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CHEMICAL SYSTEMS LABORATORY TECHNICAL REPORT
ARCSL-TR-80013

THE ACUTE EFFECTS OF SINGLE EXPOSURES
TO WHITE PHOSPHORUS SMOKE IN RATS AND GUINEA PIGS

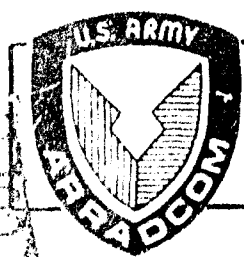
by

Bernard J. Brown
John T. Weimer
Garnet E. Affleck
Richard L. Farrand
Dale H. Heitkamp
Fred K. Lee

Research Division

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PREFACE

The work described in this report was authorized under Project 1L162622A554 (TA 4-5), Smoke Toxicology. The work was started in May 1978 and completed in December 1978. The experimental data are contained in notebook 9645.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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CONTENTS

	<u>Page</u>
I. INTRODUCTION	7
II. EXPERIMENTAL PROCEDURES	7
A. Chamber Operation and Sample Collection	7
B. Inhalation Toxicity Studies in Rats and Guinea Pigs	8
C. Oral Toxicity in Rats	8
D. Intravenous Toxicity in Rats	8
E. Skin Sensitization in Guinea Pigs	8
F. Mutation Test Using <i>Drosophila Melanogaster</i> (Fruit Flies)	8
III. RESULTS	9
A. Chamber Conditions for Inhalation Studies	9
B. Acute Inhalation Toxicity	9
1. LCt'50's	9
2. Toxic Signs	9
3. Hematology and Blood Chemistry	10
4. Pathology	10
a. Sprague-Dawley Albino Rats	10
b. Hartley Guinea Pigs	10
5. Pulmonary Resistance in Guinea Pigs	11
C. Oral Toxicity in Rats	11
D. Intravenous Toxicity of White Phosphorus/Felt Smoke Cloud Condensate in Rats	11
F. Mutation Potential	11
IV. DISCUSSION	11
V. CONCLUSIONS	12
LITERATURE CITED	13
APPENDIX A	15
APPENDIX B	17
APPENDIX C	19

	<u>Page</u>
APPENDIX D	23
APPENDIX E	25
DISTRIBUTION LIST	47

THE ACUTE EFFECTS OF SINGLE EXPOSURES TO WHITE PHOSPHORUS SMOKE IN RATS AND GUINEA PIGS

I. INTRODUCTION.

Toxicity studies were conducted to determine the acute effects of single exposures to a white phosphorus screening smoke. The smoke was generated by burning pieces of a white phosphorus/felt formulation.

The following studies were performed to obtain an acute toxicity profile: (1) oral toxicity of single doses in rats, (2) intravenous toxicity of single injections in rats, (3) sensitization potential on guinea pig skin, (4) mutagenic potential in fruit flies, (5) and inhalation toxicity of single doses in rats and guinea pigs.

II. EXPERIMENTAL PROCEDURES.

A. Chamber Operation and Sample Collection.

Animals for the inhalation studies were placed in compartmented stainless steel wire-mesh cages. The cages were put on racks in a 20-cubic-meter exposure chamber.

Military grade white phosphorus was forced under pressure into thick pieces of wool felt. This material was cut into cubes having specific weights. A cube of the white phosphorus/felt (WP/felt) on a weighed aluminum-foil pan was placed on an unlit electric hot plate within the chamber. The hot plate was a fast-heating unit capable of reaching temperatures in excess of 700°F.

The chamber door was closed and sealed, and the chamber exhaust system was shut down. Within 2 minutes after the hot plate was plugged in, the WP/felt ignited, producing a dense white smoke of phosphorous pentoxide (P_2O_5) and a number of lower oxides, of which two species are well established (P_2O_3 , PO_2). All three of these oxides are deliquescent and can contribute various aqueous droplets to the smoke. The reaction of these oxides with atmospheric moisture will produce o-phosphoric and o-phosphorous acids.^{1, 2} The burning time of the WP/felt ranged from 2 to 7½ minutes, depending on the size of the sample. The smoke was contained within the chamber for varying lengths of time and was sampled at regular intervals. A chamber exhaust system removed the smoke cloud at the end of the desired exposure times.

Samples were collected on Gelman Type A glass-fiber filter pads. At the rate of 3 liters per minute, 12 liters of chamber air were drawn through the filter pads contained in an air sampler. The filter pads were placed in Erlenmeyer flasks. Particulate matter was extracted with distilled water, boiled for 10 minutes, and titrated for phosphoric acid content (see appendix A).

The liquid particles making up the smoke cloud had to be collected for use in oral, intravenous, sensitization, and mutagenic studies. Air samples were drawn into an electrostatic precipitator, the liquid particles were deposited on aluminum tubes, and then were poured into collection bottles.

B. Inhalation Toxicity Studies in Rats and Guinea Pigs.

Five male and five female Sprague-Dawley albino rats, or a like number of Hartley albino guinea pigs, were used for each exposure. Eight males and eight females of each species were used as controls. All animals were weighed before exposure, and 1 day, 1 week, and 2 weeks after exposure. After the 2-week postexposure period, animals were selected to be bled, sacrificed, and submitted for pathological evaluation. Blood samples were analyzed for the following hematological and blood chemistry changes: (1) red blood cell, (2) white blood cell, (3) hematocrit, (4) hemoglobin, (5) differential white blood cell count, (6) glucose, (7) urea nitrogen, (8) creatinine, (9) sodium, (10) potassium, (11) chloride, (12) carbon dioxide, (13) uric acid, (14) total protein, (15) albumin, (16) globulin, (17) calcium, (18) phosphate, (19) cholesterol, (20) triglycerides, (21) alkaline phosphatase, (22) glutamic oxalacetic transaminase, (23) glutamic pyruvic transaminase, (24) lactic dehydrogenase, and (25) total bilirubin. Gross and microscopic pathological changes were noted. A group of exposed and control guinea pigs was tested for pulmonary abnormalities using the techniques described in appendix B.

C. Oral Toxicity in Rats.

Twenty-four Sprague-Dawley rats, three males and three females per dose, were used. The animals, weighing between 200 and 300 grams, were fasted overnight prior to dosing. The liquid used in these oral LD50 studies (section II. A.) was 65% phosphoric acid. Oral toxicity is discussed more fully in a comprehensive report which is to be published.*

D. Intravenous Toxicity in Rats.

Twenty-four Sprague-Dawley rats, three males and three females per dose, were used for the study. The liquid used for the intravenous LD50 studies was the same as that used for the oral toxicity study. This study is discussed in detail elsewhere.*

E. Skin Sensitization in Guinea Pigs.

Hartley albino male guinea pigs, weighing 243 to 369 grams, were tested for skin sensitization using a modified Lansteiner method as described in appendix C.

