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MAMMALIAN TOXICOLOGIC EVALUATION OF HEXACHLOROETHANE SMOKE MIXTURE AND RED PHOSPHORUS

Final Report

Prepared by

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Fort Detrick, Frederick, MD 21701

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Litton Bionetics, Inc.
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Four red phosphorus samples, three containing oil, were chemically analyzed for conformation to specifications. A hexachloroethane smoke mixture was burned in a laboratory apparatus to confirm that organic combustion products were present. The acute toxicity of one oiled red phosphorus sample was determined. This sample did not produce irritation in rabbits' eyes at a dose of 100 mg. It was also nonirritating to intact or abraded skin of rabbits when applied at doses of 0.5 g per application site under a patch for 24 hours. Dermal application to

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20. Abstract (Continued)

guinea pigs did not produce skin sensitization. Intradermal treatment of guinea pigs produced a slight irritation response but not sensitization. The oral LD50 in Fischer 344 male and female rats was greater than 10 g/kg.

EXECUTIVE SUMMARY

The objectives of this program are to provide confirmation of the organic combustion products from burning of a hexachloroethane (HC) smoke mixture and to define the acute toxicologic effects of uncombusted red phosphorus (RP) to mammalian systems. The toxicologic evaluation of RP was initiated in response to the proposed establishment by the US Army of an RP onshore production facility. Chemical analyses of four red phosphorus samples, three samples of which contained light lubricating or mineral oil showed that all samples did not meet the complete list of specifications. The total phosphorus content was below the specified 98.75 percent. The oil content was either above or below the required 1.25 percent. None of the samples met the criteria for particle size and the oiled samples had a tendency to agglomerate. All samples contained less white phosphorus than the allowed maximum of <0.01 percent.

Attempts to produce an aerosol in an inhalation chamber were unsuccessful. An aerosol of oiled red phosphorus could not be sustained with a fluid bed generator, Laskin aerosol system, or a Wright Dust Feed mechanism. The dust feed was able to generate a low chamber concentration but jammed frequently due to the clumping of the oiled material. The majority of the oiled red phosphorus would not pass through a sieve which excludes particles above 150 micrometers Less than 0.5 percent of the material passed through a sieve with a 38 micrometers cutoff. These studies indicated that the oiled red phosphorus contains very few respirable particles and would not be a potential industrial inhalation hazard.

Gastric intubation of 1,000, 3,610, and 6,810 mg/kg did not produce lethality. After administration of 10,000 mg/kg to five Fischer 344 rats per sex, one male rat died within 24 hours. This experiment was repeated using 10 rats per sex and one female died 7 days after treatment. This animal gave signs of an infection. Other toxic signs at the high-dose level were failure to gain body weight or dose of weight during the 14-day observation period.

The oiled red phosphorus did not elicit an irritation response when applied as a 0.5 g dose on intact or abraded rabbit skin. The instillation of 100 mg of the test material into rabbit eyes did not produce any irritation or injury. Intradermal injection into guinea pigs produced signs of irritation but not skin sensitization. Application of the test material to guinea pig skin did not result in irritation or sensitization responses.

Sampling of the gas phase from a burn of the hexachloroethane smoke mixture (zinc oxide and aluminum) showed the presence of phosgene, tetrachloroethylene, carbon tetrachloride, hexachloroethane, and hexachloro-1,3-butadiene.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Uses of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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INTRODUCTION

Chemical smokes/obscurants are used by the military to conceal personnel, materiel, or installations from direct visual observation. Army personnel may be exposed to these chemical smokes when they are released into the environment in training or combat operations. The duration of exposures may vary from minutes to several hours within a given day and exposures may be repeated over consecutive days or intermittently over weeks. The manufacture and assembly of smoke munitions may also expose industrial workers to vapors and/or particulate aerosols of the chemical compounds loaded into smoke munitions. Exposure durations for munition workers are the typical industrial occupational periods--6 to 8 hours per day, 5 days per week over a working lifetime. Although the exposure routes are similiar for the two occupational groups--inhalation and dermal--the chemical compounds to which they are exposed may be different. Industrial workers can be exposed to the chemical compounds which are incorporated into the smoke munitions whereas field personnel will be exposed to compounds generated by combustion processes as well as unreacted chemicals. Evaluations of the potential hazards posed by chemicals loaded into smoke munitions and combustion products of these chemical mixtures are a necessary portion of the data base required to establish comprehensive occupational health criteria.

The objectives of this program are to provide confirmation of the organic combustion products from burning of a hexachloroethane (HC) smoke mixture and to define the acute toxicologic effects of uncombusted red phosphorus (RP) to mammalian systems. The HC smoke munition contains a solid mixture composed of 9 percent grained aluminum, 46.5 percent zinc oxide, and 44.5 percent hexachloroethane. When the HC mixture is heated in a smoke pot, a self-propagating reaction is set up which is based upon the tendency of aluminum to react with hexachloroethane to form aluminum chloride. The aluminum chloride reacts with zinc oxide to form zinc chloride and aluminum oxide. Zinc chloride is the major component of the smoke generated by burning the HC mixture. Recent studies supported by US Army Medical Research and Development Command have shown that the smoke also contains unreacted hexachloroethane as well as other organic compounds.

The toxicologic evaluation of RP was initiated in response to the proposed establishment by the US Army of an RP onshore production facility. The RP presently used to produce smoke munitions is purchased from the United Kingdom. With an onshore production facility, there is the potential for occupational exposure of United States munition workers to RP. Since the RP to be produced would be identical to that presently purchased from the United Kingdom (UK), samples of UK red phosphorus were used in this study. The toxicologic evaluation was limited to acute toxicity tests by the dermal and oral routes.

MATERIALS AND METHODS

RED PHOSPHORUS

Chemical and Physical Analyses

Four samples of technical grade RP were supplied by US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL). These samples were identified as Erco (unoiled) and three oiled samples: American Hoechst HN and HB-100, and Albright and Wilson, Ltd. Lot LT-22. The oiled samples contained 1.2 to 1.25 percent of oil. For the American Hoechst lots, this was a light lubricating oil, general purpose, with the following physical and chemical requirements:

Viscosity, centistokes, 37.8°C	17-20
Flash point, minimum at °C	149
Pour point, maximum, °C	-34
ASTM, color, maximum	3.0
Neutralization number, maximum	0.10
Copper corrosion, ASTM classification maximum at 100°C	1
Viscosity index, minimum	30

The Albright and Wilson, Ltd. sample contained a refined mineral oil with the following requirements:

Viscosity, centistokes, at 100°C	16-24
Flash point (closed), minimum, °C	149
Pour point, maximum, °C	-17.8
Inorganic acidity	Nil
Organic acidity, ng potassium hydroxide per g, maximum	0.1
Saponifiable matter, maximum	1.0
Ash percent, maximum	0.01
Sulfur (corrosive)	Nil

Specification requirements for technical grade RP are not available. The US specification MIL-P-211B is presently being revised to include a class 3 RP. This class would be comparable to UK specification CS 5062, 30 April 1959, Phosphorus, Amorphorus, Grade 1 and the latter specification was used for RP analyses. Test methods used to determine conformity to specification requirements are contained in Appendix A and were provided by USAMBRDL. The samples were analyzed for:

Total phosphorus
Particle size
Acidity to mixed indicator
Acidity to phenolphthalein
Alkalinity to mixed indicator
Volatile matter
Oil content
Iron content
Water soluble material
White phosphorus
Aqua regia insoluble material

Biologic Studies

The Albright and Wilson, Ltd. Lot LT-22, oiled RP, was used in the biologic studies.

Acute Oral Toxicity

Male and female Fischer 344 (F344) albino rats were obtained from Charles River Breeding Laboratories, Inc., Portage, Michigan. Animals were acclimated to laboratory conditions for 1 week. They were individually housed in wire-bottom cages, in quarters maintained at temperatures of 72° to 76°F and 39 to 54 percent relative humidity. A 12-hour light cycle was maintained with artificial illumination. Acidified water (pH 2.5) and Purina Rat Chow were provided ad libitum except 18 to 24 hr before treatment when food was withheld. The oiled RP was suspended in corn oil and administered by oral intubation at concentration levels to provide 1.0 mL per 100 g body weight. Animals were identified by ear tags. Male rats weighing between 190 and 240 g and female rats with body weights of 146 to 172 g, 10 to 13 weeks of age were used in the range-finding studies. These studies were performed with two males and two females at each of four dose levels. Three additional male and three female rats were administered the high-dose level 6 days after the first experiment. To confirm the results of the second treatment with the high-dosage level, an additional 10 male (184 to 210 g body weight) and 10 female (163 to 174 g body weight) rats, 9 to 13 weeks of age, were given this dose. Animals were observed for 14 days following treatment.

Irritation Studies

Primary skin and eye irritation potentials of RP were determined with 10-week-old female New Zealand white rabbits obtained from B and H Rabbitry, Rockville, MD. The animals were acclimated to laboratory conditions for 1 to 2 days. Throughout the study, the rabbits were individually housed in wire-bottom cages. Temperature, humidity, light cycle, and feeding regimen were the same as provided for rats. For the skin studies, 0.5 g of the RP was

mixed with 0.5 mL sterile isotonic saline solution to form a paste-like suspension. To a 1-inch square on the left side of the rabbit's back, 0.5 g of RP was applied. A 1-inch square on the right side of the back was abraded and 0.5 g of RP was applied to this surface. For eye irritation studies, the eyes of the rabbits were examined after applying one drop of 2 percent sodium fluorescein opthalmic solution on the cornea to ensure that animals were without eye defects or irritation. The animal was held firmly and 100 mg of the dry material was applied to the eye by pulling out the lower eyelid to form a cup. Only one eye was treated, the other served as a control; three rabbits were treated in the right eye, three rabbits in the left eye. The test material was washed out of the eye with sterile isotonic saline solution 24 hours after treatment. Test methods for conducting these studies are given in Appendix B.

