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WATER QUALITY CRITERIA FOR WHITE PHOSPHORUS

FINAL REPORT

Kowetha A. Davidson
Patricia S. Hovatter
Catherine F. Sigmon

August 1987

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Data obtained from a review of the literature concerning the environmental fate and aquatic and mammalian toxicity of white phosphorus are presented in order to derive Water Quality Criteria for the protection of humans and aquatic organisms and their uses. Laboratory and field studies indicate that white phosphorus is quite toxic to aquatic organisms, with fish being more sensitive than macroinvertebrates. In dynamic bioassays with fishes, Lepomis macrochirus (bluegill) was the most sensitive species, with a 96-hr LC_{50} of 7.4 µg/L (nominal concentration). The most sensitive life stages for Pimephales promelas (fathead minnow) are 30-day-old and 60-day-old fry. Aquatic macroinvertebrates are more

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resistant, with 48-hr EC_{50} values (based on immobilization) ranging from 30 μg/L for Daphnia magna to > 560 μg/L for Asellus militaris. LT_{50} data for fish and invertebates indicate that the toxicity of white phosphorus is cumulative. Chronic studies with P. promelas indicate significant adverse reproductive effects at concentrations as low as 0.4 μg/L.

Bioaccumulation is rapid and extensive, with the greatest uptake in the liver and muscle of fish and the hepatopancreas of lobster; however, depuration occurs within 7 days postexposure. Other toxic effects to aquatic organisms include cardiovascular and histological changes. Field studies indicate that effluents containing white phosphorus adversely affect receiving aquatic systems by decreasing diversity and increasing mortality of select species.

Certain data required by the U.S. Environmental Protection Agency guidelines to calculate water quality criteria are unavailable; a tentative Criterion Maximum Concentration of 1.1530 μg/L was calculated from the available data. Sufficient data were not available to calculate a Criterion Continuous Concentration. However, since chronic studies show toxic effects at 0.4 μg/L, further research is strongly recommended.

Acute exposure to white phosphorus causes similar effects in laboratory animals and humans. In the absence of medical treatment, the estimated minimal lethal dose of white phosphorus in humans is 100 mg (1.4 mg/kg), whereas the acute oral LD_{50} in rats and mice ranges from 3 to 4 mg/kg, suggesting that humans are more sensitive to acute effects than laboratory animals. Following ingestion, organs damaged by white phosphorus are the gastrointestinal tract, liver, kidney, brain, and cardiovascular system. Dermal contact with white phoshphorus dissolved in oil does not cause irritation or sensitization, but dermal contact in the absence of a vehicle causes severe burns.

Chronic and subchronic exposure of laboratory animals to white phosphorus by oral or subcutaneous routes results in reduced growth, reduced survival at high doses, increased survival at low doses, and bone pathology. Humans chronically exposed to white phosphorus in the occupational environmental develop a specific lesion (different from that observed in laboratory animals) called phosphorus necrosis of the jawbone or "phossy jaw." Strict medical and dental surveillance of workers exposed to white phosphorus has led to a decreased incidence and severity of this disease through early diagnosis and treatment. Therefore, necrosis of the jawbone is no longer an occupational hazard.

There were no data on the carcinogenicity, mutagenicity, and teratogenicity of white phosphorus in humans. White phosphorus is not mutagenic, teratogenic, or carcinogenic in laboratory animals. Oral administration of 0.075 mg/kg of white phosphorus is toxic to pregnant females, causing a cluster of maternal deaths within 2 days of parturition. This study was used to calculate an interim water quality criterion for the protection of human health. The proposed criterion is 4.303 μg/L, which should protect the general population and the sensitive subpopulation, pregnant women. The proposed criterion for the protection of aquatic life, 1.1530 μg/L, should be adequate for protecting the sensitive human subpopulation.

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WATER QUALITY CRITERIA FOR WHITE PHOSPHORUS

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Chemical Effects Information Task Group
Information Research and Analysis Section
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EXECUTIVE SUMMARY

White phosphorus is a highly reactive allotrope (the P_4 tetrahedra) of elemental phosphorus. It is produced commercially by the electric arc process. The primary military use of white phosphorus is in the production of smoke munitions that are deployed to obscure movement of personnel.

The environmental release of white phosphorus and its degradation products can occur during the production of white phosphorus and during the manufacture and combustion of white phosphorus/felt munitions. Indirect release can occur via volatilization from aquatic and terrestrial systems and remobilization from sinks in anaerobic soils and aquatic sediments. The effluent from white phosphorus production and manufacturing facilities is known as "phossy water" and contains both suspended (colloidal) and dissolved white phosphorus. The adverse abiotic effects of release into aquatic systems are reduction in concentration of dissolved oxygen, reduction in pH (acidification), and increased deposition of fine particulates. White phosphorus is primarily transformed in air, water, and soil by oxidation. In water, the oxidation rate can be affected by dissolved oxygen concentration, temperature, pH, salinity, and the presence of metals. In soil, oxidation is dependent upon the amount of available oxygen.

Laboratory and field studies indicate that white phosphorus is quite toxic to aquatic organisms. In dynamic bioassays with fishes, bluegill sunfish was the most sensitive species, with a 96-hr LC_{50} of 2.4 $\mu g/L$ The most sensitive life stages for fathead minnows are 30- and 60-day-old fry. Aquatic macroinvertebrates are more resistant to white phosphorus, with 48-hr EC_{50} values (based on immobilization) ranging from 30 $\mu g/L$ for Daphnia magna to > 560 $\mu g/L$ for Asellus militaris. LT_{50} data for fish and invertebrates indicate that the toxicity of white phosphorus is cumulative.

Chronic studies with fathead minnows indicate that concentrations of 3.4 and 1.5 $\mu g/L$ for 150 days stunt growth so that internal and external evidence of sexual maturity is absent. Survival in this species is reduced at 1.5 $\mu g/L$, and at 0.4 $\mu g/L$ hatchability is significantly reduced. In critical-life-stage studies with fathead minnows, 30-day exposures to 1.5 $\mu g/L$ significantly reduced length. In chronic studies with Daphnia magna, exposure to 8.7 $\mu g/L$ significantly reduced survival.

Algal toxicity studies revealed variable results, with growth stimulated in two species of blue-green-algae and growth inhibited in a green algal species and a diatom species.

Bioaccumulation studies indicate rapid and extensive uptake in the liver and muscle of fish and in the hepatopancreas of lobster. Bioconcentration factors range from 9 to 2,000 in fishes, 10 to 1,267 in invertebrates, and 22 in seaweed. Depuration is also rapid, with white phosphorus falling to undetectable levels within 7 days postexposure.

Other toxic effects to aquatic organisms resulting from exposure to white phosphorus include cardiovascular changes (redness, hemolysis) and histological changes indicating that phosphorus acts as a potent cytotoxin by disrupting cell and nuclear membranes.

Field studies indicate that effluents containing white phosphorus adversely affect the receiving aquatic systems. Releases have altered the structure of benthic communities by decreasing diversity and by selective mortality. Mass fish mortalities have occurred following white phosphorus release.

A tentative Criterion Maximum Concentration (CMC) of 1.1530 μg/L was calculated from the available data; however, it should be stressed that certain data required by the U.S. Environmental Protection Agency (USEPA) guidelines are unavailable. Although a Criterion Continuous Concentration (CCC) could not be determined, due to gaps in the available data for calculating final chronic, plant, and residue values, chronic studies with fathead minnows indicate significant adverse reproductive effects at concentrations as low as 0.4 μg/L.

White phosphorus is moderately absorbed from the gastrointestinal tract of laboratory animals and humans. There is no convincing evidence, however, that white phosphorus is absorbed from the respiratory tract or skin in sufficient quantities to produce systemic effects in either laboratory animals or humans.

