawley derived (Charles River CD®) albino rats were received from the Charles River Laboratories (Portage, Michigan). Animals were individually caged in suspended wire-mesh cages throughout the study. All animals were quarantined for at least 7 days. Each rat was individually identified with a numbered Monel® metal ear tag. During the quarantine and post-exposure periods, the rats were housed in rooms where temperature, relative humidity and photoperiod (12 hours light/12 hours dark) were controlled, in accordance with standards outlined in the "Guide for the Care and Use of Laboratory Animals" (DHEW No. (N.I.H.) 85-23, 1985). Purina Certified Pelleted Rodent Chow® #5002 and tap water were available *ad libitum*, except during actual exposures.

The age at time of exposure, purchase order number, and date of receipt for the animals are tabulated below:

Group Number	Sex	Age at Time of Exposure (days)	Purchase Order No.	Date Received
I	Male	46	1551	4-24-89
	Female	46	1551	4-24-89
II	Male	55	1843	6-16-89
	Female	55	1843	6-16-89
III	Male	55	1852	6-27-89
	Female	55	1852	6-27-89

B. TEST MATERIAL ADMINISTRATION

1. Animal Exposure

Animals were exposed in either a 160 L stainless steel and glass chamber (Group I) or a 54 L all glass chamber (Groups II and

III). The supply air for chamber ventilation was dry, filtered air from the in-house compressed air system, or from tanks of compressed breathing air. The chamber exhaust was discharged into a fume hood. Chamber temperature, relative humidity and air flow rate were recorded at intervals of approximately one-half hour during the exposure. The results of the measurements are presented below:

Group Number	Chamber Air Flow Rate (L/min)		Temperature (°C)		Relative Humidity (%)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
I	110	0.0	22	0.5	42	2.5
II	98	0.0	21	2.4	-	-
III	98	0.0	21	0.0	-	-

Humidity measurements for Groups II and III could not be obtained since the high test material concentrations obscured the humidity gauge.

2. Generation of Exposure Atmospheres

Figure 1 presents a schematic diagram of a typical generation and exposure system used for Group I. The system operated as follows: test material was dispensed at a known and constant rate by the auger dust feed to an aspirator-dispersion device. The dust, entrained in a high velocity air stream, converges with a counter-current air flow at the device outlet, where deagglomeration and dispersion into the chamber occurred. Operating parameters for this generation system were as follows:

Group Number	Auger Diameter (CM)	Auger Speed (RPM)	Counter-Current Air Flow (L/min)	Total Chamber Air Flow (L/min)
I	1.3	19	15	110

Figure 2 presents a schematic diagram of a typical generation and exposure system used for Groups II and III. The system operated as follows: Test material was dispensed at a known and constant rate by an auger dust feed to an air-micronizer (Fluid Energy, Model 00). Dust was drawn into the micronizer by an aspirator, recirculated in the grinding chamber where particle-to-particle impact reduced the size of the dust until the size was small enough to follow the air flow out of the micronizer. The resulting aerosol was piped to the exposure chamber. Operating parameters for this generation system were as follows:

Group Number	Auger Diameter (CM)	Auger Speed (RPM)	Aspirator Pressure (psig)	Grinding Pressure (psig)	Dilution Air Flow (L/min)
II	1.3	10-27	36	90	-
III	1.3	1-6	36	90	-

3. Analysis of Exposure Atmospheres

a. Nominal Exposure Concentration

Exposure concentrations were determined on a nominal basis by dividing the weight of test material used during the exposure by the total volume of air that flowed through the exposure chamber during the exposure.

b. Actual Exposure Concentrations

Exposure concentrations were determined using standard gravimetric methods. Samples of the aerosol atmosphere were collected on 25 mm glass-fiber filters, held in open face filter holders. Samples were drawn through the filters at a flow rate of 2.6 or 2.9 L/min for 3 minutes. Each filter was weighed prior to and again after sample collection. The concentration was calculated as the difference in filter weight divided by the total sample volume.

c. Aerosol Particle Size

Particle size distribution of the test material aerosol was determined with an Andersen® 8-stage cascade impactor. The chamber atmosphere was sampled at a rate of 28.3 L/min for a suitable duration, and the amount of aerosol collected on each stage was determined gravimetrically. The cumulative weight percent of particles with aerodynamic diameters smaller than the stage cut-off values were derived and plotted by computer. The Equivalent Aerodynamic Diameter (EAD) and Geometric Standard Deviation (GSD) were calculated by a method similar to that described by Raabe (Environ. Sci. Technol. 2:1162-1167, 1978).

C. GENERAL OBSERVATIONS

1. Appearance, Behavior and Mortality

Animals were observed for pharmacotoxic signs immediately after the exposure. The animals were observed twice daily during the 14-day post-exposure period, once for pharmacotoxic signs and once for mortality only.

2. Body Weights

Body weights were recorded before the exposure and at 7 and 14 days post-exposure.

D. PATHOLOGY

1. Necropsy

All animals that died, or were sacrificed at termination were subjected to a necropsy. Sacrifice was accomplished by intraperitoneal injection of sodium pentobarbitol followed by exsanguination from the abdominal aorta. The tracheas were exposed and clamped so the lungs could be removed and observed in an inflated state. All major organs in the abdominal and thoracic cavities were observed for macroscopic abnormalities by trained prosectors. Carcasses were discarded.

VII. RESULTS

A. NOMINAL EXPOSURE CONCENTRATIONS

Results of the determinations of the nominal exposure concentrations are shown in the following table:

Group Number	Weight of Test Material (g) Pre-Exposure	Post-Exposure	Difference	Total Volume of Air (L)	Nominal Exposure Concentration (mg/L)
I	1088	127	961	26400	36
II	323.8	26.1	297.7	23520	12.7
III	223.7	107.6	116.1	23520	4.9

B. ACTUAL EXPOSURE CONCENTRATIONS

Results of the determinations of the actual exposure concentrations are shown in the following table:

Group Number	Concentration (mg/L)	Standard Deviation
I	4.4 neat	1.0
II	4.9 nucronized	3.1
III	2.0 nucronized	1.3

Table 1 presents individual sample data.

C. AEROSOL PARTICLE SIZE

The following table presents the aerosol particle size data in terms of the Equivalent Aerodynamic Diameter (EAD) and the Geometric Standard Deviation (GSD):

Group Number	EAD (mcm)	GSD
I	10	2.10
II	4.4	2.20
III	4.2	2.07

Figures 3, 4 and 5 present graphical representations of the aerosol particle size data.

D. GENERAL OBSERVATIONS

1. Appearance and Behavior

Table 2 presents a summary of pharmacotoxic signs observed immediately after exposure for males and females, respectively. Table 3 presents a summary of pharmacotoxic signs observed during the 14-day post-exposure period for males and females, respectively. Individual animal data for both observation periods times can be found in Appendix A.

Two animals (one male and one female) from Group II were found dead immediately after the exposure. Two additional females from Group II were found dead on the day after the exposure. All animals from groups I and III survived to study termination.

The most significant pharmacotoxic signs noted, either immediately after exposure or during the 14-day post-exposure period, were gasping and labored breathing noted in all groups, and corneal opacities noted in Group I.

2. Body Weights

The individual and group mean (±S.D.) body weights are presented in Appendix B. The following table summarizes this data:

Group Number	Sex	Pre-Exposure	Days Post-Exposure 7	14
I	M	215	209	262
	F	154	158	188
II	M	275	252	290
	F	186	185	201
III	M	292	301	339
	F	185	202	221

Except for Group III females, which gained body weight normally throughout the study, mean body weights for both males and females were lower at both post-exposure intervals than expected based on historical data.

E. PATHOLOGY

1. Necropsy and Macroscopic Observations

Macroscopic observations at necropsy are presented in Appendix C.

No exposure related abnormalities were noted in any animals from Groups I and III, or those animals from Group II which survived until termination at 14 days post-exposure. Animals from Group II which died on study exhibited various abnormalities including white material in the trachea (probably test material or edema fluid), gas filled intestines, red mottled lungs and dark kidneys.

IX. SIGNATURES

Laboratory Supervisor: _____ 2-20-90
Benjie A. Culp
Unit Supervisor
Inhalation Toxicology
Date

Reviewed By: _____ 2-20-90
John G. Drummond, Ph.D.
Manager, Inhalation Toxicology
Date

X. CONCLUSION

The test material G0539.04 was acutely toxic to rats at a concentration of 4.9 mg/L (aerosol particle size of 4.4 microns). The LC_{50} was probably slightly greater than 4.9 mg/L.