Skin Sensitization Studies

The potential of RP to produce skin sensitization was evaluated by two methods, intradermal injections or externally applied patches containing the test material. Female Hartley strain albino guinea pigs obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA, were used for the first method. The animals were acclimated to laboratory conditions for 21 days and were approximately 7 weeks of age at initiation of treatment. Males of the same strain obtained from the same source were used for the range-finding and sensitization studies where the test agent was applied by the patch method. The male animals were received in one shipment: for the range-finding studies, animals 6 to 8 weeks of age were used; the males were 9 weeks of age for the skin sensitization study. All guinea pigs were individually housed: the females in wire-bottom cages, the males in polycarbonate cages on AB-SORB-DRI(R) bedding. Water and Purina Guinea Pig Chow were provided ad libitum. Temperature, humidity, and illumination conditions were the same as for the other species.

The oiled red phosphorus was suspended in sterile isotonic saline. For the intradermal injections, 0.1 mL of a 0.1 percent (w/v) suspension was used. The suspensions' concentrations for the patch test range-finding studies were 1.0, 2.0, and 5.0 percent. In the skin sensitization studies, 10.0 percent w/v suspensions were used. All animals used in the patch tests received 0.5 mL of the test material. The positive control in both studies was 2,4-dinitro-1-chlorobenzene (Scientific Products, Inc., Lot No. F7A), 0.1 mL applied as 0.05 percent (w/v) solution.

The method for conducting skin sensitization studies in guinea pigs included application of the test material or positive control chemical to a shaved area of the flank or back 3 times per week (Monday, Wednesday, Friday) for 10 applications. A challenge application of the test material was made to a site other than the sensitization site 15 days after the last application. Immediately before each sensitizing application, the reaction from the previous application was evaluated according to the numerical scoring system for skin irritation responses (Appendix B). The site of the challenge application was evaluated 24 and 48 hours after the challenge. Reaction to the test material at the application site was determined by measurement of the diameter with calipers and estimation of the height and color. The guinea pigs were observed daily for toxic signs.

Topical application for the patch test was accomplished with a dosing band. The band consisted of two 3-inch long strips of 1-inch wide cloth adhesive tape overlapped 0.25 inch. The adhesive side was lined with gauze. A window 2 x 2 cm square was cut in the band and it was attached to the shaved back of the guinea pig with adhesive tape. The test material was applied to the back of the animal through the dosing window and the window was then covered with filter paper. The test material remained in contact with the skin for 6 hours and was then removed by wiping the area first with cotton soaked in glycerin and then with a gauze pad. In the range-finding studies, 2 males per group were treated with 1.0, 2.0, or 5.0 percent w/v suspensions of oiled RP once daily for 5 days. Three groups of 10 guinea pigs each were used in the skin sensitization studies. Group 1 received 0.1 mL of a 0.05 percent w/v solution of 2,4-dinitro-1-chlorobenzene in acetone. Group 2 was treated with 10 applications of 0.5 mL 10 percent w/v suspension of oiled RP in sterile saline. Group 3 received 0.5 mL of a 20 percent RP suspension at the challenge application only.

HC Smoke Mixture

The methods used for laboratory generation of combustion products from the HC smoke mixture were the same as developed by IIT Research Institute.[1] The composition of the HC mixture was based on the average concentration of each component found in a number of lots of HC smoke pots by Katz et al. The HC mixture used in the combustion product studies was prepared from reagent grade chemicals and had the following composition.

Hexachloroethane	45.04% (Aldrich Chemical Co., Lots HC 050987 and PB 1014771)
Zinc Oxide	45.81% (Fisher Scientific, ACS certified Lot 790234)
Aluminum	9.25% (Fisher Scientific, ACS certified Lot 784016, as a wire)

The schematic diagrams for smoke generation and cryogenic trapping are shown in Figure 1. The HC mixture (1 g) was ignited in a closed 5-liter flask at ambient (ca. 25°C) and relative humidity of 40 to 50 percent. The hot-wire ignitor was aligned such that the heating coil was completely buried in the HC mixture. After the reaction was completed, the aerosol was introduced into an evacuated 250-mL glass gas sampling bulb. A 1-mL sample was taken from this bulb through a septum by means of a 2-mL gas-tight syringe equipped with an on-off valve. The gas sample was injected into the gas chromatograph (GC, Finnigan 9610) inlet of the GC/MS system for qualitative identification. Organic components in the aerosol were trapped in a 2-mL glass U-tube which was transferred to the GC inlet of the GC/MS (Fig. 2). Conditions for the GC included a 10 ft x 1/8 in OD stainless steel column packed with 3 percent

[1] Katz, S., A. Snelson, R. Farlow, R. Welker, and S. Mainer. 1980. Physical and Chemical Characterization of Fog Oil Smoke and Hexachloroethane Smoke. Final Report, AD A0 80936. IIT Research Institute, Chicago, IL. DAMD17-78-C-8085.

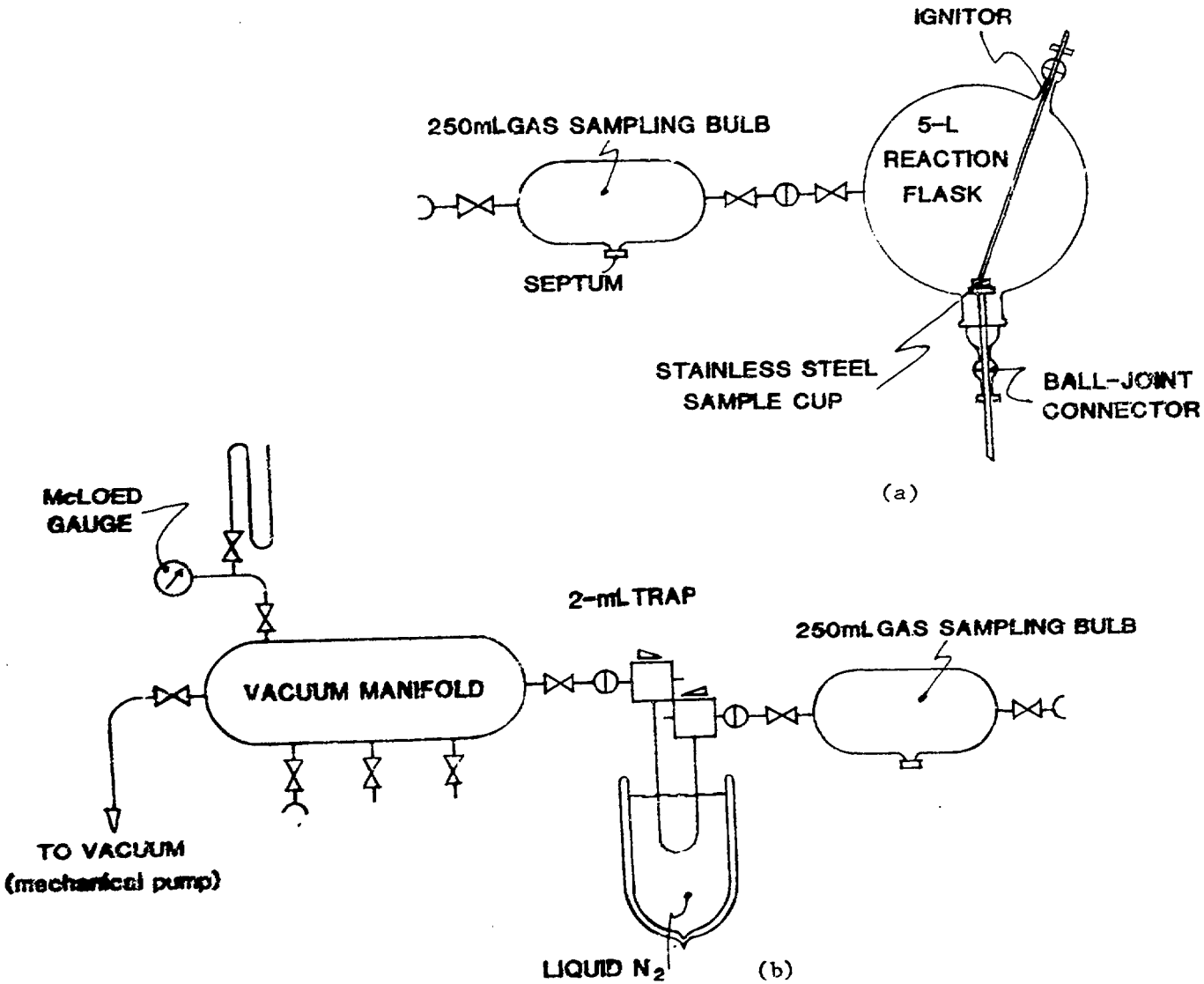

Figure 1. Schematic Diagrams for (a) HC Smoke Generation and (b) Cryogenic Trapping.