F. Mutation Test Using *Drosophila Melanogaster* (Fruit Flies).

The liquid collected from the WP smoke cloud by an electrostatic precipitator was examined for mutagenic potential. The fruit fly sex-linked recessive lethal test that was used is discussed in appendix D.

* Manthei, James H., ARCSL-TR-79056. Preliminary Toxic Screening Studies of 11 Smoke Candidate Compounds. April 1980.

III. RESULTS.

A. Chamber Conditions for Inhalation Studies.

When the WP/felt was ignited, a dense white smoke-like mist was produced. The droplets of this mist had a mass median diameter of 0.5 micron.

From 2.5 to 60 grams of the WP/felt were ignited. Of these amounts, an average of 76.3% burned (73%-83%) leaving a charred residue (23.7% of original weight) on the aluminum-foil pan.

Five of the six rat exposures were for 60 minutes; the other exposure lasted 90 minutes. Times varied from 5 to 60 minutes for the guinea pig exposures.

By varying the sample weight and the exposure time, a range of exposure concentrations was obtained. Rat exposure concentrations ranged from 505 to 2018 mg/cu m with concentrations X time (Ct's) from 30,300 to 181,620 mg min/cu m. Guinea pig exposure concentrations ranged from 88 to 801 mg/cu m with Ct's from 545 to 48,060 mg min/cu m. All exposure Ct's, exposure concentrations, exposure times, mortality responses, times to death, and statistical analyses are presented in tables E-1 through E-4.*

Chamber temperatures ($76^{\circ} \pm 6^{\circ}\text{F}$) and relative humidities ($68\% \pm 16\%$) were recorded.

B. Acute Inhalation Toxicity.

1. LCt'50's.

Bliss statistical analysis of the dose (Ct) response data produced the following:

<u>Species</u>	<u>LCt50</u>	<u>95% Confidence limits</u>	<u>Slope</u>
	mg min/cu m	mg min/cu m	
Rat	94,126	77,199 - 114,763	5.5
Guinea pig	5,321	4,749 - 5,962	15.99

2. Toxic Signs.

Animals were not visible for observation until the smoke cloud was evacuated from the chamber. When examined, guinea pigs exposed to Ct's ranging from 545 to 3840 mg min/cu m showed no ill effects. At 5280 mg min/cu m 1 of 5 guinea pigs died 30 minutes postexposure; the remaining animals appeared normal. At 5410 mg min/cu m, 6 of 10 guinea pigs died, 2

* Tables E-1 through E-21 are in appendix E.

during exposure, and 4 within 35 minutes. Until death, these animals showed severe respiratory distress. One of the survivors appeared to have some respiratory trouble, but within 3 hours, all survivors appeared normal. At the 4 higher Ct's, all guinea pigs exhibited some respiratory distress with only 2 of 40 animals surviving.

At 71,753 mg min/cu m (797 mg/cu m for 90 minutes) some rats started gasping and became ataxic, however, all recovered. Another test at almost the same Ct, though concentration and duration differed (1196 mg/cu m for 60 minutes), caused two deaths during exposure. Surviving animals appeared normal. At slightly higher Ct's (77,700 and 83,640 mg min/cu m), better than 50% of the rats died during or within 2 hours 45 minutes after exposure. Deaths were delayed in two cases, one rat dying > 24 < 48 hours and the other dying 3 to 4 days after exposure. Some of the surviving animals exhibited respiratory distress but recovered within 24 hours, and 30% appeared normal for the entire time. At the highest Ct (181,620 mg min/cu m), nine animals died during exposure. The surviving animal had respiratory problems for 1 hour and then gradually recovered.

3. Hematology and Blood Chemistry.

No agent-related changes were produced in the blood chemistry and hematology analyses of either rats or guinea pigs. "T" test evaluation indicated that there were no significant agent-related differences between control and exposed values. Mean and standard error of all blood constituents analyzed are presented in tables E-5 through E-8.

4. Pathology.

a. Sprague-Dawley Albino Rats.

Twenty-one exposed animals (12 males and 9 females) and 6 control animals (3 of each sex) were submitted for histopathological examination. Two of four rats exposed to the high dose (181,620 mg min/cu m) died in the exposure chamber. All other animals that were examined survived the lower exposure concentration and the 2-week holding period. All lesions except those found in the high-dose animals were probably not agent related. Fibrin thrombi were observed in the heart and lungs of both high-dose males. Acute diffuse congestion and focal perivascular edema were observed in the lungs of all high-dose rats. Hemorrhage was observed in the lungs of both male rats. The acute histopathological lesions found in the heart and lungs of the high-dose animals may have been agent related, but were probably related to the agonal death induced by the agent.

b. Hartley Guinea Pigs.

Seventeen exposed animals (6 females and 11 males) and six control animals (all female) were submitted for histopathological examination. At the five exposure levels studied, the WP/felt smoke did not cause pathologic lesions in the examined animals.

Incidence of lesions in animals submitted for pathological examination is presented in tables E-9 and E-10.

5. Pulmonary Resistance in Guinea Pigs.

There were no significant differences between exposed and control guinea pigs at the two exposure levels studied. The respiratory resistance of the control female animals is on the low end of the normal range; however, this mean value was lowered by one animal which was much smaller than the rest and had a very low resistance. When the results of this animal were removed, the mean for the remaining three animals was increased from 39.02 ± 6.54 to 45.50 ± 0.89 cmH₂Oℓ/second. Table E-11 summarizes the mean values obtained.

C. Oral Toxicity in Rats.

Rats affected by oral doses of the 65% phosphoric acid solution became lethargic with apparent gastric distress. Some animals became prostrate and subsequently died, usually within 24 hours. The oral 24-hour LD50 is 2346.8 mg/kg, and the 14-day oral LD50 is 2184.5 mg/kg. Details of this study are presented in tables E-12 through E-14.

D. Intravenous Toxicity of White Phosphorus/Felt Smoke Cloud Condensate in Rats.

Rats affected by the intravenous doses of the 65% phosphoric acid solution showed toxic signs within 30 seconds after injection. Rats became ataxic, with convulsions, prostration, and some deaths. Death was rapid, within 11 minutes for all but one animal that died 144 minutes after injection. Animals surviving beyond this time survived for the 14-day holding period. The 24-hour LD50 was 209.6 mg/kg. Details of this study are presented in tables E-15 and E-16.