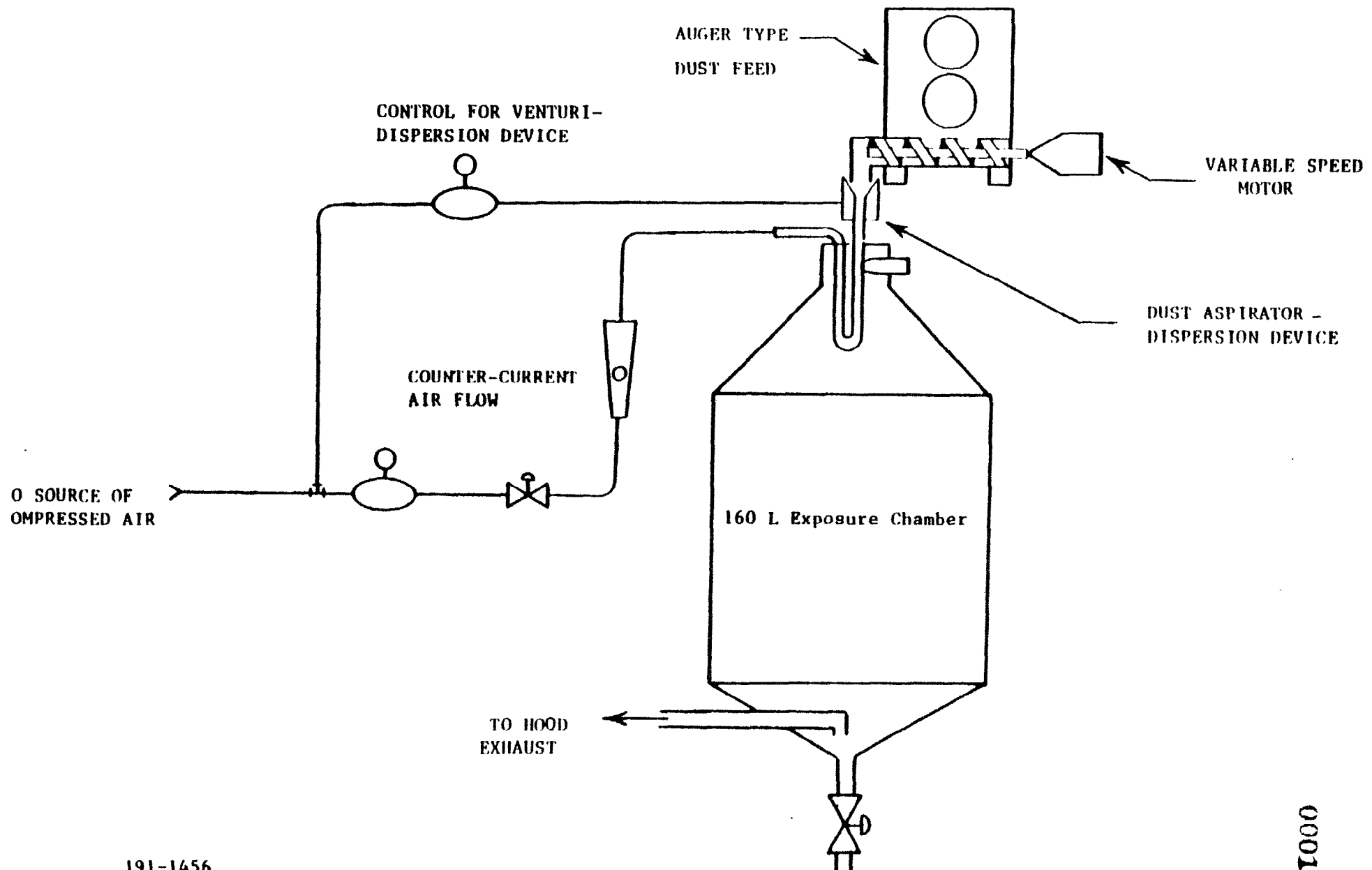
To the best of the signers' knowledge, there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality or integrity of the study. This study was conducted in conformance with the Good Laboratory Practice Regulations. This report accurately reflects the raw data obtained during the performance of the study.

C. E. Ulrich

Charles E. Ulrich, B.S.
Scientific Director, Inhalation Toxicology
Study Director