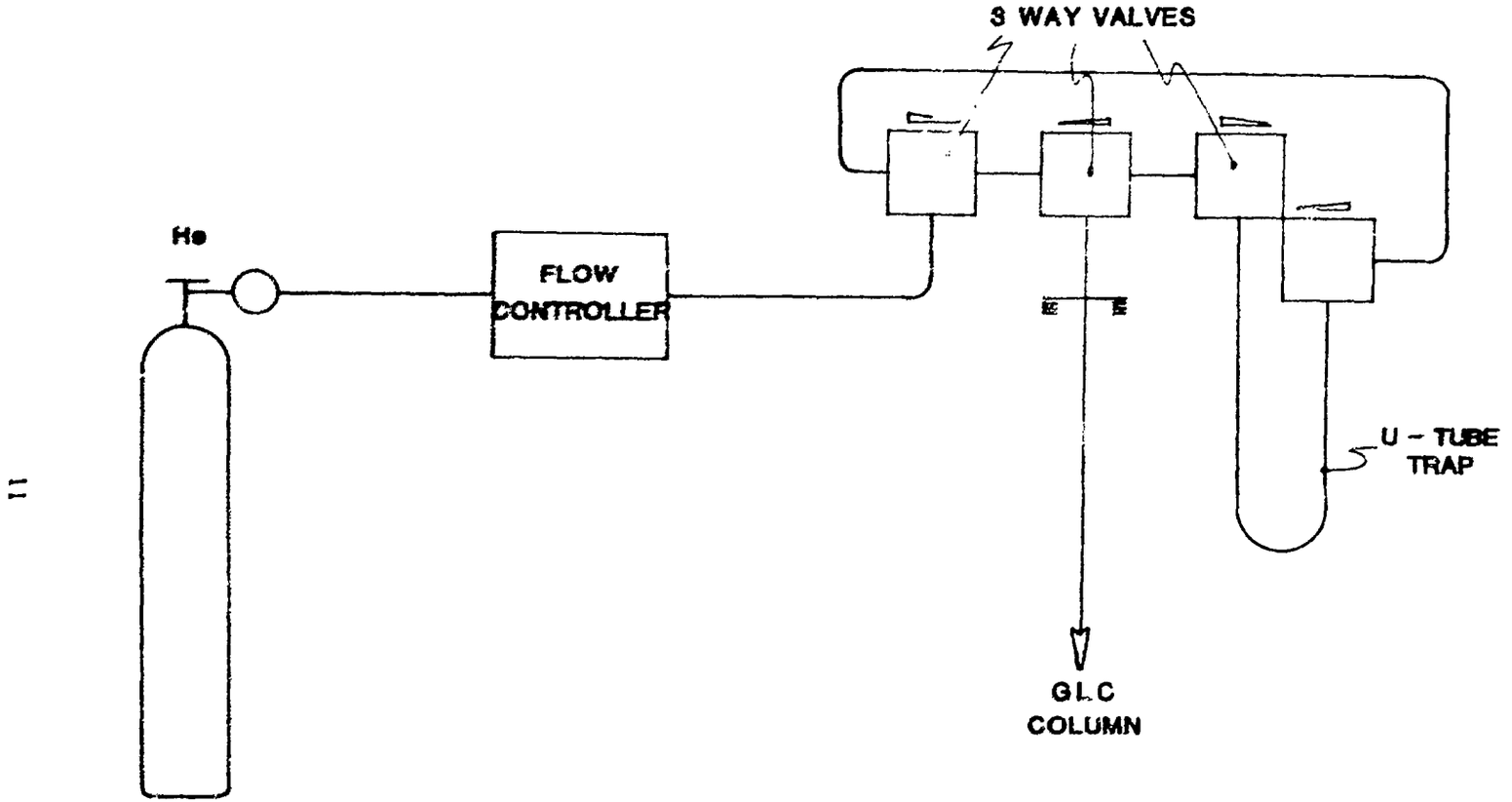

Figure 2. Schematic Diagram for Connecting the U-Tube Trap to the GC Inlet.

SP-2401 on 100/120 Supelcoport. The column was temperature programmed from -10° to 150°C.

The 5-liter flask and 250-mL bulb were washed with methylene chloride for measurement of less volatile organic reaction products. The wash solutions were analyzed by GC with a flame ionization detector. A 6 ft x 2 mm ID glass column packed with 3 percent OV-1 provided a stable baseline. Temperature was 25° to 180°C at 15°/min.

RESULTS

CHEMICAL ANALYSES

Red Phosphorus

The results of chemical analyses of the four red phosphorus samples are presented in Tables 1 through 4. A summary of the data for all four samples is given in Table 5 as well as the specifications as guidelines for comparison. The total phosphorus content of all samples was below the specified, 98.75 percent, although the Erco unoiled sample at 98.4 percent was close to specifications. The oil contents of the three oiled red phosphorus samples were all outside specifications. The Hoechst HN sample contained the greatest amount of oil, 5.5 percent, whereas the Albright and Wilson sample contained only 1.0 percent. None of the samples met the criteria for particle size.

Only the Hoechst samples were within the specification for acidity to mixed indicator. For acidity to phenolphthalein, the Albright and Wilson sample exceeded the limit of 0.5 percent. Both Hoechst samples exceeded the limit of 0.1 percent for alkalinity to mixed indicator.

The maximum for volatile matter of 0.5 percent was met by all four samples. Only the Hoechst HN sample exceeded the limit of 0.1 percent for iron content. All four samples contained more than the maximum specification of 1.0 percent for water-soluble material. Most of the samples contained much less white phosphorus than the maximum of <0.01 percent; the Albright and Wilson sample contained 0.006 percent.

Aqua regia insoluble material in the Erco sample was below the specified maximum of 0.3 percent. Analyses of the oiled samples were attempted, but quantitative recoveries could not be obtained. Treatment of large amounts of sample with aqua regia converted the oil to a gummy residue which could not be completely removed from the sides of the reaction vessel.

HC Mixture Combustion Products

Three laboratory smoke generation experiments were conducted. Data from the first experiment are not included in this report since part of the aerosol was lost due to technical malfunction. The chemical reactions of the second and third burns appeared adequate. After the voltage was applied to the ignitor, a light stream of white smoke was observed. Shortly thereafter, the HC mixture ignited and a thick cloud of gray smoke filled the flask. The reaction lasted for only a few seconds. A large portion of smoke particles may be free carbon since the black particles were not soluble in any organic

TABLE 1. ANALYSES OF ERCO RED PHOSPHORUS (UNOILED)

Characteristic	Percent by Weight	Mean ± S.D.
Total Phosphorus	103.0 95.8 96.3	98.4 ± 4.02
Particle Size:		
Retained on No. 60 Sieve	7.19 4.95 3.90	5.35 ± 1.68
Retained on No. 80 Sieve	7.34 5.20 4.12	5.55 ± 1.64
Acidity to Mixed Indicator (as H_3PO_4)	0.27 0.31 0.29	0.29 ± 0.020
Acidity to Phenolphthalein (as H_3PO_4)	0.50 0.51 0.48	0.50 ± 0.015
Alkalinity to Mixed Indicator (as Na_2CO_3)	0.00 0.00 0.00	0.00 ± 0.000
Volatile Matter	0.093 0.076 0.084	0.084 ± 0.0085
Oil Content	0.02 0.02 0.12	0.05 ± 0.06
Iron Content	0.04 0.04 0.04	0.04 ± 0.000
Water-soluble Material	3.60 3.52 4.10	3.74 ± 0.31
White Phosphorus	0.0010 0.0010 0.0011	0.0010 ± 0.00006
Aqua Regia Insoluble Material	0.27 0.32 0.28	0.29 ± 0.026

TABLE 2. ANALYSES OF AMERICAN HOECHST HN RED PHOSPHORUS

Characteristic	Percent by Weight	Mean ± S.D.
Total Phosphorus	96.2 93.8 94.1	94.7 ± 1.31
Particle Size:		
Retained on No. 60 Sieve	9.41 10.45 8.10	9.32 ± 1.18
Retained on No. 80 Sieve	12.44 13.13 10.64	12.07 ± 1.28
Acidity to Mixed Indicator (as H_3PO_4)	0.00 0.00 0.00	0.00 ± 0.00
Acidity to Phenolphthalein (as H_3PO_4)	<0.01 <0.01 <0.01	<0.01 ± 0.00
Alkalinity to Mixed Indicator (as Na_2CO_3)	0.32 0.30 0.32	0.31 ± 0.012
Volatile Matter	0.047 0.064 0.063	0.058 ± 0.010
Oil Content	5.89 5.83 4.88	5.53 ± 0.57
Iron Content	0.11 0.13 0.12	0.12 ± 0.010
Water-soluble Material	3.20 3.09 4.00	3.43 ± 0.50
White Phosphorus	0.0006 0.0005 0.0006	0.006 ± 0.00006

TABLE 3. ANALYSES OF AMERICAN HOECHST HB-100 RED PHOSPHORUS

Characteristic	Percent by Weight	Mean ± S.D.
Total Phosphorus	97.4 101.0 94.4	97.6 ± 3.30
Particle Size:		
Retained on No. 60 Sieve	2.97 2.73 4.84	3.51 ± 1.16
Retained on No. 80 Sieve	4.28 4.32 6.67	5.09 ± 1.37
Acidity to Mixed Indicator (as H_3PO_4)	0.00 <0.01 0.00	<0.01 ± 0.006
Acidity to Phenolphthalein (as H_3PO_4)	0.04 0.04 0.04	0.04 ± 0.00
Alkalinity to Mixed Indicator (as Na_2CO_3)	0.17 0.18 0.15	0.17 ± 0.015
Volatile Matter	0.052 0.071 0.033	0.052 ± 0.019
Oil Content	2.83 2.03 1.99	2.28 ± 0.47
Iron Content	0.07 0.08 0.07	0.07 ± 0.006
Water-soluble Material	2.96 2.88 3.55	3.13 ± 0.36
White Phosphorus	0.0001 0.0001 0.0001	0.0001 ± 0.00000

TABLE 4. ANALYSES OF ALBRIGHT AND WILSON, LTD. LOT LT-22 RED PHOSPHORUS

Characteristic	Percent by Weight	Mean ± S.D.
Total Phosphorus	93.4 95.8 94.6	94.6 ± 1.20
Particle Size:		
Retained on No. 60 Sieve	4.75 4.51 3.95	4.40 ± 0.41
Retained on No. 80 Sieve	6.16 7.12 7.85	7.04 ± 0.85
Acidity to Mixed Indicator (as H_3PO_4)	0.41 0.39 0.41	0.40 ± 0.012
Acidity to Phenolphthalein (as H_3PO_4)	0.76 0.81 0.73	0.77 ± 0.040
Alkalinity to Mixed Indicator (as Na_2CO_3)	0.00 0.00 0.00	0.00 ± 0.000
Volatile Matter	0.040 0.080 0.121	0.080 ± 0.0405
Oil Content	1.00 0.99 1.00	1.00 ± 0.01
Iron Content	0.09 0.08 0.07	0.08 ± 0.010
Water-soluble Material	4.14 4.19 4.82	4.38 ± 0.38
White Phosphorus	0.0043 0.0045 0.0078	0.0055 ± 0.0020

