

Microfiche No.

OTS0200512

New Doc I.D.

88-7800185

Old Doc I.D.

BEHQ-0678-0185D

Date Produced

6/09/78

Date Received

6/21/78

TSCA section

8E

Submitting Organization

VELSICOL CHEM CORP

Contractor

Document Title

LETTER FROM VELSICOL CHEMICAL CORP TO US EPA REGARDING
SUBMISSIONS FOR FIREMASTER PHT4 WITH ATTACHMENTS

Chemical Category

TETRABROMO BISPHENOL A

88-7800185

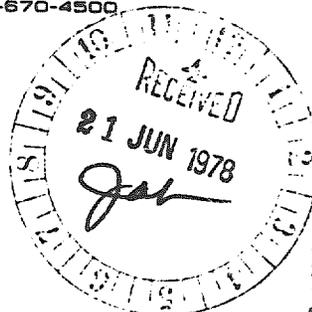
8EHQ-0678-0185

Declassified

VELSICOL CHEMICAL CORPORATION

341 EAST OHIO STREET • CHICAGO, ILLINOIS 60611 • 312-670-4500

June 9, 1978



Ms. Joan Urquhart
Document Control Officer
Chemical Information Division
OTS (WH-557)
U.S. EPA
401 M St. S. W.
Washington, D.C. 20460

Re: Firemaster PHT⁴, trade name for Tetrabromo Bisphenol A,
CAS # 79-94-7

Dear Ms. Urquhart:

The attached documents are submitted for your files and in compliance with TSCA section 8(e) if the latter is necessary.

In summary, the documents report on toxicology studies.

For the record, the document is identified as follows:

- ✓ A) "Mutagenicity Evaluation of 859-82-4 Final Report", LBI Project No. 2683, March, 1977.
- ✓ B) Letter From Irving Levenstein To Michigan Chemical Corporation, St. Louis, Michigan Assay No. 73500, Date Received February 17, 1958.
- ✓ C) Letter From Irving Levenstein To Michigan Chemical Corporation, St. Louis Michigan Assay No. 73501, Date Received February 17, 1958.
- ✓ D) "Acute Toxicity Studies on Tetrabromophthalic Anhydride" Hill Top Research Institute, Inc. January 14, 1964.
- ✓ E) "Primary Skin Irritation Study in Albino Rabbits" IRDC # 134-026, November 27, 1974.

VELSICOL CHEMICAL CORPORATION

-2-

June 9, 1978

PAR 0004

- ✓ P/ "Eye Irritation Study in Albino Rabbits." IRDC #134-027, November 29, 1974.
- ✓ G/ "Acute Inhalation Toxicity in the Albino Rat" IRDC # 134-025, December 6, 1974.
- ✓ H/ "Dermal Sensitization Study in the Albino Guinea Pig" IRDC #134-028, February 17, 1975.
- ✓ J/ "Acute Inhalation Toxicity in the Albino Rat After Pyrolysis" IRDC # 134-033, March 12, 1975.
- ✓ J/ "Acute Inhalation Toxicity in the Albino Rat After Pyrolysis" IRDC # 134-035, March 12, 1975.
- ✓ K/ "Acute Inhalation Toxicity in the Albino Rat After Pyrolysis." IRDC# 134-052, March 26, 1975.
- ✓ L/ "Twenty-Eight day Dermal Toxicity Study in Rabbits." IRDC # 134-030, April 25, 1975.
- ✓ M/ "Twenty-one Day Inhalation Toxicity Study in Rats." IRDC # 134-029, June 24, 1975.
- ✓ N/ "Mutagenicity Evaluation of 859-74-4 (FM PHT4) Final Report", LBI Project No. 2547, May 25, 1976.
- ✓ O/ "Human Repeated Insult Patch Test With Firemaster PHT4, P.O. No. 24681-A-C," December 16, 1976. IBT No. 8537-9430.
- ✓ P/ "Pilot Teratology Study in Rats" IRDC # 163-543," March 14, 1978.
- ✓ Q/ "The Acute Toxicity of Firemaster, PHT4 Lot No. 6332-B To The Bluegill Sunfish, Lepomis Macrochirus Rafinesque", UCES Proj. No. 11506-03-62, April 20, 1978.
- ✓ R/ "The Acute Toxicity of Firemaster, PHT4 Lot No. 6332-B To the Rainbow Trout, Salmo gairdneri Richardson", UCES Proj. No. 11506-03-63, April 20, 1978.
- ✓ S/ "The Acute Toxicity of Firemaster PHT4 To Water Flea, Daphnia magna Straus," UCES Project No. 11506-03-75, May 1, 1978.

VELSICOL CHEMICAL CORPORATION

-3-

June 9, 1978

We make no judgement as to whether these documents contain information concerning a significant hazard. We reserve our right to contest the propriety of TSCA section 8(e).

Velsicol has not yet completely developed internal procedures for compiling all the information required at FR43: 11113, section 1X. When those procedures are developed we plan to submit them to EPA for comment. In the meantime, if you desire further information on the subject covered by this report, please contact me at 312 - 670-4764.

REF 0005

Sincerely,

VELSICOL CHEMICAL CORPORATION

Thomas R. Loy
Thomas R. Loy
Manager,
Regulatory Activities

TRL;br

88-7800185

0678 - 0185

A)

PAGE 0121

MUTAGENICITY EVALUATION

OF

859-82-4

FINAL REPORT

SUBMITTED TO

VELSICOL CHEMICAL CORPORATION
1975 GREEN ROAD
ANN ARBOR, MICHIGAN 48105

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2683

MARCH, 1977



BIONETICS

TABLE OF CONTENTS

1.	OBJECTIVE	1
2.	MATERIALS	1
	A. Test Compound	1
	B. Indicator Microorganisms	1
	C. Activation System.	1
	D. Positive Control Chemicals	2
	E. Solvent.	2
3.	EXPERIMENTAL DESIGN	3
	A. Plate Test (Overlay Method)	3
	B. Recording and Presenting Data	3
4.	SUMMARY OF PLATE TEST RESULTS	4
5.	INTERPRETATION OF RESULTS AND CONCLUSIONS	5
	A. Toxicity	5
	B. Nonactivation Test Results	5
	C. Activation Test Results	5
	D. Conclusions	5
6.	EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS	6
	A. Surviving Populations	6
	B. Dose Response Phenomena	6
	C. Control Tests	7
	D. Evaluation Criteria for Ames Assay	7
	E. Relationship Between Mutagenicity and Carcinogenicity	8

PBF 0122

SPONSOR: Velsicol Chemical Corporation

MATERIAL: 859-82-4

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

REC'D
0123

2. MATERIALS

A. Test Compound

1. Date Received: February 14, 1977
2. Description: Pale yellowish gel

B. Indicator Microorganisms

Salmonella typhimurium, strains: TA-1535 TA-98
TA-1537 TA-100
TA-1538

Saccharomyces cerevisiae, strain: D4

C. Activation System (Ames et al., Mutation Research 31:347, 1975)

1. Reaction Mixture

<u>Component</u>	<u>Final Concentration/ml</u>
TPN	4 μ moles
Glucose-6-phosphate	5 μ moles
Sodium phosphate (dibasic)	100 μ moles
MgCl ₂	8 μ moles
KCl	33 μ moles
Homogenate fraction equivalent to 25 mg of wet tissue	0.1-0.15 ml 9,000 x g supernatant of rat liver

2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

2. MATERIALS (Continued)

D. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

<u>ASSAY</u>	<u>CHEMICAL</u> ^a	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY</u>
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS ^b
	2-Nitrofluorene (NF)	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard (QM)	Water or saline	FS ^b
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide ^c	BPS ^b
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide ^c	FS ^b

^aConcentrations given in Results Section

^bBPS = Base-pair substitution

FS = Frameshift

^cPreviously shown to be nonmutagenic

E. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in the Results Section.



BIONETICS

3. EXPERIMENTAL DESIGN

A. Plate Test (Overlay Method*)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the $9,000 \times g$ liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

*Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames overlay method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.



BIONETICS

PAGE 0125

4.---SUMMARY OF PLATE TEST RESULTS

LITTON BIOMETRICS, INC.

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 059-02-4
 9. SOLVENT: DMSO
 C. TEST DATE: FEB. 15, 1977
 NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	TISSUE	B-E-V-E-B-I-A-N-I-S		P-F-R		E-L-A-I-E	
			1	2	1	2	1	2
NONACTIVATION								
SOLVENT CONTROL	---	---	20	13	29	30	190	34
POSITIVE CONTROL**	---	---	950	733	679	>1000	823	54
TEST COMPOUND	0.00100 UL	---	26	11	21	35	192	40
	0.01000 UL	---	20	10	24	26	150	31
	0.10000 UL	---	20	16	30	28	148	25
	1.00000 UL	---	14	6	30	42	171	13
	5.00000 UL	---	15	8	26	34	127	18
ACTIVATION								
SOLVENT CONTROL	RAT	LIVER	22	17	36	23	142	63
POSITIVE CONTROL***	RAT	LIVER	312	>1000	>1000	>1000	577	466
TEST COMPOUND	0.00100 UL	RAT	17	18	46	20	141	63
	0.01000 UL	RAT	20	20	50	21	151	52
	0.10000 UL	RAT	21	15	40	23	145	71
	1.00000 UL	RAT	22	13	29	22	122	70
	5.00000 UL	RAT	16	17	33	17	96	48

* TRY* CONVERTANTS PER PLATE

** TA-1535	MNNG	10 UG/PLATE	100 UG/PLATE
TA-1537	OM	10 UG/PLATE	100 UG/PLATE
TA-1538	NF	100 UG/PLATE	100 UG/PLATE
TA-9A	NF	100 UG/PLATE	100 UG/PLATE
TA-100	MNNG	10 UG/PLATE	100 UG/PLATE
04	MNNG	10 UG/PLATE	100 UG/PLATE
SOLVENT	DMSO	2.5 %/PLATE	100 MICROMOLES/PLATE
			2.5 %/PLATE

- INDICATES TEST WAS NOT DONE

PART 0126

5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.001 μ l to 5 μ l per plate.

PAGE 0127

B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

D. Conclusions

The test compound, 859-82-4, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:

David J. Brusick
David J. Brusick, Ph.D.
Director
Department of Genetics

3/15/77
Date

Reviewed by:

Robert J. Weir
Robert J. Weir, Ph.D.
Vice President

3/15/77
Date



BIONETICS

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

PLATE 0128



6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a



6. EVALUATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/microsome test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al. (Proc. Nat. Acad. Sci. USA, 72:5135-5139, 1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluation and interpretation of the data presented in this report are based only on the demonstration of or lack of mutagenic activity.

PAGE 0130

STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.

PAGE 0131



BIONETICS

Lifton

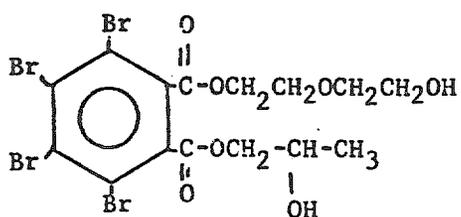
859-82-4

PHT-4 diol

Lot #757-97-2

PAGE 0132

Structure



Analytical Data

No record

88-7800185

0678 - 0185

A

8)

LEBERCO LABORATORIES



123 HAWTHORNE STREET - ROSELLE PARK, N. J.

CHESTNUT 5-1933

MARCH 11, 1958

PAGE 0133

SUBMITTED TO: MICHIGAN CHEMICAL CORPORATION
 ST. LOUIS, MICHIGAN

ASSAY NUMBER: 73500

DATE RECEIVED: FEBRUARY 17, 1958

TEST MATERIAL: 1 - SAMPLE TETRABROMOPHTHALIC ANHYDRIDE // 4

METHOD OF ASSAY: I.C.C. REGULATION 73.343

TEN NORMAL, HEALTHY RATS OF THE HOLTZMAN STRAIN, WEIGHING 200 TO 300 GRAMS EACH, WERE USED IN THIS EXPERIMENT.

THE TEST MATERIAL WAS MADE UP TO A CONCENTRATION OF 5 MILLIGRAMS PER ML. WITH DISTILLED WATER AND ADMINISTERED INTRAGASTRICALLY AT A LEVEL OF 10 ML. PER KILOGRAM OF BODY WEIGHT. FOLLOWING THIS SINGLE FEEDING THE ANIMALS WERE OBSERVED FOR A FORTY-EIGHT HOUR PERIOD FOR SIGNS OF TOXICITY AND DEATH.

THROUGHOUT THIS OBSERVATION PERIOD THE ANIMALS WERE MAINTAINED ON THEIR REGULAR DIET OF FOX BLOX AND WATER AD LIBIDUM AND WERE HOUSED IN INDIVIDUAL WIRE MESH CAGES,

RESULTS:

ANIMAL #	BODY WEIGHT IN GRAMS	MILLIGRAMS OF TEST MATERIAL ADMINISTERED PER RAT	RESULTS
1	296	14.8	ALIVE
2	280	14.0	ALIVE
3	280	14.0	ALIVE
4	280	14.0	ALIVE
5	285	14.3	ALIVE
6	201	14.3	ALIVE
7	217	10.0	ALIVE
8	276	10.85	ALIVE
9	207	13.5	ALIVE
10	280	10.35	ALIVE
		14.0	ALIVE

24

CONCLUSION:

WHEN THE TEST MATERIAL WAS ADMINISTERED INTRAGASTRICALLY AT A LEVEL OF 50 MILLIGRAMS PER KILOGRAM OF BODY WEIGHT OF RAT, NO DEATHS OCCURRED WITHIN THE FORTY-EIGHT HOUR OBSERVATION PERIOD.

LEBERCO LABORATORIES

Anne Wolven

ANNE WOLVEN, A.S.

Irving Levenstein

IRVING LEVENSTEIN, PH. D.
DIRECTOR

IL:ER

PAGE 0134

88-7800185

0678-0185

A



LEBERCO LABORATORIES

123 HAWTHORNE STREET - ROSELLE PARK, N. J.

C

CHESTNUT 5-1933

MARCH 26, 1958

PAGE 0135

SUBMITTED TO: MICHIGAN CHEMICAL CORPORATION
ST. LOUIS, MICHIGAN

ASSAY NUMBER: 73501

DATE RECEIVED: FEBRUARY 17, 1958

TEST MATERIAL: 1 - SAMPLE TETRABROMOPHTHALIC ANHYDRIDE # 4.

METHOD OF ASSAY: I.C.C. REGULATIONS 73.343

TEN NORMAL, HEALTHY, FEMALE RABBITS, WEIGHING TWO KILOGRAMS EACH, WERE USED FOR THIS EXPERIMENT.

PRIOR TO EXPOSURE TO THE TEST MATERIAL THE TRUNK OF EACH ANIMAL WAS CLIPPED FREE OF ALL HAIR USING ELECTRIC CLIPPERS. A DOSE LEVEL OF 200 MILLIGRAMS PER KILOGRAM OF BODY WEIGHT WAS APPLIED TO EACH ANIMAL'S CLIPPED BACK. THE ANIMALS WERE IMMOBILIZED IN AN ANIMAL HOLDER (DRAIZE, J.H. ET.AL. J. OF PHARM. & EXPER. THERAP. VOL. 82, NO. 4, DEC. 1944) WITH PATCHES SECURED IN PLACE BY STRIPS OF ADHESIVE TAPE. AFTER TWENTY-FOUR HOURS OF EXPOSURE THE PATCHES WERE REMOVED AND THE ANIMALS OBSERVED FOR A FORTY-EIGHT HOUR PERIOD FOR SIGNS OF TOXICITY OR DEATH.

THROUGHOUT THIS OBSERVATION PERIOD THE ANIMALS WERE MAINTAINED ON THEIR REGULAR DIET OF FOX KRUMS AND WATER AD LIBIDUM.

RESULTS:

ANIMAL #	BODY WEIGHT IN KILOGRAMS	MILLIGRAMS OF TEST MATERIAL ADMINISTERED	RESULTS
1	2.1	420	ALIVE
2	2.2	440	ALIVE
3	2.1	420	ALIVE
4	2.2	440	ALIVE
5	2.4	480	ALIVE
6	2.2	440	ALIVE
7	2.2	440	ALIVE
8	2.1	420	ALIVE
9	2.4	480	ALIVE
10	2.3	460	ALIVE

26

CONCLUSION:

WHEN THE PATCHES WERE REMOVED AT THE END OF TWENTY-FOUR HOURS NONE OF THE ANIMALS SHOWED ANY SIGNS OF AN ERYTHEMA OR EDEMA RESULTING FROM EXPOSURE TO THE TEST MATERIAL.

WHEN THE TEST MATERIAL WAS APPLIED AS DESCRIBED ABOVE, AT A TEST LEVEL OF 200 MILLIGRAMS PER KILOGRAM OF BODY WEIGHT, ALL OF THE ANIMALS SURVIVED THE FORTY-EIGHT HOUR OBSERVATION PERIOD.

LEBERCO LABORATORIES

Anne Wolven
ANNE WOLVEN, A.B.

Irving Leveisein
IRVING LEVEISEIN, PH. D.
DIRECTOR

PAGE 0136

IL:ER

88-7800185

0678-0185

A

HILL TOP RESEARCH INSTITUTE, INC.
Miami, Ohio

D)

January 14, 1964

N-364

ACUTE TOXICITY STUDIES ON TETRABROMOPHTHALIC ANHYDRIDE

For Michigan Chemical Corporation

PURPOSE

To evaluate the acute oral toxicity and the acute dermal toxicity and irritative potential of tetrabromophthalic anhydride.

TEST MATERIAL

The initial sample of tetrabromophthalic anhydride (ISO 5756, Lot 149) used in these studies was received from Michigan Chemical Corporation on November 5, 1963. An additional supply of the material was received on November 16, 1963. Tetrabromophthalic anhydride is a white powder with a faint pungent odor.

PROCEDURE

1. Acute Oral Administration - Rats

The test sample was administered orally by stomach tube to six groups, each composed of five male albino rats (Dublin Sprague-Dawley strain, weight range 201 to 291 grams). The sample was administered as a 50% weight/volume suspension in water. The dosage levels tested were 0.215, 0.464, 1.00, 2.15, 4.64, and 10.0 gm/kg of body weight. Food was withheld from the rats for approximately 18 hours prior to dosage. Following dosage, food, consisting of commercial pellets, and water were available ad libitum. The rats were housed in groups in wire mesh cages suspended above the droppings. All animals were observed closely for gross signs of systemic toxicity and mortality several times during the day of dosage, and at frequent intervals thereafter for a total of 14 days. At the end of the 14-day observation period the rats were weighed, sacrificed by cerebral concussion, and gross autopsies were performed.

2. Acute Dermal Application - Rabbits

The test sample was applied to the skin of four groups, each composed of four albino rabbits (weight range 1344 to 2310 grams). The sample was applied moistened with sufficient water to form a paste. The dosage levels tested were 1.00, 2.15, 4.64 and 10.0 gm/kg of body weight. The sample was applied to the intact abdominal skin area from which the fur had been previously removed with electric clippers. The sample was applied under a binder of rubber dental damming which was placed around the trunk of the animal. The trunks of the animals were wrapped securely with gauze and adhesive tape to keep the binder and test material in contact with the skin and to prevent ingestion of the applied

PAGE 0137

1/4/64

material. After an exposure period of 24 hours the binders were removed and any unabsorbed material remaining on the skin was removed by gentle sponging with a moistened towel.

The rabbits were observed for gross signs of systemic toxicity at several intervals during the day of application of the test material and for gross signs of dermal irritation and systemic toxicity daily thereafter for a total of 15 days. Throughout the experiment the animals were housed individually in metal cages elevated above the droppings. Food, consisting of Purina Rabbit Pellets, and water were available at all times.