2-20-90
Date

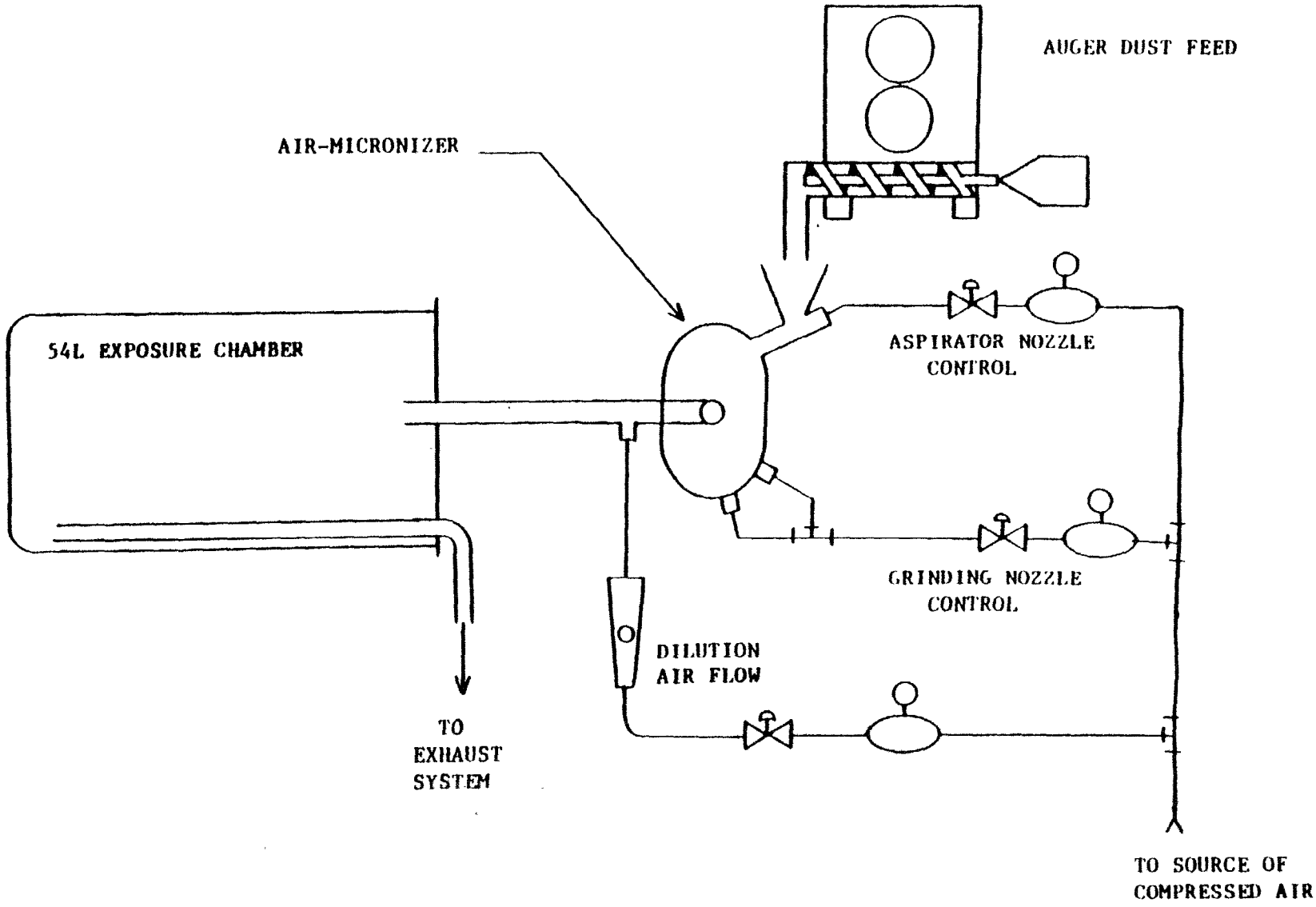
FIGURE NO. 1. - SCHEMATIC DIAGRAM OF GENERATION AND EXPOSURE SYSTEM



191-1456

000132

FIGURE 2. - SCHEMATIC DIAGRAM OF GENERATION AND EXPOSURE SYSTEM



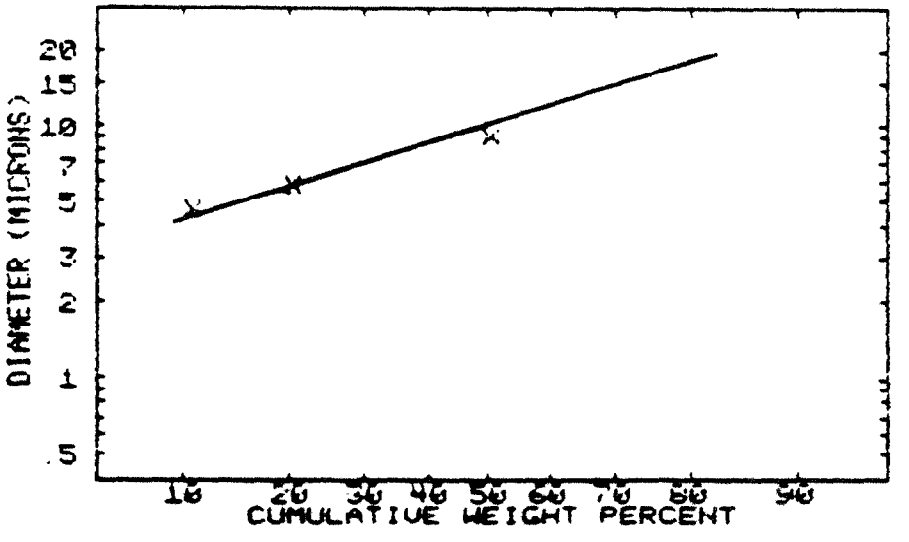
191-1456

000133

FIGURE 3. - Graphical Representation of Aerosol Particle Size for Group I

PARTICLE SIZE DISTRIBUTION FOR G0539.04
STUDY NUMBER : 191-1456
GROUP I, SAMPLE COLLECTED ON 5-5-89

STAGE NUMBER	ECD (MICRONS)	WEIGHT PERCENT	CUMULATIVE WEIGHT PERCENT
0	9	49.7	50.3
1	5.8	30.1	20.3
2	4.7	9.5	10.7
3	3.3	6.4	4.4
4	2.1	2.7	1.6
5	1.05	1.1	0.5
6	.62	0.3	0.3
7	.44	0.3	0.0
FILTER	<.44	0.0	0.0



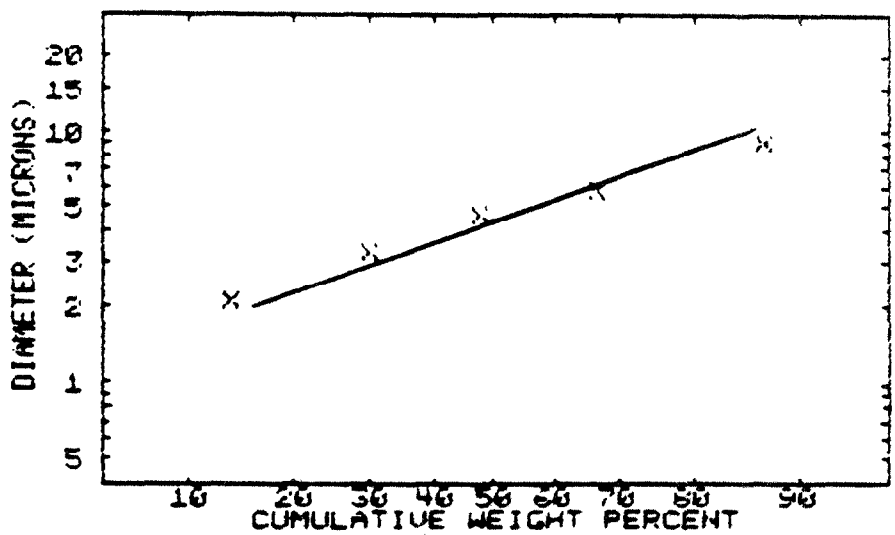
THE EQUIVALENT AERODYNAMIC DIAMETER IS 10 MICRONS
THE GEOMETRIC STANDARD DEVIATION IS 2.1

ECD = EFFECTIVE CUTOFF DIAMETER

FIGURE 4. - Graphical Representation of Aerosol Particle Size for Group II

PARTICLE SIZE DISTRIBUTION FOR G0539.04
STUDY NUMBER : 191-1456
GROUP II, SAMPLE COLLECTED ON 6-29-89

STAGE NUMBER	ECD (MICRONS)	WEIGHT PERCENT	CUMULATIVE WEIGHT PERCENT
0	9	12.8	87.2
1	5.8	20.6	66.6
2	4.7	18.9	47.7
3	3.3	17.8	29.9
4	2.1	16.4	13.5
5	1.05	8.6	4.8
6	.62	3.2	1.6
7	.44	0.9	0.6
FILTER	<.44	0.6	0.0



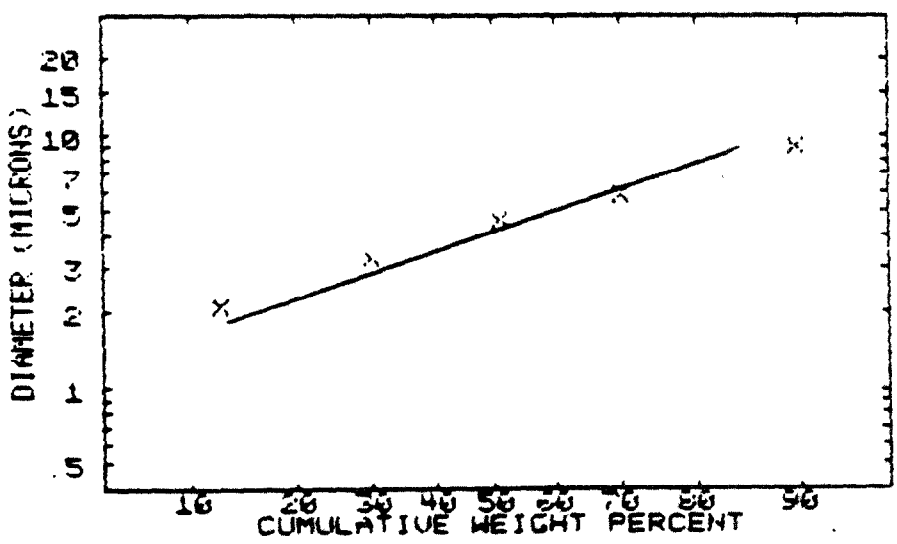
THE EQUIVALENT AERODYNAMIC DIAMETER IS 4.4 MICRONS
THE GEOMETRIC STANDARD DEVIATION IS 2.2

ECD = EFFECTIVE CUTOFF DIAMETER

FIGURE 5. - Graphical Representation of Aerosol Particle Size for Group III

PARTICLE SIZE DISTRIBUTION FOR G0539.04
STUDY NUMBER : 191-1456
GROUP III, SAMPLE COLLECTED ON 7-10-89

STAGE NUMBER	ECD (MICRONS)	WEIGHT PERCENT	CUMULATIVE WEIGHT PERCENT
0	9	10.3	89.7
1	5.8	19.4	70.3
2	4.7	19.4	50.8
3	3.3	21.1	29.7
4	2.1	17.3	12.4
5	1.05	8.0	4.5
6	.62	2.9	1.5
7	.44	1.3	0.3
FILTER	<.44	0.3	0.0



THE EQUIVALENT AERODYNAMIC DIAMETER IS 4.2 MICRONS
THE GEOMETRIC STANDARD DEVIATION IS 2.07

ECD = EFFECTIVE CUTOFF DIAMETER

TABLE 1. Individual Sample Data

Group Number	Sample Number	Weight Collected on Filter (mg)	Actual Exposure Concentration (mg/L)
I	1	17.4	3.0
	2	47.4	5.4
	3	39.0	4.5
	4	39.7	4.6
II	1	26.6	3.4
	2	37.3	4.8
	3	34.3	4.4
III	1	32.3	3.7
	2	6.4	0.7
	3	12.4	1.4
	4	20.1	2.3

TABLE 2.