TABLE 5. SUMMARY OF ANALYSES OF RED PHOSPHORUS SAMPLES

Characteristic	Specification	Percent by Weight — Sample: ERCO	Hoechst: HN	Hoechst: HB-100	A + W, Ltd. Lot LT-22
Total Phosphorus	Min. 98.75	98.4	94.7	97.6	94.6
Particle Size					
Retained on No. 60 Sieve	Max. 0.0	5.4	9.3	3.5	4.4
Retained on No. 80 Sieve	Max. 1.0	5.6	12.1	5.1	7.0
Acidity to Mixed Indicator	Max. 0.05	0.29	0.0	<0.01	0.4
Acidity to Phenolphthalein	Max. 0.5	0.5	<0.01	0.04	0.77
Alkalinity to Mixed Indicator	Max. 0.1	0.0	0.3	0.17	0.0
Volatile Matter	Max. 0.5	0.08	0.06	0.05	0.08
Oil Content	1.25	(0.05)	5.5	2.3	1.00
Iron Content	Max. 0.1	0.04	0.12	0.07	0.08
Water Soluble Material	Max. 1.0	3.7	3.4	3.1	4.4
White Phosphorus	Max. <0.01	0.001	0.0006	0.0001	0.006
Aqua Regia Insoluble Material	Max. 0.3	0.29	--	--	--

solvents, concentrated HCl, concentrated HNO_3 or dilute acids. At least half of the particles had settled within 1/2 hour.

Analysis of gas samples taken directly from the reaction mixture by GC/MS showed the presence of the following volatile organic products: phosgene ($COCl_2$), tetrachloroethylene (C_2Cl_4), carbon tetrachloride (CCl_4), hexachloroethane (C_2Cl_6) and hexachloro-1,3-butadiene (C_4Cl_6). Figures 3 and 4 show the results obtained from the second and third burns.

The methylene chloride washes of the glassware in the system were analyzed by gas chromatography with a flame ionization detector. Hexachlorobenzene was the only compound found in the wash solution from the 5-liter flask and the needle valve. Hexachloroethane was present in the stainless steel sample cup and the 250-mL sampling bulb. The concentrations of both compounds were very low.

523902: 1 mL Gas from 250 mL Bulb (Fresh): -10 to 150°C

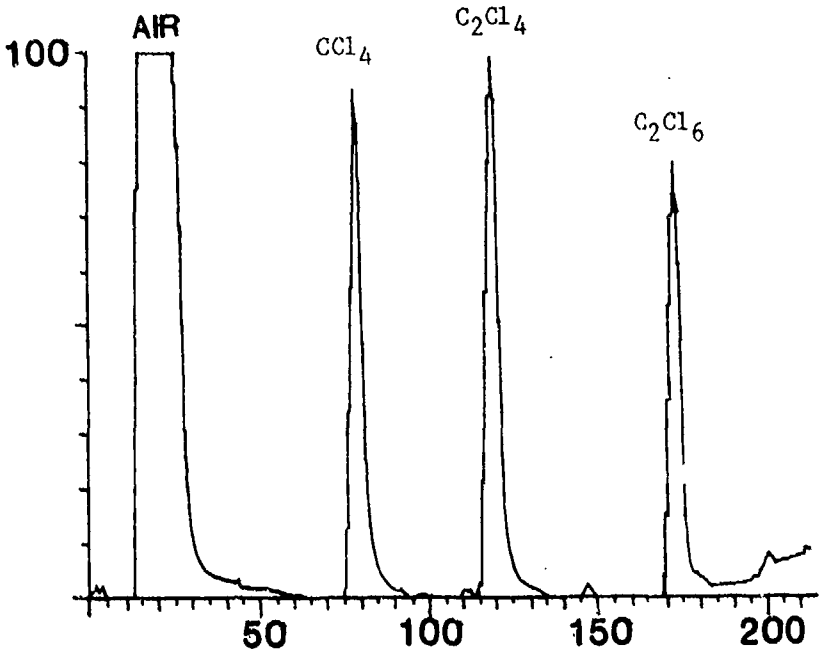

Figure 3. GC Analysis of Combustion Products from HC Mixture, Experiment 2.

524901: 1 mL Gas from 250 mL Bulb: -10 to 150°C

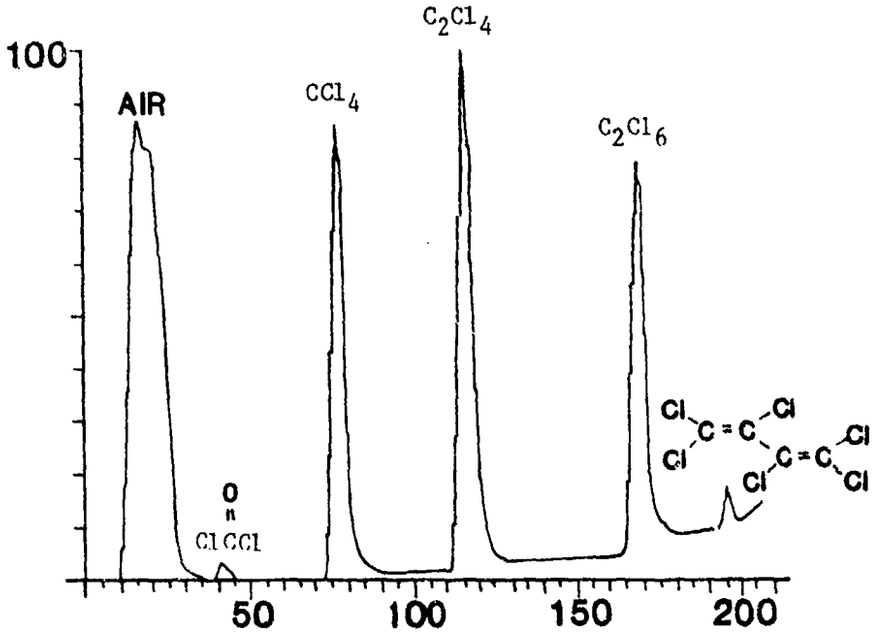

Figure 4. GC Analysis of Combustion Products from HC Mixture, Experiment 3.

RED PHOSPHORUS AEROSOLIZATION

Generation of an oiled red phosphorus aerosol for inhalation exposures could not be accomplished. An aerosol of the material could not be sustained with a fluid bed generator, Laskin aerosol system or a Wright Dust Feed mechanism. The dust feed was able to generate a low chamber concentration but jammed frequently due to the clumping of the oiled material. Two samples were taken with an Anderson 2000 Cascade Impactor to determine particle size distribution (Tables 6 and 7).

The jamming of the Wright Dust Feed was due in part to large particles bridging and blocking smaller particles. The oiled RP was sieved in a series of stainless steel NMB sieves. The majority of the material would not pass through a 100-mesh sieve which excludes particles above 150 microns. Less than 0.5 percent of the RP passed through a 400-mesh sieve with a 38-micron cutoff. These studies indicated that the oiled RP contained very few respirable particles and work on generation of inhalation chamber aerosols was terminated.

ACUTE MAMMALIAN TOXICITY

Oral Toxicity

The results of the range-finding study, employing two rats per sex per dose, suggested that oiled RP did not produce lethality at doses of 1,000, 3,160, and 6,810 mg/kg (Table 8). Rats of both sexes gained body weight during the 14-day observation period. Intubation of 10,000 mg/kg RP to five rats per sex produced lethality in one male rat. Necropsy findings were gas-filled distended intestines. Although no additional deaths were observed in these groups, the body weights of one male and one female at the end of the observation period were lower than their body weights before treatment. Another female lost body weight between days 7 and 14.

Oral administration of the high dose to an additional 10 rats per sex, did not produce as marked a toxic effect on body weight although some rats of both sex did not gain weight between days 7 and 14. This reduced body weight gain was most apparent in females. The one female which died on day 7 may have had an infection. The lungs were dark red and fluid-filled, and the rat had shown dyspnea.

Skin Irritation Study

Application of 0.5 g of oiled RP to intact and abraded skin for 24 hours did not produce signs of irritation (Table 9). The primary irritation score was 0.2 which indicates that the test material does not have irritation potential. Clinical signs indicative of systemic toxicity were not observed during the course of this study.

TABLE 6. PARTICLE SIZE DISTRIBUTION OF RED PHOSPHORUS AEROSOL (SAMPLE 1)

Stage	Weights Pre (g)	Final (g)	Net (g)	% in Size Range	Cumulative % Less than Size Range	Size Range Micrometers	ECD[a] Micrometers
0	0.38820	0.38840	0.00020	10.0	90.0	9.0 - 10.0	9.0
1	0.38530	0.38565	0.00035	17.5	72.5	5.8 - 9.0	5.8
2	0.42460	0.42485	0.00025	12.5	60.0	4.7 - 5.8	4.7
3	0.42235	0.42265	0.00030	15.0	45.0	3.3 - 4.7	3.3
4	0.42105	0.42125	0.00020	10.0	35.0	2.1 - 3.3	2.1
5	0.41855	0.41880	0.00025	12.5	22.5	1.1 - 2.1	1.1
6	0.42045	0.42060	0.00015	7.5	15.0	0.7 - 1.1	0.7
7	0.42410	0.42415	0.00005	2.5	12.5	0.4 - 0.7	0.4
Backup Filter	0.42185	0.42210	0.00025	12.5	0	0 - 0.4	0
Total			0.00200				

a. Effective Cutoff Diameter.