A gross autopsy was performed on each animal that died. At the end of the 15-day observation period the surviving rabbits were weighed, sacrificed by air embolism, and a gross autopsy was performed on each animal.

RESULTS

1. Acute Oral Administration - Rats

One rat at the 0.464 gm/kg dosage level was found dead on the third experimental day. A gross autopsy performed on this rat showed approximately 10 ml of fluid in the pleural cavity and adhesions between the lungs and pleural lining. This death was attributed to accidental misplacement of the dose and was unrelated to the test sample. There were no other mortalities during the 14-day experimental period at any level tested. Therefore, the oral LD₅₀ of tetrabromophthalic anhydride for male albino rats is greater than 10.0 gm/kg of body weight.

With the exception of wheezing, which was observed sporadically in all dosage groups, the rats at each level exhibited normal behavior and appearance throughout the 14-day observation period.

Average body weight gains for the rats during the 14-day experimental period are shown below.

<u>Dose</u> gm/kg	<u>Average Body Weight</u>		<u>Gain</u> gm
	<u>Start</u> gm	<u>Finish</u> gm	
0.215	254	337	83
0.464	230	341	111
1.00	243	329	86
2.15	249	350	101
4.64	233	314	81
10.0	248	331	83

PART 0138

1/4/64

The average body weight gain for each group is within normal limits for rats of the age, sex, and strain employed in this study.

Gross autopsies performed at termination showed slight congestion of the kidneys among the rats at the lower dosage levels, and moderate or marked congestion of the kidneys at the highest dosage level.

2. Acute Dermal Application - Rabbits

Between the tenth and thirteenth experimental days four rabbits died. These deaths were distributed as follows: one rabbit from the 1.00 and 4.64 gm/kg groups and two rabbits from the 2.15 gm/kg group. Three of these animals showed losses in body weight of from approximately 100 to 300 grams, and the remaining animal gained approximately 200 grams. Death in each rabbit was preceded by diarrhea; these deaths were attributed to enteritis, a common syndrome in laboratory rabbits, rather than to application of the test sample.

There were no other mortalities. The acute dermal LD₅₀ of tetrabromophthalic anhydride for albino rabbits is therefore greater than 10.0 gm/kg of body weight.

Eleven of the twelve surviving rabbits showed overall weight gains ranging from approximately 100 to 500 grams. The remaining rabbit essentially maintained its initial body weight. Transient diarrhea was noted in eight of these rabbits; this appeared to bear no relationship to application of the test sample.

During the 24-hour exposure period and daily thereafter the rabbits at the three lower dosage levels exhibited normal appearance and behavior. The animals at the 10.0 gm/kg level appeared depressed and showed depressed righting and placement reflexes during the exposure period. Daily thereafter these animals appeared grossly normal.

On removal of the binders at the end of the 24-hour exposure period, the abdomens of the rabbits were covered with dry test material, indicating little or no dermal absorption of the applied material. A single application of the material produced no gross signs of dermal irritation at any level tested.

Gross autopsies performed on the four animals that died were complicated by autolytic changes. Gross autopsies performed on the surviving rabbits at termination showed no significant gross pathology.

MFC 0129

SUMMARY

The acute oral LD50 of tetrabromophthalic anhydride for male albino rats was found to be greater than 10 gm/kg of body weight.

The acute dermal LD50 of tetrabromophthalic anhydride for albino rabbits was found to be greater than 10 gm/kg of body weight. A single application of the moistened material produced no gross evidence of dermal irritation.

HILL TOP RESEARCH INSTITUTE, INC.

Submitted by Robert L. Doyle
Robert L. Doyle, B.S.

John R. Elsea
John R. Elsea, Ph.D.
Director of Toxicology

REC 0140

X

30

30

88-7800185

0678-0145

E)

A

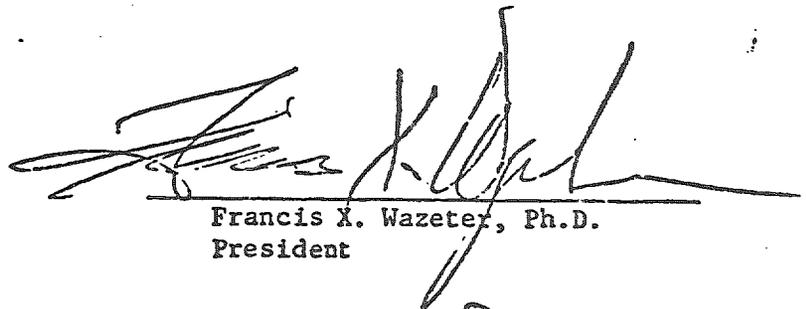
International Research and Development Corporation

PAGE 0013

SPONSOR: Michigan Chemical Corporation

COMPOUND: FM PH74 (micronized), Lot No. 6332-B

SUBJECT: Primary Skin Irritation Study
in Albino Rabbits.



Francis X. Wazeter, Ph.D.
President

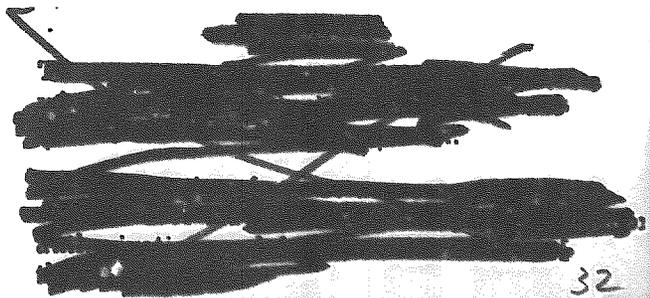


Edwin I. Goldenthal, Ph.D.
Director of Research

Collaborator:

W. P. Dean

Date: November 27, 1974



Document 134-026
Declassified
8-22-78

International Research and Development Corporation

TABLE OF CONTENTS

	Page
I. Synopsis.	1
II. Compound.	2
III. Primary Skin Irritation in Albino Rabbits	3
A. Method.	3
B. Results	3

REF 0014

Table No.

1. Summation of Primary Skin Observations.	5
2. Primary Irritation Score.	6

International Research and Development Corporation

Page 1

I. SYNOPSIS

FM PHT4 (micronized), Lot No. 6332-B was examined for primary skin irritation in albino rabbits in accordance with the regulations of the Federal Hazardous Substances Act.

FM PHT4 (micronized), Lot No. 6332-B, based upon a computed primary irritation score of 0.1, would not be considered a primary skin irritant nor would this material present a corrosive hazard to the skin when employed in the manner described.

PHT 0015

134-026

34

International Research and Development Corporation

Page 2

II. COMPOUND

The compound was received from the Michigan Chemical Corporation, St. Louis, Michigan on October 24, 1974.

It was identified as "7.5 lbs. FM PHT4 (micronized), Lot No. 6332 and was received as 2 bags of white powder, total of 15 lbs.

REF 0016

134-026

35

III. PRIMARY SKIN IRRITATION IN ALBINO RABBITS

A. METHOD:

Three male and 3 female New Zealand White rabbits weighing from 2793 to 3504 grams were used for this test.

The hair was removed from the back of each rabbit with an electric clipper. The skin of 3 of the rabbits was abraded with a scalpel blade. Food and water were available ad libitum.

500 milligrams of FM PHT4 (micronized), Lot No. 6332-B was applied to the back of each rabbit. The area of application was then wrapped with a gauze bandage and occluded with Saran Wrap. Twenty-four hours later the bandages were removed and the area was washed with tepid water and examined for skin irritation in accordance with the scale on the following page. These examinations were repeated at 72 hours.

B. RESULTS:

A summation of the primary skin observations are presented in Table 1. The computation of the primary irritation score is shown in Table 2.

Based upon the computed primary irritation score of 0.1, FM PHT4 (micronized), Lot. No 6332-B would not be considered a primary skin irritant nor would this material present a corrosive hazard to the skin when employed in the manner described.

Erythema and Eschar Formation:

	<u>Value*</u>
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

PAGE 0018

Edema Formation:

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1.0 mm)	3
Severe edema (raised more than 1.0 mm extending beyond the area of exposure)	4

*The "Value" recorded for each reading is the average value of six or more animals subjected to the test.

The values for erythema and eschar formation at 24 hours and at 72 hours for the intact animals' skin were added to similar values obtained for the abraded skin animals (a total of 4 values).

Similarly, the values for edema formation at 24 and 72 hours for intact and abraded skin animals were added together (a total of 4 values). The primary irritant score is the sum of the 8 values divided by 4. As scored by this method, a primary irritant is a substance which is not corrosive, but which results in a score of 5 or more. (Section 191.1 (g) (2) of the regulations of the Federal Hazardous Substances Act.)

FM PHT4 (micronized),
 Lot No. 6332-B: Primary Skin Irritation in the Albino Rabbit.

TABLE 1. Summation of Primary Skin Observations.

Observation	Examination Interval (No. Reacting/No. Dosed)			
	Intact Sites		Abraded Sites	
Erythema and Eschar Formation	24 hrs	72 hrs	24 hrs	72 hrs
No erythema	3/3	3/3	3/3	2/3
Very slight erythema				1/3
Well defined erythema				
Moderate to severe erythema				
Severe erythema				
Edema Formation				
No edema	3/3	3/3	3/3	3/3
Very slight edema				
Slight edema				
Moderate edema				
Severe edema				

FM PHT4 (micronized),
Lot No. 6332-B:

Primary Skin Irritation in the Rabbit.

PAGE 0020

TABLE 2.

Primary Irritation Score

Dermal Irritation	Observation Time	"Value"
Erythema and eschar formation:	Hours	
Intact skin	24	0
	72	0
Abraded skin	24	0
	72	0
Subtotal		<u>0.2</u>
		0.2
Edema formation:		
Intact skin	24	0
	72	0
Abraded skin	24	0
	72	0
Subtotal		<u>0</u>
		<u>0</u>
Total		<u><u>0.2</u></u>

Primary irritation score is $0.2 \div 4 = 0.1$

International Research and Development Corporation

T A B L E O F C O N T E N T S

	<u>Page</u>
I. Synopsis.	1
II. Compound.	1
III. Eye Irritation in Albino Rabbits.	6022
A. Method.	2
B. Results	7

Table No.

1. Eye Irritation in the Albino Rabbit (Observations).	6
2. Eye Irritation in the Albino Rabbit (Average Scores).	7

International Research and Development Corporation

Page 1

I. SYNOPSIS

FM PHT4 (micronized), Lot No. 6332-B was evaluated for eye irritation in accordance with the regulations under the Federal Hazardous Substance Act.

Based upon the results obtained, FM PHT4 (micronized) would be considered an eye irritant.

RF 0023

134-027

42

0 0 3 5

International Research and Development Corporation

Page 2

II. COMPOUND

The compound was received from the Michigan Chemical Corporation, St. Louis, Michigan on October 24, 1974.

It was identified as "7.5 lbs. FM PHT4 (micronized), Lot No. 633024B" and was received as 2 bags of white powder, total of 15 lbs.

PREP
024

134-027

43

PART 0025

III. EYE IRRITATION IN ALBINO RABBITS (Unwashed Technique)

A. METHOD:

Three male and 3 female New Zealand White rabbits were used in this test. The rabbits weighed from 2675 to 3240 grams at the beginning of the study. Food and water were available ad libitum.

Prior to compound administration, the eyes of each rabbit were examined with ultraviolet light after instillation of one drop of a 2.0 percent sodium fluorescein solution. This procedure is employed routinely so that only those rabbits with normal eyes are used in Eye Irritation Studies.

100 milligrams of the test material was instilled into the conjunctival sac of the right eye of each rabbit.

Examinations were made for ocular irritation at 24, 48 and 72 hours, and at 7 days. At the 72 hour examination, sodium fluorescein and ultraviolet light were used again to aid in revealing possible corneal injury.

The scale for scoring ocular irritation appears on the following page.

B. RESULTS:

Examination at 72 hours with sodium fluorescein and ultraviolet light did not reveal corneal damage in any of the rabbits tested.

International Research and Development Corporation

Scale for Scoring Ocular Lesions*

(1)	Cornea		
	(A)	Opacity-degree of density (area most dense taken for reading)	
		No opacity	0
		Scattered or diffuse area, details of iris clearly visible	1
		Easily discernible translucent areas, details of iris slightly obscured.	2
		Opalescent areas, no details of iris visible, size of pupil barely discernible.	3
		Opaque, iris invisible	4
	(B)	Area of cornea involved	
		One quarter (or less) but not zero	1
		Greater than one quarter, but less than half	2
		Greater than half, but less than three quarters.	3
		Greater than three quarters, up to whole area.	4
		Score equals A x B x 5	Total maximum = 80
(2)	Iris		
	(A)	Values	
		Normal	0
		Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
		No reaction to light, hemorrhage, gross destruction (any or all of these)	2
		Score equals A x 5	Total maximum = 10
(3)	Conjunctivae		
	(A)	Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)	
		Vessels normal	0
		Vessels definitely injected above normal	1
		More diffuse, deeper crimson red, individual vessels not easily discernible	2
		Diffuse beefy red	3
	(B)	Chemosis	
		No swelling	0
		Any swelling above normal (includes nictitating membrane).	1
		Obvious swelling with partial eversion of lids	2
		Swelling with lids about half closed	3
		Swelling with lids about half closed to completely closed	4
	(C)	Discharge	
		No discharge	0
		Any amount different from normal (does not include small amounts observed in inner canthus of normal animals).	1
		Discharge with moistening of the lids and hairs just adjacent to the lids	2
		Discharge with moistening of the lids and hairs, and considerable area around the eye	3
		Score equals (A + B + C) x 2	Total maximum = 20

PMT 0-026

The maximum total score is the sum of all scores obtained for the cornea, iris, and conjunctivae. Total maximum score possible = 110

* Draize, J. H., Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, Assoc. Food and Drug Officials of the U. S., Austin, Texas, 1959, p. 51, Modified according to revision in 1964. Edited by A. J. Lehman.

International Research and Development Corporation

Page 5

Table 1 presents a summary of the results obtained at each examination period. The average scores for each examination period are presented in Table 2.

Based upon the results obtained, FM PHT4 (micronized) Lot No. 6332-B would be considered an eye irritant.

REF 0021

134-027

46

TABLE 1.

Observations.

Observation	Examination Interval (No. Positive/No. Dosed)			
	Hours			Day
	24	48	72	7
Cornea:				
Cornea Normal	6/6	6/6	6/6	
Dulling normal corneal luster				
Corneal opacity: very slight				
slight				
moderate				
marked				
Iris:				
Iris Normal	5/6	6/6	6/6	6/6
Iridal Irritation	1/6			
Conjunctivae:				
Redness:				
normal			2/6	6/6
very slight				
slight		3/6	3/6	
moderate	5/6	3/6	1/6	
marked	1/6			
Chemosis:				
normal		5/6	4/6	6/6
very slight	3/6	1/6	2/6	
slight	1/6			
moderate	2/6			
marked				
Discharge:				
normal		6/6	6/6	6/6
very slight	2/6			
slight	1/6			
moderate	1/6			
marked	2/6			
Purulent Discharge				

6332-028

Eye Irritation in the Albino Rabbit.

TABLE 2. Average Scores.

Ocular Area		Average Scores (Range)			
		Observation Period			
		24 Hrs.	48 Hrs.	72 Hrs.	7 Days
Cornea	A	0	0	0	0
	B	0	0	0	0
Cornea Score		0	0	0	0
Iris	A	0.2 (0-1.0)	0	0	0
Iris Score		1.0	0	0	0
Conjunctivae	A	2.2 (2.0-3.0)	1.5 (1.0-2.0)	0.8 (0-2.0)	0
	B	1.8 (0.5-3.0)	0.1 (0-0.5)	0.2 (0-0.5)	0
	C	1.7 (0.5-3.0)	0	0	0
Conjunctivae Score		11.4	3.2	2.0	0
Total Score		12.4	3.2	2.0	0

PHT 0029

88-78 00185

0678-0185

G

A

International Research and Development Corporation

(G)

SPONSOR: Michigan Chemical Corporation

COMPOUND: FM PHT4 (micronized),
Lot No. 6332-B

SUBJECT: Acute Inhalation Toxicity
in the Albino Rat.

PAGE 0030

Francis X. Wazeter, Ph.D.
President

Edwin I. Goldenthal, Ph.D.
Director of Research

Collaborator:

W. P. Dean

Date: December 6, 1974

134-025

Document
declassified
8-22-78

International Research and Development Corporation

TABLE OF CONTENTS

	<u>Page</u>
I. Synopsis	1
II. Compound	1
III. Method	1
A. General Procedure	1
B. Compound Administration	3
IV. Results	4
A. Pharmacodynamic Signs	4
B. Necropsy Findings	4
C. Acute Inhalation Toxicity	4

II. COMPOUND

The test compound was received from the Michigan Chemical Corporation, St. Louis, Michigan on October 24, 1974. It was identified as "7.5 lbs. FM PHT4 (micronized), Lot No. 6332-B" and was received as 2 bags of white powder, total of 15 lbs.

REF 0032

III. METHOD

A. General Procedure:

Five male rats weighing from 160 to 192 grams and 5 female rats weighing from 220 to 230 grams were used in this test. All rats were of the Spartan strain. The rats were housed by sex in groups of 5 in metal cages above the droppings and maintained in temperature and humidity controlled quarters throughout the pre-exposure and post-exposure period. Purina Laboratory Chow and water were available ad libitum.

During the 4 hour exposure to the test compound, the rats were observed continuously for changes in behavior and/or appearance. Immediately following the exposure, the rats were examined closely for pharmacodynamic and/or toxic signs. All rats which died on study were subjected to a gross necropsy examination, as were all surviving animals at the end of the 14 day observation period.

All rats were weighed just prior to the initiation of the exposure period and again at 14 days.

B. Compound Administration:

The group of 10 rats was placed in a sealed 59.1 liter glass chamber and exposed for 4 hours to a dynamic atmosphere containing the dust of the test material. In order to prevent "piling up" during the exposure, the rats were separated into 4 units of 2 or 3 rats each.

Addition of the test compound to the test chamber atmosphere was controlled by a Wright Dust Feeder. Dried and filtered air was passed through the mechanism and directly into the exposure chamber. Airflow was regulated by means of a flowmeter¹.

¹Gelman Instrument Company, Ann Arbor, Michigan, Model No. 8221

PAR 003

52

17745

The calculated atmospheric concentration administered was approximately 10.92 mg/L.* of FM PHT4 (micronized), Lot No. 6332-B.

MR 030

IV. RESULTS

A. Pharmacodynamic Signs:

All of the rats exposed to the 10.92 mg/L* atmospheric concentration of FM PHT4 (micronized), Lot No. 6332-B survived the 4 hour exposure period, and also the subsequent 14 day observation period. Signs seen during the 4 hour exposure period included decreased motor activity, eye squint, slight dyspnea and erythema.

At 24 hours, one rat exhibited nasal porphyrin discharge. The remaining 9 rats were normal at 24 hours. At 48 hours and through 9 days, all rats appeared normal. At 10 through 14 days, several rats exhibited diarrhea. All rats exhibited normal body weight gains during the study period.

B. Necropsy Findings:

No gross lesions considered compound related were seen at necropsy in any of the rats sacrificed at the end of the observation period. An incidental finding in one female was hydrometra.

C. Acute Inhalation Toxicity:

In accordance with the results obtained, the acute inhalation toxicity of FM PHT4 (micronized), Lot No. 6332-B would be greater than 10.92 mg/L.*

*The physical properties of the test compound precluded administration of the test material at a higher atmospheric concentration.