E. Skin Sensitization Potential of White Phosphorus/Felt Smoke Cloud Condensate in Guinea Pigs.

There was no indication of skin sensitization in guinea pigs receiving intradermal injections of the WP/felt smoke cloud condensate. The positive controls, however, showed a fairly strong sensitization response, indicating that this group of guinea pigs is immunologically competent to respond to a sensitizer. Tables E-17 through E-20 show results of these studies.

F. Mutation Potential.

Three concentrations of the WP/felt smoke condensate (1%, 0.1%, and 0.01%) were used for these studies. A 10% concentration was lethal to all of the fruit flies. Mutations were not produced at any of the tested concentrations. Results of these studies are shown in tables E-21 and E-22.

IV. DISCUSSION.

Pathological findings in rats exposed to the WP/felt smoke were similar to those found in earlier WP studies by White and Armstrong² though at lower concentrations. Pulmonary congestion and edema were found in rats that died from exposure. Pulmonary effects are not unexpected since WP smoke is largely phosphoric acid. The question is: At what level will these effects occur?

From various reports of human exposure it has been estimated that the minimum harassing concentration of WP smoke is about 700 mg/cu m.³ In the documentation for Threshold Limit Values from 1971⁴ it has been reported that concentrations of 100 mg/cu m were unendurable except to hardened workers. A short-term exposure limit of 3 mg/cu m has been established by the American Conference of Governmental Industrial Hygienists.⁵ This is the maximum concentration to which workers can be exposed for up to 15 minutes at a time without suffering from irritation and chronic or irreversible tissue changes. It is thought that a man exposed to 100 mg/cu m or more would mask or remove himself from the smoke cloud.⁶

Although not observed in animal exposures, it has been reported that the smoke is irritating to the eyes and nose in moderate concentrations.⁷

V. CONCLUSIONS.

Results indicate that the white phosphorus smoke is moderately toxic in rats (16% mortality at Ct of 62,200 mg min/m³; 99% mortality at Ct of 248,250 mg min/m³) and highly toxic in guinea pigs (16% mortality at Ct of 4600 mg min/m³; 99% mortality at Ct of 7439 mg min/m³). Surviving animals appear fully recovered 2 weeks after exposure, based on appearance, blood chemistries, lung function tests, and pathology.

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APPENDIX A

CHEMICAL ANALYSIS OF WHITE PHOSPHORUS SMOKE*

The exposure chamber air samples which were received were diluted with distilled water, shaken, and boiled 10 minutes to convert the phosphorus acids to orthophosphoric acid; then they were cooled to room temperature. Sample volume was measured after boiling. Using a pH meter as an indicator, the acid content was determined by titration against 0.10043 N sodium hydroxide to a pH of 9.6. After measuring the amount of 0.10043 N sodium hydroxide required to adjust the sample pH to 9.6, the following formula was used to determine normality of the orthophosphoric acid:

$$1. \quad N_{\text{sample}} \times V_{\text{sample}} = N_{\text{NaOH}} \times V_{\text{NaOH}}$$

N = Normality

V = Volume of sample in milliliters

The normality of the acid is multiplied by 32.66 (milligram per milligram-equivalents of orthophosphoric acid).

$$2. \quad \frac{\text{milligram equivalents}}{\text{milliliter}} \times \frac{32.66 \text{ milligram}}{\text{milligram equivalents}} = \frac{\text{milligram}}{\text{milliliter of orthophosphoric acid}}$$

(normality of sample)

$$3. \quad \frac{\text{milligram}}{\text{milliliter}} \times \text{milliliter} = \text{milligram of orthophosphoric acid in sample}$$

* Pearce, B. P. Porton Technical Paper 154. The Stability of Red Phosphorus Compositions. June 1974.

APPENDIX B

GUINEA PIG RESPIRATORY MEASUREMENTS

The guinea pig is placed in a restraining device that isolates the animal's head from its trunk, using a heavy rubber collar around the neck. This restrainer is placed in a larger plastic box that is sealed from the outside. Respiratory flow is measured using a pneumotachometer attached to the head portion of the restrainer. Airway pressure is also measured from this point when the pneumotachometer opening is obstructed. Plethysmographic pressures are taken from the larger sealed container. The animal remains in this apparatus from 3 to 5 minutes while measurements are being recorded. Pressure changes within the plethysmograph were measured using a Statham differential pressure transducer (± 0.05 psid). Respiratory flow and airway pressure were measured with a ± 0.2 psid Statham differential transducer. The signals from the transducers are amplified using Honeywell bridge amplifiers (Accudata 218) and are recorded on a Honeywell 1858 CRT Visocorder.

Respiratory resistance is calculated in the following manner:

$$\text{Resistance (CmH}_2\text{O/liter/sec)} = \frac{\frac{[\text{Airway pressure (mmH}_2\text{O} \times \text{plethysmographic pressure (mmH}_2\text{O)}]}{\text{Plethysmographic pressure during airway pressure measurement (mmH}_2\text{O)}}}{\text{Flow (ml/sec)}} \times 100$$

Statistical comparisons of the data are based on the "t" test, comparing the exposed animals to their own control and age group.

These animals were sacrificed immediately after measurements for gross and light histopathological examination. In addition, the lungs were examined by electron microscopic techniques.

APPENDIX C

PROCEDURE FOR SENSITIZATION TESTING IN GUINEA PIGS

The electrostatically precipitated white phosphorus/felt (WP/felt) smoke cloud condensates were diluted in isotonic saline after hygroscopic stability was reached. The condensate, which contained approximately 65% phosphoric acid, was diluted to 0.1% (v/v) mixture before injection regimen began. This was the highest attainable concentration that would not cause a primary irritation when 0.05 ml was injected intradermally into guinea pigs.

Dinitrochlorobenzene (DNCB), a potent sensitizer used as a positive control, was dissolved in propylene glycol to make a 3% concentration. This was mixed in enough distilled and deionized water to make a 0.1% suspension.

On a Monday, 10 guinea pigs were injected intradermally on the upper right scapular area with 0.05 ml of the 0.1% WP smoke cloud condensate. The same volume of the vehicle was injected into the upper left scapular area. Later, at 24 and 48 hours, the reactions to these injections were measured and scored according to the grading system described on the following pages. Nine additional injections of 0.1 ml of the WP condensate were given in the lumbosacral area on consecutive Wednesdays, Fridays, and Mondays. The last injection was administered on the third Monday following the initial injection.

The 10 guinea pigs to be used with the positive control (DNCB) were prepared and injected in a manner identical to and concurrent with that of the test group. Five guinea pigs served as negative control for each exposed group.