TABLE 7. PARTICLE SIZE DISTRIBUTION OF RED PHOSPHORUS AEROSOL (SAMPLE 2)

Stage	Weights Pre (g)	Final (g)	Net (g)	% in Size Range	Cumulative % Less than Size Range	Size Range Micrometers	ECD[a] Micrometers
0	0.38725	0.38732	0.00007	2.7	97.3	9.0 - 10.0	9.0
1	0.38737	0.38748	0.00011	4.2	93.1	5.8 - 9.0	5.8
2	0.40212	0.40289	0.00077	29.5	63.6	4.7 - 5.8	4.7
3	0.39941	0.39976	0.00035	13.4	50.2	3.3 - 4.7	3.3
4	0.39694	0.39702	0.00008	3.1	47.1	2.1 - 3.3	2.1
5	0.40504	0.40596	0.00092	35.2	11.9	1.1 - 2.1	1.1
6	0.39972	0.39985	0.00013	5.0	6.9	0.7 - 1.1	0.7
7	0.40088	0.40100	0.00012	4.6	2.3	0.4 - 0.7	0.4
Backup Filter	0.40600	0.40606	0.00006	2.3	0	0 - 0.4	0
Total			0.00261				

a. Effective cutoff diameter.

TABLE 8. ACUTE ORAL TOXICITY OF OILED RED PHOSPHORUS IN F344 RATS

Dose (mg/kg)	Mean Body Weight (g) Days after Treatment			Deaths Days after Treatment					Total Mortality Deaths/Treated
	0	7	14	0	1	2-6	7	8-14	
Males									
1,000	204	229	247	-	-	-	-	-	0/2
3,160	199	219	233	-	-	-	-	-	0/2
6,810	211	241	264	-	-	-	-	-	0/2
10,000	220	234	226	-	1	-	-	-	1/5
10,000	203	230	241	-	-	-	-	-	0/10
Females									
1,000	154	162	165	-	-	-	-	-	0/2
3,160	151	159	166	-	-	-	-	-	0/2
6,910	156	167	174	-	-	-	-	-	0/2
10,000	162	170	171	-	-	-	-	-	0/5
10,000	166	172	175	-	-	-	1	-	1/10

TABLE 9. SKIN IRRITATION STUDY OF RED PHOSPHORUS IN RABBITS

Rabbit Number	Erythema and Eschar Formation[a]				Edema Formation			
	Intact Skin		Abraded Skin		Intact Skin		Abraded Skin	
	24 hr	72 hr	24 hr	72 hr	24 hr	72 hr	24 hr	72 hr
23540	0	0	1	0	0	0	1	0
23550	0	0	1	0	0	0	1	0
23560	0	0	1	0	0	0	0	0
23570	0	0	1	0	0	0	0	0
23580	0	0	1	0	0	0	0	0
23590	0	0	1	0	0	0	0	0
Mean	0	0	1	0	0	0	0.1	0

a. Scoring methods given in Appendix B.

Eye Irritation Study

The instillation of 100 mg of oiled red phosphorus into rabbit eyes did not produce any irritation or injury to the cornea or iris. The conjunctivae of all rabbits showed a slight redness 1 hour after treatment (Table 10). In one rabbit, a slight redness was also apparent at 24 hours. Five of six rabbits treated with the test material showed slight swelling at 1 hour post-treatment which was not present at 24-hours posttreatment. From these results it was concluded that oiled RP was neither corrosive or irritating to the eye.

TABLE 10. CONJUNCTIVAE IRRITATION STUDY OF OILED RED PHOSPHORUS IN RABBITS

Rabbit Number	Redness					Chemosis				
	Hours				Day	Hours				Day
	1	24	48	72	7	1	24	48	72	7
2360D	<1	0	0	0	0	1	0	0	0	0
2361D	<1	0	0	0	0	1	0	0	0	0
2362D	<1	<1	0	0	0	1	0	0	0	0
2363D	<1	0	0	0	0	1	0	0	0	0
2364D	<1	0	0	0	0	0	0	0	0	0
2365D	<1	0	0	0	0	1	0	0	0	0

Skin Sensitization Study

Observation of the injection sites after intradermal injection of oiled RP into guinea pigs' skin revealed hard, slightly red areas after two doses. These areas became larger after each dose (Appendix C). Slight hair loss occurred after five doses. The reactions to the challenge dose were ambiguous. Of the eight guinea pigs, three reacted to the challenge dose with a greater response than seen on sensitization, three reacted with a lesser response, and two showed the same response. In contrast, the positive control guinea pigs' reactions during sensitization were encrustment after four to five doses. Within 24 hours after the challenge doses, the injection sites were hard, red, and crusty.

Because the guinea pigs treated intradermally with oiled RP may have shown an irritation response to particles in suspension, a patch test was used; the test material was applied to the skin. The skin and surrounding hair were stained the same red shade as RP which was not the same color as erythema. There was no response to the challenge dose of RP.

APPENDIX A

CHEMICAL PROCEDURES FOR RED PHOSPHORUS ANALYSES

TOTAL PHOSPHORUS CONTENT

Principle:

A weighed amount of red phosphorus (RP) is solubilized in aqua regia and diluted to a suitable volume with water. An aliquot of this solution is oxidized with ammonium persulfate and sulfuric acid to convert all forms of phosphorus to orthophosphate. After reaction with molybdovanadate reagent, the amount of phosphate is determined spectrophotometrically.

Reagents:

Aqua regia
Prepare by mixing carefully 18 parts concentrated nitric acid and 82 parts concentrated hydrochloric acid, both reagent grade
Ammonium persulfate, reagent grade
Dihydrogen potassium phosphate, primary standard
Deionized water
Perchloric acid, 70-72%, reagent grade
Ammonium molybdate solution
Dissolve by heating 10 gm ammonium molybdate tetrahydrate in 100 mL water
Ammonium metavanadate solution
Dissolve by heating 0.5 gm ammonium metavanadate in 65 mL water. Cool and add 50 mL perchloric acid
Molybdovanadate reagent
Carefully mix the ammonium molybdate and ammonium vanadate solutions Dilute to 500 mL with water
Concentrated sulfuric acid, reagent grade

Materials and Apparatus:

Analytical balance accurate to 0.01 mg
Rubber spatula
125 mL Erlenmeyer flasks
Hot plate
Assorted laboratory glassware, including volumetric pipettes and flasks
Double-beam spectrophotometer capable of reading in the 360 to 700 mm range
Spectrophotometric cuvettes, 1.0 cm pathlength

Preparation of Standard Solutions:

Dry approximately 1 g of dihydrogen potassium phosphate 105°C for 1 hr
Prepare a stock standard solution by weighing, to the nearest 0.01 mg, 100 mg of the compound and transfer to a 500 mL volumetric flask. Dissolve in water and dilute to the mark. Based on a

phosphorus content of 22.76%, determine the concentration, which should be approximately 50 ppm phosphorus.

Prepare a series of calibration standards by pipetting 0.0, 1.0, 2.0, 6.0, and 12.0 mL of the stock standard into 50 mL volumetric flasks. These are equivalent to 0, 50, 100, 300, and 600 µg phosphorus

To each flask, add 2 mL perchloric aid

Preparation of Samples:

Weigh, in triplicate, 25 mg RP to the nearest 0.01 mg and transfer to a 125 mL Erlenmeyer flask

Add 5 mL aqua regia; heat gently on hot plate to initiate dissolution

After dissolution is completed, transfer quantitatively with water to a 500 mL volumetric flask. Dilute to mark and mix well.

Prepare a reagent blank by diluting 5 mL aqua regia to 500 mL with water

Transfer a 5.0 mL aliquot from each flask into a 125 mL Erlenmeyer flask and bring up to approximately 50 mL with water. Add 0.5 mL H_2SO_4 and 0.4 g ammonium persulfate.

Gently boil the mixture on a hot plate for approximately 40 min; do not allow to go to dryness.

Cool and quantitatively transfer with water to a 50 mL volumetric flask.

Add 1.5 mL perchloric acid to each flask

To samples, blank and standards, add 10 mL of molybdovanadate reagent.

Spectrophotometric Analysis:

Within 5 min, dilute to the mark (50 mL) with water and mix well. Let stand 15 min.

Determine the absorbance of the solutions in 1.0 cm cuvettes at 400 nm, using the 0.0 ppm standard in the reference cell.

Construct a standard curve, plotting absorbance of each standard versus µg phosphorus.

Correct the sample response for the response of the reagent blank. Determine the µg phosphorus in the sample solution from the standard curve.

Calculation:

$$\% \text{ phosphorus in RP} = \frac{P \times 10}{W}$$

where P = µg phosphorus in sample solution
W = Weight of sample in mg

PARTICLE SIZE

Principle:

A weighed portion of red phosphorus (RP) is placed in a sieve and tapped until no more material passes through. The RP remaining in the sieve is then weighed.

Materials and Apparatus:

Analytical balance sensitive to 1 mg
Rubber spatula
Sieves: Number 60 (250 micron)
Number 80 (180 micron)

Procedure:

Weigh a 10 g sample of RP to the nearest 1 mg and transfer to the sieve.
Tap the sieve until no more RP passes through. Brush the bottom of the sieve to remove any adhering particles.
Transfer the RP in the sieve to a tared weighing paper and weigh to the nearest 1 mg.

Calculation:

$$\text{Percent material retained} = \frac{R \times 100}{W}$$

where R = amount of RP retained by sieve in g
W = weight of sample in g.

ACIDITY TO MIXED INDICATOR

Principle:

A weighed sample of red phosphorus (RP) is extracted with ethanol-water (20% v/v) and filtered. The filtrate is titrated with standardized sodium hydroxide to the end point of the mixed indicator.