The minimum lethal oral dose of white phosphorus in humans is estimated to be 100 mg (1 [illegible] mg/kg), but it may be as low as 50 mg (0.7 mg/kg). An oral dose of 1 mg (0.2 mg/kg) may cause toxic effects. The estimated minimum harassing concentration is 700 mg/m^3 in working humans and 1,000 mg/m^3 in resting humans, but exposure to doses as low as 185 mg/m^3 for 5 min may cause respiratory tract irritation.

The acute oral LD50's for white phosphorus are 3.76 and 3.03 mg/kg in male and female rats, respectively, and 4.85 and 4.82 mg/kg in male and female mice, respectively. In contrast, the acute oral LD50 for white phosphorus/felt smoke condensate in rats is 2,346.8 mg/kg at 24 hr and 2,184.5 mg/kg at 14 days. The acute intravenous LD50 for white phosphorus/felt smoke condensate is 209.6 mg/kg (or 0.252 mL of the undiluted condensate) in rats.

The effects of acute oral exposure in laboratory animals and humans are similar. The major target organs damaged are the gastrointestinal tract, liver, kidney, brain, and cardiovascular system. White phosphorus is not irritating to rabbit skin and eyes, and it does not sensitize guinea pig skin. Undiluted white phosphorus/felt smoke condensate is a severe irritant to both rabbit skin and rabbit eyes.

Subchronic and chronic exposure to white phosphorus causes different effects in laboratory animals and humans. In laboratory animals, white phosphorus administered either orally or subcutaneously causes reduced growth, reduced survival at high doses, increased survival at low doses, and bone pathology consisting of thickening of the epiphyseal

line and extension of the trabeculae into the shaft. Liver pathology is usually absent. Subchronic inhalation of white phosphorus smoke causes laryngitis, tracheitis, bronchitis, and congestion.

Humans occupationally exposed to white phosphorus develop necrosis of the jawbone, a specific suppurative lesion that can result in the loss of some or all of the upper and/or lower jawbones. Necrosis of the jawbone may appear as early as 3 months or as late as 23 years after initiating exposure. The incidence and severity of this disease have been reduced through strict medical and dental surveillance.

White phosphorus is not carcinogenic in laboratory animals. White phosphorus and white phosphorus/felt smoke condensate are not mutagenic in bacteria, and white phosphorus/felt smoke is not mutagenic in fruit flies or in rats. White phosphorus/felt smoke is also not teratogenic in rats. Oral administration of white phosphorus induces a cluster of deaths in pregnant female rats within 2 days of parturition; therefore, it has reproductive toxic effects.

There is no information on the genotoxicity, teratogenicity, carcinogenicity, or reproductive toxicity of white phosphorus in humans.

The acceptable daily intake (ADI) of 0.0105 mg/day for a 70-kg person was calculated by using an uncertainty factor of 100 and a no observed adverse effect level (NOAEL) of 0.015 mg/kg/day obtained from the Monsanto study (Monsanto 1985) showing reproductive toxicity in pregnant rats. White phosphorus in corn oil (0.075 mg/kg/day) administered by gavage to female rats prior to and during mating, gestation, and lactation induced a cluster of deaths within 2 days of parturition. Doses of 0.015 and 0.005 mg/kg/day caused no effects. An interim water quality criterion for the protection of human health, calculated according to USEPA guidelines, of 4.303 μg/L is proposed. Because pregnant females appear to be the most sensitive population, this criterion should also protect the general population.

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1. INTRODUCTION

White phosphorus is a highly reactive allotrope (the P_4 tetrahedra) of elemental phosphorus. It oxidizes spontaneously in air at environmental temperatures. Although phosphorus is the twelfth most abundant element in nature, it occurs almost exclusively as salts of phosphoric acid. Phosphorus only occurs naturally as white phosphorus in some meteorites and in the mineral schreibersite (Van Wazer 1982). White phosphorus is also called yellow phosphorus, which will be used synonymously with white phosphorus throughout this document.

White phosphorus is used in the production of phosphorus sulfides, phosphorus halides, phosphorus pentoxide, phosphoric acid, and red phosphorus. It is used in ferrous metallurgy and in roach and rodent poisons. The military uses, which are of particular interest in this discussion, include the production of mortar shells, artillery shells, and hand and rifle grenades deployed to produce smoke that obscures movement of military personnel (Van Wazer 1982).

Ammunition loading is a potential source of environmental release of white phosphorus (Wang et al. 1982). According to Wang et al. (1982), loading operations use either "dip-filled" or "dry-filled" lines. Wastewaters, often called "phossy water," include overflow water from dip tanks, water from flushing pipes, spray from wetting conveyors, and waters from cleaning. Phossy water contains soluble, colloidal, and particulate white phosphorus and oxides of phosphorus (Sullivan et al. 1979). Dry-fill operations, which use nitrogen and carbon dioxide to prevent exposure of white phosphorus to air, greatly reduce the production of phossy water.

The environmental release of white phosphorus may cause visible changes in aquatic ecosystems. White phosphorus is toxic in humans and other mammals and in aquatic organisms. This summary evaluates the available health and environmental data in order to calculate criteria for the protection of aquatic life and human health according to U.S. Environmental Protection Agency (USEPA) guidelines. In the absence of sufficient data, recommendations for further research are made.

1.1 PHYSICAL AND CHEMICAL PROPERTIES

Elemental phosphorus exists in three allotropes: white, red, and black. Commercial white phosphorus is 99.9 percent pure and has a slightly yellow color, hence the synonym yellow phosphorus that is often used in the literature. The yellow color has been attributed to the presence of traces of red phosphorus, the other commercially important allotrope. Of the three allotropes, white phosphorus, which ignites spontaneously in air, is the most reactive (Van Wazer 1982). This reactivity may result from white phosphorus maintaining its tetrahedral structure as a solid, liquid, and gas (Corbridge et al. 1966, as reported in Sullivan et al. 1979). At room temperature, white

phosphorus exists as cubic crystals, called the alpha form. At -79.6°C, it converts to hexagonal crystals (Windholz et al. 1983).

A summary of the general physical and chemical properties of white phosphorus is presented in Table 1, and the chemical and physical properties influencing environmental fate are presented in Table 2.

White phosphorus is soluble in organic solvents but shows only limited solubility in water. The high octanol/water partition coefficient reveals potential for bioaccumulation, and the high volatility indicates some potential for release to the atmosphere.

1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES

The electric arc process is the important commercial means of producing white phosphorus. Phosphate rock is ground, formed into pellets, and dried. Dried coke, silica, and phosphate rock pellets are then charged into furnaces and heated electrically to 1,200 to 1,500°C (Van Wazer 1982). White phosphorus vaporizes, is cleaned by passing through an electrostatic precipitator, and condenses. After it liquifies, it is stored under warm water until shipment (Jangaard 1970). This general process is also called the electric furnace method (Chemical & Engineering News 1952).

Analytical methods for the detection of white phosphorus are of three basic types, in descending order of sensitivity: neutron activation analysis, gas-liquid chromatography, and colorimetry. An overview of different analytical methods for white phosphorus is given by Gorzny (1972). Lai and Rosenblatt (1977a) and Lai (1979a) developed a neutron activation technique for measuring ultramicro quantities in water, and Krishnan and Gupta (1970) developed a neutron activation technique for detecting phosphides and white phosphorus in biological materials. Lai (1981) modified his method in order to measure white phosphorus in sediment and reported a sensitivity of 1 to 2 μg/kg. For concentrations of 7.5 μg/kg and greater, measured values were within 10 percent of theoretical values. In the method of Lai and Rosenblatt (1977a), white phosphorus was extracted from water using benzene, converted to phosphate, and back-extracted in nitric acid. The samples were irradiated with thermal neutrons, the irradiated phosphate in each sample was separated using radiochemical methods, and the radioactivity was measured in a low-background proportional counter. This technique measured concentrations in water as low as 0.01 ppb with an accuracy of 10 percent. The detection limit could be extended to 0.001 ppb by further reducing background phosphorus levels in the reagents.