Fourteen days after the last injection, the 10 guinea pigs that received the WP condensate were challenged with 0.05 ml of the 0.1% dilution injected intradermally in the lower right scapular region. The five negative control animals were given the challenge dose in the same area to determine the effects of aging on skin response. All 15 guinea pigs were injected in the lower left scapular area with 0.05 ml of the vehicle.

The challenge dose was compared to the initial dose according to the numerical evaluation system and formulae outlined on the following pages.

GRADING OF SKIN REACTIONS IN THE GUINEA PIG SENSITIZATION TEST

The grading system is designed so that the intensity of the skin reaction is represented by a proportionate numerical value and, also, any reaction elicited by the vehicle ("control substance") is subtracted from the reaction produced by the test substance and the vehicle combined.

The product of the width and length (in millimeters) of the wheal is multiplied by the following reaction scores:

- 0 = Needle puncture ("np"), no wheal
- 1 = Very faint pink ("vfp"), no value is recorded for this reaction
- 2 = Faint pink ("fp")
- 3 = Pink ("p")
- 4 = Red ("r")
- 5 = Bright red ("R")
- 6 = Edema - < 1 mm in height ("e")
- 7 = Edema - > 1 mm in height ("E")
- 8 = Necrosis - < 1 sq mm ("nec")
- *9 = Necrosis - > 1 sq mm ("NEC")

* The product of width and length of the necrotic area multiplied by 8 or 9 is added to the numerical value of any of the foregoing reactions that are present.

Calculation of Numerical Values from Skin Reaction Scores

The numerical values of the 24-hour readings are calculated from the following equations.

$$\begin{aligned}G_2 - G_1 &= a \\G_4 - G_3 &= b \\b - a &= \text{final grade}\end{aligned}$$

where

- G_1 = 24-hour reaction score from initial injection of vehicle
- G_2 = 24-hour reaction score from challenge injection of vehicle
- G_3 = 24-hour reaction score from initial injection of test substance
- G_4 = 24-hour reaction score from challenge injection of test substance

The numerical values of the 48-hour readings are calculated from the following equations.

- G_5 = 48-hour reaction score from initial injection of vehicle
- G_6 = 48-hour reaction score from challenge injection of vehicle
- G_7 = 48-hour reaction score from initial injection of test substance
- G_8 = 48-hour reaction score from challenge injection of test substance

A final grade of 25 or less indicates no sensitizing potential, and a final grade of 100 indicates a moderate sensitization potential in guinea pigs, according to the Lansteiner Guinea Pig Sensitization Test, as modified by the Chemical Hygiene Fellowship, Mellon Institute. July 1967.

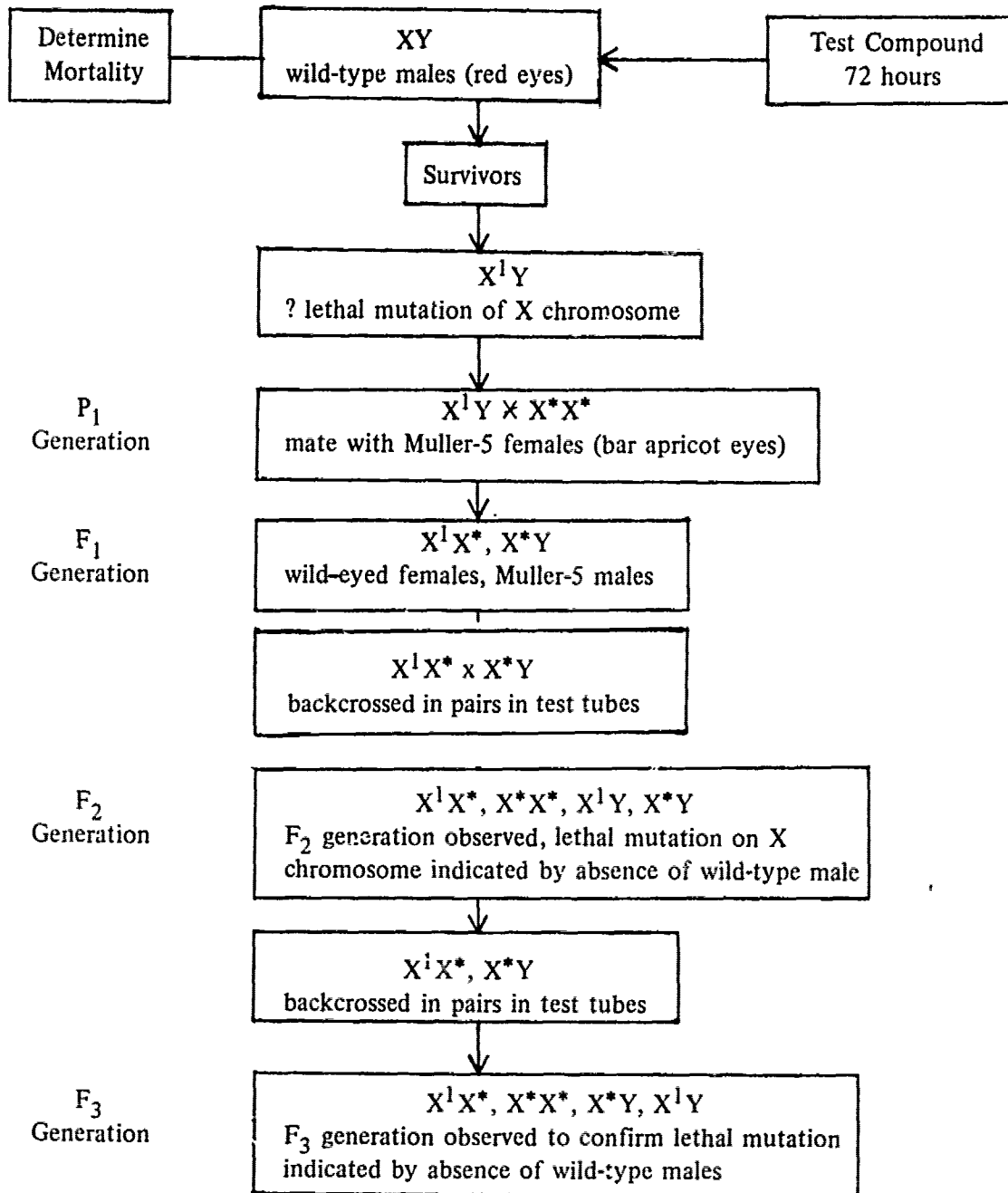
APPENDIX D

THE FRUIT FLY SEX-LINKED RECESSIVE LETHAL MUTATION TEST

The liquid white phosphorus smoke-cloud condensate was mixed in distilled water. Five grams of dry food and 15 ml of the test solution was placed in plastic culture vials. Wild-type Oregon-K strain *Drosophila Melanogaster* red-eyed males (age 1 to 2 days) were put in the vials which were placed in a controlled light, temperature, and humidity incubator. The food mixture was changed every 24 hours. After 72 hours of exposure mortality was determined.