International Research and Development Corporation

TABLE OF CONTENTS

I. Synopsis	Page 1
II. Compound	2
III. Method	3
A. General Procedure.	3
B. Compound Administration.	3
1. FM PHT4.	4
2. 2,4-Dinitro-1-Chlorobenzene.	4
3. 0.9 Percent Sodium Chloride Solution	4
IV. Results.	5
A. General Behavior and Appearance.	5
B. Skin Reactions to Compound Administration.	5
1. FM PHT4.	5
2. 2,4-Dinitro-1-Chlorobenzene.	5
3. 0.9 Percent Sodium Chloride Solution	6
<u>Table No.</u>	
1. FM PHT4.	7-8
2. Positive Control, 2,4-Dinitro-1-Chlorobenzene, Reaction (Wheal and Flare)	9

I. SYNOPSIS

FM PHT4 was evaluated for dermal sensitization in the albino guinea pig.

Based upon the results obtained, the test compound would be considered a probable sensitizing agent in man which may produce sensitization in some individuals.

MR 0037

International Research and Development Corporation

Page 2

II. COMPOUND

The test compound was received from the Michigan Chemical Corporation, St. Louis, Michigan on October 24, 1974.

It was identified as "7.5 lbs. FM PHT4 (micronized), Lot No. 6332-B" and was received as a white powder.

REF 0038

134-028

57

III. METHOD

A. General Procedure:

Twelve male albino guinea pigs, weighing from 326 to 393 grams, were used for this study. The animals were divided into 2 groups consisting of a positive control group of 4 guinea pigs and 1 treated group of 8 guinea pigs.

The animals were housed individually in metal cages suspended above the droppings in an air conditioned room. Food and water were available ad libitum.

Observations were made daily for dermal and pharmacotoxic signs.

B. Compound Administration:

Each control and test compound was injected intradermally into a prepared area on the back and flanks of the respective guinea pigs. The backs were prepared by shaving with electric clippers and were further shaved throughout the study as necessary.

The control or test compounds were injected every other day, three times each week, until a total of ten sensitizing doses had been given. The volume for the first sensitizing dose was 0.05 ml and thereafter for the remaining nine doses a volume of 0.10 ml was used.

In no case was the same injection site used more than once (each site was identified with a marking pen, directly below the site of injection). The test and control compounds were injected on the right flank of each animal in the respective control or treated groups and an identical volume of the vehicle (0.9 percent sodium chloride solution) was injected on the left side of each animal in all groups.

134-028

58

0 0 5 1

The injection sites were read and scored for diameter and intensity of erythema (flare) and height of edema (wheal) at 24 and 48 hours following each injection.

Two weeks following the administration of the tenth sensitizing dose, a challenge dose, at a volume of 0.05 ml was given by intradermal injection of the respective control or test compounds. Reactions to the challenge dose were read and scored at 24 and 48 hours as in the case of the sensitizing injections.

In the event that the score for a challenge dose was greater than the average score of the ten sensitizing doses, the control or test compound was considered to have produced dermal sensitization in the guinea pig.

All injections were made with a 26 gauge sterile needle.

The control and test compounds used in this study were as follows:

1. FM PHT4:

The compound was administered to a group of eight guinea pigs at a concentration of 0.1 percent in 0.9 percent sodium chloride solution.

2. 2,4-Dinitro-1-Chlorobenzene:

This chemical was used as the positive control, and was administered to a group of 4 guinea pigs at a concentration of 0.1 percent in a 0.9 percent sodium chloride solution.

3. 0.9 Percent Sodium Chloride Solution:

This solution was used as the control vehicle and was administered to the left flank of all the animals in this study.

The materials were prepared fresh each day just prior to injection.

IV. RESULTS

A. General Behavior and Appearance:

All of the guinea pigs used in this study appeared normal at all times. All animals exhibited normal body weight gains during the study period.

B. Skin Reactions to Compound Administration:

1. FM PHT4 (Table 1):

All eight guinea pigs responded to the challenge dose. Four of eight guinea pigs exhibited an average flare response to the challenge dose which was greater than twice the average response obtained in the sensitizing doses. The remaining four guinea pigs exhibited responses which were approximately 158 to 186 per cent of the average response obtained during the sensitizing period. No significant effect was noted in the wheal response to the challenge dose when compared to the response obtained in the sensitizing doses.

Comparison of the average of the individual mean values (flare) of the sensitizing doses to the average mean values (flare) of the challenge doses shows a range of from 58 to 178 per cent increase in the magnitude of the values obtained with the challenge doses. This response exceeds that response obtained with the positive control material.

Based upon the results obtained, the test compound would be considered a probable sensitizing agent in man which may produce sensitization in some individuals.

2. 2,4-Dinitro-1-Chlorobenzene (Table 2):

All four guinea pigs in this group exhibited a response to the challenge dose that was greater than that obtained in the sensitizing

MOF 0041

doses. The per cent range of challenge result obtained was from approximately 121 to 194 per cent of the average values obtained during the sensitization period of the study.

3. 0.9 Per Cent Sodium Chloride Solution:

Skin reactions observed in all of the guinea pigs tested (treated or control groups) were essentially negative following the sensitizing or challenge doses with 0.9-per cent sodium chloride solution.

REF 0042

doses. The per cent range of challenge result obtained was from approximately 121 to 194 per cent of the average values obtained during the sensitization period of the study.

3. 0.9 Per Cent Sodium Chloride Solution:

Skin reactions observed in all of the guinea pigs tested (treated or control groups) were essentially negative following the sensitizing or challenge doses with 0.9-per cent sodium chloride solution.

REF 0042

Dermal Sensitization Study in the Guinea Pig.

TABLE 1.

FM PHT4.

Reaction (Wheal and Flare)														
Sensitizing Dose	Dose (ml)	Reading Time (Hrs)	Guinea Pig No. 1142			Guinea Pig No. 1143			Guinea Pig No. 1144			Guinea Pig No. 1145		
			Dia. (mm)	Hgt. (mm)	Color	Dia. (mm)	Hgt. (mm)	Color	Dia. (mm)	Hgt. (mm)	Color	Dia. (mm)	Hgt. (mm)	Col (mm)
1	0.05	24	2	T	P	2	T	P	0	0	N	2	T	P
		48	T	0	P	2	T	P	0	0	N	T	P	
2	0.10	24	10	1	P	8	1	P	11	1	P	10	T	P
		48	9	1	P	7	T	P	4	1	T	7	T	P
3	0.10	24	6	T	P	5	T	P	9	T	P	8	T	P
		48	6	2	P	4	2	P	6	1	P	8	2	P
4	0.10	24	5	1	P	4	2	P	5	1	P	10	0	P
		48	5	1	P	4	2	P	4	3	P	5	2	P
5	0.10	24	7	T	P	4	2	P	7	1	P	6	T	P
		48	7	1	P	4	1	P	4	1	P	6	2	P
6	0.10	24	5	T	P	4	1	P	7	1	P	8	T	P
		48	5	T	P	4	T	P	6	2	P	7	2	P
7	0.10	24	7	T	P	5	T	P	5	2	P	15	3	P
		48	5	T	P	4	2	P	4	2	P	4	2	P
8	0.10	24	10	2	P	11	2	P	12	2	P	10	2	P
		48	9	1	P	8	2	P	9	1	Pa	15	1	Pa
9	0.10	24	7	1	P	10	1	P	8	2	P	7	1	R
		48	5	1	P	5	1	P	5	1	Pa	5	1	P
10	0.10	24	8	1	P	4	1	P	15	1	Pa	7	T	P
		48	5	1	R	8	1	P	5	1	Pa	9	1	P
Mean Value Sensitizing Doses			6.2			5.4			6.3			7.5		
Challenge Dose	0.05	24	24	T	P	25	T	P	25	T	P	14	T	P
		48	10	T	P	5	T	P	5	T	P	10	1	P
Mean Value Challenge Dose			17.0			15.0			15.0			12.0		

Color Code: N - Normal P - Pink
 T - Trace (not measurable) R - Red
 a - Scab on center of inflamed area

Dermal Sensitization Study in the Guinea Pig.

TABLE 1. continued

FM PHT4.

Reaction (Wheal and Flare)														
Sensitizing Dose	Dose (ml)	Reading Time (Hrs)	Guinea Pig No. 1151			Guinea Pig No. 1152			Guinea Pig No. 1153			Guinea Pig No. 1154		
			Dia. (mm)	Hgt. (mm)	Color	Dia. (mm)	Hgt. (mm)	Color	Dia. (mm)	Hgt. (mm)	Color	Dia. (mm)	Hgt. (mm)	Color
1	0.05	24	3	1	P	4	T	P	0	0	N	2	T	P
		48	0	0	N	T	0	P	0	0	N	0	0	N
2	0.10	24	10	2	P	8	1	P	7	2	P	9	2	P
		48	7	1	P	5	1	T	6	T	T	13	1	P
3	0.10	24	10	T	P	5	T	P	6	T	P	7	T	P
		48	5	1	P	5	1	P	6	T	P ^a	7	1	P
4	0.10	24	3	1	P	10	T	P	5	T	R ^a	4	2	P
		48	4	1	P	4	1	P	5	T	R ^a	3	3	P
5	0.10	24	12	1	P	5	T	P	30	T	pa	7	1	P
		48	8	2	R	6	1	P	7	1	pa	7	2	P
6	0.10	24	8	2	R	6	T	P	20	T	pa	8	1	P
		48	7	2	P	5	T	P	10	T	pa	5	2	P
7	0.10	24	6	1	P	15	2	P	35	T	P ^a	7	1	P
		48	6	1	P	7	1	P	6	T	pa	5	2	T
8	0.10	24	10	2	P	8	1	P	10	2	pa	11	2	P
		48	12	1	P	7	1	P	11	1	pa	8	1	P
9	0.10	24	15	2	P	8	1	P	30	2	pa	8	1	P
		48	12	1	pa	5	1	pa	12	1	pa	9	1	P
10	0.10	24	9	2	P	8	1	P	8	T	pa	10	2	P
		48	10	1	pa	7	1	P	11	1	pa	7	1	P
Mean Value Sensitizing Doses			7.9			6.4			11.3			6.9		
Challenge Dose	0.05	24	15	T	P	18	T	P	32	1	P	25	T	P
		48	10	1	P	5	1	P	10	1	P	3	T	P
Mean Value Challenge Dose			12.5			11.5			21.0			14.0		
Color Code:			N - Normal					P - Pink						
			T - Trace (not measurable)					R - Red						
			a - Scab on center of inflamed area											

Dermal Sensitization Study in the Guinea Pig.

TABLE 2. Positive Control, 2,4-Dinitro-1-Chlorobenzene.

Reaction (Wheal and Flare)														
Sensitizing Dose	Dose (ml)	Reading Time (Hrs)	Guinea Pig No. 1146			Guinea Pig No. 1147			Guinea Pig No. 1149			Guinea Pig No. 1150		
			Dia. (mm)	Hgt. (mm)	Color	Dia. (mm)	Hgt. (mm)	Color	Dia. (mm)	Hgt. (mm)	Color	Dia. (mm)	Hgt. (mm)	Co.
1	0.05	24	4	T	P	4	T	P	5	T	P	2	T	P
		48	2	O	P	0	O	N	2	O	P	0	O	N
2	0.10	24	5	1	P	6	T	T	3	T	T	6	1	P
		48	4	T	P	3	T	P	2	T	P	3	T	P
3	0.10	24	5	T	P	5	T	P	6	1	P	5	T	R
		48	4	1	P	4	T	T	5	1	P	5	1	R
4	0.10	24	10	O	P	5	1	P	6	T	P	4	2	R
		48	10	T	P	5	T	T	6	1	P	4	2	P
5	0.10	24	9	T	R ^a	9	T	P ^a	8	T	P	7	1	P
		48	11	1	R ^a	7	1	P	6	T	P	10	1	P
6	0.10	24	12	1	R ^a	10	1	P ^a	5	T	P	7	1	P
		48	10	1	R	11	T	P	7	T	P	7	1	P
7	0.10	24	13	1	P ^a	10	T	P ^a	10	T	P	5	1	P
		48	14	T	P	10	T	P	10	T	P	5	T	P
8	0.10	24	13	1	P	12	2	P ^a	10	1	P	7	T	P
		48	13	1	R ^b	12	1	P ^a	10	T	P	6	T	P
9	0.10	24	10	1	P ^b	11	1	P ^a	11	T	P ^a	9	1	P
		48	10	1	P ^b	9	1	P	10	T	P	9	1	P
10	0.10	24	11	1	P ^a	11	1	P	11	1	P	9	1	P
		48	9	T	P	8	T	P	8	T	P	6	T	P
Mean Value Sensitizing Doses			9.0			7.6			7.1			5.8		
Challenge Dose	0.05	24	20	T	P	12	T	P	12	1	R	10	1	P
		48	15	T	P	9	T	P	12	T	P	4	0	P
Mean Value Challenge Dose			17.5			10.5			12.0			7.0		

Color Code: N - Normal P - Pink
 T - Trace (not measurable) R - Red
 a - Blanched spot in center of inflamed area.
 b - Scab in center of inflamed area.

88-7800185

0678-0185

A

I

International Research and Development Corporation

I

SPONSOR: Michigan Chemical Corporation
 COMPOUND: HIPS Resin./ Sb₂O₃
 SUBJECT: Acute Inhalation Toxicity in
 the Albino Rat After Pyrolysis.

REF 0046

Francis X. Wazeter, Ph.D.
President

Edwin I. Goldenthal, Ph.D.
Director of Research

Collaborators:

Dr. R. G. Geil, D.V.M.,
Vice President - Director
of Pathology

W. P. Dean

Date: March 11, 1975

~~_____~~
~~_____~~
~~_____~~
~~_____~~
~~_____~~

134-033

Declassified
8-22-78

65

005A

International Research and Development Corporation

TABLE OF CONTENTS

	<u>Page</u>
I. Synopsis	1
II. Compound	2
III. Method	3
A. General Procedure	3
B. Compound Administration	3
IV. Results	5
A. Pharmacodynamic Signs	5
B. Necropsy Findings	5

PRE 0047

I. SYNOPSIS

HIPS Resin/Sb₂O₃ was evaluated for acute inhalation toxicity in albino rats after pyrolysis. No deaths occurred during the 4 hour exposure period or during the subsequent 14 day period of observation.

REF 0040

The pharmacodynamic and/or toxic signs observed are recorded in the body of this report.

Necropsy findings observed at terminal necropsy conducted at 14 days revealed 7 of 10 rats showing congestion of the lungs and one rat which exhibited petechiation of the lungs.

II. COMPOUND

The test compound was received from the Michigan Chemical Corporation, St. Louis, Michigan on November 29, 1974. It was identified as "HIPS Resin/Sb₂O₃, Lot No. 853-11-1" and was received as white cylindrical chunks.

MM 0009

III. METHOD:

A. General Procedure:

Five male and 5 female albino rats of the Spartan strain, weighing from 208 to 240 grams, were exposed to an atmosphere containing pyrolysis products of the test material.

The rats were housed by sex in groups of 5 rats each in wire mesh cages elevated above the droppings and maintained in temperature and humidity controlled quarters throughout the pre-exposure and post-exposure periods. Food and water were available ad libitum.

During the 4 hour exposure the rats were observed for changes in behavior and/or appearance. Immediately following the exposure, they were examined closely for pharmacodynamic and/or toxic signs. The rats were observed for a period of 14 days and then sacrificed and necropsied.

B. Compound Administration:

The rats were placed in a sealed 59.1 liter glass chamber and exposed for 4 continuous hours to a dynamic atmosphere containing pyrolysis products of the test compound. In order to prevent crowding during the exposure, the rats were separated into 4 units of two or three rats each.

The test chamber atmosphere was prepared by passing an airflow of 10 LPM through a 500 ml 3 necked flask (Pyrex No. 4950) containing 100.02 grams of HIPS Resin/Sb₂O₃. The flask was immersed in an oil bath. Airflow through the flask was not initiated until the test compound temperature reached at least 450°F. Two 300 mm Liebig condensers (Pyrex No. 2400) were used in line between the flask and

REC 0050

International Research and Development Corporation

Page 4

exposure chamber. Throughout the 4 hour exposure period, the temperature of the oil bath was monitored at from 488 to 503°F; the test compound at from 450 to 458°F; and the atmosphere of the exposure chamber at from 63 to 69°F. For a very brief interval during the 4 hour exposure period the temperature of the test compound was at 446°F, but was readjusted to at least 450°F.

The flask containing the test compound was weighed before and after the exposure and sent to the sponsor. From this data the percent weight loss was calculated.

MR 0051

134-033

70

IV. RESULTS:

A. Pharmacodynamic Signs:

All of the rats exposed to the pyrolysis products of the test compound survived the 4 hour exposure period, and also the subsequent 14 day observation period.

Signs seen during the 4 hour exposure period included decreased motor activity, eye squint, slight dyspnea, and lacrimation.

At 24 hours and throughout the 14 day observation period, most rats appeared normal. Signs that were observed included slight dyspnea in one rat at 24 hours and through 4 days; and soft stool which was exhibited by a few rats from 3 through 7 days and by several rats from 9 through 14 days.

All rats exhibited normal body weight gains during the study period.

The percent weight loss of compound in the flask, after pyrolysis following 4 hours was calculated to be 0.27 percent.

B. Necropsy Findings:

Necropsy findings observed at terminal necropsy conducted at 14 days revealed 7 of 10 rats showing congestion of the lungs and one rat which exhibited petechiation of the lungs.

REC 0052

International Research and Development Corporation

TABLE OF CONTENTS

	<u>Page</u>
I. Synopsis	1
II. Compound	2
III. Method	3
A. General Procedure	3
B. Compound Administration	3
IV. Results	5
A. Pharmacodynamic Signs	5
B. Necropsy Findings	5

PAGE 0054

I. SYNOPSIS

HIPS Resin/PHT4/Sb₂O₃ was evaluated for acute inhalation toxicity in albino rats after pyrolysis. No deaths occurred during the 4 hour exposure period or during the subsequent 14 day period of observation.

The pharmacodynamic and/or toxic signs observed are recorded in the body of this report.

No gross lesions were obtained at terminal necropsy following 14 days of observation.

REF 055

International Research and Development Corporation

Page 2

II. COMPOUND

The test compound was received from the Michigan Chemical Corporation, St. Louis, Michigan on November 29, 1974. It was identified as "HIPS Resin/PHT4/Sb₂O₃, Lot No. 853-11-3" and was received as off-white cylindrical chunks.

PNF 0056

134-035

75

III. METHOD:

A. General Procedure:

Five male and 5 female albino rats of the Spartan strain, weighing from 234 to 284 grams, were exposed to an atmosphere containing pyrolysis products of the test material.

The rats were housed by sex in groups of 5 rats each in wire mesh cages elevated above the droppings and maintained in temperature and humidity controlled quarters throughout the pre-exposure and post-exposure periods. Food and water were available ad libitum.

During the 4 hour exposure the rats were observed for changes in behavior and/or appearance. Immediately following the exposure, they were examined closely for pharmacodynamic and/or toxic signs. The rats were observed for a period of 14 days and then sacrificed and necropsied.

B. Compound Administration:

The rats were placed in a sealed 59.1 liter glass chamber and exposed for 4 continuous hours to a dynamic atmosphere containing pyrolysis products of the test compound. In order to prevent crowding during the exposure, the rats were separated into 4 units of two or three rats each.

The test chamber atmosphere was prepared by passing an airflow of 10 LPM through a 500 ml 3 necked flask (Pyrex No. 4950) containing 100.05 grams of HIPS Resin/PHT4/Sb₂O₃. The flask was immersed in an oil bath. Airflow through the flask was not initiated until the test compound temperature reached at least 450°F. Two 300 mm Liebig condensers (Pyrex No. 2400) were used

International Research and Development Corporation

Page 4

in line between the flask and exposure chamber. Throughout the 4 hour exposure period, the temperature of the oil bath was monitored at from 492 to 512°F; the test compound at from 459 to 500°F; and the atmosphere of the exposure chamber at from 64 to 72°F.