In the method of Krishnan and Gupta (1970), phosphine, zinc phosphide, and white phosphorus in biological tissues were separated by acidification, distillation, and ion exchange chromatography and measured by neutron activation analysis. As in the method of Lai and Rosenblatt (1977a), the detection limit could be lowered from 10 to 7 ng by further purification of the reagents to remove background phosphorus contamination.

TABLE 1. PHYSICAL AND CHEMICAL PROPERTIES OF WHITE PHOSPHORUS

Property	Value	Reference
CAS No.	12185-10-3	Van Wazer 1982
Synonyms	α-white phosphorus, yellow phosphorus	Van Wazer 1982
Molecular formula	P_4	Corbridge et al. 1966
Molecular weight	123.90	Weast et al. 1985
Appearance and purity	Colorless or white waxy solid;	Van Wazer 1982
	Commercial, 99.9% pure	Van Wazer 1982
	Electronic grade 99.9999% pure	Hawley 1981
Cystalline form	α- is cubic and exists at normal temperatures; vapor and liquid are tetrahedral	Corbridge et al. 1966
Melting point (°C)	44.1	Van Wazer 1982
Boiling point (°C)	280	Weast et al. 1985
Density	1.82 g/cm^2	Van Wazer 1982
Refractive index (n_D^{20})	2.144	Weast et al. 1985
Enthalpy of solution	6 kcal/mol (estimate)	Spanggord et al. 1985
Heat capacity (cal/mol·deg)	22.18 at 25°C	Berkowitz et al. 1981
	22.73 at 44.1°C	Berkowitz et al. 1981
Heat of fusion	600 ± 3/mole of P_4	Berkowitz et al. 1981
Heat of combustion (kcal/mole)	710.2 ± 1.0	Berkowitz et al. 1981
Critical pressure	82.2 atm	Berkowitz et al. 1981
Critical temperature (°C)	695	Berkowitz et al. 1981
Reactivity	Ignites spontaneously in air at 30°C; phosphoresces at room temperature	Hawley 1981

TABLE 2. PHYSICAL AND CHEMICAL PROPERTIES OF PHOSPHORUS INFLUENCING ENVIRONMENTAL FATE

Property	Value	Reference
Solubility in water	2.4 mg/L at 15°C	Spanggord et al. 1985
	3.0 mg/L at 15°C	Berkowitz et al. 1981
	4.1 mg/L at 25°C	Spanggord et al. 1985
	5.0 mg/L at 35°C	Spanggord et al. 1985
Solubility in organic solvents	Soluble in ether, benzene, and other organic solvents;	Van Wazer 1982
	2.5 g/L ethanol, 10 g/L ether, 25 g/L methylene chloride, 125 g/L carbon disulfide;	Berkowitz et al. 1981
	0.3 g/100 mL alcohol;	Weast et al. 1985
	880 g/100 mL carbon disulfide at 10°C	Weast et al. 1985
Octanol/water partition coefficient	1200 ± 100	Spanggord et al. 1985
Vapor pressure	0.023 torr[a] at 25°C	Spanggord et al. 1985
	1 atm at 76.6°C	Berkowitz et al. 1981
Henry's constant	1600 L-torr/mole at 25°C	Spanggord et al. 1985

a. Spanggord et al. (1985) considered this estimate low because of transformation to red phosphorus during the determination.

The most commonly used analytical technique in aquatic surveys and bioassays is gas-liquid chromatography. The method of Addison and Ackman (1970) provided the basis for the variations in analytical technique used in studies of aquatic biota. Either benzene or isooctane was used to extract white phosphorus from water, sediment, or tissues. The extract was concentrated in order to increase sensitivity. A gas chromatograph equipped with a flame photometric detector (FPD) and a flame ionization detector allowed measurement of elemental phosphorus in the presence of organic contaminants. This technique also detected the presence of phosphine. Addison and Ackman (1970) stated that the FPD could detect about 10^{-12} g of phosphorus without the concentration step. Sullivan et al. (1979) stated that the practical detection limit of the technique was 0.1 to 0.2 µg/L. The sensitivity, however, was increased 25 or 50 times by the concentration step.

Ackman et al. (1970) modified the technique of Addison and Ackman (1970) to use on fish samples. The detection limit was approximately 1 ppb expressed on a wet weight basis. Concentrating the organic solvent to 10 percent of its original volume gave a detection limit of approximately 0.1 ppb.

The least sensitive method for measuring white phosphorus in water is by extraction with an organic solvent and colorimetric determination by the molybdenum blue procedure. Rushing (1962) modified this general procedure for phosphorus determinations in air by adding a separation step for removing oxides. The detection limit was 1 to 2 μg per aliquot tested. Zitko et al. (1970) used the general procedure to measure yellow phosphorus in water. The detection limit was not given.

Bullock and Newlands (1969) also used a colorimetric technique, but they quantified phosphorus as the yellow phosphovanadomolybdate anion. The lower limit of detection by this technique was 40 ppb.

2. ENVIRONMENTAL EFFECTS AND FATE

2.1 ABIOTIC ENVIRONMENTAL EFFECTS

Three abiotic effects are potential consequences of the release of white phosphorus: increased acidity, decreased dissolved oxygen (DO), and increased sedimentation. Increased acidity and decreased DO could result from the oxidation of white phosphorus to hypophosphorous, phosphorous, and phosphoric acids, which are aqueous hydrolysis products of white phosphorus (Lai and Rosenblatt 1977b). The half-life of the conversion from the elemental form to the lower oxy-acids is on the order of minutes to hours (Berkowitz et al. 1981). Because the half-life of conversion from these acids to phosphates is on the order of weeks to months (Berkowitz et al. 1981), significant decline in pH could result from the accumulation of acids.

Pearson et al. (1976) reported a slight decrease in DO in the receiving creek during filling operations at the Pine Bluff Arsenal. During a nonproduction period the DO was 10.87 mg/L, and during a production period it was 9.80 mg/L. Ackman et al. (1971), citing the work of Bohn et al. (1970), suggested that the anoxic conditions in the sediments of Long Harbor, Placentia Bay, Newfoundland, may have been associated with the slow oxidation of white phosphorus.

Peer (1972) described the bottom sediments near a phosphorus plant effluent. Precipitation of phosphorus near the effluent discharge resulted in significant local changes in sediment characteristics. A fine sediment blanketed the natural coarse gravel and formed an 18-inch layer near the effluent pipe, thinning to a fine layer farther from the pipe. Analysis of the sediment revealed the greatest fraction of phosphorus in the coarse to medium and fine sand. Individual particles with a mean diameter of 275 μm were identified as yellow phosphorus.

2.2 ENVIRONMENTAL FATE

Figure 1 illustrates potential sources, fates, and sinks of white phosphorus in the environment. White phosphorus and its degradation products are directly and indirectly released into the environment. Direct release occurs during the production of white phosphorus (Idler 1969) and during the manufacture and combustion of white phosphorus/felt smoke-producing devices (75.3 $\pm$ 3.5 percent white phosphorus) (Bentley et al. 1978). Indirect release occurs via the volatilization of white phosphorus from aquatic and terrestrial systems. During the deployment of munitions, about 10 percent of the white phosphorus is not oxidized; thus, it is available for release into the environment (Spanggord et al. 1985). Although hydrolysis and volatilization can have significant effects on the environmental fate of white phosphorus, its fate in air, soil, and water is generally determined by oxidative processes (Berkowitz et al. 1981, Spanggord et al. 1985). Anaerobic sediments and soils can serve as sinks for white phosphorus that, in turn, can serve as long-term sources for mobilization into the environment (Lai 1981).