Survivors of the 72-hour exposure were used for mutagenicity testing employing the Muller-5 technique (see schematic diagram). A negative control of food and distilled water and a positive control of the mutagen methylnethanesulfonate (MMS) were used. The surviving males were held for 1 day and then were mated with virgin Muller-5 bar-eyed female. Each mated pair (P_1) was kept in a 7-ml test tube. After 7 days, the mating adults were discarded. The emerging offspring (F_1) were taken and backcrossed in pairs in 7-ml test tubes. The mating adults of the F_1 generation were discarded after 7 days. The emerging F_2 generation flies were examined for absence of wild-type males. Those tubes in which wild-type males were absent, and contained 10 or more Muller-5 males, were positive. Tubes containing no wild-type males, but with less than 10 Muller-5 males, were designated as questionable positive. Those tubes containing wild-type males were scored negative. The positive and questionable positive F_2 generation flies were backcrossed in pairs and handled as were the earlier generation. Scoring of the emerging F_3 generation was the same as the F_2 scoring. A complete lack of wild-type males confirmed a mutation.

**SCHEMATIC DIAGRAM OF TEST PROCEDURE
EMPLOYING *DROSOPHILA MELANOGASTER***



APPENDIX E

DETAILS OF TOXICOLOGICAL EVALUATION

Table E-1. Inhalation Exposure Doses and Mortalities in Rats Exposed to White Phosphorus/Felt

Exposure Ct mg min/m ³	Average exposure concentration mg/m ³	Exposure time min	Mortality	Time to death
30,300	505	60	0/10	--
71,753	797	90	0/10	--
71,760	1196	60	2/10	2 died during exposure
77,700	1295	60	5/10	3 died during exposure 1 died 5 minutes postexposure 1 died > 24 < 48 hours
83,640	1394	60	6/10	4 died during exposure 1 died 165 minutes postexposure 1 died > 24 hours
181,620	2018	90	9/10	9 died during exposure

Table E-2. Bliss Statistical Analyses of Rat Dose-Response Data
for Acute Inhalation of White Phosphorus/Felt

Exposure Ct* mg min/m ³	95% Confidence limits	Mortality %
35,688	18,741 - 67,959	1
62,180	47,601 - 81,223	16
75,642	63,199 - 90,536	30
94,126	77,199 - 114,763	50
142,484	91,199 - 222,610	84
248,253	106,667 - 577,777	99
Slope 5.5		

* Concentrations based on acids of phosphorus.

Table E-3. Inhalation Exposure Doses and Mortalities in Guinea Pigs Exposed to White Phosphorus/Felt Smoke

Exposure Ct	Average exposure concentration	Exposure time	Mortality	Time to death
mg min/m ³	mg/m ³	min		
545	109	5	0/10	--
616	88	7	0/10	--
920	92	10	0/10	--
3,840	128	30	0/10	--
5,280	176	30	1/5	1 died within 30 minutes
5,410	541	10	6/10	2 died during exposure 2 died at 30 minutes 2 died at 35 minutes
6,560	656	10	9/10	1 died during exposure 2 died at 5 minutes 3 died at 10 minutes 1 each died at 30, 95, 120 minutes
13,520	451	30	9/10	8 died during exposure 1 died on 3rd day
14,310	477	30	10/10	8 died during exposure 2 died at 15 minutes
48,060	801	60	10/10	9 died during exposure 1 died at 15 minutes

Table E-4. Bliss Statistical Analyses of Guinea Pig Dose-Response Data for Acute Inhalation of White Phosphorus/Felt

Exposure Ct*	95% Confidence limits	Mortality
mg min/m ³		%
3,806	2,477 - 5,848	1
4,611	3,663 - 5,804	16
4,934	4,176 - 5,830	30
5,321	4,749 - 5,962	50
6,140	5,282 - 7,138	84
7,439	5,310 - 10,420	99
Slope 15.99		

* Concentrations based on acids of phosphorus.

Table E-5. Hematology in Rats Bled 2 Weeks after Exposure to White Phosphorus/Felt Smoke

Exposure Ct	Red blood cell			White blood cell			Hematocrit			Hemoglobin		
	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error
mg min/m ³ control	12	7.25	.73	12	9583.33	2150.13	12	41.13	3.59	12	15.53	1.22
30,300	10	6.59*	.59	10	9650.00	2313.11	10	38.80	3.67	10	13.78*	1.35
71,753	9	6.48*	.57	9	9077.78	2072.45	9	38.36	3.15	9	14.37*	1.13
71,760	8	6.64	.64	8	7950.00	1757.13	8	38.76	4.21	8	14.01*	1.23
77,700	5	6.87	.46	5	11560.00	2935.03	5	40.34	2.45	5	14.78	.90
83,640	4	5.43*	.30	4	7025.00*	798.04	4	31.87*	1.46	4	11.75*	.75
181,620	1	6.71		1	10600.00		1	38.90		1	15.40	

Exposure Ct	Neutrophils			Band cells			Lymphocytes			Eosinophils		
	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error
mg min/m ³ control	4	2.75	1.09	1	1.00		4	97.00	1.22			
71,753	3	7.33	3.40				3	92.00	4.32	1	2.00	
71,760	1	7.00					1	93.00				
83,640	1	4.00					1	96.00				

* Significantly lower than control based on statistical "t" test.

NOTE: Statistical evaluation of differential white count components: monocytes, eosinophils, basophils, and band cells may be meaningless since normal occurrences range from 1% to 10%.