The flask containing the test compound was weighed before and after the exposure and sent to the sponsor. From this data the percent weight loss was calculated.

PAP 0058

IV. RESULTS:

A. Pharmacodynamic Signs:

All of the rats exposed to the pyrolysis products of the test compound survived the 4 hour exposure period and the subsequent 14 day observation period.

Signs observed during the 4 hour exposure period included decreased motor activity, eye squint and lacrimation.

At 24 and 48 hours, decreased motor activity was observed. In addition, soft stool was exhibited by one rat at 48 hours. At 72 hours and throughout the remainder of the observation period, all rats appeared normal, with the exception of one or two rats exhibiting soft stool on 3, 5, 7, 8, 9, 11 and 14 days.

All rats exhibited normal body weight gains during the study period.

The percent weight loss of compound in the flask, after pyrolysis, following 4 hours was calculated to be 2.14 percent.

B. Necropsy Findings:

No gross lesions were obtained at terminal necropsy following 14 days of observation.

PAE 0059

88-7800185

0678 - 0188

K

International Research and Development Corporation

K

SPONSOR: Michigan Chemical Corporation

COMPOUND: HIPS Resin/PHT4/Sb₂O₃

SUBJECT: Acute Inhalation Toxicity in
the Albino Rat After Pyrolysis.

REF 0060

Francis X. Wazeter, Ph.D.
President

Edwin I. Goldenthal, Ph.D.
Director of Research

Collaborators:

Dr. R. G. Geil, D.V.M.,
Vice President - Director
of Pathology

W. P. Dean

Date: March 26, 1975

134-052

Declassified
8-22-78

79

International Research and Development Corporation

TABLE OF CONTENTS

	<u>Page</u>
I. Synopsis	1
II. Compound	2
III. Method	3
A. General Procedure	3
B. Compound Administration	3
IV. Results	5
A. Pharmacodynamic Signs	5
B. Necropsy Findings	5

1900-061

I. SYNOPSIS

HIPS Resin/PHT4/Sb₂O₃ was evaluated for acute inhalation toxicity in albino rats after pyrolysis. No deaths occurred during the 6 hour exposure period or during the subsequent 14 day period of observation.

The pharmacodynamic and/or toxic signs observed are recorded in the body of this report.

No gross lesions which were considered compound related were observed at terminal necropsy following 14 days of observation.

PAR 0062

II. COMPOUND

The test compound was received from the Michigan Chemical Corporation, St. Louis, Michigan on November 29, 1974. It was identified as "HIPS Resin/PHT4/Sb₂O₃, Lot No. 853-11-3" and was received as off-white cylindrical chunks.

PAGE 0063

III. METHOD:

A. General Procedure:

Five male and 5 female albino rats of the Spartan strain, weighing from 204 to 228 grams, were exposed to an atmosphere containing pyrolysis products of the test material.

The rats were housed by sex in groups of 5 rats each in wire mesh cages elevated above the droppings and maintained in temperature and humidity controlled quarters throughout the pre-exposure and post-exposure periods. Food and water were available ad libitum.

During the 6 hour exposure, the rats were observed for changes in behavior and/or appearance. Immediately following the exposure, they were examined closely for pharmacodynamic and/or toxic signs. The rats were observed for a period of 14 days and then sacrificed and necropsied.

B. Compound Administration:

The rats were placed in a sealed 59.1 liter glass chamber and exposed for 6 continuous hours to a dynamic atmosphere containing pyrolysis products of the test compound. In order to prevent crowding during the exposure, the rats were separated into 4 units of two or three rats each.

The test chamber atmosphere was prepared by passing an airflow of 8 LPM through a 500 ml 3 necked flask (Pyrex No. 4950) containing 200.03 grams of HIPS Resin/PHT4/Sb₂O₃. The flask was immersed in an oil bath. Airflow through the flask was not initiated until the test compound temperature reached at least 450°F. The pyrolysis products were passed from the flask directly into the exposure chamber.

International Research and Development Corporation

Page 4

Throughout the 6 hour exposure period, the temperature of the oil bath was monitored at from 490 to 520°F; the test compound at from 474 to 498°F; and the atmosphere of the exposure chamber at from 69 to 84°F. The temperatures of the atmosphere in the exposure chamber were obtained from three locations within the chamber, (i.e., at the center and at both ends of the chamber).

The flask containing the test compound was weighed before and after the exposure and sent to the sponsor. From this data the percent weight loss was calculated.

PAGE 0065

134-052

84

IV. RESULTS:

A. Pharmacodynamic Signs:

All of the rats exposed to the pyrolysis products of the test compound survived the 6 hour exposure period and the subsequent 14 day observation period.

Signs observed during the 6 hour exposure period included eye squint, lacrimation, salivation, slight dyspnea and a white deposit around the nares. At the termination of the 6 hour exposure period, two rats exhibited nasal porphyrin discharge.

At 24 through 72 hours, eye squint and clear ocular discharge were observed. In addition, one rat exhibited tachypnea at both 24 and 48 hours.

At 4 days, eye squint was observed in two rats. From 5 days through the remainder of the 14 day study period, most rats appeared normal. At 6 days only, two rats exhibited very slight corneal opacity; and from 10 through 14 days, one or two rats exhibited soft stool.

All rats exhibited normal body weight gains during the study period.

The percent weight loss of compound in the flask, after pyrolysis, following 6 hours was calculated to be 1.89 percent.

B. Necropsy Findings:

No gross lesions which were considered compound related were observed at terminal necropsy following 14 days of observation.

88-7800185

(4)

International Research and Development Corporation

SPONSOR: Michigan Chemical Corporation

COMPOUND: FM PHT4 (micronized)

SUBJECT: Twenty-Eight Day Dermal Toxicity Study in Rabbits.

PAGE 0067

Francis X. Wazeter, Ph.D.
President

Edwin I. Goldenthal, Ph.D.
Director of Research

Collaborators:

R. G. Geil, D.V.M., Director
of Pathology

B. W. Benson, B.S., Director
of Small Animal Toxicology

Date: April 25, 1975

International Research and Development Corporation

TABLE OF CONTENTS

	Page
I. Synopsis	1
II. Compound	2
III. Clinical Studies	3
A. Method	3
1. General Procedure	3
2. Compound Administration	3
3. Observations	3
4. Laboratory Tests	4
a. Hematology	4
b. Biochemistry	4
c. Urinalysis	4
5. Bromine Analysis	4
B. Results	4
1. General Behavior, Appearance and Survival	4
2. Body Weights	4
3. Laboratory Tests	5
a. Hematology	5
b. Biochemistry	5
c. Urinalysis	5
4. Bromine Analysis	5
IV. Pathological Studies	7
A. Methods	7
1. Gross Pathology	7
2. Histopathology	7
B. Results	7
1. Gross Pathology and Organ Weights	7
2. Histopathology	8

Page 0-018

International Research and Development Corporation

T A B L E O F C O N T E N T S
(Continued)

<u>Table No.</u>		<u>Page</u>
1.	Individual Body Weights	10
2.	Summary of Hematological Values	11
3- 5.	Individual Hematological Values	12-14
6.	Summary of Biochemical Values	15
7- 9.	Individual Biochemical Values	16-18
10-12.	Individual Urinalysis Values	19-21
13.	Bromine Content	
14.	Necropsy Observations	
15.	Absolute and Relative Organ Weights	
16.	Histomorphologic Observations	25-26

REF ID: A68

I. SYNOPSIS

In a 28 day dermal study in New Zealand White rabbits, FM PHT⁴ (micronized) was applied at dosage levels of 50, 500 and 5000 mg/kg/day, 5 days/week for 4 weeks. Three male and 3 female rabbits were used at each dosage level and also as a control group. The rabbits were observed daily and body weights were recorded weekly. Hematological, biochemical and urinalysis studies were conducted once in the control period and at 14 and 26 days of study.

Very slight to slight and occasionally moderate erythema was noted for control rabbits and for rabbits at the 50 mg/kg/day dosage level. Very slight to moderate erythema was noted for rabbits at the 500 and 5000 mg/kg/day dosage level. Moderate desquamation also was noted for 3 of the rabbits at the 5,000 mg/kg/day dosage level. Rabbits at the 5000 mg/kg/day dosage level showed losses of body weight prior to death. All of the rabbits at the 5,000 mg/kg/day dosage level died or were sacrificed in extremis.

At 14 days of study, 1 rabbit at the 5000 mg/kg/day dosage level showed a moderate increase in urea nitrogen. At 26 days of study the 1 surviving rabbit at the 5000 mg/kg/day dosage level showed a neutrophilia with a lymphopenia, nucleated erythrocytes, marked increases in glucose and urea nitrogen and albumin in the urine.

Deaths or the declining condition which necessitated early sacrifice of all rabbits from the 5000 mg/kg/day group were considered due to compound effect. Pale liver, accentuated liver lobulation and gastric irritation in several rabbits from the 5000 mg/kg/day level may have been compound related. Microscopically, the only lesion seen in animals from the 50 or 500 mg/kg/day groups which was considered compound related was very slight hyperkeratosis of the application site in one rabbit from the 500 mg/kg/day group.

PRO 0071

90

II. COMPOUND

The compound was received from Michigan Chemical Corporation, St. Louis, Michigan on October 24, 1974. The compound was a white powder and was identified as "FM PHT4 (micronized) Lot No. 6332-B."

PAGE 0070

III. CLINICAL STUDIES

A. METHOD:

1. General Procedure:

Twelve male (weighing from 2521 to 3453 grams) and 12 female (weighing from 2815 to 3301 grams) New Zealand White rabbits were used in this study. The rabbits were housed individually in metal cages and maintained in a temperature and humidity controlled room. Purina Rabbit Chow and water were available ad libitum.

PAGE 072

Following 28 days of study, all of the rabbits were sacrificed and necropsied. Selected tissues were collected for histopathology.

2. Compound Administration:

FM PHT4 (micronized) was applied dermally at dosage levels of 50, 500 and 5000 mg/kg/day, 5 days/week for 4 weeks. Three male and 3 female rabbits were used at each dosage level and also in a control group. The control rabbits received saline only at a volume of 12 ml on the same regimen as treated rabbits.

The dorsal skin of each rabbit was shaved with electric clippers as necessary during the study. The skin of one-half of the rabbits was abraded twice a week. The compound was mixed with a small amount (maximum 12 ml) of saline to form a paste. The paste was then spread over the skin with a glass rod. The rabbits were held in wooden stocks during the 6 hour compound administration following which the backs were washed with tepid tap water and the rabbits were returned to their individual cages. Individual daily doses were based upon the body weights obtained weekly.

3. Observations:

The rabbits were observed daily for changes in general behavior and appearance. Observations for dermal irritation were done prior to

and following the 6 hour compound administration period. Individual body weights were recorded weekly.

4. Laboratory Tests:

Once in the control period and at 14 and 26 days of study, blood and urine samples were obtained from all rabbits for analysis.

a. Hematology:

Hematological studies included hemoglobin¹, hematocrit², total erythrocyte count³ and total³ and differential leucocyte counts.

b. Biochemistry:

Biochemical studies included glucose⁴, urea nitrogen⁴, serum glutamic oxalacetic and pyruvic transaminase activities⁴ and serum alkaline phosphatase activity⁴.

c. Urinalysis:

Urinalysis included measurement of volume, pH⁵ and specific gravity; description of color and appearance; and qualitative tests for albumin⁵, glucose⁵, bilirubin⁵ and occult blood⁵.

5. Bromine Analysis:

Samples of liver, fat, kidney, skin and blood were collected from all rabbits at the terminal necropsy. All samples collected from the control rabbits and rabbits at the 50 and 500 mg/kg/day dosage levels were analyzed for bromine content by neutron activation analysis.

B. RESULTS:

1. General Behavior, Appearance and Survival:

Very slight to slight and occasionally moderate erythema was noted for control rabbits and for rabbits at the 50 mg/kg/day dosage level. Very slight to moderate erythema was noted for rabbits at the

PAGE 0013

92

500 and 5000 mg/kg/day dosage level. Moderate desquamation also was noted for 3 of the rabbits at the 5,000 mg/kg/day dosage level. One rabbit at the 5,000 mg/kg/day dosage level showed marked ataxia, unable to lift head or right itself and dyspnea and was sacrificed in extremis. All of the rabbits at the 5,000 mg/kg/day dosage level died or were sacrificed in extremis.

2. Body Weights (Table 1):

Changes in body weight were similar for control rabbits and rabbits at the 50 and 500 mg/kg/day dosage level. Rabbits at the 5000 mg/kg/day dosage level showed losses of body weight prior to death.

3. Laboratory Tests (Tables 2-12):

a. Hematology:

At 26 days of study, the 1 surviving rabbit at the 5,000 mg/kg/day dosage level showed a neutrophilia with a lymphopenia and nucleated erythrocytes. No other changes were seen in hematological studies.

b. Biochemistry:

At 14 days of study, 1 rabbit at the 5000 mg/kg/day dosage level showed a moderate increase in urea nitrogen. At 26 days of study the 1 surviving rabbit at the 5000 mg/kg/day dosage level showed a marked increase in glucose and urea nitrogen. No other changes were seen in biochemical studies.

c. Urinalysis:

At 26 days of study, the 1 surviving rabbit at the 5000 mg/kg/day dosage level showed a 2+ for albumin in the urine. No other changes were seen in urinalysis.

4. Bromine Analysis:

The results of the bromine analysis are presented in Table 13.

PARF 0074

0 0 8 6

Examination of the bromine data reveals a marked increase in the concentration of bromine found in the skin at the 50 and 500 mg/kg/day dosage levels. Increases in bromine were also noted in liver samples obtained from the 50 and 500 mg/kg/day dosage levels (these increases were slight in degree), in kidney and blood samples at the 50 and 500 mg/kg/day levels (both kidney and blood samples produced very slight increases at the 50 mg/kg/day levels). An increase in the bromine content of fat samples also was noted at the 500 mg/kg/day dosage level.

PAGE 0175

Tissue and blood samples were not retained for analysis from animals which died or were sacrificed on study from the 5000 mg/kg/day dosage level.

Statistical analysis of the increases noted above revealed statistically significant increases in the skin samples at the 50 mg/kg/day dosage level and in the skin and blood samples at the 500 mg/kg/day dosage level. (See Table 13).

All statistical analyses compared the treatment groups with the control group. The bromine content in the liver, fat, kidney, skin and blood were compared by analysis of variance (one-way classification) as described by Steel and Torrie⁶. Then Bartlett's test for homogeneity of variances as described by Steel and Torrie⁶ was applied to the respective parameters. The appropriate t-test (for equal or unequal variances) as described by Steel and Torrie⁶ was used to judge the significance of differences between the means, based upon Dunnett's⁷ multiple comparison tables.



IV. PATHOLOGICAL STUDIES

A. METHODS:

1. Gross Pathology:

At the completion of the compound application period, all rabbits were sacrificed with intravenous air embolization and necropsied. Selected organs were weighed and representative tissues from each rabbit were collected in buffered neutral 10% formalin.

2. Histopathology:

The following tissues from each rabbit from the control and 500 mg/kg/day groups were paraffin embedded, sectioned, stained with hematoxylin and eosin and examined microscopically:

skin	ileum	nerve, muscle
regional	jejunum	bone/marrow
lymph node	colon	thymus
mesenteric	liver	heart
lymph node	gallbladder	lung
spleen	adrenals	thyroid, parathyroid
pancreas	kidneys	eye
stomach	urinary bladder	brain
duodenum	ovaries/testes	pituitary

Hematoxylin and eosin stained paraffin sections of skin, liver, kidneys and bone marrow from rabbits from the 50 mg/kg/day group also were prepared and examined.

B. RESULTS:

1. Gross Pathology (Table 14) and Organ Weights (Table 15):

Deaths or the declining condition which necessitated early sacrifice of all rabbits from the 5000 mg/kg/day group were attributed to compound effect. In rabbits from this group, pale liver, accentuated liver lobulation and evidence of gastric irritation in a few rabbits may have been due to compound effect. No gross lesions considered compound related were seen in

910076

95

any rabbits from the 50 or 500 mg/kg/day groups which were sacrificed at termination.

No compound related organ weight effects were observed in rabbits from the experimental groups.

2. Histopathology (Table 16):

Except for very slight hyperkeratosis of the application site one rabbit in the 500 mg/kg/day group, no microscopic lesions considered compound related were seen in any tissues examined from rabbits from the 50 or 500 mg/kg/day groups. Microscopic lesions in these animals were those which commonly occur spontaneously and were not significant.

PAGE 08 77

References

1. Cyanmethemoglobin Method, John B. Miale, 3rd Ed., 1967, The C. V. Mosby Company, p. 1143.
2. Microhematocrit, John B. Miale, 3rd E., 1967, The C. V. Mosby Company, p. 1154.
3. Coulter Particle Size Counter, Model A., Coulter Electronics, 590 W. 20th Street, Hialeah, Florida.
4. Technicon Auto Analyzer 6/60 Micro Methodology.
5. "Bililabstix" (Ames Reagent Strips).
6. Steel, R. G. D. and Torrie, J. H. (1960), Principles and Procedures of Statistics, McGraw-Hill Book Company, Inc., New York, New York.
7. Dunnett, C. W. (1964), New Tables for Multiple Comparisons With a Control, Biometrics, 482-491.

PAGE 0079

FM PHT4:

Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 1. Individual Body Weights, grams.

Group, Rabbit No.	Sex	Compound Administration Weeks					
		-1	0	1	2	3	4
Control:							
21164	M	2578	2641	2517	2702	2829	2806
21168	M	2684	2801	2664	2630	2692	2748
21216	M	3407	3453	3286	3360	3351	3414
21165	F	3315	3301	3142	2925	3210	3339
21167	F	2826	2815	2678	2659	2830	2888
21169	F	2451	2981	2827	2728	2892	2908
Mean		2877	2999	2852	2834	2967	3017
50 mg/kg/day:							
21170	M	3125	3133	3086	2978	3210	3223
21172	M	2643	2955	2716	2653	2952	3008
21174	M	2659	2811	2670	2639	2738	2735
21173	F	3128	3084	2566	2555	2793	2802
21217	F	2097	2967	2793	2750	2950	3070
21223	F	2839	2948	2685	2658	2720	3049
Mean		2884	2983	2753	2706	2894	2981
500 mg/kg/day:							
21176	M	3040	2973	2786	2670	2661	2770
21212	M	2504	2639	2588	2607	2658	2714
21214	M	3313	3338	3156	3219	3444	3476
21177	F	3141	3272	2907	2812	3032	2872
21181	F	2751	2854	2692	2591	2863	3135
21221	F	2836	2878	2732	2677	2790	3002
Mean		2931	2992	2810	2763	2908	2995
5000 mg/kg/day:							
21184	M	2352	2521	2304	Died, day 11		
21186	M	2962	3010	2911	2929	2589	died, day 26
21188	M	3198	3362	3109	Sacrificed, day 11		
21183	F	3013	3116	2770	Died, day 10		
21185	F	2915	3058	2751	Died, day 10		
21187	F	2881	2910	2779	2627	Died, day 17	
Mean		2887	2996	2771	2778	2589	

PHT 0019

FM PHTA:

Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 2.

Summary of Hematological Values.