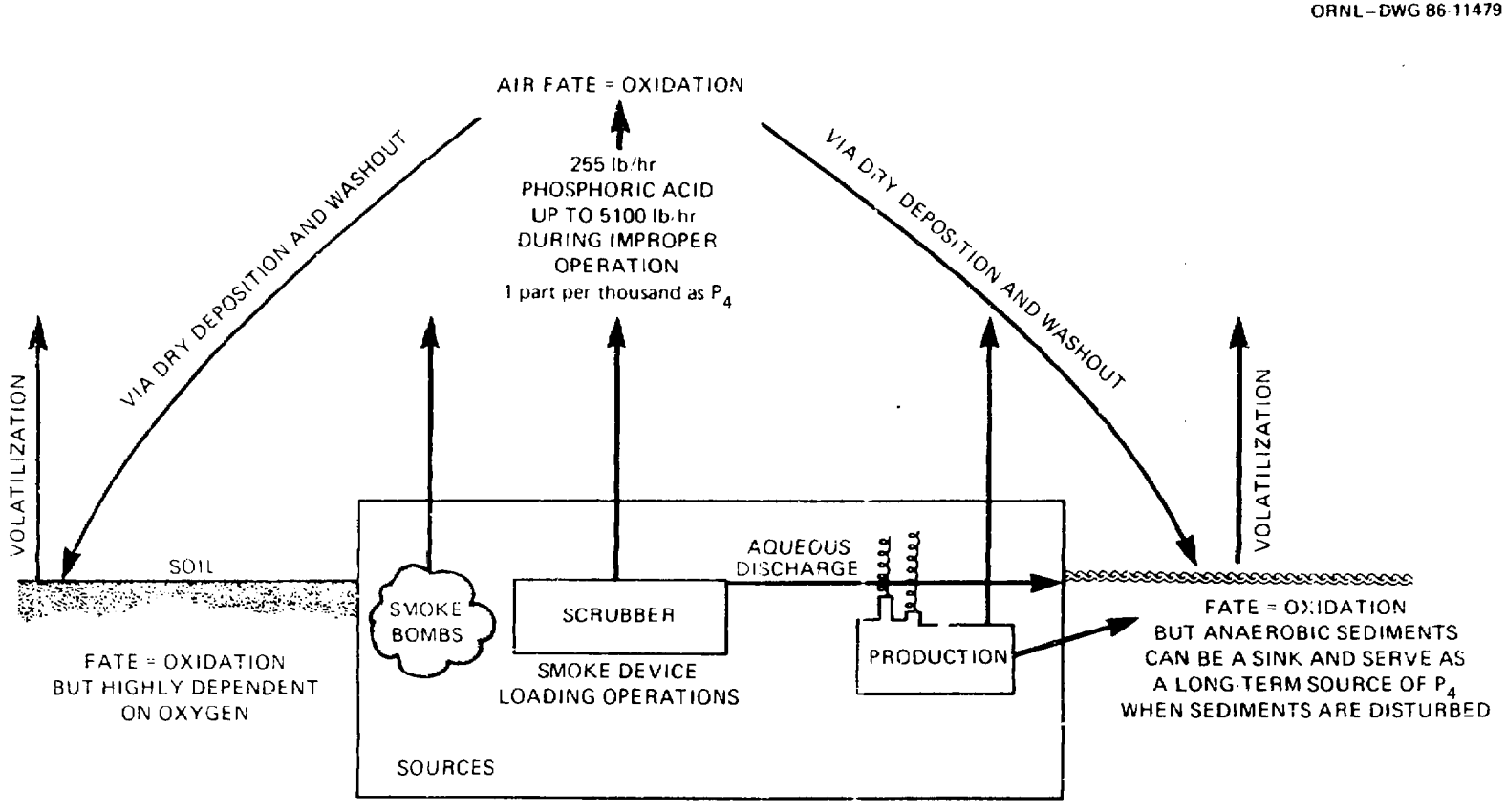

Figure 1. Summary of Transport, Transformation, and Fate of White Phosphorus in the Environment.

2.2.1 Sources of White Phosphorus and Degradation Products

The appearance of white phosphorus in effluents and in environmental samples serves as evidence of its release. This is best exemplified by the white phosphorus pollution crisis at the Long Harbour Inlet -- Placentia Bay, Newfoundland in 1969. During the initial stages of operation, the ERCO production plant discharged 68 to 91 kg/day of colloidal yellow phosphorus into the bay. Concentrations in bottom sediments ranged from 5000 ppm near the effluent pipe to 1 ppm at a distance of 2.4 km (Ackman et al. 1971).

The effluent from white phosphorus production and manufacturing facilities, which contains both suspended and dissolved white phosphorus, is known as "phossy water." White phosphorus/felt smoke munitions are manufactured at the Pine Bluff Arsenal, Arkansas. Prior to the implementation of a wastewater treatment and recycling system at the arsenal, Pearson et al. (1976) reported concentrations of 16.0 to 53.4 mg/L of white phosphorus in discharge water during filling operations. Lai (1979a) reported concentrations of yellow phosphorus in Yellow Lake (located within the arsenal grounds) ranging from 0.005 to 0.010 μg/L. A concentration as high as 13.8 μg/L was reported in White Creek, a feeder stream of Yellow Lake. Concentrations of 0.003 to 0.004 μg/L were reported in the Arkansas River, which fronts the arsenal.

Berkowitz et al. (1981) estimated the release of white phosphorus during a simulated breakdown in the phossy water treatment and recycling system at the Pine Bluff Arsenal. If the white phosphorus concentration is as great as 40 mg/L and 100 percent is transported to Yellow Lake, then 1 ppb of white phosphorus (threshold toxicity concentration) would occur in the lake water after the discharge of 5,000 gallons of phossy water, which is approximately equal to the volume discharged during an 8-hr production shift. Therefore, continuous discharge would have a serious impact on the water quality of Yellow Lake and receiving waters. However, even with the water pollutant control system operating under optimum conditions, small amounts of white phosphorus would still be released.

During production of the white phosphorus/felt munitions at the Pine Bluff Arsenal, white phosphorus in the stack emissions would be converted primarily to phosphoric acid or phosphates in the ambient air. Berkowitz et al. (1981) report that under normal operations of venturi scrubbers, 255 lb of phosphoric acid per hour would be emitted; however, in the event of a scrubber breakdown, emissions could be as high as 5,100 lb per hr. White phosphorus has been reported in stack emissions at concentrations of 1 part per thousand, probably due to the formation of a passivating surface oxide layer that would retard oxidation. A white phosphorus emission of 1 mg/kg would result in an estimated 1-hr maximum exposure of 0.5 $\mu g/m^3$.

Poston et al. (1986) studied the combustion products released upon ignition of white phosphorus/felt munitions. Phosphorus oxides ranging up to the pentoxide are formed upon complete ignition. In oxygen-limiting situations, the lower oxides (trioxides) are enhanced.

2.2.2 Transformation and Transport of White Phosphorus

The processes by which white phosphorus is transformed in air, water, and soil are oxidation, hydrolysis, and volatilization. White phosphorus is not transformed by photolysis, and it is also resistant to biodegradation by anaerobic organisms (Spanggord et al. 1985).

In air, white phosphorus spontaneously reacts to form phosphorus oxides; consequently, it is usually stored under a blanket of water (Berkowitz et al. 1981). White phosphorus that is emitted into the air may be covered with an oxide passivating layer, which would prevent oxidation. White phosphorus released in air would probably reach an aquatic or soil system via dry deposition or rainout (Berkowitz et al. 1981).