Summary of Clinical Findings
MALES

Observation	Interval: 1a - 1a Day Group I Day of Exposure (5)	Group II Day of Exposure (5)	Group III Day of Exposure (5)
APPEARANCE AND CONDITION			
Found dead	0	1	0
BODY SURFACE			
Urine stained abdomen	0	4	0
RESPIRATION			
Gasping	0	2	1
Labored breathing	2	2	5

191 1456

( ) = Number of animals observed at start of interval
a = Immediately following exposure
a = Immediately following exposure

000135

TABLE 2. Cont.

Summary of Clinical Findings
MALES

Observation	Interval: 1a - 1a Day Group I Day of Exposure (5)	Group II Day of Exposure (5)	Group III Day of Exposure (5)
ORAL/NASAL			
Increased salivation	0	0	3
EYES			
Material around eye	5	0	0

191 1456

( ) = Number of animals observed at start of interval
a = Immediately following exposure
a = Immediately following exposure

0:0139

TABLE 2. Cont.

Summary of Clinical Findings
FEMALES

Observation	Interval; 1a - 1a Day Group I Day of Exposure (5)	Group II Day of Exposure (5)	Group III Day of Exposure (5)
APPEARANCE AND CONDITION			
Found dead	0	1	0
Prostration	0	1	0
RESPIRATION			
Gasping	0	4	1
Labored breathing	4	4	5
ORAL/NASAL			
Increased salivation	0	0	3
Material around nose	0	0	1

191 1456

( ) = Number of animals observed at start of interval
a = Immediately following exposure
a = Immediately following exposure

0.0140

TABLE 2. Cont.

Summary of Clinical Findings
FEMALES

Observation	Interval: 1a - 1a Day Group I Day of Exposure (5)	Group II Day of Exposure (5)	Group III Day of Exposure (5)
EYES			
Material around eye	4	0	1
Eyes pale	0	0	2

191-1458

( ) = Number of animals observed at start of interval
a = Immediately following exposure
a = Immediately following exposure

TABLE 3.

Summary of Clinical Findings
MALES

Observation	Interval: 1 - 14 Day Group I Post Exposure (5)	Group II Post Exposure (4)	Group III Post Exposure (5)
APPEARANCE AND CONDITION			
No visible abnormalities for entire interval	0	0	0
Portion external ear missing	1	0	0
BODY SURFACE			
Alopecia	5	4	1
Urine stained abdomen	5	4	5
RESPIRATION			
Gasping	0	1	0
Labored breathing	0	4	3

191 1456 ( ) = Number of animals observed at start of interval

TABLE 3. Cont.

Summary of Clinical Findings
MALES

Observation	Interval: 1 - 14 Day Group I Post Exposure (5)	Group II Post Exposure (4)	Group III Post Exposure (5)
ORAL/NASAL			
Increased salivation	0	2	0
Material around mouth	5	4	5
Material around nose	5	4	5
EYES			
Corneal abnormality	1	0	0
Material around eye	5	4	4
Eyes pale	0	3	0
Eye closed	2	2	0
Corneal opacity	2	0	0

191 1456 ( ) = Number of animals observed at start of interval

TABLE 3. Cont.

Summary of Clinical Findings
FEMALES

Observation	Interval: 1 - 14 Day Group I Post Exposure (5)	Group II Post Exposure (4)	Group III Post Exposure (5)
APPEARANCE AND CONDITION			
No visible abnormalities for entire interval	0	0	0
Portion external ear missing	1	0	0
Died prior to first det obs	0	2	0
BODY SURFACE			
Alopecia	5	2	5
Scabbed area	0	0	1
Sore	1	0	0
Urine stained abdomen	5	2	5

191 1456

( ) = Number of animals observed at start of interval
det obs = detailed observation

0001.44

TABLE 3. Cont.

Summary of Clinical Findings
FEMALES

Observation	Interval: 1 - 14 Day Group I Post Exposure (5)	Group II Post Exposure (4)	Group III Post Exposure (5)
RESPIRATION			
Labored breathing	1	2	5
ORAL/NASAL			
Material around mouth	5	2	5
Material around nose	5	2	5
EYES			
Material around eye	5	2	5
Eyes pale	0	2	3
Eye closed	3	0	1
Corneal opacity	1	0	0
Lacrimation	2	0	0

191 1456

( ) = Number of animals observed at start of interval

APPENDIX A

Individual Clinical Findings

det = detailed

Individual Clinical Findings
Male

Group, Rat Number		Day of Study Onset	Day of Study Duration	Frequency
Group 1 Day of Exposure:				
58856	Material around eye, dry, red, both	1a	1a	1
58857	Material around eye, red, both	1a	1a	1
58858	Labored breathing	1a	1a	1
	Material around eye, dry, red, both	1a	1a	1
58859	Material around eye, dry, red, both	1a	1a	1
58860	Labored breathing	1a	1a	1
	Material around eye, dry, red, both	1a	1a	1

191 1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = Immediately following exposure

0001.17

Individual Clinical Findings
Female

Group, Rat Number		Day of Study Onset	Day of Study Duration	Frequency
Group I Day of Exposure:				
58861	Labored breathing	1a	1a	1
58862	Material around eye, dry, red, both	1a	1a	1
58863	Labored breathing	1a	1a	1
	Material around eye, dry, red, both	1a	1a	1
58864	Labored breathing	1a	1a	1
	Material around eye, dry, red, both	1a	1a	1
58865	Labored breathing	1a	1a	1
	Material around eye, dry, red, both	1a	1a	1

191 1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = Immediately following exposure

0001.19

Individual Clinical Findings
Male

Group, Rat Number		Day of Study Onset	Duration	Frequency
Group II Day of Exposure:				
59051	Urine stained abdomen	1a	1a	1
	Labored breathing	1a	1a	1
59052	Urine stained abdomen	1a	1a	1
	Labored breathing	1a	1a	1
59053	Urine stained abdomen	1a	1a	1
	Gasping	1a	1a	1
59054	Found dead	1a	1a	1
59055	Urine stained abdomen	1a	1a	1
	Gasping	1a	1a	1

191-1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = Immediately following exposure

Individual Clinical Findings
Female

Group, Rat Number		Day of Study Onset	Duration	Frequency
Group II Day of Exposure:				
59056	Prostration	1a	1a	1
	Gasping	1a	1a	1
	Labored breathing	1a	1a	1
59057	Gasping	1a	1a	1
	Labored breathing	1a	1a	1
59058	Gasping	1a	1a	1
	Labored breathing	1a	1a	1
59059	Found dead	1a	1a	1
59060	Gasping	1a	1a	1
	Labored breathing	1a	1a	1

191 1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = Immediately following exposure

000150

Individual Clinical Findings
Male

Group, Rat Number		Day of Study Onset	Day of Study Duration	Frequency
Group III Day of Exposure:				
59081	Labored breathing	1a	1a	1
	Increased salivation	1a	1a	1
59082	Labored breathing	1a	1a	1
59083	Labored breathing	1a	1a	1
	Increased salivation	1a	1a	1
59084	Gasping	1a	1a	1
	Labored breathing	1a	1a	1
	Increased salivation	1a	1a	1
59085	Labored breathing	1a	1a	1

191 1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = Immediately following exposure

000151

Individual Clinical Findings
Female

Group, Rat Number		Day of Study Onset	Duration	Frequency
Group III Day of Exposure:				
59086	Labored breathing	1a	1a	1
	Increased salivation	1a	1a	1
59087	Gasping	1a	1a	1
	Labored breathing	1a	1a	1
59088	Labored breathing	1a	1a	1
	Material around nose, brown	1a	1a	1
	Increased salivation	1a	1a	1
	Material around eye, brown, left	1a	1a	1
59089	Labored breathing	1a	1a	1
	Eyes pale, both	1a	1a	1
59090	Labored breathing	1a	1a	1
	Increased salivation	1a	1a	1
	Eyes pale, both	1a	1a	1

191 1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = Immediately following exposure