Hematology	Days	Control	50 mg/ kg/day	500 mg/ kg/day	5000 mg/ kg/day
Erythrocytes, 10^6 /cmm	Control	4.83	4.96	5.22	4.93
	14	5.62	5.61	5.74	-
	28	5.60	5.60	5.81	-
Hemoglobin, gm/100 ml	Control	11.2	11.7	11.8	11.2
	14	12.0	11.7	12.0	-
	28	12.6	12.5	12.8	-
Hematocrit, %	Control	35	37	39	37
	14	40	40	41	-
	28	39	39	34	-
Leucocytes, 10^3 /cmm	Control	8.44	7.24	8.76	9.01
	14	8.41	7.32	8.03	-
	28	8.85	8.19	9.27	-
Neutrophils, %	Control	26	24	36	28
	14	28	41	46	-
	28	32	40	35	-
Lymphocytes, %	Control	71	73	62	69
	14	71	58	51	-
	28	66	59	64	-
Eosinophils, %	Control	2	2	1	2
	14	0	1	2	-
	28	1	1	1	-
Monocytes, %	Control	1	1	1	1
	14	1	0	1	-
	28	1	0	0	-
Basophils, %	Control	0	0	0	0
	14	0	0	0	0
	28	0	0	0	0

FM PH14: Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 3. Individual Hemtological Values - Control.

Group, Rabbit No.	Sex	Erythro- cytes 10^6 /cmm	Hemo- globin gm/100 ml	Hemato- crit %	Leuco- cytes 10^3 /cmm	Neutrophils Seg. Non-Seg.	Lympho- cytes %	Eosino- phils %	Mono- cytes %	Baso- phils %
Control:										
21164	M	4.87*	10.9	33	8.55	28	0	67	5	0
21168	M	4.84	11.5	36	8.44	21	0	77	1	0
21216	M	4.57	11.3	35	8.29	40	0	55	4	0
21165	F	5.12	11.6	36	7.42	23	0	74	0	0
21167	F	4.55	10.6	34	9.36	33	0	65	1	0
21169	F	5.02	11.5	36	8.57	11	0	86	2	0
Mean		4.83	11.2	35	8.44	26	0	71	2	0
50 mg/kg/day:										
21170	M	5.53	13.0	42	8.00	17	0	81	2	0
21172	M	4.64	10.8	34	7.87	12	0	86	1	0
21174	M	4.71	11.1	35	9.13	18	0	81	1	0
21173	F	5.18	12.7*	38*	6.74	21	0	77	2	0
21217	F	4.60	11.0	36	6.18	43	0	55	2	0
21223	F	5.12	11.8	38	5.54	30	0	66	2	0
Mean		4.96	11.7	37	7.24	24	0	73	2	0
500 mg/kg/day:										
21176	M	5.53	12.6	41	10.99	55	0	41	2	1
21212	M	4.76	11.3	37	7.71	35	0	63	2	0
21214	M	5.61	12.2	41	6.87	50	0	50	0	0
21177	F	4.87	10.9	36	8.34	21	0	77	1	0
21181	F	4.76	11.0	35	9.18	29	0	69	0	0
21221	F	5.76	12.9	43	9.47	26	0	73	0	0
Mean		5.22	11.8	39	8.76	36	0	62	1	0
5000 mg/kg/day:										
21184	M	5.01	10.6	37	10.23	18	0	76	5	0
21186	M	4.80	11.3	37	8.25	18	0	77	2	0
21188	M	4.98	10.8	35	8.73	56	0	44	0	0
21183	F	4.76	11.3	35	7.26	31	0	63	5	0
21185	F	4.78	11.0	36	11.11	22	0	76	1	0
21187	F	5.24	11.9	39	8.47	20	0	79	1	0
Mean		4.93	11.2	37	9.01	28	0	69	2	0

PRF 0082

*Repeat Determination

134-030

Twenty-Night Day Dermal Toxicity Study in Rabbits.

TABLE 4. Individual Hematological Values - 14 Days.

Group, Rabbit No.	Sex	Erythro- cytes 10 ⁶ /cmm	Hemo- globin gm/100 ml	Hemato- crit %	Leuco- cytes 10 ³ /cmm	Neutrophils Seg. Non-Seg.	Lympho- cytes %	Eosino- phils %	Mono- cytes %	Huso- phils %
<u>Control:</u>										
21164	M	5.47	11.3	38	8.25	17	82	1	0	0
21168	M	5.54	11.9	38	11.10	39	60	1	0	0
21216	M	5.55	11.5	38	7.27	23	77	0	0	0
21165	F	5.61	12.2	41	9.86	43	55	0	2	0
21167	F	5.68	12.1	40	6.55	11	89	0	0	0
21169	F	5.85	12.8	42	7.45	32	66	0	2	0
Mean		5.62	12.0	40	8.41	28	71	0	1	0
<u>500 mg/kg/day:</u>										
21170	M	5.88	12.4	42	6.53	46	52	1	1	0
21172	M	5.53	11.5	39	8.72	33	67	0	0	0
21174	M	5.83	11.4	38	11.35	63	37	0	0	0
21173	F	5.56	12.1	43	5.93	38	62	0	0	0
21217	F	5.41	11.5	38	5.28	27	73	0	0	0
21223	F	5.43	11.5	38	6.08	36	61	3	0	0
Mean		5.61	11.7	40	7.32	41	58	1	0	0
<u>500 mg/kg/day:</u>										
21176	M	6.15	13.1	43	10.25	39	58	2	1	0
21212	M	5.83	12.2	43	8.08	42	56	2	0	0
21214	M	5.68	11.8	40	8.13	62	36	0	2	0
21177	F	5.42	11.6	40	7.29	58	42	0	0	0
21181	F	5.24	10.2	36	6.46	32	65	3	0	0
21221	F	6.14	13.3	42	7.98	43	54	2	1	0
Mean		5.74	12.0	41	8.03	46	51	2	1	0
<u>5000 mg/kg/day:</u>										
21184	M	Died, day 11								
21186	M	5.60	11.9	41	7.48	32	68	0	0	0
21188	M	Sacrificed, day 11								
21183	F	Died, day 10								
21185	F	Died, day 10								
21187	F	5.84	11.6	40	7.50	28	71	0	0	0

Twenty-Eight Day Dermal Toxicity Study in Rabbits.

Individual Hematological Values - 28 Days.

Group, Rabbit No.	Sex	Erythro-cytes 10 ⁶ /cmm	Hemo-globin gm/100 ml	Hemato-crit %	Leuco-cytes 10 ³ /cmm	Neutrophils Seg. Non-Seg.	Lympho-cytes %	Eosino-phils %	Mono-cytes %	Maso-phils %
Control:										
21164	M	5.34	11.9	36	10.16	35	65	0	0	0
21168	M	5.84	12.9	42	8.42	30	68	2	0	0
21216	M	5.43	12.5	38	10.95	40	57	1	2	0
21165	F	5.48	12.0	36	7.17	25	72	2	1	0
21167	F	5.44	12.6	39	7.74	14*	86	0	0	0
21169	F	6.06	13.7	42	8.66	48	49	3	0	0
Mean		5.60	12.6	39	8.85	32	66	1	1	0
50 mg/kg/day:										
21170	M	6.20	13.9	41	8.21	41	58	1	0	0
21172	M	5.64	12.5	39	10.32	49	50	0	1	0
21174	M	5.29	12.0	38	7.73	55	44	1	0	0
21173	F	5.65	13.0	40	8.32	30	68	2	0	0
21217	F	5.08	11.5	36	7.01	34	66	0	0	0
21223	F	5.72	12.3	39	7.57	30	69	0	.1	0
Mean		5.60	12.5	39	8.19	40	59	1	0	0
500 mg/kg/day:										
21176	M	6.52	14.1	45	9.21	51	46	2	1	0
21212	M	5.78	12.9	41	11.29	28	71	0	1	0
21214	M	5.69	12.1	39	7.61	14*	85	1	0	0
21177	F	5.54	11.9	38	11.01	47	0	0	0	0
21181	F	5.26	11.8	38	7.00	44	53	3	0	0
21221	F	6.07	13.7	43	9.47	24	75	1	0	0
Mean		5.81	12.8	41	9.27	35	64	1	0	0
5000 mg/kg/day:										
21184	M	Died, day 11								
21186	M	5.01	11.9	34	14.92	88**	12	0	0	0
21188	M	Sacrificed, day 11								
21183	F	Died, day 10								
21185	F	Died, day 10								
21187	F	Died, day 17								

PAF 0083

*Repeat Determination
**22 nucleated RBC/ 100 WBC

134-030

102

FM PHT4: Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 6. Summary of Biochemical Values.

Biochemistry	Days	Control	50 mg/ kg/day	500 mg/ kg/day	5000 mg/ kg/day
Glucose, mg/100 ml	Control	134	139	139	135
	14	79	94	87	-
	28	122	130	129	-
B.U.N., mg/100 ml	Control	18.8	16.2	17.7	19.7
	14	17.3	15.0	15.1	-
	28	18.3	17.3	17.0	-
Alkaline Phosphatase, international units/ml	Control	155	176	189	135
	14	95	91	122	-
	28	71	67	91	-
S.G.O.T., international units/ml	Control	19	29	26	18
	14	24	19	21	-
	28	16	14	16	-
S.G.P.T., international units/ml	Control	24	19	30	26
	14	27	27	34	-
	28	41	44	54	-

134-030

1800

103

0096

FM PHT4:

Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 7. Individual Biochemical Values - Control.

Group, Rabbit No.	Sex	Glucose mg/100 ml	B.U.N. mg/100 ml	Alkaline Phos. int'l Units/ml	S.G.O.T. int'l Units/ml	S.G.P.T. int'l Units/ml
<u>Control:</u>						
21164	M	116	13.0	169	18	26
21168	M	122	15.5	200	20	30
21216	M	151	23.0	167	21	15
21165	F	126	21.0	162	25	27
21167	F	115	20.1	109	13	34
21169	F	134	20.5	125	20	10
Mean		134	18.8	155	19	24
<u>50 mg/kg/day:</u>						
21170	M	130	15.0	223	20	16
21172	M	136	16.0	220	25	20
21174	M	129	12.1	110	23	17
21173	F	136	20.0	187	25	17
21217	F	145	19.0	149	30	19
21223	F	161	15.0	167	52	26
Mean		139	16.2	176	29	19
<u>500 mg/kg/day:</u>						
21176	M	117	21.0	189	38	35
21212	M	144	17.0	197	19	32
21214	M	141	15.8	193	26	38
21177	F	139	17.5	145	24	16
21181	F	146	18.0	224	22	32
21221	F	146	17.1	185	29	30
Mean		139	17.7	189	26	30
<u>5000 mg/kg/day:</u>						
21184	M	125	18.5	153	25	25
21186	M	117	18.0	95	11	15
21188	M	133	13.8	150	23	33
21183	F	139	20.0	173	18	38
21185	F	139	24.9	124	11	20
21187	F	155	23.0	118	22	25
Mean		135	19.7	135	18	26

PAGE 005

FM PHT4: Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 8. Individual Biochemical Values - 14 Days.

Group, Rabbit No.	Sex	Glucose mg/100 ml	B.U.N. mg/100 ml	Alkaline Phos. int'l Units/ml	S.G.O.T. int'l Units/ml	S.G.P.T. int'l Units/ml
<u>Control:</u>						
21164	M	81	10.5	109	21	35
21168	M	79	12.0	105	18	26
21216	M	80	17.5	131	39	20
21165	F	74	21.1	85	29	25
21167	F	89	20.1	73	19	38
21169	F	71	22.5	66	17	17
Mean		79	17.3	95	24	27
<u>50 mg/kg/day:</u>						
21170	M	82	16.2	121	6	19
21172	M	75	14.9	116	19	19
21174	M	99	16.0	65	19	26
21173	F	71	13.9	79	43	45
21217	F	115	15.9	104	20	26
21223	F	119	13.1	58	9	28
Mean		94	15.0	91	19	27
<u>500 mg/kg/day:</u>						
21176	M	95	11.0	66	20	32
21212	M	93	13.9	174	21	37
21214	M	84	12.6	110	17	35
21177	F	91	16.0	105	17	22
21181	F	68	19.1	136	36	45
21221	F	91	18.1	140	17	31
Mean		87	15.1	122	21	34
<u>5000 mg/kg/day:</u>						
21184	M	Died, day 11				
21186	M	87	14.1	55	10	17
21188	M	Sacrificed, day 11				
21183	F	Died, day 10				
21185	F	Died, day 10				
21187	F	91	59.0	47	17	47

PAGE 0006

105

FM PHT4:

Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 9. Individual Biochemical Values - 28 Days.

Group, Rabbit No.	Sex	Glucose mg/100 ml	B.U.N. mg/100 ml	Alkaline Phos. int'l Units/ml	S.G.O.T. int'l Units/ml	S.G.P.T. int'l Units/ml
<u>Control:</u>						
21164	M	116	13.2	80	17	59
21168	M	128	13.9	79	20	43
21216	M	128	17.9	84	20	34
21165	F	125	22.1	72	14	52
21167	F	120	21.2	59	15	31
21169	F	116	21.5	50	11	28
Mean		122	18.3	71	16	41
<u>50 mg/kg/day:</u>						
21170	M	146	19.0	70	15	34
21172	M	122	18.9	93	15	37
21174	M	120	16.0	64	13	44
21173	F	107	15.1	56	16	47
21217	F	151	14.0	59	11	37
21223	F	135	21.0	61	12	66
Mean		130	17.3	67	14	44
<u>500 mg/kg/day:</u>						
21176	M	131	15.2	63	17	62
21212	M	132	17.1	96	15	51
21214	M	146	25.5	140	18	65
21177	F	131	12.9	84	20	61
21181	F	117	16.0	75	12	44
21221	F	115	15.0	89	16	39
Mean		129	17.0	91	16	54
<u>5000 mg/kg/day:</u>						
21184	M	Died, day 11				
21186	M	498*	175.0	29	80	61
21188	M	Sacrificed, day 11				
21183	F	Died, day 10				
21185	F	Died, day 10				
21187	F	Died, day 17				

ME0007

FM PHT4:

Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 10. Individual Urinalysis Values - Control.

Group, Rabbit No.	Sex	Vol. ml.	Color & Appear.	Albu- min	Glucose	Occult Blood	Bili- rubin	Spec. Grav.	pH
<u>Control:</u>									
21164	M	18	Normal	N	N	N	N	1.028	8.2
21168	M	120	Normal	N	N	N	N	1.016	8.4
21216	M	120	Normal	N	N	N	N	1.035	8.3
21165	F	155	Normal	N	N	N	N	1.022	7.1
21167	F	130	Normal	N	N	N	N	1.020	8.3
21169	F	70	Normal	N	N	N	N	1.021	8.5
<u>50 mg/kg/day:</u>									
21170	M	230	Normal	N	N	N	N	1.016	8.2
21172	M	135	Normal	N	N	N	N	1.031	8.0
21174	M	140	Normal	N	N	N	N	1.014	8.6
21173	F	165	Normal	N	N	N	N	1.015	8.3
21217	F	220	Normal	N	N	N	N	1.015	7.8
21223	F	240	Normal	N	N	N	N	1.013	9.0
<u>500 mg/kg/day:</u>									
21176	M	165	Normal	N	N	N	N	1.014	8.5
21212	M	250	Normal	N	N	tr	N	1.011	8.0
21214	M	215	Normal	N	N	N	N	1.011	7.3
21177	F	390	Normal	N	N	N	N	1.014	7.0
21181	F	285	Normal	N	N	N	N	1.014	9.0
21221	F	130	Normal	N	N	N	N	1.019	8.1
<u>5000 mg/kg/day:</u>									
21184	M	245	Normal	N	N	N	N	1.017	7.0
21186	M	90	Normal	N	N	N	N	1.027	8.7
21188	M	90	Normal	N	N	N	N	1.022	9.0
21183	F	215	Normal	N	N	tr	N	1.010	6.8
21185	F	210	Normal	N	N	N	N	1.015	6.7
21187	F	175	Normal	N	N	1+	N	1.023	8.1

134-030

Code: N - Negative
tr - Trace
1+ - Trace to slight

2+ - Slight to moderate
3+ - Moderate
4+ - Marked

FM PHT4:

Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 11. Individual Urinalysis Values - 14 Days.

Group, Rabbit No.	Sex	Vol. ml.	Color & Appear.	Albu- min	Glucose	Occult Blood	Bili- rubin	Spec. Grav.	pH
<u>Control:</u>									
21164	M	90	Normal	N	N	2+	N	1.019	9.0
21168	M	120	Normal	N	N	2+	N	1.011	7.8
21216	M	100	Normal	N	N	N	N	1.042	8.9
21165	F	130	Normal	N	N	3+	N	1.024	8.1
21167	F	120	Normal	N	N	tr	N	1.023	8.9
21169	F	205	Normal	N	N	tr	N	1.012	8.8
<u>50 mg/kg/day:</u>									
21170	M	250	Normal	N	N	tr	N	1.011	9.0
21172	M	260	Normal	N	N	2+	N	1.013	9.0
21174	M	175	Normal.	N	N	1+	N	1.013	8.9
21173	F	205	Normal	N	N	tr	N	1.025	9.0
21217	F	80	Normal	N	N	tr	N	1.028	8.4
21223	F	70	Normal	N	N	1+	N	1.028	8.6
<u>500 mg/kg/day:</u>									
21176	M	290	Normal	N	N	2+	N	1.011	8.5
21212	M	350	Normal	N	N	tr	N	1.014	9.0
21214	M	85	Normal	N	N	4+	N	1.026	9.0
21177	F	230	Normal	N	N	3+	N	1.015	8.1
21181	F	390	Normal	N	N	1+	N	1.014	8.8
21221	F	50	Normal	N	N	2+	N	1.031	8.6
<u>5000 mg/kg/day:</u>									
21184	M	Died, day 11							
21186	M	85	Normal	N	N	N	N	1.023	9.0
21188	M	Sacrificed, day 11							
21183	F	Died, day 10							
21185	F	Died, day 10							
21187	F	Q.N.S.							

134-030

Code: N - Negative
 tr - Trace
 1+ - Trace to slight
 QNS - Quantity not sufficient

2+ - Slight to moderate
 3+ - Moderate
 4+ - Marked

108

FM PHT4: Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 12. Individual Urinalysis Values - 28 Days.

Group, Rabbit No.	Sex	Vol. ml.	Color & Appear.	Albu- min	Gluc- cose	Occult Blood	Bili- rubin	Spec. Grav.	pH
<u>Control:</u>									
21164	M	135	Normal	N	N	tr	N	1.020	8.9
21168	M	85	Normal	N	N	tr	N	1.015	8.8
21216	M	130	Normal	N	N	tr	N	1.015	8.6
21165	F	160	Normal	N	N	1+	N	1.028	8.5
21167	F	280	Normal	N	N	4+	N	1.013	7.8
21169	F	170	Normal	N	N	4+	N	1.010	8.0
<u>50 mg/kg/day:</u>									
21170	M	140	Normal	N	N	3+	N	1.015	8.8
21172	M	200	Normal	N	N	3+	N	1.015	8.9
21174	M	20	Normal	N	N	1+	N	1.028	7.2
21173	F	300	Normal	N	N	N	N	1.010	7.9
21217	F	50	Normal	N	N	N	N	1.015	8.6
21223	F	300	Normal	N	N	tr	N	1.010	8.1
<u>500 mg/kg/day:</u>									
21176	M	180	Normal	N	N	tr	N	1.011	8.6
21212	M	85	Normal	N	N	N	N	1.011	8.0
21214	M	300	Normal	N	N	2+	N	1.014	8.0
21177	F	85	Normal	N	N	N	N	1.011	8.2
21181	F	145	Normal	N	N	tr	N	1.011	9.0
21221	F	55	Normal	N	N	N	N	1.023	9.0
<u>5000 mg/kg/day:</u>									
21184	M	Died, day 11							
21186	M	175	Normal	2+*	4+*	tr	N	1.020	6.0
21188	M	Sacrificed, day 11							
21183	F	Died, day 10							
21185	F	Died, day 10							
21187	F	Died, day 17							

134-030

Code: N - Negative
tr - Trace
1+ - Trace to slight
*Repeat Determination

2+ - Slight to moderate
3+ - Moderate
4+ - Marked

109

FM PHT4:

Twenty-Eight Day Toxicity Study in Rats.