Reagents:

Deionized water
Ethanol, denatured, reagent grade
0.1% methyl orange
Dissolve 25 mg methyl orange in 25 mL water
0.04% bromocresol green
Dissolve 10 mg of bromocresol green (sodium salt) in 0.7 mL 0.01N NaOH and dilute to 25 mL with water.
Mixed indicator
Combine methyl orange and bromocresol green solutions prepared as above.
0.05N NaOH
Dissolve 2.15 g reagent grade NaOH (carbonate-free) in 1000 mL of water.
0.085N H_3PO_4 certified standard (Harleco)

Materials and Apparatus:

Analytical balance sensitive to 0.01 mg
Rubber spatula
250 mL Erlenmeyer flasks

Whatman #5 filter paper
Buchner funnels
500 mL vacuum flasks
10.0 mL burette graduated to 0.05 mL
Magnetic stirrer
Assorted volumetric pipettes and flasks

Titration Procedure:

Add four drops of mixed indicator.
Place on magnetic stirrer and titrate with standardized NaOH solution to a green end point free of any yellow color.
Record volume of NaOH solution used.

Analysis of reagent blanks:

Prepare in triplicate 250 mL Erlenmeyer flasks each containing 5 mL ethanol and 75 mL water.
Titrate as described above and calculate the mean volume of NaOH solution used

Standardization of NaOH solution:

Prepare in triplicate 250 mL Erlenmeyer flasks each containing 5 mL 0.085 H_3PO_4 and 70 mL water.
Titrate as described above, correcting the volume of NaOH used with the mean reagent blank titration volume.
Calculate normality of NaOH solution from the following formula:

$$N = \frac{0.085 \times 5}{V}$$

where V = corrected volume in mL of NaOH solution used.
Calculate the mean normality of the NaOH solution.

Analysis of samples:

Weigh, in triplicate, 10 g of RP to the nearest mg and transfer to 250 mL Erlenmeyer flasks.
Add 5 mL ethanol and 25 mL water.
Mix well and filter through Whatman #5 paper with the aid of vacuum.
Rinse the flask twice with 25 mL portions of water, passing each rinse through the same filter.
Transfer the filtrate to a 250 mL Erlenmeyer flask and titrate as described above.
Correct the volume of NaOH solution used with the mean reagent blank titration volume.
Calculate

$$\% \text{ acidity } (H_3PO_4) = \frac{A \times N \times 9.8}{W}$$

where A = corrected volume of NaOH solution used in mL
N = mean normality of NaOH solution
W = weight of sample in g.

ACIDITY TO PHENOLPHTHALEIN

Principle:

A weighed sample of red phosphorus (RP) is extracted with ethanol-water (20% v/v) and filtered. The filtrate is titrated with standardized sodium hydroxide to the end point of phenolphthalein.

Reagents:

Deionized water
Ethanol, denatured, reagent grade
1% phenolphthalein
  Dissolve 0.25 g phenolphthalein in 15 mL ethanol and dilute to 25 mL with water.
  0.05N NaOH
  Dissolve 2.15 g reagent grade NaOH (carbonate-free) in 1000 mL of water.
  0.085 N H_3PO_4 certified standard (Harleco)

Materials and Apparatus:

Analytical balance sensitive to 0.01 mg
Rubber spatula
250 mL Erlenmeyer flasks
Whatman #5 filter paper
Buchner funnels
500 mL vacuum flasks 10.0 mL burette graduated to 0.05 mL
Magnetic stirrer
Assorted volumetric pipettes and flasks

Titration Procedure:

Add 5 drops of phenolphthalein indicator.
Place on magnetic stirrer and titrate with standardized NaOH solution to the first color change (pink).
Record volume of NaOH solution used.

Analysis of reagent blanks:

Prepare in triplicate 250 mL Erlenmeyer flasks each containing 5 mL ethanol and 75 mL water.
Titrate as described above and calculate the mean volume of NaOH solution used.

Standardization of NaOH solution:

Prepare in triplicate 250 mL Erlenmeyer flasks each containing 5 mL ethanol, 5.0 mL 0.085 H_3PO_4 and 70 mL water.
Titrate as described above, correcting the volume of NaOH used with the mean reagent blank titration volume.
Calculate normality of NaOH solution from the following formula:

$$N = \frac{0.085 \times 5}{V}$$

where V = corrected volume in mL of NaOH solution used.
Calculate the mean normality of the NaOH solution.

Analysis of RP samples:

Weigh, in triplicate, 10 g of RP to the nearest mg and transfer to 250 mL Erlenmeyer flasks.
Add 5 mL ethanol and 25 mL water.
Mix well and filter through Whatman #5 paper with the aid of vacuum.
Rinse the flask twice with 25 mL portions of water, passing each rinse through the same filter.
Transfer the filtrate to a 250 mL Erlenmeyer flask and titrate as described above.
Correct the volume of NaOH solution used with the mean reagent blank titration volume.

Calculate

$$\% \text{ acidity } (H_3PO4) = \frac{A \times N \times 9.8}{W}$$

where A = corrected volume of NaOH solution used in mL
N = mean normality of NaOH solution
W = weight of sample in g

ALKALINITY TO MIXED INDICATOR

Principle:
A weighed sample of red phosphorus (RP) is extracted with ethanol-water (20% v/v) and filtered. The filtrate is titrated with standardized hydrochloric acid to the end point of the mixed indicator.

Reagents:

Deionized water
Ethanol, denatured, reagent grade
0.1% methyl orange
Dissolve 25 mg methyl orange in 25 mL water
0.04% bromocresol green
Dissolve 10 mg of bromocresol green (sodium salt) in 0.7 mL 0.02N NaOH and dilute to 25 mL with water
Mixed indicator
Combine methyl orange and bromocresol green solutions prepared above
0.1N HCl
Mix 8.5 mL concentrated HCl in water and dilute to 1000 mL
0.5N Na_2CO_3
Weigh to the nearest 0.01 mg, 3 g of $Na_2CO_3.HgO$ and transfer to a 100 mL volumetric flask. Dissolve in water and dilute to the mark.
Calculate the normality of the solution as follows:

$$N = \frac{W}{6.2}$$

where W = weight of $Na_2CO_3.HgO$ in g

Materials and Apparatus:

Analytical balance sensitive to 0.01 mg
Rubber spatula
250 mL Erlenmeyer flask
Whatman #5 filter paper
Buchner funnels
500 mL vacuum flasks
10.0 mL burette graduated to 0.05 mL
Magnetic stirrer
Assorted volumetric pipettes and flasks

Titration Procedures:

Add four drops of mixed indicator.
Place on magnetic stirrer and titrate with standardized HCl solution to a yellow end point free of green color.
Record volume of NaOH solution used.

Analysis of reagent blanks:

Prepare in triplicate 250 mL Erlenmeyer flasks each containing 5 mL ethanol and 75 mL water.
Titrate as described above and calculate the mean volume of HCl solution used.

Standardization of HCl solution:

Prepare in triplicate 250 mL Erlenmeyer flasks each containing 5 mL ethanol, 1.0 mL Na_2CO_3 solution and 74 mL water.
Titrate as described above, correcting the volume of HCl used with the mean reagent blank titration volume.
Calculate the normality of the HCl solution from the following formula:

$$N = \frac{N^1 \times 1.0}{V}$$

where N^1 = normality of $Na_2CO_3.H_2O$
V = corrected volume in mL of HCl solution used.
Calculate the mean normality of the HCl solution.

Analysis:

Weigh, in triplicate, 10 g of RP to the nearest mg and transfer to 250 mL Erlenmeyer flasks.
Add 5 mL ethanol and 25 mL water.
Mix well and filter through Whatman #5 paper with the aid of vacuum.
Rinse the flask twice with 25 mL portions of water, passing each rinse through the same filter.

Transfer the filtrate to a 250 mL Erlenmeyer flask and titrate as described above.
Correct the volume of HCl solution used with the mean reagent blank titration volume.
Calculate the alkalinity of the sample as follows:

$$\% \text{ alkalinity } (Na_2CO_3) = \frac{A \times N \times 10.6}{W}$$

where A = corrected volume of HCl solution used in mL
N = mean normality of HCl solution
W = Weight of sample in mg

VOLATILE MATTER

Principle:

A weighed sample of RP is kept in a vacuum dessicator for 24 hr. The loss in weight is determined gravimetrically.

Materials and Apparatus:

Analytical balance sensitive to 1 mg
Rubber spatula
Porcelain crucibles with covers
Drying ovens
Vacuum dessicator containing activated silica gel
Drying trap containing activated silica gel

Procedure:

Heat covered crucibles in drying oven for 30 min at 105°C.
Place in vacuum dessicator for 30 min and weigh to nearest 1 mg.
Immediately place 5 g RP in crucible, cover and weigh to nearest 1 mg (perform triplicate determinations).
Place sample, with cover removed, in vacuum dessicator, maintaining vacuum for 24 hr.
Release vacuum through drying trip, cover samples and reweigh.

Calculation:

$$\text{Percent volatile matter} = \frac{L \times 100}{W}$$

where L = loss in weight in g
W = sample weight in g

OIL CONTENT

Principle:

A weighed sample of RP is extracted by stirring with carbon tetrachloride.
The RP is removed by filtration and the solvent in the filtrate removed by evaporation. The oil residue is determined gravimetrically.