Table E-6. Hematology in Guinea Pigs Bled 2 Weeks after Exposure to White Phosphorus/Felt Smoke

Exposure Ct	Red blood cell			White blood cell			Hematocrit			Hemoglobin		
	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error
mg min/m ³ control	12	X 10 ⁶ mm ³ 4.27	.51	12	per mm ³ 6841.67	1694.33	12	% 36.83	3.77	12	mg/100 ml 13.41	.58
920	9	4.27	.38	9	6766.67	1128.42	9	36.63	3.26	9	13.19	.83
3,840	4	3.90	.89	4	7625.00	1989.19	4	34.55	7.89	4	12.45	3.18
5,410	4	3.70	.74	4	5650.00	1458.60	4	32.70	6.28	4	11.50	2.12
6,560	1	4.11		1	6700.00		1	35.60		1	11.90	

Exposure Ct	Neutrophils			Lymphocytes			Monocytes			Eosinophils		
	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error
mg min/m ³ control	1	% 30.00		1	% 68.00		1	% 1.00		1	% 1.00	
5,140	1	3.00		1	97.00		1	1.00		1	1.00	

Table E-7. Blood Chemistry in Rats Bled 2 Weeks after Exposure to White Phosphorus/Felt Smoke

Exposure Ct	Glucose			Blood urea nitrogen			Creatinine			Sodium		
	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error
mg min/m ³ control	12	185.33	19.82	12	20.92	2.02	12	.60	.06	12	144.42	2.18
30,300	10	163.60*	15.47	10	25.20**	3.63	10	.71**	.05	10	142.40*	.66
71,753	9	184.00	22.43	9	20.11	2.23	9	.53	.11	9	144.56	2.36
71,760	8	190.12	24.02	8	22.37	2.18	8	.50*	.05	8	140.12*	4.70
77,700	5	170.60	14.71	5	21.60	2.65	5	.50	.11	5	141.20*	.98
83,640	3	173.33	16.50	3	20.00	.82	3	.50	.14	3	144.67	.94
181,620	1	187.00		1	26.00		1	.60		1	152.00	

Exposure Ct	Potassium			Chloride			Carbon dioxide			Uric Acid		
	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error
mg min/m ³ control	12	4.37	.75	12	104.58	2.22	12	19.42	5.44	12	1.77	.92
30,300	10	4.24	.39	10	102.80	2.09	10	20.70	3.82	10	1.04*	.51
71,753	9	4.49	1.29	9	102.89	1.66	9	20.22	7.66	9	1.26	.88
71,760	8	5.01	1.88	8	102.50	3.77	8	20.25	3.99	8	1.24	.50
77,700	5	3.96	.63	5	102.60	1.85	5	22.40	5.28	5	.64*	.14
83,640	3	4.20	.64	3	107.33**	.47	3	19.00	.82	3	1.40	.59
181,620	1	3.70		1	109.00		1	17.00		1	.90	

* Significantly lower than control based on statistical "t" test.
 ** Significantly higher than control based on statistical "t" test.

Table E-7. Continued

Exposure Ct	Total protein			Albumin			Globulin			Calcium		
	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error
mg min/m ³ control	12	5.97	.45	12	4.23	1.00	12	1.73	.88	12	10.15	.41
30,300	10	5.63	.30	10	5.14**	.18	10	.49*	.20	10	10.20	.44
71,753	9	5.48*	.30	9	4.80	.79	9	.68*	.79	9	9.93	.63
71,760	8	5.61	.31	8	5.09**	.28	8	.52*	.19	8	9.81	.86
77,700	5	5.74	.34	5	5.00**	.19	5	.74*	.23	5	10.10	.62
83,640	3	5.17	.63	3	4.87	.48	3	.30*	.16	3	9.33	.60
181,620	1	6.20		1	3.80		1	2.40		1	11.10	

Exposure Ct	Phosphate			Cholesterol			Triglycerides			Alkaline phosphatase		
	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error
mg min/m ³ control	12	6.48	1.15	12	73.00	6.00	12	58.00	34.97	10	344.20	77.72
30,300	10	6.00	.81	10	77.30	5.92	10	64.50	39.29	10	435.10	144.15
71,753	9	6.37	1.02	9	72.00	8.35	9	65.11	26.28	9	341.33	112.26
71,760	8	6.62	1.72	8	77.00	9.25	8	43.37	23.31	7	358.57	105.01
77,700	5	5.94	2.17	5	84.60**	3.98	5	54.60	36.35	3	325.33	75.57
83,640	3	5.60	.33	3	86.67**	4.92	3	36.00	27.07	3	306.67	87.86
181,620	1	5.50		1	86.00		1	62.00		1	447.00	

* Significantly lower than control based on statistical "t" test.
 ** Significantly higher than control based on statistical "t" test.

Table E-7. Continued

Exposure Ct	Glutamic oxalacetic transaminase			Glutamic pyruvic transaminase			Lactic dehydrogenase			Total bilirubin		
	No. of animals	Mean μ/l	Standard error	No. of animals	Mean μ/l	Standard error	No. of animals	Mean μ/l	Standard error	No. of animals	Mean mg/dl	Standard error
mg min/m ³ control	11	177.45	123.18	12	67.08	38.08	11	612.82	583.20	12	.11	.03
30,300	10	134.80	56.38	10	71.00	35.41	10	519.60	215.90	10	.10	.00
71,753	9	223.89	229.10	9	85.00	96.65	8	796.25	1105.13	9	.10	.00
71,760	6	94.83	13.62	7	62.00	27.76	6	275.33	50.85	8	.11	.03
77,700	4	81.25*	20.40	4	54.00	26.49	4	239.00	100.60	4	.10	.00
83,640	3	129.67	4.99	3	59.33	28.08	2	455.50	37.50	3	.10	.00
181,620	1	102.00		1	35.00		1	308.00		1	.10	

* Significantly lower than control based on statistical "t" test.

Table E-8. Blood Chemistry in Guinea Pigs Bled 2 Weeks after Exposure to White Phosphorus/Felt Smoke

Exposure Ct	Glucose		Blood urea nitrogen		Creatinine		Sodium		
	No. of animals	Mean mg/dl	Standard error	No. of animals	Mean mg/dl	Standard error	No. of animals	Mean meq/l	Standard error
mg min/m ³ control	12	149.58	10.11	12	18.92	3.80	12	138.83	1.77
920	10	171.00**	9.90	10	13.90*	2.30	10	137.90	1.81
3,840	3	156.00	7.12	4	18.00	2.45	4	140.50	1.50
5,410	4	197.00	24.05	4	18.75	1.92	4	140.75	1.48
6,560	1	148.00		1	12.00		1	135.00	

Exposure Ct	Potassium		Chloride		Carbon dioxide		Uric acid		
	No. of animals	Mean meq/l	Standard error	No. of animals	Mean meq/l	Standard error	No. of animals	Mean mg/dl	Standard error
mg min/m ³ control	12	4.67	1.33	12	107.42	2.29	12	.75	.24
920	10	4.38	.29	10	105.40*	1.20	10	.82	.25
3,840	4	4.45	.71	4	108.25	3.34	4	1.25	.27
5,410	4	5.37	1.41	4	107.00	2.12	4	1.77	.31
6,560	1	5.30		1	104.00		1	1.40	

* Significantly lower than control based on statistical "t" test.