Individual Clinical Findings
Male

Group, Rat Number		Day of Study Onset - Duration	Frequency
Group 1 Post Exposure:			
58856	Urine stained abdomen	1 - 9	9
	Alopecia, both eyes	11 - 14	4
	Alopecia, nose	11 - 14	4
	Alopecia, mouth	11 - 14	4
	Material around mouth, brown	1 - 10	10
	Material around nose, brown	1 - 10	10
	Corneal abnormality	1 - 2	2
	Material around eye, dry, brown, both	1 - 10	10
	Eye closed, right	1 - 1	1
	Corneal opacity, right	2 - 2	1
58857	Urine stained abdomen	1 - 9	9
	Alopecia, both eyes	11 - 14	4
	Alopecia, nose	11 - 14	4
	Alopecia, mouth	11 - 14	4
	Material around mouth, brown	1 - 10	10
	Material around nose, brown	1 - 10	10
	Material around eye, dry, brown, both	1 - 10	10
	Eye closed, right	1 - 1	1
	Corneal opacity, left	1 - 2	2
58858	Portion external ear missing, right	12 - 14	3
	Urine stained abdomen	1 - 9	9
	Alopecia, both eyes	11 - 14	4
	Alopecia, nose	11 - 14	4
	Alopecia, mouth	11 - 14	4
	Material around mouth, brown	1 - 10	10
	Material around nose, brown	1 - 10	10
	Material around eye, dry, brown, both	1 - 10	10
58859	Urine stained abdomen	1 - 9	9
	Alopecia, nose	11 - 14	4
	Alopecia, mouth	11 - 14	4
	Material around mouth, brown	1 - 10	10
	Material around nose, brown	1 - 10	10
	Material around eye, dry, brown, tan	1 - 10	10

191-1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

Individual Clinical Findings
Male

Group, Rat Number		Day of Study Onset - Duration	Frequency
Group I Post Exposure:			
58860	Urine stained abdomen	1 - 9	9
	Alopecia, both eyes	11 - 14	4
	Alopecia, nose	11 - 14	4
	Alopecia, mouth	11 - 14	4
	Alopecia, right lateral abdomen	13 - 14	2
	Alopecia, left lateral abdomen	13 - 14	2
	Material around mouth, brown	1 - 10	10
	Material around nose, brown	1 - 10	10
	Material around eye, brown	1 - 11	11

191 1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

000153

Individual Clinical Findings
Female

Group, Rat Number		Day of Study Onset	-	Duration	Frequency
Group I Post Exposure:					
58861	Urine stained abdomen	1	-	9	9
	Alopecia, dorsal head	5	-	12	8
	Alopecia, both eyes	11	-	14	4
	Alopecia, abdominal	11	-	14	4
	Alopecia, nose	11	-	14	4
	Alopecia, mouth	11	-	14	4
	Alopecia, ventral thorax	14	-	14	1
	Material around mouth, brown	1	-	10	10
	Material around nose, brown	1	-	10	10
	Material around eye, brown, both	1	-	9	9
	Eye closed, left	1	-	2	2
58862	Urine stained abdomen	1	-	6	6
	Alopecia, both eyes	11	-	14	4
	Material around mouth, brown	1	-	10	10
	Material around nose, brown	1	-	10	10
	Material around eye, brown, both	1	-	9	9
58863	Urine stained abdomen	1	-	10	10
	Alopecia, both eyes	10	-	14	5
	Alopecia, nose	11	-	14	4
	Alopecia, abdominal	11	-	14	4
	Alopecia, mouth	11	-	14	4
	Alopecia, ventral neck	11	-	14	4
	Labored breathing	4	-	4	1
	Material around mouth, brown	1	-	10	10
	Material around nose, brown	1	-	10	10
	Lacrimation	1	-	5	4
	Material around eye, brown, both	1	-	11	11
	Corneal opacity, left	1	-	9	8
	Eye closed, right	2	-	5	4
58864	Urine stained abdomen	1	-	9	9
	Alopecia, ventral neck	7	-	14	8
	Alopecia, both eyes	11	-	14	4
	Alopecia, nose	11	-	14	4
	Alopecia, mouth	11	-	14	4
	Alopecia, anogenital region	13	-	14	2

191-1456

Onset - Day first observed
Duration = Day last observed
Frequency = Number of days observed

000155

Individual Clinical Findings
Female

Group, Rat Number			Day of Study Onset	-	Duration	Frequency
Group I Post Exposure:						
58864	(Continued)	Material around mouth, brown	1	-	10	10
		Material around nose, brown	1	-	10	10
		Lacrimation	1	-	1	1
		Material around eye, brown	1	-	9	9
58865		Portion external ear missing, right	14	-	14	1
		Urine stained abdomen	1	-	9	9
		Sore, right forelimb	7	-	9	3
		Alopecia, nose	10	-	14	5
		Alopecia, abdominal	10	-	14	5
		Alopecia, mouth	10	-	14	5
		Alopecia, ventral neck	10	-	14	5
		Alopecia, both forelimbs	11	-	14	4
		Alopecia, right inguinal	13	-	14	2
		Alopecia, left inguinal	13	-	14	2
		Alopecia, anogenital region	13	-	14	2
		Material around mouth, brown	1	-	10	10
		Material around nose, brown	1	-	10	10
		Material around eye, brown, both	1	-	9	9
		Eye closed, both	1	-	1	1

191 1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

Individual Clinical Findings
Male

Group, Rat Number		Day of Study Onset	-	Duration	Frequency
Group II Post Exposure:					
59051	Urine stained abdomen	1	-	10	10
	Alopecia, nose	11	-	14	4
	Alopecia, mouth	11	-	14	4
	Gasping	1	-	1	1
	Labored breathing	1	-	8	8
	Material around mouth, brown	1	-	11	11
	Material around nose, brown	1	-	11	11
	Increased salivation	1	-	1	1
	Material around eye, dry, black, both	1	-	10	10
	Eyes pale, left	1	-	11	8
	Eye closed, right	1	-	2	2
	Eyes pale, both	3	-	5	3
59052	Urine stained abdomen	1	-	12	12
	Alopecia, anterior dorsal region	7	-	14	8
	Alopecia, nose	11	-	14	4
	Alopecia, mouth	11	-	14	4
	Alopecia, ventral neck	12	-	14	3
	Alopecia, abdominal	13	-	14	2
	Labored breathing	1	-	7	7
	Material around mouth, brown	1	-	10	10
	Material around nose, brown	1	-	10	10
	Increased salivation	1	-	1	1
	Material around eye, dry, black, both	1	-	7	7
59053	Urine stained abdomen	1	-	10	10
	Alopecia, nose	11	-	14	4
	Alopecia, mouth	11	-	14	4
	Alopecia, anterior dorsal region	14	-	14	1
	Labored breathing	1	-	8	8
	Material around mouth, brown	1	-	10	10
	Material around nose, brown	1	-	10	10
	Material around eye, brown, both	1	-	6	6
	Eyes pale, left	1	-	2	2
	Eye closed, right	1	-	2	2
	Eyes pale, both	3	-	11	9

191-1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

000157

Individual Clinical Findings
Male

Group, Rat Number		Day of Study Onset - Duration	Frequency
Group II Post Exposure:			
59u55	Urine stained abdomen	1 - 10	10
	Alopecia, nose	11 - 14	4
	Alopecia, mouth	11 - 14	4
	Labored breathing	1 - 7	7
	Material around mouth, brown	1 - 10	10
	Material around nose, brown	1 - 10	10
	Material around eye, dry, black, both	1 - 6	6
	Eyes pale, both	1 - 8	8

191 1456

Onset - Day first observed
Duration = Day last observed
Frequency = Number of days observed

000158

Individual Clinical Findings
Female

Group, Rat Number		Day of Study Onset - Duration	Frequency
Group II Post Exposure:			
59056	Died prior to first det observation	1 - 1	1
59057	Died prior to first det observation	1 - 1	1
59058	Urine stained abdomen	1 - 12	12
	Alopecia, both eyes	9 - 14	6
	Alopecia, nose	11 - 14	4
	Alopecia, mouth	11 - 14	4
	Labored breathing	1 - 7	7
	Material around mouth, brown	1 - 5	5
	Material around nose, brown	1 - 5	5
	Material around eye, wet, black, both	1 - 10	10
	Eyes pale, both	3 - 11	9
	Eyes pale, right	13 - 14	2
59060	Urine stained abdomen	1 - 11	11
	Alopecia, dorsal head	6 - 14	9
	Alopecia, both eyes	9 - 14	6
	Alopecia, anterior dorsal region	11 - 14	4
	Alopecia, nose	11 - 14	4
	Alopecia, mouth	11 - 14	4
	Labored breathing	1 - 8	8
	Material around mouth, brown	1 - 5	5
	Material around nose, brown	1 - 5	5
	Material around eye, dry, black, both	1 - 8	8
	Eyes pale, both	1 - 13	11