In aquatic systems, oxidation can be affected by the concentration above and below the solubility limit (3 mg/L). Addison (1971) described the oxidation of 1 ppm (below the solubility limit) of aqueous white phosphorus to phosphate according to first-order kinetics with a half-time of 0.85 hr at 30°C. Bullock and Newlands (1969) found that colloidal suspensions of white phosphorus at concentrations up to 50 mg/L oxidized with half-times of 80 hr at 30°C and 280 hr at 0°C. Lai and Rosenblatt (1977b) identified several intermediate oxidation states of orthophosphorus formed during the oxidation of phossy water (contains both dissolved and colloidal white phosphorus). These lower oxides are rapidly hydrolyzed to hypophosphorous acid (H_3PO_2), phosphorous acid (H_3PO_3), and phosphoric acid (H_3PO_4). Complete oxidation to phosphoric acid is slow. Approximately 6 to 9 percent of the initial phosphorus is transformed to highly toxic phosphine (PH_3); however, phosphine exhibits low water solubility and is either further oxidized or rapidly released to the air (Lai and Rosenblatt 1977b).

Several investigators observed that the oxidation rate in aqueous systems can be affected by dissolved oxygen concentration, temperature, pH, salinity, and the presence of metals. Lai and Rosenblatt (1977b) showed that the oxidation of white phosphorus was approximately ten times slower under an argon-purged solution (DO = 1.5 ppm) than under an air-purged solution (DO = 5.9 to 8.9 ppm). Lai (1979b) demonstrated that the oxidation rate increased proportionally with temperature, even in the presence of higher concentrations of dissolved oxygen. In solutions initially containing 44 μg/L of white phosphorus, the half-lives ranged from 268 hr at 3°C to 11.4 hr at 93°C. Lai (1979b) also studied the effect of pH on oxidation rate. At pH values between 2 and 6, the oxidation rate was not affected. At pH 6, 83.0 percent of the initial 44 μg/L of white phosphorus was oxidized within 69 hr, whereas at pH 8, 95.9 percent was oxidized within the same time. The authors suggested that this increased activity was probably due to the increase in the concentration of hydroxide ions.

Zitko et al. (1970) reported that in seawater and at concentrations below the solubility limit, white phosphorus was oxidized with a half-time of 2 hr at 10°C. Bullock and Newlands (1969) reported oxidation rates of colloidal suspensions that were 1.5 times faster in freshwater than in saltwater. The authors suggested that the salts coagulated the colloidal particles, thereby retarding exposure to oxygen.

Data from Monsanto (1972, as reported in Sullivan et al. 1979) indicated that ionic copper, at a concentration of 1 ppm, catalyzed the oxidation reaction, whereas iron, by providing a resistant coating, extended the half-life of white phosphorus by an order of magnitude.

Spanggord et al. (1985) suggested that volatilization is a mechanism for the release of white phosphorus in turbulent and shallow aquatic systems. The authors estimated a volatilization half-life of 5.6 hr for a water body such as a pond.

The primary pathway of the degradation of white phosphorus in soil is also by oxidation, with the rate dependent upon the available oxygen. Based on the observation of heat evolved, Bohn et al. (1970) reported that white phosphorus oxidized to the more stable red allotrope within 2 hr. Berkowitz et al. (1981) stated that, within weeks to months, white phosphorus will be predominantly oxidized to phosphates. Warnock (1972) reported that 50 ppm of white phosphorus in soils at 18 to 24°C was oxidized within 3 to 7 days; however, small quantities (0.004 to 0.5 percent) escaped into the atmosphere through volatilization. The authors also found that volatilization was reduced by deeper placement in the soil (up to 10 cm). Spanggord et al. (1985) suggested that because most soils are anaerobic short distances below the surface, the half-life of white phosphorus may be extremely long.

The uptake and release of white phosphorus from sediments in aquatic systems are important in the long-term dynamics of phosphorus. In experiments to determine partitioning between water and sediments, Lai (1981) showed that at a water-column concentration of 0.07 to 0.46 $\mu g/mL$ of white phosphorus and at water/sediment ratios of 1 to 7 mL/g, more than 90 percent of the white phosphorus was removed within 24 hr. The percentage of white phosphorus removed by the sediments was greater for lower water-to-sediment ratios. Lai stated that much of the white phosphorus undergoes oxidation during the partitioning phase. In aquatic systems, when white phosphorus is finally oxidized to phosphate, partitioning between sediments and water is 90 percent and 10 percent, respectively (Edzwald 1977, as reported in Berkowitz et al. 1981).

Peer (1972) examined the sediments in Placentia Bay near the ERCO wharf and found that the white phosphorus occurred in a particulate form, approximately 275 μm in diameter, which did not separate from the mineral particles. Ackman et al. (1971) determined that the surface deposits of a few parts per million probably oxidized quickly; whereas deeper deposits of higher concentration could be stable for years. These deposits could provide a source of long-term release to the water column. Lai (1981) disturbed sediments, containing 10 mg/kg of white phosphorus, in an experimental system and showed an initial surge of release until a maximum water concentration was reached, followed by a rapid decline to an asymptotic level. Pearson et al. (1976) noted that phosphorus levels in fish of Yellow Lake, Pine Bluff Arsenal, Arkansas, rose following rain that disturbed the sediment.

Spanggord et al. (1985) calculated the equilibrium partitioning among soil-air, soil-water, and soil-solids. The authors stated that

partitioning between soil and water is high enough to allow "appreciable leaching" of white phosphorus and that partitioning between soil and air is high enough to allow "modest volatilization." Despite the limited mobility of white phosphorus in soil, Spanggord et al. (1985) concluded that a more detailed investigation of the mobility is not required, because the time scale of oxidation is rapid relative to that of leaching or of volatilization.

Spanggord et al. (1985) investigated the dissolution of phosphorus from soil and estimated a dissolution rate of 6.6 μg/hr using oxygen-saturated water and a flow rate of about 3 mL/min. At steady state, dissolution rates were the same for aged phosphorus solutions, fresh phosphorus solutions, nitrogen-purged water, and oxygen-saturated water. The authors estimated that a 1-cm^3 sphere of phosphorus with a surface area of 4.8 cm^2 dissolving at a rate of 5.8 μg/hr would lose 2.8 percent of its mass in 1 year. The persistence of this particle would be orders of magnitude longer than the persistence of dissolved phosphorus in soil water (Spanggord et al. 1985).

Katz et al. (1981) and Poston et al. (1986) studied the degradation of white phosphorus during combustion of white phosphorus/felt munitions. White phosphorus is oxidized to phosphorus oxides ranging up to a pentoxide. Upon contact with water, the oxides form linear polyphosphates and smaller amounts of cyclic metaphosphates (acidic species), that are then hydrolyzed to phosphoric acid; total hydrolysis is slow. The inherent alkaline nature of the receiving waters (pH ≥ 6.0) would neutralize the phosphoric acid and form a precipitate that could blanket the substrate, subsequently having an adverse affect on the aquatic system (Poston et al. 1986). The formation of trioxides provides a potential pathway for the formation of highly toxic phosphine gas; however, phosphine is further oxidized or volatilized due to its low water solubility.

2.3 SUMMARY

White phosphorus and its degradation products can be released into the environment during the production of white phosphorus and during the manufacture and combustion of white phosphorus/felt munitions. Indirect release may occur via volatilization from aquatic and terrestrial systems. The effluent from white phosphorus production and manufacturing facilities, containing both suspended (colloidal) and dissolved white phosphorus, is known as "phossy water." In 1969, releases of white phosphorus from the ERCO production plant into Placentia Bay, Newfoundland, resulted in concentrations in bottom sediments ranging from 5,000 ppm near the effluent pipe to 1 ppm at a distance of 2.4 km. The manufacture of felt munitions at the Pine Bluff Arsenal, Arkansas, has resulted in concentrations of white phosphorus as high as 13.8 μg/L in receiving streams.