** Significantly higher than control based on statistical "t" test.

Table 8. Continued

Exposure Ct	Total protein			Albumin			Globulin			Calcium		
	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error
mg min/m ³ control	12	4.20	.29	12	3.08	.22	12	1.12	.39	12	10.11	.53
920	10	3.78*	.28	10	3.26**	.16	10	.52*	.20	10	9.80	.40
3,840	4	4.38	.55	4	3.13	.18	4	1.25	.60	4	9.20	.67
5,410	4	4.32	.59	4	2.92	.29	4	1.40	.62	4	10.30	.58
6,560	1	4.10		1	3.20		1	.90		1	10.30	

Exposure Ct	Phosphate			Cholesterol			Triglycerides			Alkaline phosphatase		
	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error
mg min/m ³ control	12	5.90	.56	12	39.00	6.43	12	48.00	15.42	12	323.67	51.10
920	10	5.72	.73	10	32.70*	5.46	10	48.00	20.87	10	292.60	52.43
3,840	3	6.00	.50	4	43.75	6.61	4	37.00	18.45	4	350.00	65.73
5,410	4	7.17	.50	4	43.00	11.90	4	62.50	12.74	4	302.75	48.77
6,560	1	7.80		1	43.00		1	22.00		1	289.00	

* Significantly lower than control based on statistical "t" test.

** Significantly higher than control based on statistical "t" test.

Table E-8. Continued

Exposure Ct	Glutamic oxalacetic transaminase			Glutamic pyruvic transaminase			Lactic dehydrogenase			Total bilirubin		
	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error	No. of animals	Mean	Standard error
mg min/m ³ control	12	89.33	78.23	12	41.17	24.70	12	229.17	204.29	12	.11	.03
920	10	86.60	42.23	10	33.60	4.98	10	191.10	75.85	10	.10	.00
3,840	4	63.00	17.99	4	48.00	7.25	4	235.00	84.31	1	.10	
5,410	4	180.00	20.31	4	50.75	6.98	4	444.50	61.91	3	.10	.00
6,560	1	96.00		1	36.00		1	260.00		1	.10	

Table E-9. Incidence of Lesions in Rats Exposed to White Phosphorus/Felt Smoke

Lesions	Control		Exposure Ct (mg min/m ³)											
	181,620		83,640		77,700		71,753		30,300					
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
LUNG: (animals examined)														
Peribronchial lymphoid aggregates	3	3	2	2	1	3	2	2	2	2	2	2	2	2
Perivascular lymphoid aggregates	3	3	2	2	1	3	2	2	2	2	2	2	2	2
Fibrin thrombi	3	3	2	1		2			1			1	1	1
Congestion, acute, diffuse			2	2		1			1			1		1
Edema, perivascular, focal			2	2	1	1			1					
Hemorrhage, perivascular, focal			2	2	1	1								
Hemorrhage, intra-alveolar, focal			2	2										
Macrophages, intra-alveolar, focal		1		1		2	1					2	1	
Pneumonia, interstitial, eosinophilic, focal		1		1		1				1		2	2	1
Pneumonia, acute, focal												1		
Pneumonia, interstitial, suppurative, focal												1		
Granulomas, focal (MNCC)						1						2		
LIVER:														
Granulomas, focal (MNCC)	3	3	2	2	1	3	2	2	2	2	2	2	2	2
Simusoidal congestion, diffuse			2	2										
Eosinophilic focus, solitary		1												
Hepatocellular vacuolation, periportal, diffuse									1					
Triaditis, subacute, eosinophilic, focal														
KIDNEYS:														
Proteinuria, focal	3	3	2	2	1	3	2	2	2	2	2	2	2	2
Concretions, intratubular	1	1	1	1	2	2	2	2	1	1	1	1	1	1
Congestion, focal	3	3	1	1	1	1	1	1	1	1	1	1	1	1
Glomerular adhesions, focal			2	2	1	1	1	1	1	1	1	1	1	1

Table E-9. (Continued)

Lesions	Control		Exposure Ct (mg min/m ³)											
	181,620		83,640		77,700		71,753		30,300					
	M	F	M	F	M	F	M	F	M	F	M	F		
Nephritis, interstitial, chronic, focal					2	2							1	1
Dilated tubules, focal					1	1								
Pyelitis, chronic, focal					1	1								
Pelvic epithelial hyperplasia, focal			1	1										
Pelvic epithelial mineralization focal			1	1										
HEART:														
Congestion, diffuse	3	3	2	2	2	3	2	2	2	2	2	2	2	2
Fibrin thrombi			2	2										
SPLEEN:														
Lymphoid hyperplasia, focal	3	3	2	2	2	3	2	2	2	2	2	2	2	2
RE* cell hyperplasia, diffuse														
TURBINATES:														
Rhinitis, chronic-active	3	3	1	2	2	3	2	2	2	2	2	2	2	2
TRACHEA:	2	3	1	2	2	3	2	2	2	2	2	2	2	2
Mucosal epithelium - ICIB**	3	2	1	2	2	3	2	2	2	2	2	2	2	2
Tracheitis, chronic-active	2	1	1	1	2	2	2	2	2	2	2	2	1	1

* RE - Reticulo endothelial.

** ICIB - Intracytoplasmic inclusion.

Table E-10. Incidence of Lesions in Guinea Pigs Exposed to White Phosphorus/Felt Smoke

Lesions	Control		Exposure Ct (mg min/m ³)											
	M	F	920		3,840		5,280		5,410		6,560			
			M	F	M	F	M	F	M	F	M	F		
LUNG:														
Peribronchial lymphoid aggregates	3	3	2	2	2	2	2	2	1	1	3	0	1	
Perivascular lymphoid aggregates	3	3	2	2	2	2	2	1	1	3				
Pneumonia, interstitial, focal	3	3	2	2	2	2	2	1	1	3			1	
Congestion, focal	3	3	1	2	2	2	2	2	1	3			1	
Macrophages, alveolar, focal	2	3	2	2	2	2	2	2	2	2				
Hemorrhage, alveolar, focal		1	1	1	2	1	1	1	2	2				
Granulomas, focal (MNGC)		1							1	1				
Pneumonia, chronic-active focal					2	1								
Adenomatous hyperplasia, solitary														
KIDNEY:														
Glomerular adhesions	3	3	2	2	2	2	2	2	2	1	3	0	1	
Tubular epithelial mineralization	3	2	2	2	2	2	2	2	2	1	3		1	
Proteinuria		1	1	1	1	1	1	1	1	1	1			
Interstitial, nephritis, focal														
Pyelitis, subacute, focal														
LIVER:														
Hepatocellular, vacuolation, diffuse (large vacuoles)	3	3	2	2	2	2	2	2	2	1	3	0	1	
Hepatocellular necrosis, chronic, focal														
Pericholangitis, chronic, focal			2	1										