191-1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

000159

Individual Clinical Findings
Male

Group, Rat Number		Day of Study Onset	Duration	Frequency
Group III Post Exposure:				
59081	No visible abnormalities	8	14	7
	Urine stained abdomen	1	3	3
	Alopecia, ventral neck	4	6	3
	Material around mouth, brown	1	7	7
	Material around nose, brown	1	7	7
59082	No visible abnormalities	7	14	8
	Urine stained abdomen	1	4	3
	Labored breathing	1	2	2
	Material around mouth, brown	1	6	6
	Material around nose, brown	1	6	6
	Material around eye, black, both	1	3	3
59083	No visible abnormalities	4	14	11
	Urine stained abdomen	1	3	3
	Material around mouth, brown	1	3	3
	Material around nose, brown	1	3	3
	Material around eye, black, both	1	2	2
59084	No visible abnormalities	5	14	10
	Urine stained abdomen	1	2	2
	Labored breathing	4	4	1
	Material around mouth, brown	1	3	3
	Material around nose, brown	1	3	3
	Material around eye, brown, right	2	2	1
59085	No visible abnormalities	7	14	8
	Urine stained abdomen	1	5	5
	Labored breathing	1	2	2
	Material around mouth, brown	1	3	3
	Material around nose, brown	1	3	3
	Material around eye, brown, both	1	6	6

191 1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

Individual Clinical Findings
Female

Group, Rat Number		Day of Study Onset	Duration	Frequency
Group III Post Exposure:				
59086	No visible abnormalities			
	Urine stained abdomen	9	14	6
	Alopecia, ventral neck	1	5	5
	Labored breathing	5	8	4
	Material around mouth, brown	1	6	4
	Material around nose, brown	1	3	3
	Material around eye, brown, both	1	3	3
59087	Urine stained abdomen	1	5	5
	Alopecia, both eyes, slight	6	14	9
	Alopecia, anterior dorsal region	8	10	3
	Labored breathing	1	7	7
	Material around mouth, brown	1	6	6
	Material around nose, brown	1	6	6
	Material around eye, black, right	1	5	5
	Eyes pale, right	1	1	1
	Eye closed, left	1	1	1
59088	Urine stained abdomen	1	9	8
	Alopecia, both eyes	4	14	11
	Alopecia, anterior dorsal region	7	9	3
	Alopecia, ventral neck	11	14	4
	Labored breathing	1	7	4
	Material around mouth, brown	1	7	7
	Material around nose, brown	1	7	7
	Material around eye, black, both	1	5	5
59089	Urine stained abdomen	1	10	10
	Alopecia, both eyes	4	14	11
	Labored breathing	1	7	6
	Material around mouth, brown	1	4	4
	Material around nose, brown	1	4	4
	Material around eye, black, both	1	5	5
	Eyes pale, both	1	6	6

91-1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

000161

Individual Clinical Findings
Female

Group, Rat Number		Day of Study Onset - Duration	Frequency
Group III Post Exposure:			
59090	Urine stained abdomen	1 - 7	7
	Alopecia, both eyes	4 - 14	11
	Alopecia, nose	5 - 12	8
	Alopecia, mouth	5 - 12	8
	Scabbed area, tail	11 - 14	4
	Labored breathing	1 - 10	10
	Material around mouth, brown	1 - 4	4
	Material around nose, brown	1 - 4	4
	Material around eye, black, both	1 - 5	5
	Eyes pale, both	1 - 9	9
	Material on surface of eye, dry, right	11 - 13	3

191 1456

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

001132

APPENDIX B

Individual Body Weights

Individual Body Weights (grams)

Animal Number	Sex	Pre-exposure	Post-exposure Day 7	Post-exposure Day 14
Group I:				
58856	M	209	235	279
58857	M	233	214	279
58858	M	224	216	270
58859	M	208	200	247
58860	M	201	180	235
Mean		215	209	262
S.D.		13.1	20.4	20.0
58861	F	151	146	179
58862	F	158	161	189
58863	F	163	168	209
58864	F	150	162	190
58865	F	150	153	174
Mean		154	158	188
S.D.		5.9	8.6	13.4
Group II:				
59051	M	270	240	274
59052	M	274	236	287
59053	M	283	269	291
59054	M	269	-	-
59055	M	280	264	308
Mean		275	252	290
S.D.		6.1	16.7	14.0
59056	F	192	-	-
59057	F	183	-	-
59058	F	182	184	204
59059	F	187	-	-
59060	F	184	185	198
Mean		186	185	201
S.D.		4.0	0.7	4.2

S.D. - Standard deviation

Individual Body Weights (grams)

Animal Number	Sex	Pre-exposure	Post-exposure Day 7	Post-exposure Day 14
Group III:				
59081	M	291	303	345
59082	M	290	297	330
59083	M	294	302	349
59084	M	292	305	334
59085	M	294	296	337
Mean		292	301	339
S.D.		1.8	3.9	7.8
59086	F	184	197	218
59087	F	183	205	221
59088	F	190	202	219
59089	F	184	185	208
59090	F	184	220	241
Mean		185	202	221
S.D.		2.8	12.7	12.1

S.D. - Standard deviation

191-1456

000166

APPENDIX C

Individual Post-Mortem Observations

Individual Post-Mortem Observations

Dosage Level Rat Number	Sex	Fate	Day	Site	Observation
Group I Post Exposure:					
58856	M	S	(14)	-	NVA
58857	M	S	(14)	-	NVA
58858	M	S	(14)	-	NVA
58859	M	S	(14)	-	NVA
58860	M	S	(14)	-	NVA
58861	F	S	(14)	-	NVA
58862	F	S	(14)	-	NVA
58863	F	S	(14)	-	NVA
58864	F	S	(14)	-	NVA
58865	F	S	(14)	-	NVA
Group II Post Exposure:					
59051	M	S	(14)	-	NVA
59052	M	S	(14)	-	NVA
59053	M	S	(14)	Kidney	white spots, left
59054	M	D	(a)	Trachea	white in color, white dust present
				Intestines	gas filled, slight
				Liver	dark in color
59055	M	S	(14)	-	NVA
59056	F	D	(1)	Intestines	gas filled, marked
				Trachea	white in color, foam-like fluid
				Lungs	mottled, red in color
				Kidney	dark spots on exterior, right
				Liver	uneven in color
59057	F	D	(1)	Intestines	gas filled, slight
				Trachea	foam like fliud
59058	F	S	(14)	-	NVA
59059	F	D	(a)	Trachea	white in color, white dust present
				Lungs	red in color, mottled
				Intestines	gas filled, very slight
59060	F	S	(14)	-	NVA

191 1456

D = Found dead
S = Scheduled sacrifice
NVA = No visible abnormalities

a - Died during exposure

000167

Individual Post-Mortem Observations

Dosage Level Rat Number	Sex	Fate	Day	Site	Observation
Group III Post Exposure:					
59081	M	S	(14)	-	NVA
59082	M	S	(14)	-	NVA
59083	M	S	(14)	-	NVA
59084	M	S	(14)	-	NVA
59085	M	S	(14)	-	NVA
59086	F	S	(14)	-	NVA
59087	F	S	(14)	-	NVA
59088	F	S	(14)	-	NVA
59089	F	S	(14)	-	NVA
59090	F	S	(14)	-	NVA

191 1458

S = Scheduled sacrifice
NVA = No visible abnormalities

000169

APPENDIX D
Protocol

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL REVISION OR CLARIFICATION

Protocol Sheet No. 1 Study No. 191-1456 (DRD# BY0874) (TSIN# G0539.04)

TITLE: OECD ACUTE INHALATION TOXICITY EVALUATION IN RATS

ITEM	JUSTIFICATION
1	Issuance of protocol.

ITEM	PROTOCOL REVISION OR CLARIFICATION
1	Conduct study in accordance with attached protocol.

Study Director Charles E. Ulrich, B.S.

C.E. Ulrich 5/02/89

Signature Date

IR90-49-5

I. STUDY TITLE

OECD Acute Inhalation Toxicity Evaluation in Rats

II. PURPOSE OF THE STUDY

The purpose of this study is to evaluate the acute toxicity of an experimental compound when administered via the inhalation route according to the Guidelines of the Organization for Economic Coordination and Development issued June, 1981.