The three potential abiotic effects of the environmental release of white phosphorus are reduction in dissolved oxygen, reduction in pH, and increased deposition of fine particulates.

The processes by which white phosphorus are transformed in air, water, and soil are oxidation, hydrolysis, and volatilization. In air, white phosphorus spontaneously reacts to form phosphorus oxides. However, white phosphorus that is emitted to the air may be covered with an oxide passivating layer that retards oxidation and allows the phosphorus to reach aquatic or terrestrial systems in an unchanged state either by dry deposition or rainout.

In water, oxidation of white phosphorus to phosphate at concentrations below the solubility limit proceeds according to first-order kinetics, with a half-time of 0.85 hr at 30°C. Oxidation of colloidal suspensions of white phosphorus at concentrations up to 50 mg/L occur at half-times of 80 hr at 30°C and 280 hr at 0°C, with the formation of several intermediate oxidation states of orthophosphorus. These lower oxides are hydrolyzed to hypophosphorous acid, phosphorous acid, and phosphoric acid. The oxidation rate is affected by the concentration of dissolved oxygen, temperature, pH, salinity, and the presence of metals. Oxidation is increased with increased dissolved oxygen and temperature. At pH values above 6.0, oxidation is substantially increased, probably due to the increase in the concentration of hydroxide ions. The oxidation rate of colloidal suspensions is 1.5 times faster in freshwater than in saltwater, due to coagulation of the colloidal particles. Ionic copper catalyzes the oxidation reaction, whereas iron provides a coating resistant to oxidation.

The uptake and release of white phosphorus from sediments in aquatic systems are important in the long-term dynamics of white phosphorus. When white phosphorus is finally oxidized to phosphate, partitioning between sediments and water is 90 percent and 10 percent, respectively. Surface deposits oxidize quickly, whereas deeper deposits of higher concentrations could be stable for years and could provide a source of long-term release to the water column.

White phosphorus in soil is primarily oxidized to phosphate, depending upon the available oxygen. Small quantities of white phosphorus will escape from the soil into the atmosphere through volatilization. Partitioning to soil-water is high enough to allow "appreciable leaching," and partitioning to air is high enough to allow "modest volatilization." Based on dissolution rates, the persistence of colloidal white phosphorus in soil is orders of magnitude longer than the persistence of dissolved phosphorus in soil water. Most soils are anaerobic short distances below the surface, thereby extending the half-life of phosphorus deposits and serving as a sink that could provide a long-term source of mobilization into the environment.

The environmental release of white phosphorus combustion products occurs with the deployment of white phosphorus/felt munitions. Upon contact with water, the combustion products are finally transformed to phosphoric acid, which may influence the pH of the receiving waters. However, at a pH of around 6.0, the inherent alkaline nature of the receiving waters neutralizes the acid, forming a precipitate that could adversely affect the aquatic system by blanketing the substrate.

3. AQUATIC TOXICOLOGY

3.1 ACUTE TOXICITY TO AQUATIC ANIMALS

3.1.1 Fishes

The earliest study of the toxicity of white phosphorus to fish was Isom's (1960) determinations of TL_m values (median tolerance limit) for Lepomis macrochirus (bluegill sunfish). He was especially interested in determining if the toxic component in white phosphorus solutions was soluble or colloidal phosphorus. Samples of industrial wastes from which colloidal phosphorus had been removed by filtration were not toxic, but the unfiltered wastes caused 100 percent mortality within 24 hr. Isom concluded that colloidal phosphorus was more toxic than soluble phosphorus and suggested that the TL_m for colloidal phosphorus was not greater than 0.105 mg/L at 48 hr nor less than 0.025 mg/L at 163 hr. The study was limited by the lack of measured phosphorus concentrations. In contrast, Krasnov (1970) ranked the toxicity of different forms of phosphorus to carp, perch, Leuciscus rutilus (roach), and Leuciscus leuciscus (dace) as follows: emulsion > solution > suspension > colloid.

Bentley et al. (1978) determined the acute toxicity of elemental phosphorus to Ictalurus punctatus (channel catfish), bluegill sunfish, Pimephales promelas (fathead minnow), and Salmo gairdneri (rainbow trout). The five or more test concentrations (which were not specified) used to measure LC50 (lethal concentration causing 50 percent mortality) values were determined from preliminary range-finding tests. Static tests were conducted according to methods described by the USEPA (1975, as reported in Bentley et al. 1978). White phosphorus was more toxic to bluegill than to the other species tested (Table 3). LC50 values (nominal concentrations) varied among the four fish species from 6 μg/L for bluegill to 73 μg/L for catfish.

The static 96-hr LC50 for bluegill from tests using aged solutions of white phosphorus ranged from 4 μg/L at time 0 of aging (95 percent confidence interval = 2-7) to 9 μg/L after 96 hr of aging (95 percent confidence interval = 6-12). Although the percentage of white phosphorus declined with aging, the toxicity to bluegill was not significantly affected (Bentley et al. 1978).

Bentley et al. (1978) determined the effect of variations in water quality on bluegill, the most sensitive species studied. Temperatures of 15-25°C, hardness of 35 and 100 mg/L $CaCO_3$, and pH values of 7 and 8 did not affect the toxicity, with 96-hr LC50's ranging from 2 to 8 μg/L (nominal concentrations). However, very hard water (250 mg/L $CaCO_3$) and a pH of 6 decreased the toxicity by an order of magnitude, with 96-hr LC50's of 86 μg/L and 69 μg/L, respectively.

Pearson et al. (1978) determined LC50 values (measured concentrations) for bluegill and Gambusia affinis (mosquito fish) in static tests using phossy water from the white phosphorus munitions filling plant at

TABLE 3. ACUTE TESTS FOR IMMOBILIZATION[a] OR MORTALITY[b] OF AQUATIC SPECIES FOLLOWING EXPOSURE TO WHITE PHOSPHORUS

Species	Test Method	Test Duration	LC_{50}/EC_{50}[c] (µg/L)	Genus Mean Acute Value (µg/L)	Reference
Arthropoda					
Crustacea					
Daphnidae					
Daphnia magna[a,d]	S[e]	48 hr	30 (25-37)[f]	30	Bentley et al. 1978
Daphnia magna[a,d]	FT[g]	48 hr	> 50		Bentley et al. 1978
Gammaridae					
Gammarus fasciatus[a,h]	S	48 hr	250 (190-310)	250	Bentley et al. 1978
Asellidae					
Asellus militaris[a,h]	S	48 hr	> 560	> 560	Bentley et al. 1978
Palaemonetidae					
Palaemonetes kadiakensis[a]	S	48 hr	59.6 (30.9-88.4)	-	Pearson et al. 1978
Insecta					
Chironomidae					
Chiropomus tentans[a,i]	S	48 hr	140 (110-190)	124.6585	Bentley et al. 1978
Chironomus tentans[a,i]	FT	48 hr	111 (31-399)		Bentley et al. 1978
Chordata					
Osteichthyes					
Centrarchidae					
Lepomis macrochirus[b,j]	S	96 hr	29.0 (19.3-43.7)	-	Pearson et al. 1978
Lepomis macrochirus[b,j]	S	96 hr	6 (4-9)	3.795	Bentley et al. 1978
Lepomis macrochirus[b,k]	FT	96 hr	2.4 (1.7-3.5)		Bentley et al. 1978
Salmonidae					
Salmo gairdneri[b,l]	S	96 hr	22 (15-32)	22	Bentley et al. 1978
Ictaluridae					
Ictalurus punctatus[b,m]	S	96 hr	73 (53-99)	73	Bentley et al. 1978
Ictalurus punctatus[b,n]	FT	96 hr	> 19		Bentley et al. 1978
Poeciliidae					
Gambusia affinis[b]	S	96 hr	63.0 (45.1-81.9)	-	Pearson et al. 1978
Cyprinidae					
Pimephales promelas[b,o]	S	96 hr	20 (16-25)	20	Bentley et al. 1978