TABLE 13.

Bromine Content, ppm

Group, Rat Number	Sex	Compound Administration Week 4				
		Liver	Fat	Kidney	Skin	Blood
Control:						
21164	M	2.4	1.1	7.5	107.0	13.1
21168	M	4.6	2.5	5.9	34.0	13.3
21216	M	1.9	3.1	14.0	13.3	13.3
21165	F	3.0	2.4	7.4	42.3	7.8
21167	F	3.6	1.7	7.6	28.1	8.8
21169	F	6.5	0.61	8.7	12.6	7.7
Mean		3.7	1.9	8.5	39.6	8.7
50 mg/kg/day:						
21170	M	4.2	2.2	12.9	695.0	9.4
21172	M	2.6	2.1	8.9	361.0	13.4
21174	M	2.5	2.2	11.2	1090.0	7.7
21173	F	7.8	4.0	11.1	1310.0	8.7
21217	F	3.1	2.1	10.1	250.0	10.6
21223	F	5.7	<1.0	9.7	566.0	10.4
Mean		4.3	2.3	10.7	712.0 ^a	10.0
500 mg/kg/day:						
21176	M	5.3	5.6	19.5	471.0	21.5
21212	M	0.68	32.7	15.3	863.0	10.8
21214	M	4.5	3.4	17.8	608.0	11.8
21177	F	7.6	1.4	11.2	648.0	10.4
21181	F	5.1	6.8	43.8	629.0	13.3
21221	F	5.1	4.0	16.4	1140.0	11.7
Mean		4.7	9.0	20.7	726.5 ^b	13.3 ^a
5000 mg/kg/day:						
21184	M	Died, day 11*				
21186	M	Died, day 26*				
21188	M	Sacrificed, day 11*				
21183	F	Died, day 10*				
21185	F	Died, day 10*				
21187	F	Died, day 17*				

*Tissues were not retained for Bromine Analysis

^aSignificantly different than control group mean, p<0.05^bSignificantly different than control group mean, p<0.01

FM PHT4:

Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 14.

Necropsy Observations.

Group, Rabbit No.	Sex	no gross lesions	accessory adrenal on right adrenal	congestion, lung	edema, lung	localized atelectasis, lung	mucosal petechiation, stomach	few scattered erosions and ulcers, stomach	nematodes, cecum	impaction, cecum, colon	pale liver	accentuated lobulation, liver	5 mm dark, depressed area right kidney	pale kidneys	hemorrhage in fascia under application site
<u>Control:</u>															
21164	M	x													
21168	M	x													
21216	M														x
21165	F	x													
21167	F	x													
21169	F														x
<u>50 mg/kg/day:</u>															
21170	M								x						
21172	M	x													
21174	M	x													
21173	F	x													
21217	F	x													
21223	F														x
<u>500 mg/kg/day:</u>															
21176	M	x													
21212	M	x													
21214	M			x									x		
21177	F	x													
21181	F	x													
21221	F	x													
<u>5000 mg/kg/day:</u>															
21184*	M			x	x						x	x			
21186*	M						x								
21188*	M					x		x	x	x	x			x	
21183*	F			x	x										
21185*	F			x	x										
21187*	F			x											

-030

*died or sacrificed in extremis

Twenty-Eight Day Dermal Toxicity Study in Rabbits.

PH 4744:

TABLE 15. Absolute (Grams) and Relative (% Body Weight) Organ Weights.

Group, Rabbit No.	Sex	Body Wt. gm	Spleen		Liver		Adrenals		Kidneys		Testes/Ovaries		Thyroid Parathyroid		Brain	
			gm	%x10 ²	gm	%	gm	%x10 ²	gm	%	gm	%x10 ²	gm	%x10 ²	gm	%
Control:																
21164	M	2806	0.92	3.28	107.86	3.84	1.09	3.88	18.81	0.67	5.68	0.20	0.330	1.18	8.51	0.30
21168	M	2748	0.50	1.82	115.67	4.21	0.50	1.82	17.01	0.62	5.90	0.21	0.240	0.87	9.45	0.34
21216	M	3414	0.99	2.90	134.63	3.94	0.46	1.35	21.42	0.63	5.20	0.15	0.473	1.39	9.61	0.28
21165	F	3339	1.20	3.59	132.33	3.96	0.51	1.53	21.52	0.64	0.359	1.08	0.338	1.16	9.31	0.28
21167	F	2888	1.15	3.98	102.30	3.54	0.51	1.77	20.82	0.72	0.286	0.99	0.309	1.07	9.11	0.32
21169	F	2908	1.68	5.78	86.98	2.99	0.49	1.69	15.11	0.52	0.365	1.26	0.335	1.15	9.21	0.32
500 mg/kg/day:																
21170	M	3223	1.42	4.41	124.48	3.86	0.73	2.26	18.81	0.58	6.91	0.21	0.408	1.27	9.10	0.28
21172	M	3008	1.50	4.99	107.00	3.56	0.40	1.33	18.11	0.60	3.36	0.11	0.320	1.06	10.51	0.35
21174	M	2735	0.67	2.45	97.19	3.55	0.60	2.19	14.21	0.52	5.00	0.18	0.439	1.61	8.71	0.32
21173	F	2802	1.42	5.07	101.15	3.61	0.90	3.21	21.72	0.78	0.220	0.79	0.399	1.42	9.21	0.33
21217	F	3070	0.80	2.61	134.58	4.38	1.12	3.65	19.62	0.64	0.380	1.24	0.440	1.43	8.68	0.28
21223	F	3049	1.22	4.00	151.60	4.97	0.57	1.87	21.62	0.71	0.199	0.65	0.378	1.24	8.51	0.28
5000 mg/kg/day:																
21176	M	2770	0.80	2.89	80.78	2.92	0.60	2.17	15.81	0.57	6.10	0.22	0.279	1.01	8.61	0.31
21212	M	2714	1.10	4.05	108.91	4.01	0.40	1.47	18.11	0.67	6.41	0.24	0.390	1.44	8.01	0.30
21214	M	3476	1.20	3.45	159.46	4.59	0.51	1.47	21.42	0.62	5.30	0.15	0.324	0.93	8.61	0.25
21177	F	2872	1.20	4.18	99.30	3.46	0.40	1.39	16.51	0.57	0.162	0.56	0.354	1.23	8.41	0.29
21181	F	3135	1.74	5.56	107.98	3.44	1.19	3.80	21.10	0.67	0.660	2.11	0.720	2.30	9.37	0.30
21221	F	3002	1.00	3.33	97.59	3.25	0.50	1.67	15.71	0.52	0.301	1.00	0.258	0.86	9.01	0.30
5000 mg/kg/day:																
21184	M	2230*	1.00	4.50	99.81	4.50	0.83	3.74	20.39	0.92	4.17	0.19	-	-	8.32	0.37
21186	M	2630*	0.73	3.27	105.72	4.74	1.00	4.48	18.20	0.82	6.29	0.28	0.900	4.04	9.90	0.44
21188	M	2630*	1.13	4.30	112.83	4.29	0.52	1.98	23.12	0.88	-	-	0.223	0.85	8.29	0.32
21183	F	3109*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21185	F	2751*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21187	F	2377*	1.94	8.16	83.36	3.51	0.90	3.79	23.10	0.97	0.203	0.85	-	-	8.37	0.35

*died or sacrificed in extreme

134-030

MBE 0093

112

Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 16. Histomorphologic Observations.

Group, Rabbit Number	Sex	Histomorphologic Observations																				
		Adrenal	Thyroid	Heart	Lung	Kidney	Liver	Cecum	Large Intestine	Pancreas	Spleen											
Control:																						
21164	M																					
21168	M																					
21216	M																					
21165	F																					
21167	F																					
21169	F																					
50 mg/kg/day:																						
21170	M																					
21172	M																					
21174	M																					
21173	F																					
21217	F																					
21223	F																					
500 mg/kg/day:																						
21176	M																					
21212	M																					
21214	M																					
21177	F																					
21181	F																					
21221	F																					

Code: n = normal or essentially so
 x = condition present
 2 = very slight
 3 = slight
 4 = moderate
 5 = marked
 6 = extreme

NOF 0094

PAF 0095

PM PM4: Twenty-Eight Day Dermal Toxicity Study in Rabbits.

TABLE 16. Continued. Histomorphologic Observations.

Group, Rabbit Number	Sex	Skin		Peripheral Nerve														
		diffuse hyperkeratosis,		local reordering	hemorrhage	Brain	Pituitary	Spleen	Testis/Ovary	Thymus	Stomach	Small intestine	Skeletal muscle	Urinary bladder	Lymph node	Gallbladder	Eye	Bone marrow
<u>Control:</u>																		
21164	M	n		n		n		n		n		n		n		n		n
21168	M	n		n		n		n		n		n		n		n		n
21216	M	n		n		n		n		n		n		n		n		n
21165	F	n		n		n		n		n		n		n		n		n
21167	F	n		n		n		n		n		n		n		n		n
21169	F	n		n		n		n		n		n		n		n		n
<u>50 mg/kg/day:</u>																		
21170	M	n		n		n		n		n		n		n		n		n
21172	M	n		n		n		n		n		n		n		n		n
21174	M	n		n		n		n		n		n		n		n		n
21173	F	n		n		n		n		n		n		n		n		n
21217	F	n		n		n		n		n		n		n		n		n
21223	F	n		n		n		n		n		n		n		n		n
<u>500 mg/kg/day:</u>																		
21176	M	n		n		n		n		n		n		n		n		n
21212	M	n		n		n		n		n		n		n		n		n
21214	M	n		n		n		n		n		n		n		n		n
21177	F	n	2	n		n		n		n		n		n		n		n
21181	F	n		n		n		n		n		n		n		n		n
21221	F	n		n		n		n		n		n		n		n		n

Code: n - normal or essentially so 3 - slight 6 - extreme
 x - condition present 4 - moderate
 2 - very slight 5 - marked

114

88-7800185

0678-0185

M

International Research and Development Corporation

M

SPONSOR: Michigan Chemical Corporation

COMPOUND: FM PHT4 (Micronized)

SUBJECT: Twenty-one Day Inhalation Toxicity Study in Rats.

REC 0096

Francis X. Wazeter
Francis X. Wazeter, Ph.D.
President

Edwin I. Goldenthal
Edwin I. Goldenthal, Ph.D.
Director of Research

Collaborators:

R. G. Geil, D.V.M., Director of Pathology
W. P. Dean

Date: June 24, 1975

134-029

Document declassified
8-22-78

[REDACTED]

International Research and Development Corporation

TABLE OF CONTENTS

	<u>Page</u>
I. Synopsis	1
II. Compound	3
III. Clinical Studies	4
A. Method	4
1. General Procedure.	4
2. Compound Administration.	4
3. Observations	5
4. Clinical Laboratory Tests.	5
a. Hematology	5
b. Biochemistry	5
c. Urinalysis	5
5. Bromine Analysis	5
B. Results.	6
1. General Behavior, Appearance and Survival.	6
2. Body Weight.	6
3. Food Consumption	6
4. Clinical Laboratory Tests.	7
a. Hematology	7
b. Biochemistry	7
c. Urinalysis	7
5. Bromine Analysis	7
IV. Pathological Studies	8
A. Methods.	8
1. Gross Pathology.	8
2. Histopathology	8
B. Results.	8
1. Gross Pathology and Organ Weights.	8
2. Histopathology	9

PMF-0097

T A B L E O F C O N T E N T S
(Continued)

Table No.

	<u>Page</u>
1. Individual Weekly Body Weights	11
2. Individual Weekly Food Consumption	12
3. Individual Hematological Values - 21 Days.	13
4. Individual Biochemical Values - 21 Days.	14
5. Individual Urinalysis Values - 21 Days	15
6. Bromine Content.	16
7. Necropsy Observations.	17
8. Absolute and Relative Organ Weights.	18
9. Organ Weights.	19
10. Histomorphologic Observations.	20

I. SYNOPSIS

In a 21 day inhalation study, albino rats were subjected to FM PHT⁴ (Micronized) at atmospheric concentrations of 2 and 8 mg/L. Exposure of the rats in each group to their respective atmospheric concentrations was conducted for 4 hours daily, 5 days each week, for 3 weeks. A control group of rats was employed wherein only air, not containing the test compound, was introduced into the test chamber.

Observations were made daily for general physical appearance, behavior, and pharmacotoxic signs. Individual body weights and food consumption values were obtained weekly. Hematological, biochemical and urinalysis studies were conducted at 20 days. Bromine analysis by neutron activation of liver, fat, kidney, lung and blood samples were conducted on samples taken at terminal necropsy.

Clinical observations obtained during the study period revealed the following findings in the treated groups which were not found in the control group: salivation, lacrimation, nasal discharge, and nasal porphyrin discharge. Respiratory congestion was observed once only in one animal at the 8 mg/L concentration. No deaths occurred in either the control or treated groups during the 21 day period of study.

Changes in body weight were observed in treated male and female rats following 3 weeks of study. Treated animals exhibited slightly lesser body weight gains than did male and female animals in the control group. Food consumption values for rats in the control and treated groups were essentially similar throughout the study period except for the treated female groups. Treated females exhibited a slightly lesser food consumption than did control females.

Hematological, biochemical and urinalysis studies obtained at 20 days did not exhibit changes which were considered related to compound administration. The results of the bromine analysis of selected tissues

and blood by neutron activation revealed increased bromine values in the tissues and blood from animals in the 8 mg/L concentration group when compared to the corresponding samples from the untreated control animals.

No compound related gross pathologic lesions were seen at necropsy in rats from either experimental group. Decreases in liver weight and increases in lung weight at both exposure levels were considered compound effects. An increase in relative adrenal and thyroid weight in females at the 8 mg/L level may possibly have been compound related. Microscopically, an increase in inflammatory lung lesions in both experimental groups may have been compound related. No other lesions related to compound administration were seen in other tissues examined from rats at the 8 mg/L level.

REC'D 100

II. COMPOUND

The test compound was received from the Michigan Chemical Corporation, St. Louis, Michigan on October 24, 1974.

It was identified as "7.5 lbs FM PHT4 (Micronized), Lot No. 6332-B", and was received as a white powder.

REF 0101

III. CLINICAL STUDIES

A. METHOD:

1. General Procedure:

Fifteen male and 15 female rats of the Spartan strain, weighing from 214 to 262 grams, were used in this study. The rats were acclimated for a period of 7 days following which the rats were divided into 3 groups of 5 male and 5 female rats each. The rats were individually housed in metal cages suspended above the droppings and were maintained in temperature and humidity controlled quarters during the study period. Purina Laboratory Chow for rats and water were available ad libitum.

PHT 0102

Following the 21 days of study, all rats were sacrificed and necropsied. Selected tissues were collected and were processed for histopathology.

2. Compound Administration:

Each group of 5 male and 5 female rats was placed in a sealed 59.1 liter glass chamber and was exposed for 4 hours, 5 days weekly for 3 weeks, to a dynamic atmosphere containing the test material as a dust. In order to prevent "piling up" during the exposure, the rats were separated by sex into 4 units of 2 or 3 rats each.

Addition of the test compound to the test chamber atmosphere was controlled by a Wright Dust Feeder. Dried and filtered air was passed through the mechanism and directly into the exposure chamber. Air flow was regulated by means of a flowmeter.

The atmospheric concentrations employed were as follows:

Group I - Control: Air flow only, without the introduction of the test compound.

Group II - FM PHT4: 2 mg/liter

Group III - FM PHT4: 8 mg/liter

3. Observations:

Prior to and immediately following each daily exposure, each rat was observed for general physical appearance, behavior and pharmacotoxic signs. During each four hour daily exposure, each group of 10 rats was observed continually.

Individual body weights and food consumption were obtained weekly.

4. Clinical Laboratory Tests:

At day 20 of the study period, 24 hour fasting blood samples were obtained from all rats for the following analyses:

a. Hematology:

Hematological studies included hemoglobin concentration¹, hematocrit², total erythrocyte count³, and total³ and differential leucocyte counts.

b. Biochemistry:

Biochemical studies included plasma urea nitrogen⁴, fasting plasma glucose⁴, serum alkaline phosphatase activity⁴, and serum glutamic oxalacetic and pyruvic transaminase activities⁴.

c. Urinalysis:

Urinalysis studies included measurement of volume, specific gravity and pH⁵, determination of color and appearance; qualitative tests for albumin⁵, glucose⁵, bilirubin⁵ and occult blood⁵, and microscopic examination of the sediment.

5. Bromine Analysis:

Samples of liver, fat, kidney, lung and blood were collected from all animals at the terminal necropsy. All samples collected from the rats in the control and high dosage level groups were analyzed for bromine content by neutron activation analysis.

B. RESULTS:

1. General Behavior, Appearance and Survival:

Clinical observations which were noted in the control group and in the treated groups were limited to soft stool.

Observations noted in both of the treated groups but not in the control group included most animals exhibiting salivation, lacrimation, and nasal discharge. These signs were evidenced in both groups on an intermittent basis throughout the study period. One animal in the 2 mg/L dosage level group exhibited nasal porphyrin discharge during the study period as did 5 animals at the 8 mg/L dosage level. Nasal porphyrin discharge was noted in one additional high dose rat intermittently throughout the study period. Also noted at the 8 mg/L dosage level was ocular porphyrin discharge, observed intermittently in one animal and respiratory congestion noted once only in one animal.

No deaths occurred during the study period in either the control or treated groups.

2. Body Weight (Table 1):

Male and female rats in the control group exhibited slightly greater body weight gains at the end of the three week study period than did male and female rats in the treated groups.

3. Food Consumption (Table 2):

Food consumption values for rats in the control and treated groups were essentially similar throughout the study period except for the treated female groups. Both treated female groups during the treatment period exhibited slightly lesser food consumption values than they did during the control period or the control female group during the study period.

4. Clinical Laboratory Tests (Tables 3-5):

a. Hematology:

Hematology values obtained at 20 days did not reveal changes which were considered compound related.

b. Biochemistry:

Biochemical studies did not reveal any changes which were considered related to compound administration.

c. Urinalysis:

Urinalysis values obtained at 20 days did not reveal abnormalities which were considered compound related.

5. Bromine Analysis:

The results of the bromine analysis determined by neutron activation, conducted on samples of liver, fat, kidney, lung and blood from animals in the control and high dosage level groups are presented in Table 6.

The amount of bromine contained in each of the samples analyzed was greater in the group receiving FM PHT⁴ at a concentration of 8 mg/L than was noted in the corresponding control values.

PHF 0105

IV. PATHOLOGICAL STUDIES

A. METHODS:

1. Gross Pathology:

At the completion of the exposure period, all rats were sacrificed by decapitation and necropsied. Selected organs were weighed and representative tissues from each rat were collected in buffered neutral 10% formalin.

2. Histopathology:

The following tissues from each rat from the control and 8 mg/L groups were paraffin embedded, sectioned, stained with hematoxylin and eosin and examined microscopically:

nasal turbinate area	kidneys
trachea	urinary bladder
lung	ovaries/testes
spleen	bone/marrow
pancreas	heart
stomach	mediastinal lymph node
duodenum	thyroid/parathyroid
colon	eye
mesenteric lymph node	brain
liver	pituitary
adrenals	

Hematoxylin and eosin stained paraffin sections of lung from rats at the 2 mg/L group also were prepared and examined.