Reagents:

Carbon tetrachloride, reagent grade

Materials and Apparatus:

Analytical balance sensitive to 1 mg
Rubber spatula
100 mL beakers
50 mL graduated cylinder
Magnetic stirrers
Buchner funnels
500 mL vacuum flasks
Whatman #5 filter paper
Aluminum evaporating dishes
Vacuum dessicator containing suitable dessicant
Steam bath

Procedure

Weigh, in triplicate, 5 g portions of RP to the nearest mg.
Transfer to 100 mL beaker and add 50 mL carbon tetrachloride.
Stir for 30 min.
Filter through two thicknesses of Whatman #5 filter paper with the aid of vacuum.
Rinse beaker with 50 mL carbon tetrachloride and pass through same filter.
Decant filtrate into 100 mL beaker and evaporate on steam bath to approximately 3 mL.
Transfer quantitatively with rinses of carbon tetrachloride to tared aluminum evaporating dish.
Allow to air dry without heating until no odor of solvent remains.
Place in vacuum dessicator for 30 min to remove residual moisture and weigh to nearest mg.

Calculation:

$$\text{Percent oil content} = \frac{R \times 100}{W}$$

where R = Weight of oil residue in g
W = Weight of sample in g

IRON CONTENT

Principle:

A weighed portion of red phosphorus (RP) is dissolved in a minimum volume of aqua regia and the solution diluted to a suitable volume with water. The iron content is determined by atomic absorption spectophotometry.

Reagents:

Aqua regia
Preparing by mixing carefully 18 parts concentrated nitric acid with 82 parts concentrated hydrochloric acid (both reagent grade).
Deionized water
Stock standard iron solution certified for atomic absorption spectrophotometry (1000 ppm).

Materials and Apparatus:

Analytical balance accurate to 0.01 mg
Rubber spatula
50 mL graduated centrifuge tubes
Hot water bath
Assorted volumetric pipettes and flasks
Atomic absorption spectrophotometer

Preparation of Standard Solutions:

Dilute stock iron standard 1 to 100 with water to give a concentration of 10.0 ppm.
Dilute the 10.0 ppm standard with water as follows:

1 to 10 = 1.0 ppm
1 to 4 = 2.5 ppm
1 to 2 = 5.0 ppm

Procedure:

Weigh, in triplicate, 100 mg of RP to the nearest 0.01 mg and transfer to 50 mL graduated centrifuge tubes.
Add 4 mL aqua regia and heat gently in hot water bath to initiate dissolution.
After the initial reaction has ceased, heat the water bath to boiling to complete dissolution. (There will always be a small amount of insoluble material present.)
Cool and dilute carefully with water to 20.0 mL.
Prepare a reagent blank by diluting 4 mL of aqua regia to 20.0 mL with water.
Optimize the instrumental parameters of the spectrophotometer with the 10.0 ppm iron standard, and set the photometric response to zero with water.
Analyze all four iron standards, 1.0 to 10.0 ppm, to obtain a standard curve.
Correct the sample response for the response of the reagent blank.
Calculate the iron content of the samples in ppm from the standard curve.

Calculations:

$$\text{Percent Iron} = \frac{C \times 2}{W}$$

Where C = concentration (ppm) of iron in sample solution
W = weight of sample in mg

WATER-SOLUBLE MATERIAL

Principle:

A weighed amount of red phosphorus (RP) is extracted with water and filtered through a tared filter paper. The paper and its contents are dried and weighed to determine the amount of water-soluble material.

Materials and Apparatus:

Analytical balance accurate to 1 mg
Rubber spatula
125 mL Erlenmeyer flasks
50 mL graduated cylinder
Magnetic stirrer
Whatman #5 filter paper
S & S #123 filter supports
Filter funnels
Vacuum flasks
Vacuum dessicator containing suitable dessicant

Procedure:

Weigh, in triplicate, 1 g of RP to the nearest mg and transfer to a 125 mL Erlenmeyer flask.
Add 50 mL deionized water and mix on a magnetic stirrer for 30 min.
Filter through a tared (to the nearest mg) Whatman #5 paper supported by an S & S #123 filter support.
Rinse the flask several times with water and filter the washings through the same paper.
Remove the filter with its contents and allow to air dry. Place in vacuum dessicator overnight.
Weigh the dried filter and residue to the nearest mg.

Calculation:

$$\text{Percent water-soluble material} = \frac{L \times 100}{W}$$

where L = loss in weight after extraction (in g)
W = weight of sample analyzed in g

WHITE PHOSPHORUS CONTENT

Principle:

The methods for white phosphorus (WP) analyses are based on procedures in the following references:

Ackman, R.G. and Addison, R.F. Direct Determination of Elemental Phosphorus by Gas-Liquid Chromatography. Proc. Conf. Pollution, Chem. Inst. Canada, St. Mary's Univ., Halifax, N.S., Aug 24-26, 1969, pp. 140-147.

Ackman, R.G. and Addison, R.F. 1970. Direct Determination of Elemental Phosphorus by Gas-Chromatography. J. Chromatog. 47:421.

Dillon, H. Kenneth, et al. 1978. Solid Sorbent Sampler for White Phosphorus in Air. Am. Ind. Hyg. Assoc. J. 39:608.

A weighed amount of RP is extracted with benzene. The extract is clarified and analyzed for WP by gas-liquid chromatography, using an electron capture detector.

Reagents:

Benzene, nanograde distilled in glass
White phosphorus

Materials and Apparatus:

Analytical balance accurate to 0.01 mg
Rubber spatula
Cadmium-plated forceps
15 mL glass-stoppered graduated centrifuge tubes
Gas chromatograph equipped with ^{63}Ni electron capture detection system and 1.0 mV recorder
Gas chromatographic column, 1.8 x 2 mm ID glass, packed with 3% OV-1 on 80/100 mesh Supercoport
10.0 microliter syringe
Assorted volumetric pipettes and flasks

Preparation of Standard Solutions:

Weigh to the nearest 0.1 mg a 15 mL graduated centrifuge tube containing 2.0 mL of benzene.
Add, using cadmium-plated forceps, approximately 100 mg WP; mix to dissolve.
Adjust the volume of any water carried over with the WP to 1.0 mL with deionized water and weigh the tube and its contents to the nearest 0.1 mg.
Subtract 1.0000 g to correct for the weight of the water, then subtract the weight of the tube and the benzene to obtain the weight of the WP.

Adjust the volume of the benzene phase with additional benzene to 10.0 mL; the WP concentration is approximately 10,000 ppm. Mix well before making subsequent dilutions.
Dilute serially with benzene to a final concentration of about 2 ppm.

Procedure:

Weigh, in triplicate, 2 g of RP to the nearest mg and transfer to a 15 mL glass centrifuge tube containing 10.0 mL benzene.
Stopper and vortex intermittently over a 1-hr period.
Allow the RP to settle out.
Establish a standard curve by injecting 0.5, 1.0, 2.0, and 3.0 mL of the 2 ppm WP standard solution into the chromatograph. The following parameters are suggested.

Column temperature:	75°
Injection temperature	275°C
Detector temperature:	340°C
Nitrogen (carrier) flow rates:	30 mL/min (column)
	40 mL/min (detector)

Analyze the sample extract and determine the amount of any WP present by comparison of the sample response to the standard curve.

Calculation:

$$\text{Percent WP} = \frac{P}{V \times W \times 1000}$$

where P = amount of WP found in ng
V = volume of extract injected in mL
W = weight of sample in g

AQUA REGIA - INSOLUBLE MATTER

Principle:

A weighed amount of red phosphorus (RP) is added in small portions to aqua regia, heating gently to aid solution. After all the sample is added, the solution is heated to boiling. The solution is cooled and filtered with the aid of vacuum through previously tared glass fiber filters. The insoluble matter is determined gravimetrically after drying in a dessicator.

Reagents:

Aqua regia
Prepare by carefully mixing 18 parts of concentrated nitric aid and 82 parts of concentrated hydrochloric acid, both reagent grade.
Deionized water

Materials and Apparatus:

Analytical balance sensitive to 0.01 mg
Rubber spatula

600 mL beakers
Hot plate
Glass stirring rods
500 mL graduated cylinder
Buchner funnels
1000 mL vacuum flasks
Whatman GF/A glass fiber filter paper
Vacuum dessicator containing suitable dessicant

Procedure:

Weigh, in triplicate, 10 g of RP to the nearest mg.
Add in small (ca. 500 mg) portions to 300 mL aqua regia in a 600 mL beaker.
After each addition, heat gently with occasional stirring to solubilize. Take care that excessive foaming does not occur.
When all the sample has been added, heat to boiling. Cool and filter, with aid of vacuum, through glass-fiber filter which has been tared previously to the nearest 0.01 mg.
Rinse the beaker with several small portions of water; pass the rinser through the same filter.
Allow the filter and residue to air dry and place in vacuum dessicator overnight.
Weigh the filter and residue to the nearest 0.01 mg and determine the weight of residue obtained.

Calculation:

$$\text{Percent matter insoluble in aqua regia} = \frac{R}{W \times 10}$$

where R = weight of insoluble residue in mg
W = weight of sample in g

APPENDIX B

TEST METHODS FOR SKIN AND EYE IRRITATION

TEST METHODS

Primary Skin Irritants

This method is essentially that recommended by the FDA to comply with the intent of the Federal Hazardous Substances Act. It can be found in the Federal Register, 16 CFR 1500.41 91 26,141.

Six albino rabbits are used for each test compound. Before applying the material, the hair is clipped from the back in an area large enough to accommodate two 1 inch x 1 inch gauze pads without overlapping. On each animal, one of the two sites is abraded lightly, sufficient to cause minor incisions in the stratum corneum, but not deep enough to disturb the derma or to produce bleeding. Material is introduced under the gauze patch and the patch pressed down firmly to assure uniform contact with the skin. The trunks of the animals are then securely wrapped with an impervious material (plastic wrap) and the animals are placed in restraining collars.

Twenty-four hours later the patches are removed and the resulting reactions in both intact and abraded skin are evaluated on the basis of the values in the table. The results from the six animals are combined to give an average value. The observation and grading of responses is repeated after 72 hours. To obtain the primary irritation score, the average values at 24 and 72 hours are totaled and divided by four. If the score is five or greater the material is classified as an irritant and must be appropriately labeled.