a. Immobilization tests were designed to give EC_{50} values.
b. Mortality tests were designed to give LC_{50} values.
c. All concentrations were nominal, with the exception of the FT test with *I. punctatus* in which concentrations were measured.
d. Test animals were < 24 hr old at start of test.
e. S = static test.
f. Values in parentheses indicate 95% confidence interval.
g. FT = flow-through test.
h. Juveniles.
i. Test animals were second or third instars at start of test.
j. Mean weight ± SD = 1.0 g ± 0.3, mean length ± SD = 37 mm ± 6.
k. Mean weight ± SD = 1.7 g ± 0.5, mean length ± SD = 40 mm ± 3.
l. Mean weight ± SD = 0.9 g ± 0.3, mean length ± SD = 43 mm ± 4.
m. Mean weight ± SD = 1.2 g ± 0.5, mean length ± SD = 47 mm ± 11.
n. Mean weight ± SD = 0.1 g ± 0.4, mean length ± SD = 43 mm ± 8.
o. Mean weight ± SD = 2.1 g ± 0.2, mean length ± SD = 43 mm ± 3.

Pine Bluff Arsenal, Arkansas (Table 3). The 96-hr LC_{50} for bluegill (29.0 $\mu g/L$) was much greater than the value (using standard stock solutions) obtained by Bentley et al. (1978). These values cannot be used to calculate a water quality criteria, because of the use of phossy water and because the source of mosquito fish (Yellow Lake) has a history of receiving white phosphorus.

Bentley et al. (1978) also conducted acute static tests to determine the effects of elemental phosphorus on various life stages (egg, 1-hr-old fry, 7-day-old fry, 30 day-old-fry, 60-day-old fry) of fathead minnows. Eggs were exposed for 144 hr and all other life stages were exposed for 96 hr (test concentrations were not specified). The authors reported 96-hr LC_{50} values of > 560 $\mu g/L$ for eggs, 154 $\mu g/L$ for 1-hr-old fry, and 74 $\mu g/L$ for 7-day-old fry. Thirty- and 60-day-old fry were more sensitive to elemental phosphorus than the earlier life stages, based on 96-hr LC_{50} values of 21 and 18 $\mu g/L$ (nominal concentrations), respectively. The range of mean LC_{50} values calculated for 24-, 48-, and 96-hr tests was 93 to 74 $\mu g/L$ for 7-day-old fry, 26 to 21 $\mu g/L$ for 30-day-old fry, and 27 to 18 $\mu g/L$ for 60-day-old fry. LC_{50}'s for early fry and eggs did not vary much with the duration of exposure.

In addition to static tests, Bentley et al. (1978) conducted dynamic bioassays on bluegills, the most sensitive fish in their static tests, and channel catfish, the least sensitive fish in their static tests. USEPA (1975, as reported in Bentley et al. 1978) test procedures with a flow rate of 5 L/hr through 30-L aquaria were used. Both species were more sensitive to white phosphorus in the dynamic assays than in the static tests (Table 3). The 96-hr LC_{50} of 2.4 $\mu g/L$ for bluegill was the lowest value obtained for any species tested.

Incipient LC_{50} (lethal concentration for 50 percent mortality from long exposure) values were also determined in flow-through tests. The mean incipient LC_{50} for channel catfish was 4.2 $\mu g/L$ (95 percent confidence interval = 3.3 to 5.4 $\mu g/L$). A test duration of 624 hr was required to estimate the incipient LC_{50}. The incipient LC_{50} for bluegill was 0.6 $\mu g/L$ (95 percent confidence interval = 0.4 to 1.1 $\mu g/L$). The test duration was 192 hr. Because the incipient LC_{50} values were much lower in dynamic tests than in static tests and because the time required to estimate incipient LC_{50}'s was long, Bentley et al. (1978) suggested that phosphorus toxicity is cumulative.

Zitko et al. (1970) determined LT_{50} (median time to death for 50 percent of the test population) for Clupea harengus (herring) and Salmo salar (Atlantic salmon). Tests were conducted with one of four phosphorus sources: contaminated sediments, phossy water, aqueous dispersion of yellow phosphorus prepared in the laboratory, and artificially contaminated sediments prepared from yellow phosphorus mixed with mud, sand, or alumina. An aqueous dispersion was prepared by adding a saturated solution of yellow phosphorus in ethanol to distilled water. Phosphorus concentrations were measured by first extracting the phosphorus with benzene, then converting it to phosphate, and, last, quantifying it by the standard molybdate-stannous chloride method. An LT_{50} of approximately 130 hr was obtained for herring at the lowest dose

tested, 2.5 μg/L. Consequently, the incipient lethal level must be below this concentration. Because herring exposed to a suspension of contaminated mud for 3 hr before transfer to clean water showed the same LT_{50} as those continuously exposed to contaminated conditions, the authors suggested that the effects of phosphorus are cumulative. An incipient lethal level of approximately 18 μg/L was estimated for Atlantic salmon.

Fletcher et al. (1970) determined the LT_{50}'s of yellow phosphorus to yearling Salvelinus fontinalis (brook trout). Flow-through tests were conducted with measured phosphorus concentrations. Fish were acclimatized to seawater 7 to 30 days before testing. LT_{50} values were estimated using the methods of Litchfield and Wilcoxon (1949) and Litchfield (1949). The LT_{50} for the lowest concentration tested (0.5 μg/L) was 121 hr. The LT_{50} for the controls, 200 hr, was significantly ($P < 0.05$) greater than that of any of the treated groups. Increased mortality observed in the control fish was attributed to the high salinity of the seawater.

Fletcher and Hoyle (1972) determined LT_{50}'s for Atlantic salmon and Gadus morhua (Atlantic cod) in flow-through tests in which the phosphorus concentration was determined using the gas-liquid chromatographic method of Addison and Ackman (1970). Test concentrations ranged from 0.79 to 1,900 μg/L for Atlantic salmon and from 1.89 to 5,780 μg/L for Atlantic cod. No fish died in controls treated with dilution water or in controls treated in dilution water containing ethanol, the carrier used in some of the experiments. Because results from tests using phosphorus introduced in ethanol were similar to those of tests using aqueous suspensions, results were combined. The LT_{50} for Atlantic salmon maintained in saltwater was 195 hr for exposure to the lowest concentration tested, 0.79 μg/L. LT_{50}'s for Atlantic salmon maintained in freshwater were 2.82 hr for a 763-μg/L exposure, 20.9 hr for a 99.0-μg/L exposure, and 50.2 hr for a 22.8-μg/L exposure. The LT_{50} for Atlantic cod exposed continuously to 1.89 μg/L was 125 hr.

Fletcher and Hoyle (1972) also conducted brief-exposure experiments in which cod and salmon were exposed to white phosphorus at concentrations ranging from 245 to 4,030 μg/L for time periods ranging from 20 min to 3.5 hr. Regression analysis of the two data sets, continuous and brief exposures, indicated that short exposures to high concentrations were lethal within the same time period as continuous exposures to low concentrations.

Maddock and Taylor (1976) investigated the toxicity of dissolved phosphorus to Atlantic cod in a flow-through bioassay. Phosphorus concentrations were monitored at three positions in the tank during the study. The authors stated that dissolved elemental phosphorus could be maintained at any concentration between 0.25 and 30 μg/L in the test solution for periods up to 20 days with a variation of only 5 percent. The authors reported an incipient lethal level of 1 to 2 μg/L and a threshold time of exposure of about 17 hr regardless of exposure concentration.