B. RESULTS:

1. Gross Pathology (Table 7) and Organ Weights (Tables 8-9):

No compound related gross pathologic lesions were observed at necropsy in any rats from the experimental groups.

Group mean absolute and relative liver weights of female rats at both the 2 and 8 mg/L levels were significantly ($p < 0.01$) decreased from control values; group mean absolute liver weight of males at 8 mg/L also was significantly decreased ($p < 0.05$). Group mean absolute

and relative lung weights of males at the 2 and 8 mg/L levels were significantly ($p < 0.01$) increased from control values; group mean relative lung weights of females at these two levels also were significantly ($p < 0.05$) increased. These variations in liver and lung weights in the treated groups were considered compound related. An increase in group mean relative adrenal and thyroid weight ($p < 0.05$) of females at the 8 mg/L level also may have been compound related.

2. Histopathology (Table 10):

Rats from the 2 and 8 mg/L exposure levels had a higher incidence of inflammatory lung lesions than did rats in the control group. This increase in lung disease may have been compound related. No other lesions suggestive of a compound effect were seen in other tissues examined from rats at the 8 mg/L level.

10107

References

1. Cyanmethemoglobin Method, John B. Miale, 3rd Ed., 1967, The C. V. Mosby Company, p. 1143.
2. Microhematocrit, John B. Miale, 3rd Ed., 1967, The C. V. Mosby Company, p. 1154.
3. Coulter Particle Size Counter, Model A, Coulter Electronics, 590 W. 20th Street, Hialeah, Florida.
4. Technicon Auto Analyzer, 6/60 Micro Methodology.
5. Bililabstix (Ames Reagent Strips)

PAF 0108

TABLE 1. Individual Weekly Body Weights, Grams.

Group, Rat No.	Sex	Control		Compound Administration Weeks		
		-1	0	7	14	21
<u>Control:</u>						
21675	M	192	252	277	304	315
21676	M	196	262	305	340	373
21677	M	188	243	263	295	318
21678	M	194	243	289	312	325
21679	M	187	242	269	292	299
Mean		191.4	248.4	280.6	308.6	326.0
21695	F	208	219	228	242	248
21696	F	215	229	240	250	262
21697	F	212	224	240	254	264
21698	F	216	229	236	258	264
21699	F	217	229	237	250	258
Mean		213.6	226.0	236.2	250.8	259.2
<u>2. mg/L:</u>						
21680	M	192	245	260	260	304
21681	M	191	258	271	288	320
21682	M	177	227	250	272	305
21683	M	201	262	265	284	312
21684	M	194	245	259	274	298
Mean		191.0	247.4	261.0	275.6	307.8
21700	F	205	219	215	214	228
21701	F	208	227	227	235	250
21702	F	214	228	226	230	239
21703	F	216	227	225	232	245
21704	F	206	218	209	214	215
Mean		209.8	223.8	220.4	225.0	235.4
<u>8 mg/L:</u>						
21685	M	190	248	267	294	301
21686	M	201	261	296	308	327
21687	M	188	226	257	282	292
21688	M	203	261	286	302	320
21689	M	185	244	266	278	288
Mean		193.4	248.0	274.4	292.8	305.6
21705	F	213	224	224	232	224
21706	F	199	214	215	225	213
21707	F	212	223	224	232	237
21708	F	208	224	230	238	239
21709	F	206	219	221	237	234
Mean		207.6	220.8	222.8	232.8	229.0

PAGE 0109

(Micronized): Twenty-one Day Inhalation Toxicity Study in Rats.

TABLE 2. Individual Weekly Food Consumption, Grams.

Group, Rat No.	Sex	Compound Administration Weeks			
		Control 0	1	2	3
<u>Control:</u>					
21675	M	171	182	190	162
21676	M	171	202	232	205
21677	M	160	164	185	169
21678	M	153	196	194	161
21679	M	167	188	185	161
Mean		164.4	186.4	197.2	171.6
21695	F	128	131	151	142
21696	F	142	138	145	146
21697	F	136	145	148	139
21698	F	142	134	158	143
21699	F	145	127	140	135
Mean		138.6	135.0	148.4	141.0
<u>2 mg/L:</u>					
21680	M	168	155	165	166
21681	M	174	171	179	175
21682	M	150	165	180	173
21683	M	168	151	170	165
21684	M	162	155	172	159
Mean		164.4	159.4	173.2	167.6
21700	F	140	117	118	113
21701	F	166	130	134	122
21702	F	138	127	112	158
21703	F	135	132	132	136
21704	F	128	113	117	67
Mean		141.4	123.8	122.6	119.2
<u>8 mg/L:</u>					
21685	M	173	166	178	159
21686	M	172	175	169	164
21687	M	149	165	172	154
21688	M	171	153	161	154
21689	M	154	150	151	140
Mean		163.8	161.8	166.2	154.2
21705	F	142	117	126	119
21706	F	117	98	112	98
21707	F	154	128	125	113
21708	F	159	130	147	125
21709	F	142	130	141	118
Mean		142.8	120.6	130.2	114.6

FM PHT4 (Micronized):

Twenty-one Day Inhalation Toxicity Study in Rats.

TABLE 3.

Individual Hematological Values - 21 Days.

Group, Rat No.	Sex	Erythro- cytes 10 ⁶ /cmm	Hemato- crit %	Hemo- globin gm/100 ml	Leuco- cytes 10 ³ /cmm	Neutrophils		Lympho- cytes %	Eosino- phils %	Mono- cytes %	Baso- phils %
						Seg. %	Non-Seg. %				
Control:											
21675	M	7.95	53	15.9	13.39	8	0	92	0	0	0
21676	M	7.36	48	14.1	18.09	9	0	91	0	0	0
21677	M	7.04	46	14.1	13.23	6	0	94	0	0	0
21678	M	7.39	49	14.8	16.81	13	0	86	1	0	0
21679	M	7.85	50	15.3	12.47	7	0	93	0	0	0
Mean		7.52	49	14.8	14.80	9	0	91	0	0	0
21695	F	6.69	43	13.6	10.92	24	0	73	3	0	0
21696	F	7.35	46	14.8	12.05	5	0	94	1	0	0
21697	F	7.12	50	15.3	14.45	9	0	90	1	0	0
21698	F	7.32	46	15.1	9.76	8	0	91	1	0	0
21699	F	7.48	47	14.4	8.16	10	0	88	1	1	0
Mean		7.19	46	14.6	11.07	11	0	88	1	0	0
2 mg/L:											
21680	M	7.87	52	16.2	14.55	9	0	91	0	0	0
21681	M	7.64	49	15.3	16.76	7	0	93	0	0	0
21682	M	7.95	51	15.6	16.36	5	0	95	0	0	0
21683	M	7.53	49	15.3	12.96	12	0	87	1	0	0
21684	M	7.93	54	16.6	16.22	11	0	89	0	0	0
Mean		7.78	51	15.8	15.37	9	0	91	0	0	0
21700	F	7.02	47	14.8	14.13	6	0	92	2	0	0
21701	F	7.76	51	16.2	16.12	7	0	91	2	0	0
21702	F	7.84	49	15.8	14.43	5	0	94	1	0	0
21703	F	7.30	50	16.2	12.08	5	0	93	2	0	0
21704	F	7.53	51	16.4	10.61	12	0	87	1	0	0
Mean		7.49	50	15.9	13.47	7	0	91	2	0	0
8 mg/L:											
21685	M	8.11	54	16.5	19.29	9	0	91	0	0	0
21686	M	7.38	51	16.2	16.48	6	0	94	0	0	0
21687	M	7.83	53	16.2	16.46	10	0	88	2	0	0
21688	M	8.04	55	16.4	15.73	15	0	85	0	0	0
21689	M	8.08	56	16.5	16.21	5	0	94	1	0	0
Mean		7.89	54	16.4	16.83	9	0	90	1	0	0
21705	F	7.63	49	15.3	19.36	6	0	93	1	0	0
21706	F	6.96	47	14.6	21.40	11	0	87	2	0	0
21707	F	7.60	50	16.0	16.05	4*	0	96	0	0	0
21708	F	7.10	49	14.7	16.65	11	0	88	1	0	0
21709	F	7.76	51	15.8	15.34	15	0	83	2	0	0
Mean		7.41	49	15.3	17.76	9	0	90	1	0	0

PAGE 0111

134-029

*Repeat determination

130

FM.PHT4
(Micronized):

Twenty-one Day Inhalation Toxicity Study in Rats.

TABLE 4.

Individual Biochemical Values - 21 Days.

Group, Rat No.	Sex	Glucose mg/100 ml	B:U.N. mg/100 ml	Alk. Phos. int'l units/ml	S.G.O.T. int'l units/ml	S.G.P.T. int'l units/ml
Control:						
21675	M	127	17.8	195	131	36
21676	M	125	21.4	121	114	35
21677	M	118	16.8	163	137	17
21678	M	121	15.9	117	111	48
21679	M	125	17.9	145	147	32
Mean		123	18.0	148	128	34
21695	F	142	17.0	80	92	19
21696	F	141	14.9	88	121	23
21697	F	136	17.5	115	127	20
21698	F	139	18.2	70	105	28
21699	F	127	20.9	111	101	33
Mean		137	17.7	93	109	25
2 mg/L:						
21680	M	121	19.9	171	126	40
21681	M	126	14.8	146	195	21
21682	M	113	14.0	149	150	37
21683	M	141	14.1	129	111	36
21684	M	130	22.0	146	122	44
Mean		126	17.0	148	141	36
21700	F	140	19.0	100	150	31
21701	F	131	17.8	91	136	29
21702	F	117	16.1	88	105	26
21703	F	134	19.0	81	111	33
21704	F	132	18.2	101	108	34
Mean		131	18.0	92	122	31
8 mg/L:						
21685	M	122	19.2	185	134	27
21686	M	142	17.2	237	124	9
21687	M	137	14.1	169	200	33
21688	M	130	19.3	142	133	29
21689	M	135	19.2	187	155	35
Mean		133	17.8	184	149	27
21705	F	115	18.0	110	169	31
21706	F	144	16.9	126	161	21
21707	F	122	16.2	72	116	24
21708	F	136	16.1	122	158	21
21709	F	141	20.0	139	159	42
Mean		132	17.4	114	153	28

PAGE 0112

FM PHT⁴
(Micronized): Twenty-one Day Inhalation Toxicity Study in Rats.

TABLE 6. Bromine Content, ppm

Group, Rat No.	Sex	Compound Administration Week 3				
		Fat	Liver	Lung	Kidney	Blood
<u>Control:</u>						
21675	M	1.73	2.03	14.7	7.08	7.05
21676	M	0.58	3.75	12.6	7.15	6.03
21677	M	2.73	2.13	10.59	6.12	6.51
21678	M	1.33	2.34	10.12	6.27	5.44
21679	M	0.97	2.46	8.63	7.74	5.90
21695	F	1.53	3.44	12.85	8.25	9.25
21696	F	1.75	3.62	13.57	11.85	11.79
21697	F	1.23	3.72	8.29	8:30	9.49
21698	F	2.16	4.23	9.95	7.26	9.81
21699	F	0.98	3.66	15.14	12.24	7.04
Mean		1.50	3.14	11.64	8.23	7.83
<u>8 mg/L:</u>						
21685	M	11.26	8.06	31.61	21.14	19.77
21686	M	11.40	11.36	36.45	24.51	20.94
21687	M	5.65	8.41	51.64	25.87	22.82
21688	M	16.43	9.13	43.24	21.28	20.64
21689	M	7.31	-	44.43	25.50	20.81
21705	F	20.11	11.29	36.72	24.34	24.99
21706	F	13.86	11.84	-	23.96	23.05
21707	F	11.32	9.36	38.75	24.08	27.95
21708	F	20.18	8.20	32.47	30.23	27.10
21709	F	17.86	11.58	34.61	26.94	26.96
Mean		13.54	9.91	38.88	24.79	23.50

PAGE 0114

FM PHT⁴
 (Micronized): Twenty-one Day Inhalation Toxicity Study in Rats.

TABLE 7. Necropsy Observations.

Group	Sex	no gross lesions	scattered 1 mm. dark foci, lung	hydrometra
Control:	M	5		
	F	4	1	
2 mg/L:	M	5		
	F	5		
8 mg/L:	M	5		
	F	4		1

PAGE 0115

134-029

134

FM Ph⁴ (Micronized):

Twenty-one Day Inhalation Toxicity Study in Rats.

TABLE 8. Absolute (Grams) and Relative (% Body Weight) Organ Weights.

Group	Sex	Body Wt. Gm	Spleen Gm	%	Liver Gm	%	Adrenals Gm	%x10 ²	Kidneys Gm	%	Testes Gm	%	Heart Gm	%	Lung Gm	%	Thyroid/ Parathyroid Gm	%x10 ²	Brain Gm	%
Control:	M	326	0.81	0.25	13.39	4.11	0.054	1.65	2.74	0.84	3.63	1.11	1.21	0.37	1.42	0.43	0.023	0.71	1.72	0.53
	F	239	0.85	0.33	10.21	3.94	0.074	2.87	1.94	0.75			1.02	0.39	1.51	0.58	0.018	0.71	1.64	0.63
2 mg/L:	M	308	0.99	0.32	12.52	4.07	0.059	1.91	2.24	0.73	3.70	1.20	1.14	0.37	2.05**	0.67**	0.019	0.62	1.70	0.55
	F	235	0.77	0.33	8.34**	3.54**	0.074	3.16	1.89	0.80			0.88	0.37	1.87	0.79*	0.018	0.77	1.61	0.68
8 mg/L:	M	306	0.73	0.25	11.59*	3.79	0.059	1.92	2.49	0.81	3.64	1.19	1.23	0.40	2.01**	0.66**	0.021	0.69	1.59	0.52
	F	229	0.69	0.30	7.96**	3.48**	0.085	3.71*	1.76	0.77			0.96	0.42	1.85	0.81*	0.021	0.92*	1.60	0.70

*significantly different from control group mean, p < 0.05

**significantly different from control group mean, p < 0.01

134-029

PAGE 0116

135

FM PH⁴ (Micronized): Twenty-one Day Inhalation Toxicity Study in Rats.

TABLE 9. Organ Weights, grams.

Group, Rat No.	Sex	Body Wt. gm.	Spleen	Liver	Adrenals	Kidneys	Testes	Heart	Lung	Thyroid/ Parathyroid	Brain
<u>Control:</u>											
21675	M	315	0.72	11.97	0.057	2.57	3.42	1.21	1.43		1.80
21676	M	373	0.87	15.56	0.055	3.09	3.74	1.17	1.37	0.022	1.82
21677	M	318	0.80	13.49	0.053	2.40	3.52	1.16	1.22	0.021	1.75
21678	M	325	0.81	13.66	0.049	2.92	3.92	1.40	1.67	0.022	1.48
21679	M	299	0.85	12.27	0.055	2.72	3.57	1.13	1.39	0.028	1.77
21695	F	248	1.00	9.66	0.066	2.02	2.02	0.92	2.01	0.020	1.62
21696	F	262	0.79	10.79	0.080	2.08	2.08	1.16	1.35	0.020	1.79
21697	F	264	0.85	10.56	0.077	1.77	1.77	0.94	1.20	0.016	1.72
21698	F	264	0.81	10.15	0.079	2.00	2.00	1.00	1.62	0.017	1.20
21699	F	258	0.80	9.89	0.070	1.82	1.82	1.09	1.39	0.019	1.88
<u>2 mg/L:</u>											
21680	M	304	0.71	13.86	0.054	1.40	3.48	1.05	2.60	0.020	1.49
21681	M	320	1.82	12.96	0.076	2.38	4.01	1.26	1.75	0.019	1.78
21682	M	305	0.89	12.61	0.056	2.20	2.20	1.12	1.83	0.014	1.69
21683	M	312	0.81	11.78	0.058	2.70	3.41	1.13	2.12	0.020	1.74
21684	M	298	0.72	11.38	0.050	2.50	3.89	1.13	1.96	0.022	1.81
21700	F	228	0.70	7.40	0.071	1.84	1.84	0.86	1.95	0.021	1.78
21701	F	250	0.92	9.52	0.068	1.89	1.89	1.03	1.85	0.022	1.60
21702	F	239	0.74	8.02	0.082	1.91	0.89	0.89	2.01	0.016	1.60
21703	F	245	0.80	9.18	0.074	2.01	0.83	0.83	2.05	0.019	1.42
21704	F	215	0.70	7.58	0.077	1.82	0.79	0.79	1.49	0.013	1.63
<u>8 mg/L:</u>											
21685	M	301	0.81	12.28	0.054	2.49	3.38	1.52	2.50	0.017	1.75
21686	M	327	0.82	12.08	0.054	2.74	3.64	1.23	1.89	0.023	1.59
21687	M	292	0.81	11.45	0.054	2.42	3.64	1.28	1.93	0.023	1.64
21688	M	320	0.75	11.77	0.057	2.51	3.91	1.19	1.75	0.022	1.72
21689	M	288	0.69	10.38	0.075	2.29	2.29	0.92	2.00	0.020	1.23
21705	F	224	0.69	8.08	0.107	1.68	1.68	0.98	1.68	0.022	1.72
21706	F	213	0.69	7.22	0.081	1.78	0.89	0.89	1.68	0.019	1.68
21707	F	237	0.60	8.40	0.082	1.73	0.83	0.83	1.61	0.020	1.62
21708	F	237	0.68	7.99	0.085	1.80	1.02	1.02	2.35	0.018	1.68
21709	F	234	0.80	8.10	0.070	1.83	1.10	1.10	1.92	0.026	1.28

PAGE 0117

IN PM⁴ (Micronized): Twenty-one Day Inhalation Toxicity Study in Rats.

TABLE 10. Histomorphologic Observations.