Table E-10. (Continued)

Lesions	Control		Exposure Ct (mg min/m ³)											
	920		3,840		5,280		5,410		6,560					
	M	F	M	F	M	F	M	F	M	F				
HEART:	3	3	2	2	2	2	2	2	1	3	0	1		
Epicarditis, chronic-active, focal		1												
Mycocarditis, subendocardial, chronic, focal	1				2	1			1					
Mycocarditis, acute pyonecrotizing, focal		1												
Phabdomyomatosis, focal		1												
Obturator thrombus, acute, atrial		1												
TRACHEA:	3	3	2	2	2	2	2	2	1	3	0	1		
Tracheitis, chronic-active	1	3		2		1			2	3		1		
TURBINATES:	3	3	2	2	2	2	2	2	1	3	0	1		
Rhinitis, chronic-active	3	3	1	2	1	1	2	2		1				

Table E-11. Pulmonary Resistance in Guinea Pigs Exposed to Either 3840 or 5280 mg min/m³ of White Phosphorus/Felt Smoke

Animals	Number of animals	Weight		Resistance		Rate	
		Mean	SE*	Mean	SE*	Mean	SE*
Male control	5	464.6	14.0	65.02	5.08	97.0	1.6
Male exposure	6	432.5	10.0	62.29	5.85	112.0	12.4
Female control	4	372.0	17.6	39.02	6.54	124.0	17.1
Male exposure	4	390.5	19.3	57.37	3.92	105.0	10.1

* SE - standard error.

Table E-12. Oral Toxicity of White Phosphorus/Felt Smoke Cloud Condensate in Rats

Dose gm/kg	Mortality	
	24-hour	14-day
1.48	0/6	1/6
1.86	1/6	2/6
2.34	3/6	3/6
2.96	5/6	5/6

Table E-13. Bliss Probit Analysis of 24-hour Dose-Response Data

Mortality	Dose	95% Confidence limit
%	gm/kg	gm/kg
1	1.42	0.76 - 2.65
16	1.90	1.43 - 2.52
30	2.10	1.74 - 2.54
50	2.35	2.00 - 2.79
84	2.93	2.05 - 4.19
99	3.91	1.93 - 7.96

Table E-14. Bliss Probit Analysis of 72-hour* Dose-Response Data

Mortality	Dose	95% Confidence limits
%	gm/kg	gm/kg
1	0.92	0.11 - 8.09
16	1.51	0.61 - 3.74
30	1.80	1.12 - 2.91
50	2.19	1.72 - 2.79
84	3.17	1.09 - 9.20
99	5.20	0.50-53.76

* These data are the same as the 14-day data.

Table E-15. Intravenous Toxicity of White Phosphorus/Felt Smoke Cloud Condensate in Rats

Dose mg/kg	Mortality*	
	24-hour	14-day
148	0/6	0/6
186	2/6	2/6
234	4/6	4/6
370	5/6	6/6

Table E-16. Bliss Probit Analysis of Intravenous Dose-Response Data

Mortality	Dose	95% Confidence limits
%	mg/kg	mg/kg
1	138	38 - 487
16	176	108 - 286
30	191	151 - 243
50	210	171 - 260
84	253	120 - 534
99	324	70 - 1502

Table E-17. Twenty-Four Hour Sensitization Reaction in Guinea Pigs Challenged with White Phosphorus/Felt Cloud Condensate

Animal number	$G_4 - G_3 = b^*$			$G_2 - G_1 = a$			$b - a^{**}$
86	9	12	0	0	0	0	0
87	0	0	0	3	0	3	0
88	18	0	18	0	20	0	18
89	9	0	9	0	0	0	9
90	0	20	0	0	20	0	0
91	16	30	0	0	30	0	0
92	20	6	14	0	0	0	14
93	0	0	0	0	16	0	0
94	15	0	15	0	0	0	15
95	0	9	0	8	12	0	0

Table E-18. Forty-Eight Hour Sensitization Reaction in Guinea Pigs Challenged with White Phosphorus/Felt Smoke Cloud Condensate

Animal number	$G_8 - G_7 = d^*$			$G_6 - G_5 = c$			$d - c^{**}$
86	0	9	0	0	12	0	0
87	0	0	0	0	0	0	0
88	4	16	0	0	0	0	0
89	0	0	0	0	0	0	0
90	0	0	0	0	6	0	0
91	0	0	0	0	0	0	0
92	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0
94	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0

* Explanation of coding and scoring in appendix C.

** Final sensitization grade - if 25 or less results are negative.

Table E-19. Twenty-Four Hour Sensitization Reaction in Guinea Pigs Challenged with Dinitrochlorobenzene

Animal number	$G_4 - G_3 = b^*$			$G_2 - G_1 = a$			$b - a^{**}$
96	630	25	605	0	0	0	605
97	700	16	684	0	0	0	684
98	392	42	350	4	25	0	350
99	567	0	567	0	0	0	567
126	392	9	383	0	16	0	383
101	504	40	464	0	0	0	464
102	560	20	540	0	25	0	540
103	490	16	474	0	0	0	474
104	448	30	418	0	0	9	409
105	448	30	418	0	15	0	418

Table E-20. Forty-Eight Hour Sensitization Reaction in Guinea Pigs Challenged with Dinitrochlorobenzene

Animal number	$G_8 - G_7 = d^*$			$G_6 - G_5 = c$			$d - c^{**}$
96	567	16	551	0	0	0	551
97	630	0	630	0	0	0	630
98	392	75	367	0	0	0	367
99	567	16	551	0	0	0	551
126	343	25	318	0	25	0	293
101	567	0	567	0	0	0	567
102	630	12	618	0	0	0	618
103	504	0	504	0	0	0	504
104	392	0	392	0	0	0	392
105	448	0	448	0	0	0	448

* Explanation of coding and scoring in appendix C.

** Final sensitization grade - all animals sensitized.

Table E-21. Toxicity of White Phosphorus/Felt Smoke-Cloud Condensate to 1- to 2-Day Old Male Fruit Flies

Concentration	Number of exposures	72-Hour mortality
%		%
10	100	100
1	125	11
0.1	110	2
0.01	110	0

Table E-22. Induction of Lethal Mutation in 1- to 2-Day Old Male Fruit Flies

Concentration	X-chromosomes tested	Lethal	
		No.	%
%			
1	100	0	0
0.1	101	0	0
0.01	104	0	0

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