Eye Irritants

This method is essentially that recommended by the FDA to comply with the intent of The Federal Hazardous Substances Act. It can be found in The Federal Register, 16 CFR 1500.42 91 26,142.

Six albino rabbits are used for each test substance. Both eyes of each animal in the test group are examined before testing, and only those animals without eye defects or irritation are used. The animal is held firmly but gently until quiet. The test material is placed in one eye of each animal by gently pulling the lower lid away from the eyeball to form a cup into which the test substance is dropped. The lids are gently held together for one second and the animal is released. The other eye, remaining untreated, serves as a control. The eyes are not washed following instillation of test material. The eyes are examined and the grade of ocular reaction is recorded at 1, 24, 48, and 72 hours.

An animal is considered as exhibiting a positive reaction if the test substance produces at any of the readings ulceration of the cornea, opacity of the cornea, inflammation of the iris, or obvious swelling of the conjunctivae with partial evasion of the lids or a diffuse

crimson-red with individual vessels not easily discernible. The reactions are graded acording to the table.

The test is considered positive if four or more of the animals in the test group exhibit a positive reaction. If only one animal exhibits a positive reaction the test is regarded as negative. If two or three animals exhibit a positive reaction, the test is repeated using a different group of six animals. The second test is considered positive if three or more of the animals exhibit a positive reaction. If only one or two animals in the second test exhibit a positive reaction, the test is repeated with a different group of six animals. Should a third test be needed, the substance is regarded as an irritant if any animal exhibits a positive response.

EVALUATION OF SKIN REACTIONS

Erythema and Eschar Formation	Value	Edema Formation	Value
No erythema	0	No edema	0
Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)	1
Well defined erythema	2	Slight edema (edges of area well defined by definite raising)	2
Moderate to severe erythema	3	Moderate edema (raised approximately 1 millimeter)	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4	Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4

EVALUATION OF EYE REACTIONS

Cornea (before staining)	
No ulceration or opacity	0
Scattered or diffuse areas (other than a slight dulling of normal luster), details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Nacreous areas, no details of iris visible, size of pupil barely discernible	3
Complete corneal opacity, iris not discernible	4
Cornea (after staining)	
None	0
Very slight, few areas of necrosis	1
Slight, up to 50% of eye covered with shallow necrosis	2
Moderate, 50 - 100% of eye necrotic but lesions are shallow	3
Severe, marked necrosis over whole area which may result in loss of eye	4

Iris	
Normal	0
Markedly deepened folds, congestion, swelling, moderate circumcorneal injection (any 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
Conjunctiva-Redness	
Vessels normal	0
Some vessels definitely injected	1
Diffuse, crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3
Conjunctiva-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 more than half closed	4

APPENDIX C

SKIN SENSITIZATION STUDY IN GUINEA PIGS OF OILED RED PHOSPHORUS, INTRADERMAL TREATMENT

Animal Number: 3764D Positive Control

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0	0	0
	4	0	0	0
	6	1.25	0.25	Slight. Red
	8	0.75	0	Slight. Red
	11	1.0	0.1	Slight. Red
	13	1.1	0.1	Mod. Red
	15	0.7	0.1	Mod. Red
	18	1.4	0.1	Mod. Red
	20	1.6	0.1	Slight. Red
	22	1.8	0.2	Slight. Red
Challenge Dose				
0.1	36	0.5	0	Red
	37	0.5	0	Red

Animal Number: 3765D Positive Control

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0	0	0
	4	0	0	0
	6	1.25	0.25	Mod. Red
	8	1.1	0.35	Slight. Red
	11	0.6	0.1	Slight. Red
	13	1.4	0.2	Mod. Red
	15	0.5	0.1	Slight. Red
	18	0.6	0.1	Slight. Red
	20	1.3	0.1	Mod. Red
	22	1.7	0.15	Slight. Red
Challenge Dose				
0.1	36	0.8	0.15	Mod. Red
	37	0.7	0.15	Mod. Red

Animal Number: 3766D Positive Control

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0	0	0
	4	0	0	0
	6	1.0	0.25	Slight. Red
	8	0.8	0.3	Slight. Red
	11	1.6	0.1	Slight. Red
	13	1.1	0.15	Slight. Red
	15	0.7	0.1	Slight. Red
	18	0.9	0.1	Slight. Red
	20	1.3	0.15	Slight. Red
	22	1.4	0.1	Slight. Red
Challenge Dose				
0.1	36	0.8	0.15	Slight. Red
	37	0.75	0.15	Slight. Red

Animal Number: 3767D Positive Control

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0	0	0
	4	0	0	0
	6	1.25	0.25	Slight. Red
	8	1.45	0.25	Slight. Red
	11	1.7	0.2	Slight. Red
	13	1.4	0.2	Red
	15	1.3	0.15	Red
	18	1.2	0.1	Slight. Red
	20	1.7	0.1	Red
	22	1.9	0.15	Mod. Red
Challenge Dose				
0.1	36	0.8	0.1	Red
	37	0.8	0.1	Red

Animal Number: 3768D Red Phosphorus

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0	0	0
	4	0	0	0
	6	1.0	0.25	Slight. Red
	8	0.9	0.1	Slight. Red
	11	0.7	0.1	Slight. Red
	13	0.75	0.15	Slight. Red
	15	0.9	0.15	Slight. Red
	18	1.5	0.1	Slight. Red
	20	1.4	0.1	Slight. Red
	22	1.5	0.1	Slight. Red
Challenge Dose				
0.1	36	1.2	0.2	Slight. Red
	37	1.0	0.15	Slight. Red

Animal Number: 3769D Red Phosphorus

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0.5	0.5	0
	4	0.5	0.5	0
	6	0.75	0.25	Slight. Red
	8	0.5	0.2	Slight. Red
	11	0.8	0.1	Slight. Red
	13	1.1	0.2	Slight. Red
	15	1.0	0.1	Slight. Red
	18	1.1	0.1	Slight. Red
	20	1.5	0.1	Slight. Red
	22	1.4	0.2	Slight. Red
Challenge Dose				
0.1	36	1.2	0.2	Slight. Red
	37	1.1	0.1	Slight. Red

Animal Number: 3770D Red Phosphorus

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0.5	0.5	0
	4	0.5	0.5	0
	6	0.75	0.25	Slight. Red
	8	1.0	0.1	Slight. Red
	11	0.9	0.1	Slight. Red
	13	1.1	0.2	Slight. Red
	15	0.8	0.1	Slight. Red
	18	1.0	0.1	Slight. Red
	20	1.3	0.15	Mod. Red
	22	1.5	0.1	Slight. Red
Challenge Dose				
0.1	36	1.0	0.15	Slight. Red
	37	1.0	0.1	Slight. Red

Animal Number: 3771D Red Phosphorus

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0.5	0.5	0
	4	0.5	0.5	0
	6	0.75	0.25	Slight. Red
	8	1.7	0.25	Slight. Red
	11	0.9	0.1	Slight. Red
	13	0.8	0.2	Slight. Red
	15	0.8	0.1	Slight. Red
	18	0.7	0.1	Slight. Red
	20	1.6	0.15	Slight. Red
	22	1.8	0.1	Slight. Red
Challenge Dose				
0.1	36	0.8	0.1	Slight. Red
	37	0.8	0.1	Slight. Red

Animal Number: 3772D Red Phosphorus

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0	0	0
	4	0.5	0.5	0
	6	1.0	0.25	Slight. Red
	8	0.9	0.2	Slight. Red
	11	1.1	0.1	Slight. Red
	13	0.95	0.15	Slight. Red
	15	0.9	0.1	Slight. Red
	18	0.8	0.1	Slight. Red
	20	1.6	0.1	Mod. Red
	22	1.4	0.15	Slight. Red
Challenge Dose				
0.1	36	0.9	0.15	Slight. Red
	37	0.9	0.15	Slight. Red

Animal Number: 3773D Red Phosphorus

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0.75	0.5	0
	4	0.5	0.5	0
	6	0.75	0.25	Slight. Red
	8	1.0	0.2	Slight. Red
	11	0.6	0.1	Slight. Red
	13	1.1	0.15	Mod. Red
	15	0.8	0.1	Slight. Red
	18	1.4	0.1	Slight. Red
	20	1.5	0.1	Mod. Red
	22	1.8	0.05	Slight. Red
Challenge Dose				
0.1	36	1.1	0.2	Slight. Red
	37	1.0	0.1	Slight. Red

Animal Number: 3774D Red Phosphorus

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0.25	0.25	Slight. Red
	4	0.5	0.25	0
	6	0.75	0.25	Slight. Red
	8	1.0	0.3	Slight. Red
	11	0.6	0.1	Red
	13	1.15	0.15	Mod. Red
	15	0.9	0.2	Slight. Red
	18	1.9	0.1	Slight. Red
	20	2.1	0.1	Slight. Red
	22	2.4	0.1	Slight. Red
Challenge Dose				
0.1	36	1.1	0.2	Slight. Red
	37	1.0	0.2	Slight. Red

Animal Number: 3775D Red Phosphorus

Dose (mL)	Day	Observations		
		Diameter (cm)	Height (cm)	Color
0.1	1	0.5	0.5	Slight. Red
	4	0.5	0.5	0
	6	1.0	0.25	Slight. Red
	8	1.0	0.3	Slight. Red
	11	0.4	0.1	Slight. Red
	13	1.0	0.1	Mod. Red
	15	0.8	0.1	Slight. Red
	18	1.0	0.1	Slight. Red
	20	1.0	0.1	Mod. Red
	22	1.4	0.1	Slight. Red
Challenge Dose				
0.1	36	1.2	0.2	Slight. Red
	37	1.1	0.15	Slight. Red

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