Group, Rat No.	Sex	Nasal Turbinates		Lungs										Other Organs														
		exudate	macropurulent	perivascular infiltrate, primarily lymphocytic	local interstitial pneumonia	alveolar macrophages	bronchiectasis with exudate	peribronchial lymphocytic hyperplasia	Brain	Eye	Pituitary	Thyroid	Adrenals	Trachea	Lung	Heart	Spleen	Lymph Node	Bone Marrow	Stomach	Small Intestine	Large Intestine	Pancreas	Liver	Kidneys	Urinary bladder	Testes/Ovaries	
Control:																												
21675	M				3	2																						
21676	M																											
21677	M																											
21678	M																											
21679	M																											
21695	F			5	4	3																						
21696	F																											
21697	F					2																						
21698	F																											
21699	F					3																						
8 mg/L:																												
21685	M				4	3																						
21686	M				3	3																						
21687	M		4		3	3																						
21688	M																											
21689	M			2		2																						
21705	F																											
21706	F																											
21707	F																											
21708	F				3	2			2																			
21709	F			2		3			3																			
2 mg/L:																												
21680	M			3		2																						
21681	M			2		3																						
21682	M			4		3																						
21683	M			4		3																						
21684	M			3		3																						
21700	F			4		3																						
21701	F			4		3																						
21702	F			4		3																						
21703	F			4		4																						
21704	F			4		4																						

Code: n = normal or essentially so 2 = very slight 3 = slight 4 = moderate 5 = marked 6 = extreme
 x = condition present

PAGE 0118

137

88-7800185

(Handwritten signature)

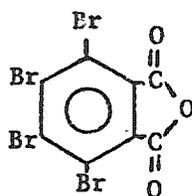
859-74-4

PHT-4

Lot #3368-B

Structure

? NOT EMPH-4



Analytical Data

M.P.	= 273.5-278.0
Neutralization Equivalent	= 229.8
Resin Gel Time	= > 1 hr
Color	= 3

PAGE 0119

(N)

PAGE 0141

MUTAGENICITY EVALUATION

OF

859-74-4 (FM PHT4)

FINAL REPORT

SUBMITTED TO

MICHIGAN CHEMICAL CORPORATION
TECHNICAL CENTER
1975 GREEN ROAD
ANN ARBOR, MICHIGAN 48105

SUBMITTED BY

LITTON BIONETICS, INC.
5516 NICHOLSON LANE
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2547

MAY 25, 1976



139

TABLE OF CONTENTS

1.	OBJECTIVE	1
2.	MATERIALS	1
	A. Test Compound	1
	B. Indicator Microorganisms	1
	C. Reaction Mixture	1
	D. Tissue Homogenates and Supernatants	2
	E. Positive Control Chemicals	2
3.	EXPERIMENTAL DESIGN	3
	A. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions	3
	B. Plate Tests (Overlay Method)	3
	C. Recording and Presenting Data	3
4.	SUMMARY OF PLATE TEST RESULTS	4
5.	INTERPRETATION OF RESULTS AND CONCLUSIONS	5
6.	EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS	6

PAGE 0142

SPONSOR: Michigan Chemical Corporation

MATERIAL: 859-74-4

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

PAGE 0143

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

1. Date Received: April 30, 1976
2. Description: White powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

1. Yeast Strain: Saccharomyces cerevisiae, strain D4
2. Bacteria Strains: Salmonella typhimurium, strains
TA-1535 TA-98
TA-1537 TA-100
TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	6 μ moles
2. Isocitric acid	35 μ moles
3. Tris buffer, pH 7.4	28 μ moles
4. MgCl ₂	2 μ moles
5. Homogenate fraction equivalent to 25 mg of wet tissue	



BIONETICS

141

2. MATERIALS (Continued)

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from the livers of Sprague-Dawley adult male rats. The animals were pretreated with Aroclor 1254 (500 mg/kg) 5 days before kill.

E. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

<u>ASSAY</u>	<u>CHEMICAL*</u>	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY</u>
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS**
	2-Nitrofluorene (NF)	Dimethylsulfoxide***	FS**
	Quinacrine mustard (QM)	Water or saline	FS**
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide***	BPS**
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide***	FS**
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide***	FS**
	Dimethylnitrosamine (DMNA)	Saline	BPS**

* Concentrations given in Results Section

** BPS = Base-pair substitution.

FS = Frameshift

*** Previously shown to be nonmutagenic

PHO 0144



BIONETICS

142

3. EXPERIMENTAL DESIGN

A. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities of tissues) were killed by cranial blow, decapitated, and bled. Organs were immediately dissected from the animal using aseptic techniques and were placed in ice-cold 0.25 M sucrose buffered with Tris at a pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4C. The organ homogenate obtained from this step was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80C. Samples from this preparation were used for the activation studies.

REF 0145

B. Plate Test (Overlay Method)

Approximately 10^9 cells from a log phase culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests the 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Four dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

C. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were transferred directly to the report form sheets and presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points.



BIONETICS

143



LITTON BIONETICS

4. SUMMARY OF PLATE TEST RESULTS

A. Name or code designation of the test compound: 859-74-4

B. Solvent: DMSO

C. Test date: May 11, 1976

D. Concentrations of the test compound: (1) .05 µg (2) .25 µg (3) .5 µg (4) 5 µg (5) 50 µg/plate

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE					
			TA-1535	TA-1537	TA-1538	TA-98	TA-100	D4*
<u>NONACTIVATION</u>								
Solvent control	---	---	24	13	13	22	131	53
Positive control**	---	---	>10 ³	>10 ³	258	520	>10 ³	179
Test compound (1) (2) (3) (4) (5)	---	---	---	---	---	---	---	---
	---	---	23	15	13	13	112	50
	---	---	23	19	12	13	122	56
	---	---	28	19	12	12	130	60
	---	---	23	15	13	9	--	67
<u>ACTIVATION</u>								
Solvent Control	Rat	Liver	17	25	10	35	206	37
Positive control***	Rat	Liver	108	636	>10 ³	>10 ³	582	44
Test compound (1) (2) (3) (4)	Rat	Liver	16	18	10	38	169	36
	Rat	Liver	17	19	15	39	197	41
	Rat	Liver	13	14	18	41	204	40
	Rat	Liver	15	23	16	33	226	39

* Try⁺ convertants per plate

** TA-1535	MNNG	10 µl/plate	*** TA-1535	ANTH	100 µg/plate
TA-1537	QM	10 µl/plate	TA-1537	AMQ	100 µg/plate
TA-1538	NF	100 µg/plate	TA-1538	AAF	100 µg/plate
TA-98	NF	100 µg/plate	TA-98	AAF	100 µg/plate
TA-100	MNNG	10 µl/plate	TA-100	DMNA	100 µg/plate
D4	MNNG	10 µl/plate	D4	DMNA	100 µmoles/plate

5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, 859-74-4, was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.25 μ g to 50 μ g per plate. A lower dose of 0.05 μ g per plate was used in the test with TA-100.

B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

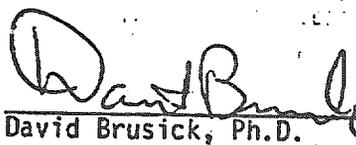
C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

D. Conclusions

The test compound, 859-74-4, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:



David Brusick, Ph.D.
Director
Department of Genetics

Reviewed by:


Robert J. Weir, Ph.D.
Vice President

PAGE 0147



BIONETICS

145

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

PAGE 0148



BIONETICS

146

0 1 3 4

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Interpretation of Results

The demonstration of dose-related increases in mutant counts is the most reliable method to demonstrate mutagenicity. Mutant increases at only one or two doses may be significant if they occur at the higher doses. Increases at low or intermediate concentrations followed by reduced mutant counts at higher doses may indicate that the test chemical has a narrow activity range or that the high dose levels were toxic and the induced revertant cells were killed. We are able to detect the latter possibility by inspecting the background growth, and the former possibility can be investigated by looking at a narrow series of dose levels bracketing the presumptive active range.

It is difficult to detect mutagens with little or no toxicity in this assay since such agents are generally weak mutagens and produce only two to threefold increases in mutant counts. Variations of two to threefold are often within normal fluctuations of the spontaneous counts, and the use of even higher concentrations is often difficult because of the likelihood of overloading the system with large quantities of the chemical. To resolve the mutagenicity of such a chemical, other assays to which statistical evaluations can be applied may be necessary.

PAGE 0149



BIONETICS

147

88-7800185

0675-0115

0

Industrial BIO-TEST Laboratories, Inc.

1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

0/

REPORT TO

MICHIGAN CHEMICAL CORPORATION

HUMAN REPEATED INSULT PATCH TEST WITH
FIREMASTER® PHT4

P.O. NO. 24681-A-C
DECEMBER 16, 1976

IBT NO. 8537-9430

PAGE 0150

Declassified
8-22-78

Industrial BIO-TEST Laboratories, Inc.
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS 60062

December 16, 1976

PAGE 0151

Mr. R. C. Nametz, Group Leader
Applications Development
Michigan Chemical Corporation
1975 Green Road
Ann Arbor, Michigan 48105

Dear Mr. Nametz:

Re: IBT No. 8537-09430 - Human Repeated Insult Patch
Test with FireMaster® PHT4 - P.O. No. 24681-A-C

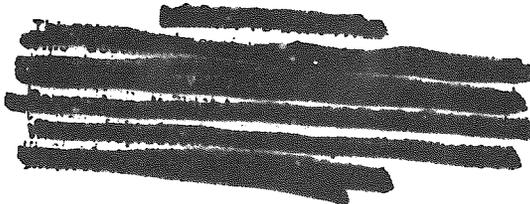
We are submitting herewith our laboratory report prepared
in connection with the above study.

Very truly yours,



J. C. Calandra
President

JCC:bp



REPORT TO

MICHIGAN CHEMICAL CORPORATION

HUMAN REPEATED INSULT PATCH TEST WITH
FIREMASTER® PHT4

P.O. NO. 24681-A-C

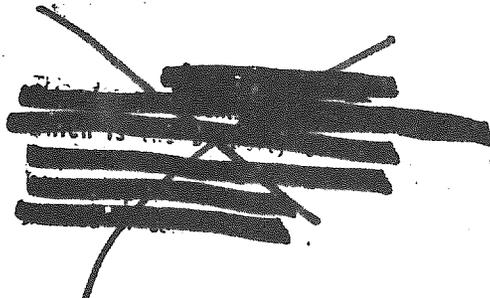
DECEMBER 16, 1976

IBT NO. 8537-9430

PAGE 0152

I. Introduction

A sample of FireMaster® PHT4 was received from Michigan Chemical Corporation for evaluation of skin irritating and sensitizing properties employing a panel of 50 human test subjects. The test material was evaluated as received.



II. Summary

A repeated insult patch test employing a panel of 50 human test subjects was conducted with FireMaster® PHT4.

The results of the study showed that no irritation reactions (all scores 0) were produced by the test material in any of the subjects during the series of induction patch applications.

There was no evidence of skin sensitization noted in any of the test subjects.

PAGE 0153

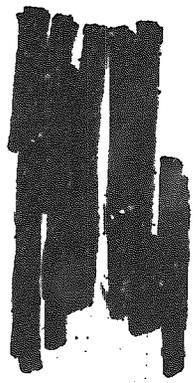
Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

Clinical Investigator: Samuel Solomon
Samuel Solomon, M.D.
Consultant Dermatologist

Report prepared by: Pamela M Wenzel
Pamela M. Wenzel, A.A.
Technician
Clinical Evaluations

Report approved by: Martin J. Garofalo
Martin J. Garofalo, B.A.
Section Head
Clinical Evaluations



Florence K. Kinoshita
Florence K. Kinoshita, Ph.D.
Technical Manager, Toxicology

M. L. Keplinger
M. L. Keplinger, Ph.D.
Manager, Toxicology

jtg

151

0 1 4 4

III. Procedure

Fifty human test subjects were employed in the investigation. The composition of the test population is shown in Table I.

The repeated insult technique was used in the patch test. The test material was evaluated as received. The upper arm (outer surface) of the male subjects was utilized for patching, while the female subjects were patched on the upper back, shoulder area.

The technique used for the patch test called for a series of 9 induction patches of the test material to be placed on each of the subjects. The series was followed 12 days later by a single challenge patch of the test material to detect skin sensitization.

The series of 9 induction patches of the test material was applied according to the following schedule. A patch was applied on Monday, Wednesday and Thursday and allowed to contact the skin for 24 hours, after which time it was removed and the skin site graded for irritation. Thursday's patch was placed immediately after removal and grading of Wednesday's application. After the ninth induction patch had been placed, a nonpatching period of 12 days elapsed before the challenge patch of the test material was applied to detect sensitization reactions. For this 24-hour patch, a new skin site was used. This site was invariably chosen adjacent to the induction site; i.e., one where repeated applications had been made during the series of patches. This site was observed at patch removal for sensitization reactions and again after 24 and 48 hours to detect possible delayed reactions.

PAGE 0154

[REDACTED]

152

TABLE I

TEST MATERIAL: FireMaster® PHT4

Human Repeated Insult Patch Test

Composition of Test Population

Sex	Age Range (years)	Race	No. of Subjects
Male	29-65	Caucasian	2
Female	24-62	Caucasian	47
		Negro	<u>1</u>
		Total -	50

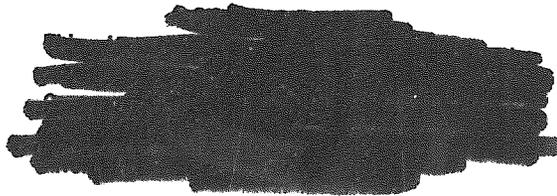
PAGE 0155

[REDACTED]

Skin applications of the test material were made using Readi-Band* clear plastic patches 1-1/2 inches square with nonwoven Webril centers. The Webril centers were premoistened with tap water just prior to application, and a sufficient amount of the test material was placed on the patch to cover the premoistened area.

PARC 0156

The scoring criteria used for skin irritation reactions are shown in Table II.



* Parke, Davis & Company, Detroit, Michigan

TABLE II

TEST MATERIAL: FireMaster® PHT4

Human Repeated Insult Patch Test

Scoring Criteria for Skin Irritation Reactions

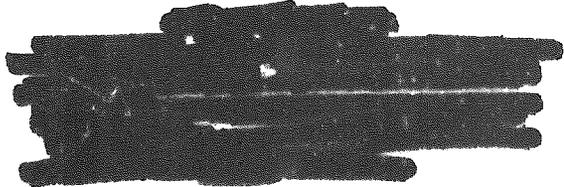
Erythema and Eschar Formation

No reaction	0
Very slight erythema (barely perceptible)	1
Mild, well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
 Total possible erythema score	 4

Edema Formation

No reaction	0
Very slight edema (barely perceptible)	1
Slight edema (edge of area well-defined by definite raising)	2
Moderate edema (area raised approximately 1 mm)	3
Severe edema (area raised more than 1 mm and extending beyond area of exposure)	4
 Total possible edema score	 4
 Total possible irritation score	 8

Paper 0157



155

IV. Results

The results of the repeated insult patch test showed that none of the subjects exhibited any erythema or edema (all reaction scores 0) during the series of induction patch applications with the test material. This data is shown in Table III and summarized in Table IV.

There was no evidence of skin sensitization noted in any of the test subjects following the challenge patch applications.

PAF 0158



TABLE III
 TEST MATERIAL: FIREMASTER® PHT4
 HUMAN REPEATED INSULT PATCH TEST
 INDIVIDUAL IRRITATION SCORES -CLOSED PATCH TECHNIQUE

SUBJECT NUMBER	RACE SEX AGE	SCORES AFTER APPLICATION NUMBER:														
		1			2			3			4			5		
		ER.	ED.	T.	ER.	ED.	T.	ER.	ED.	T.	ER.	ED.	T.	ER.	ED.	T.
1	C-M-29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	C-F-44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	C-F-60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	C-M-65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	C-F-34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	C-F-34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	C-F-48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	C-F-49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	C-F-40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	C-F-36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	C-F-29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	C-F-50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	C-F-58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	C-F-50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	C-F-57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	C-F-24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	C-F-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	C-F-34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	C-F-36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	C-F-47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	C-F-44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	C-F-39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	C-F-41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	C-F-55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	C-F-58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

PHT 150



TABLE III CONTINUED
 TEST MATERIAL: FIREMASTER® PHT4
 HUMAN REPEATED INSULT PATCH TEST
 INDIVIDUAL IRRITATION SCORES - CLOSEDPATCH TECHNIQUE

PART 01500

SUBJECT NUMBER	RACE SEX AGE	SCORES AFTER APPLICATION NUMBER:														
		1			2			3			4			5		
		ER.	ED.	T.	ER.	ED.	T.	ER.	ED.	T.	ER.	ED.	T.	ER.	ED.	T.
26	C-F-46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	C-F-49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	C-F-37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	C-F-42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	C-F-41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	C-F-43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	C-F-40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	C-F-48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	C-F-42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	C-F-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	C-F-37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	C-F-35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	C-F-42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	C-F-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	C-F-49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	C-F-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	C-F-62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	M-F-49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	C-F-34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	C-F-37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	C-F-29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	C-F-50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	C-F-42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	C-F-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	C-F-39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

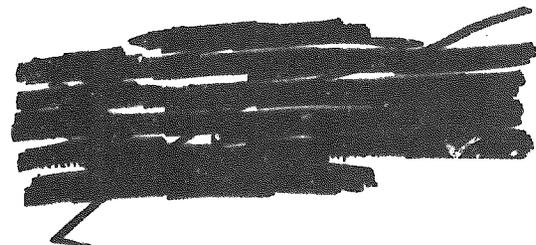


TABLE III CONTINUED
 TEST MATERIAL: FIREMASTER® PHT4
 HUMAN REPEATED INSULT PATCH TEST
 INDIVIDUAL IRRITATION SCORES - CLOSED PATCH TECHNIQUE

SUBJECT NUMBER	RACE SEX AGE	SCORES AFTER APPLICATION NUMBER:												CHALLENGE		
		6			7			8			9					
		ER.	ED.	T.	ER.	ED.	T.	ER.	ED.	T.	ER.	ED.	T.	ER.	ED.	T.
1	C-M-29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	C-F-44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	C-F-60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	C-M-65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	C-F-34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	C-F-34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	C-F-48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	C-F-49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	C-F-40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	C-F-36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	C-F-29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	C-F-50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	C-F-58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	C-F-50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	C-F-57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	C-F-24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	C-F-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	C-F-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	C-F-36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	C-F-47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	C-F-44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	C-F-39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	C-F-41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	C-F-55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	C-F-58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

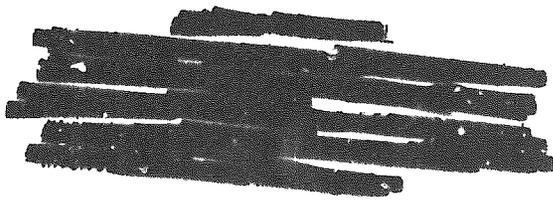
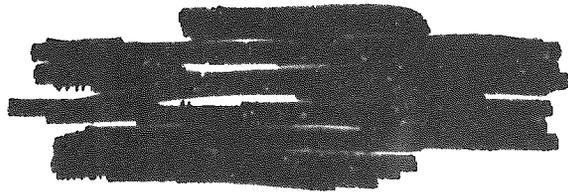


TABLE III CONTINUED
 TEST MATERIAL: FIREMASTER® PHT4
 HUMAN REPEATED INSULT PATCH TEST
 INDIVIDUAL IRRITATION SCORES -CLOSED PATCH TECHNIQUE

SUBJECT NUMBER	FACE SEX AGE	SCORES AFTER APPLICATION NUMBER:												CHALLENGE		
		6			7			8			9				ED.	T.
26	C-F-45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	C-F-49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	C-F-37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29	C-F-42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	C-F-41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31	C-F-43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32	C-F-40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33	C-F-48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	C-F-42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35	C-F-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	C-F-37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37	C-F-35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38	C-F-42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39	C-F-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40	C-F-49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
41	C-F-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	C-F-62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	N-F-49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	C-F-34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45	C-F-37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
46	C-F-29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
47	C-F-50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
48	C-F-42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
49	C-F-38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	C-F-39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0



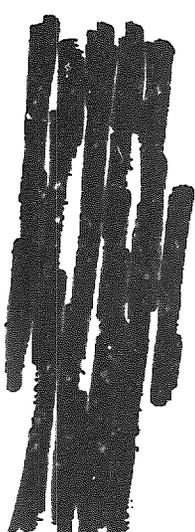


TABLE IV

TEST MATERIAL: FIREMASTER® PHT4
 HUMAN REPEATED INBUILT PATCH TEST

SUMMARY OF REACTIONS - CLOSED PATCH TECHNIQUE

		SCORES AFTER APPLICATION NUMBER								CHALLENGE	GRAND TOTALS	
		1	2	3	4	5	6	7	8	9		
REACTIONS WITH SCORES OF 1		0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0	0	0
TOTAL REACTIONS		0	0	0	0	0	0	0	0	0	0	0
TOTAL NONREACTIONS		50	50	50	50	50	50	50	50	50	50	450
TOTAL PATCHES APPLIED		50	50	50	50	50	50	50	50	50	50	450
INCIDENCE OF REACTIONS	FRACTION	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/50	0/450
	PERCENT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

*CHALLENGE APPLICATION NOT INCLUDED IN GRAND TOTALS

PAGE 0163

161