III. STUDY NUMBER

191-1456

IV. TESTING FACILITY

International Research and Development Corporation
Mattawan, Michigan 49071

V. SPONSOR

The Procter & Gamble Co.
11511 Reed Hartman Highway
Cincinnati, OH 45241

VI. SPONSOR'S REPRESENTATIVE

Dr. Greg Allgood

VII. IRDC PERSONNEL RESPONSIBILITIES

Study Director:	Charles E. Ulrich, B.S. Scientific Director, Inhalation Toxicology
Manager of Inhalation Toxicology:	John G. Drummond, Ph.D.
Manager of Test Material Control:	Mark W. Griggs, B.S.
Associate Director of Research, Scientific Director of General Toxicology Division:	Malcolm Blair, Ph.D.
Director of Quality Assurance:	Margery J. Wirth, B.S.

VIII. SCHEDULE

Proposed Starting Date of Study:	May 3, 1989
Proposed Completion Date of Study:	May 17, 1989
Proposed Date of Final Report:	July 30, 1989

IX. TEST MATERIAL DATA

A. Identification: Sponsor Identification Number=G0539.01

B. IRDC Number: 10020

C. Lot Number: Not applicable

D. Batch Number: Not applicable

E. Physico-Chemical Properties: white powder

F. Purity: 100% as determined by sponsor

G. Shelf Life: expiration date April 1990

H. Storage Conditions: room temperature

I. Safety Precautions: possible irritant

J. Stability: stable at room temperature

K. Source: The experimental compound will be provided by the Sponsor.

L. Amount Required: At least two (2) kilograms or two (2) liters will be required for solids or liquids, respectively. For gases, approximately one hundred (100) liters will be required.

For self-contained aerosol products, the exact number of cans required will depend on the product's spray rate. However sixty (60) minutes of spray provided by twelve (12) to twenty-four (24) cans will usually be adequate for Phase I.

X. TEST ANIMALS

A. Species: Rat

B. Strain: Charles River CD - Sprague Dawley derived

C. Source: The Charles River Breeding Laboratories, Inc.
9801 Shaver Road
Portage, Michigan 49081

D. Age at Start of Study: At least six (6) weeks

X. TEST ANIMALS (continued)

E. Body Weight: Individual animal body weight for each sex will be within the ranges indicated below. All individual animals of a particular sex within a given group will be within ± 20% of the mean weight for that sex and all group means for a particular sex will be within ± 20%.

Males: 200 - 300 grams
Females: 150 - 225 grams

F. Method of Identification: Individual ear tag

G. Number on Study: Between ten (10) and one hundred (100)

H. Housing: Individually caged throughout the study. Average colony room temperature and humidity will be between nineteen (19) and twenty-five (25) degrees centigrade and thirty (30) to seventy (70) percent relative humidity.

I. Quarantine: At least seven (7) days

J. Reason for Selection: The rat is a universally used model for demonstrating acute toxicity.

XI. STUDY DURATION

The time required to conduct this study will vary from approximately two (2) weeks, when only Phase I is required, up to approximately six (6) weeks when Phase II is also required.

XII. METHOD OF ADMINISTRATION OF THE TEST MATERIAL

The compound will be administered via the inhalation route utilizing whole body exposure methods. Inhalation is considered a potential route for human exposure.

XIII. EXPERIMENTAL DESIGN

This study will be divided into two (2) phases. During the first phase a single group of five (5) male and five (5) female rats will be exposed for four (4) hours to an actual concentration slightly greater than five (5) mg/L or the maximum obtainable concentration. If no animals die during a fourteen (14) day observation period (sexes combined), then the study will be terminated and reported. However, if any animals die, then a complete four (4) hour LC_{50} study will be conducted. Animals exhibiting signs of reversible toxicity at fourteen (14) days, such as recovering lost body weight, will be maintained for an extended post-exposure observaton period.

XIII. EXPERIMENTAL DESIGN (continued)

If a solvent is required to generate the material, then a solvent control group will be added to the design.

A. Animals

The rats used for this study will be selected from a colony maintained for acute and subacute work. The animals will appear healthy and free of any signs of disease prior to selection for this study. When two (2) or more groups are to be exposed on the same day, the animals will be randomized into the various groups utilizing Standard Randomization Procedure C.

When only one (1) group is to be exposed on a given day, formal randomization will not be required. Each animal will be given a permanent animal number and an ear tag with that number will be placed on the animal.

B. Basal Laboratory Diet Data

1. Diet: Certified Pelleted Rodent Chow® #5002, Ralston Purina Company, *ad libitum* except during actual exposures.

2. Identification: Each lot utilized will be identified and recorded.

3. Contaminant Levels: Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the certified diet which would interfere with the results of this study. Therefore, no analyses other than those routinely performed by the feed supplier will be conducted.

C. Drinking Water

Tap water will be supplied *ad libitum* except during actual exposures.

The drinking water used for test animals will be monitored for specified contaminants at periodic intervals according to IRDC Standard Operating Procedures. Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the drinking water which would interfere with the results of this study. Therefore, no analyses other than those mentioned in this protocol will be conducted.

XIII. EXPERIMENTAL DESIGN (continued)

D. Exposure Methods

Exposures will be conducted in fifty-four (54) liter all glass or one-hundred-sixty (160) liter stainless steel and glass exposure chambers. The 4-hour exposure will be measured from the end of the t_{99} time. The chambers will be operated under dynamic conditions, where the continuously monitored chamber air is supplied by either the HVAC system which is separate from the general laboratory systems or by in-house compressed air. The air is filtered and temperature and humidity controlled. Average chamber temperature and humidity will be monitored continuously and will be within twenty (20) to twenty-four (24) degrees centigrade and thirty (30) to seventy (70) percent RH if possible, considering the requisite exposure conditions. The oxygen content will be maintained at nineteen (19) percent or greater. Chamber ventilation rate will be at least twelve (12) airchanges per hour and will be monitored continously. All animals will be caged individually during the exposure.

E. Exposure Atmosphere Generation Methods

For solids, where a dust exposure is required, the compound will be utilized as received and only obvious large agglomerates will be broken up. At the Sponsor's request, the experimental compound can be ground and sieved to produce an aerosol of greater "respirability." [1]

For liquids, exposures will be to a liquid droplet aerosol of the compound unless a vapor exposure is specifically requested.

Details of generation system methodologies cannot be defined until the physical and chemical characteristics of the experimental compound are known. Therefore, this will be recorded in the Study Notes after preliminary methods evaluations are conducted.

F. Methods for Determination of Exposure Concentrations

Actual and nominal chamber concentrations will be determined for all exposures. For dusts, actual measured chamber concentrations will be determined by standard gravimetric methods. For liquid droplet aerosols of materials with low volatility, the standard gravimetric method can also be used for determining actual chamber concentrations. However, for materials with a significant

[1] Grind and sieve test material as needed to acheive particles of greater "respirability" (e.g. approximately two(2) microns in size) Return ten (10) grams of sample to sponsor after sieving for analytical evaluation.

XIII. EXPERIMENTAL DESIGN (continued)

vapor pressure and for all vapor exposures, a specific analytical evaluation will be required.[2] Additional costs will be incurred for these analytical determinations. All results will be evaluated in terms of the actual measured concentrations. At least three (3) determinations will be made during each exposure.

G. Methods for Determination of Aerosol Particle Size

For dust and liquid droplet aerosol exposures, particle size determinations will be conducted once (1X) during each exposure utilizing an Andersen Cascade Impactor. Aerosol size will be expressed in terms of Equivalent Aerodynamic Diameter.

H. Observations for Pharmacotoxic Signs

All animals will be observed for pharmacotoxic signs during the exposure. During the fourteen (14) day post-exposure observation period, the animals will be observed twice (2X) daily for mortality and once (1X) daily for pharmacotoxic signs. During exposure the time of death will be recorded to the nearest half (1/2) hour, while during the observation period death will be considered to have occurred on the day the animal is found dead.

I. Body Weights

Body weights will be recorded just prior to exposure on days seven (7) and fourteen (14) post-exposure. Animals will also be weighed when found dead. When an extended post-exposure observation period is required, body weights will continue to be recorded at weekly intervals.

J. Necropsy

All animals which die during the exposure, during the observation period or are sacrificed at the termination of the study, will undergo a complete necropsy. Sacrifice will be by intraperitoneal sodium pentobarbital and exsanguination via the abdominal aorta. The trachea will be exposed and clamped such that the lungs can be removed and examined in an inflated state. All major organ systems in the thoracic and abdominal cavities will be observed for gross abnormalities and then the carcass will be discarded. No tissues will be preserved.

[2] Return at least four (4) glass-fiber filters in screw-cap containers to the sponsor for analytical evaluation. Each filter should have approximately thirty (30) milligrams of test material. Chamber exposure factors (time and size) should be supplied to allow determination of exposure concentrations.

XIV. STATISTICAL ANALYSIS

When Phase II of the experiment is conducted the concentration mortality data will be statistically analyzed for the LC_{50} and its confidence limits by one of the following methods:

A simplified method of evaluation dose-effect experiments
J.T. Litchfield, Jr. and F. Wilcoxon
J. Pharmacol. and Expt. Therp.
Vol. 96, 1949

The determination of the dosage-mortality curve from small numbers
Bliss
Quart. J. Pharm. Pharmacol.
Vol. 11, 1938

XV. REPORT

The report will contain a detailed description of the experimental design and methods. Individual and mean body weight data along with standard deviations on surviving animals will be provided. Narrative or tabular style data on pharmacotoxic signs and macroscopic abnormalities observed at necropsy will be provided. Exposure concentrations will be reported as a mean and standard deviation while particle size data will be reported as a log size-probability plot. Ten (10) copies of the report will be provided.

XVI. PERSONNEL HEALTH AND SAFETY

Normal safety precautions will be employed in the handling of the test compound.

XVII. DATA RETENTION

All data generated by the conduct of this study will be retained for at least ten (10) years after completion of the study and stored in the IRDC Archives. An appropriate sample of the test material will be retained for ten (10) years following completion of the study.

XVIII. QUALITY ASSURANCE

The study will be subjected to quality assurance inspection in accordance with IRDC Standard Operating Procedures, and the final report will be reviewed by the IRDC Quality Assurance Department. Study quality assurance inspection records will be made available to the Sponsor during Sponsor visits to IRDC.

XIX. GOOD LABORATORY PRACTICES

The study will be conducted in accordance with the Good Laboratory Practice regulations.

XX. ALTERATION OF DESIGN

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the specific written request or consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, such change will be honored by IRDC. However, it then becomes the responsibility of the Sponsor to follow such verbal change with a written verification.

XXI. DECLARATION OF INTENT

This study is intended to support (Sponsor should initial where appropriate):

A. Registration or notification of a product or chemical by the U.S. Environmental Protection Agency _____

B. Application for research and/or marketing permits for a product regulated by the U.S. Food and Drug Administration mh 4/26/89 per GSA

C. Neither of the above _____

Approved by Sponsor

THE PROCTER & GAMBLE CO.

By: Dr. Greg Allgood

Title: Divisional Toxicologist

Date: 4/25/89

Issued by

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

By: C. E. Ulrich
Charles E. Ulrich, B.S.

Title: Scientific Director, Inhalation Toxicology

Date: 2-22-89

THE PROCTER & GAMBLE COMPANY
MIAMI VALLEY LABORATORIES

MAILING ADDRESS
P O BOX 398707
CINCINNATI OHIO 45239-8707

SHIPPING ADDRESS
11810 EAST MIAMI RIVER ROAD
ROSS (BUTLER COUNTY) OHIO 45013

April 26, 1989

Mr. Charles Ulrich
International Research and
Development Corporation
500 North Main St.
Mattawan, Michigan 49071

Dear Mr. Ulrich:

This is to authorize you to carry out the following study according to the attached protocol, and in agreement with the stipulations of our current Laboratory Services Agreement.

Notice: 1) This study is expected to be submitted to the following regulatory agency: FDA. The stipulations of the protocol are to be implemented in complete conformance with the FDA Good Laboratory Practice Regulations.

2) Documentation of the derivation, characterization, and stability testing of the test substance will be the responsibility of the Sponsor.

3) The test substance is to be used for research and development purposes only.

Test: OECD Acute Inhalation Toxicity Evaluation in Rats
Protocol No: Special protocol dated 4/25/89
Test Substance No: G0539.04 Doc. Req. No.: BY0874
Physical Form: Powder

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Divisional Toxicologist:

Dr. G. S. Allgood Telephone (513) 530-4098
The Procter & Gamble Company
11511 Reed Hartman Highway - Room HB2D39
Cincinnati, Ohio 45241

191-1456

Mr Ulrich
Mr Ulrich
Mr Ulrich

MAY 01 1989

THE PROCTER & GAMBLE COMPANY

Mr. Charles Ulrich
International Research and
Development Corporation
April 26, 1989
Page 2

Complete Sections III and VIII, page 1, of the protocol. Return one copy to me, along with a letter stating that you agree to do the work specified in the attached, approved protocol. Please telephone verbal results to Dr. Allgood by May 18, 1989. Three copies of the draft report are needed as soon as possible, and are to be sent to me. Also, complete the attached animal accounting form and return it as instructed.

All unused samples are to be returned to the following address (the cost of shipment should be included in the study cost):

Mr. James V. Matthews
The Procter & Gamble Company
11511 Reed Hartman Highway - Room HB2D31
Cincinnati, Ohio 45241

The invoice for this study should be sent to:

Dr. Jochen M. Quack
Hoechst AG
Marketing TH/ATA
6230 Frankfurt am Main 80
Federal Republic of Germany

Sincerely,

THE PROCTER & GAMBLE COMPANY
Research & Development Department

G. A. Nixon/nh

G. A. Nixon
Professional Standards Department

nh
cc: Study File
G. S. Allgood
J. M. Quack

Inhalation

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

DRD: BY 0874

TSIN: 60539.04

1. Test Article (Product/Ingredient): Octopirox
2. Brand Notebook Ref: HC0529-22
3. Physical Form: Powder
   Color: White
   Storage Conditions:
   pH:
   Expiration Date: 4/90
   Hazards: Possible irritant

   [x] Room Temperature [ ] Other _____

4. Stability Testing is:

   [ ] Completed [x] In Progress

5. D.O.T. Hazard Classification: Non-hazardous Regulatory Initials _____
   (See Regulatory Personnel)
   These chemicals/test articles have been checked ___ Yes ___ No
   against the TSCA inventory list.

6. Formulated Composition

Component	(C.A.S.)[b] (Number)	Nominal Component Level	Nominal Calculated Assay	Actual Calculated Assay	Stock Code No. (Lot #)
Octopirox	68890-66-4	100.0	100.0	100.0	8x288-4

(a) Use chemical or CTFA names which uniquely identifies each component. Common, non-chemical names such as (Tergitol 15-S-0 or yellow dye #10) should be indicated in parentheses following the chemical name. Non-definitive identification (e.g., BC-Base, Arquad) is not acceptable.

(b) CAS numbers should be included whenever available. They are especially important for main components of the formulation.

TSCR/1/Ct.jvm
4/89

DRD: BY 0874

TSIN: 60539.04

7. ANALYSIS

Date Submitted	Submitter Code Or Lab Notebook	Analysis	(X) *	Measured Value	Limits	Laboratory Testing Or Data Source
*4/12/89	FDP261	MCT	X	<100 cfu/g	≤100cfu 4/17/89	Microservices
2/8/88	88032021	IDIR		Pass	Pass	Analytical

* Repacked results

* Analysis required by toxicologist or microbiologist

8.
Acceptable Microbial Susceptibility: [signature] 4/17/89
(Signature) (Date)

Made By: _____
(Signature) (Phone No.) (Date Made)

Approval for Release: The test substance as made and characterized is a representative example of the intended formulation. Making records for plant-made product are being/have been obtained and evaluated by Products Research.

Process Development: [signature] 4/17/89
(Signature) (Date)

Products Research: NA —
(Signature) (Date)

GMP Quality Assurance: [signature] 4-18-89
(Signature) (Date)

(Attach batch records for GMP review)

0 finished products will be retained by Quality Assurance/GMP Raw Material
(# samples)

9.
This substance has been appropriately characterized and is acceptable for testing outlined in the attached DRD.

Division Toxicologist: [signature] 4/20/89
(Signature) (Date)

MBCR/2/C1 jam
4/89