3.1.2 Macroinvertebrates

Bentley et al. (1978) measured the acute toxicity of elemental phosphorus to Daphnia magna (water flea), Gammarus fasciatus (scud), Asellus militaris (sowbug), and Chironomus tentans (midge) in static tests (nominal concentrations). Dynamic tests were conducted with D. magna and C. tentans (nominal concentrations). All test procedures followed methods described by USEPA (1975, as reported in Bentley et al. 1978). Dynamic tests used 1.75-L aquaria with a flow rate of 4.0 L/day. Because the stock phosphorus solution was prepared in dimethyl sulfoxide (DMSO), the control chamber received DMSO equal to the highest concentration used in any treatment.

Bentley et al. (1978) reported that white phosphorus is generally less toxic to macroinvertebrates than to fishes (Table 3). However, D. magna exhibited sensitivity similar to the fishes, with a static EC_{50} (effective concentration causing 50 percent death based on immobilization) of 30 $\mu g/L$. Static EC_{50} values for the other species tested ranged from 140 $\mu g/L$ (C. tentans) to > 560 $\mu g/L$ (Asellus militaris). White phosphorus was not significantly more toxic in dynamic tests with 48-hr EC_{50}'s of > 50 $\mu g/L$ for D. magna and 111 $\mu g/L$ for C. tentans. Incipient LC_{50}'s (nominal concentrations) were substantially lower than EC_{50}'s determined in 48-hr tests (Bentley et al. 1978). For C. tentans, the incipient LC_{50} in flow-through tests was 20 $\mu g/L$; for D. magna, the value was 11 $\mu g/L$.

Pearson et al. (1978) conducted preliminary tests with Branchiuri sowerbyi, Glyptotendipes, and Chaoborus punctatus. The 48-hr EC_{50}'s for these invertebrates ranged somewhere between 12 and 120 $\mu g/L$. At 12 $\mu g/L$ the mortality rate was the same as that of the controls, and at 120 $\mu g/L$ mortality was 100 percent. The geometric mean of the bounds, 37.9 $\mu g/L$, is considered to be an estimated EC_{50} value for all three species. The authors conducted definitive toxicity tests to determine a 48-hr EC_{50} value for Paleomonetes kadiakensis (grass shrimp) from Yellow Lake, Pine Bluff Arsenal, Arkansas (Table 3). Concentrations of 0, 21, 73, 192, and 220 $\mu g/L$ (nominal concentration based on measured concentration in stock solution) were tested. The 48-hr EC_{50} was calculated to be 59.6 $\mu g/L$, with a 95 percent confidence interval of 30.9 to 88.4.

Zitko et al. (1970) determined LT_{50} values for Gammarus oceanicus (beach fleas) and Homarus americanus (lobster) in static bioassays. Although none of the tests were flow-through tests, the water was moved (without turbulence) by maintaining some test chambers on a moving platform. Lobster were maintained in tanks of continuously aerated sea water containing phosphorus-contaminated mud (0.5, 1.0, or 10 percent contaminated mud). LT_{50} values were determined from the line of best fit describing the relationship between the probit of percentage mortality and the log of exposure time.

The macroinvertebrates were substantially more resistant to the effects of phosphorus than were the fish (Zitko et al. 1970). For beach fleas, incipient lethal levels were 3 to 4 mg/L (nominal concentration) in still water and 6.5 mg/L (nominal concentration) in moving water.

Beach fleas exposed to a phosphorus concentration of 8 mg/L for 3 hr recovered when transferred to clean water. For lobster, the incipient lethal level was not clearly defined but was likely between 20 and 40 μg/L (measured concentrations). In an experiment with lobsters similar to that of Fletcher et al. (1970), Fletcher (1971) determined that the LT_{50} was 620 hr (95 percent confidence interval = 563-682 hr) for lobsters exposed continuously to a measured phosphorus concentration of 23.0 ± 7.0 μg/L in a flow-through system.

Zitko et al. (1970), using lobsters, examined the relationship between the time-concentration phenomenon and toxicity. They reported that the product of time and concentration was more important than the individual times or concentrations in determining toxicity. For example, 4-day exposure to 120 μg/L (product of exposure duration and exposure concentration = 480 μg-days/L) produced an LT_{50} of 235 days, while 7-day exposure to 50 μg/L (product of exposure duration and exposure concentration = 350-μg days/L) produced an LT_{50} of 239 days. Additional experiments were conducted in which lobsters were exposed continuously or intermittently to 50 μg/L of yellow phosphorus. Lobsters continuously exposed to 50 μg/L had an LT_{50} of 239 hr, and those exposed in alternating periods of 24 hr of exposure and 24 hr in clean water had an LT_{50} of 240 hr of contact time (480 hr elapsed time). The authors concluded that the time-concentration phenomenon was consistent with the irreversibility of the toxic action of phosphorus.

3.2 CHRONIC TOXICITY TO AQUATIC ANIMALS

3.2.1 Fishes

In addition to acute toxicity bioassays, Bentley et al. (1978) conducted chronic tests in flow-through systems with fathead minnows. In the chronic tests, solvent controls were exposed to 7.2 mg/L DMSO, the amount used with the highest concentration of phosphorus. Negative controls received only dilution water. Phosphorus concentrations in the test waters were measured weekly during the first 4 weeks and every other week thereafter for the remainder of the tests. The mean measured concentrations were 0.40, 0.71, 1.5, 3.4, and 6.0 μg/L. The mean measured phosphorus concentrations during the tests were usually 60 to 80 percent of the nominal concentrations. During the chronic tests, water quality varied little among controls and treatments. Fifty eggs from the first ten spawns in each aquarium were tested in the corresponding test concentration. Eggs were transferred from control tanks into treatment tanks in which little or no spawning had occurred. In addition, eggs spawned in treated tanks were transferred to control tanks. Fry from the first two incubated spawns in each aquarium were maintained for 30 days, when percent survival, length, and total weight of the fry group were determined. Tests were discontinued after 241 days.

Phosphorus exposure reduced both survival and size of fathead minnow fry, depending on the duration of exposure. First-generation

hatchability and survival were not affected by 30-day exposure to concentrations as great as 6.0 μg/L. At 60 days, however, fry exposed to 6.0 μg/L were only one-half the size of control fry, and their survival was significantly reduced ($P \leq 0.05$). Between 60 and 150 days, significant reductions in survival were noted in the 3.4- and 1.5-μg/L exposure groups. None of the fish in the 6.0-μg/L treatment survived more than 150 days. By day 150, when the final spawning ratios were determined, the growth of all the fish surviving exposures to 3.4 and 1.5 μg/L was so stunted that internal and external evidence of sexual maturity was absent. By day 241, all remaining fish exposed to 3.4 μg/L had died, and survival in the 1.5 μg/L-exposure group was further reduced.

Male fathead minnows exposed to concentrations ≤ 0.71 μg/L were not significantly smaller than controls. Females exposed to 0.71 and 0.40 μg/L were significantly ($P \leq 0.05$) smaller than controls. The number of spawns, total eggs, spawns per female, eggs per female, and eggs per spawn did not differ between controls and groups exposed to 0.71 and 0.40 μg/L. However, hatchabilty of eggs spawned by minnows (second-generation eggs) exposed to 0.71 and 0.40 μg/L was significantly reduced ($P \leq 0.05$) when incubated in treatment water or when incubated in control water. The highest percent hatchability (16 percent in a 0.40-μg/L exposure group) was far less than the 90 percent or greater hatchability achieved in the controls.

Bentley et al. (1978) conducted critical-life-stage studies in flow-through systems with channel catfish and fathead minnows. Eggs and fry were exposed to each of five concentrations of elemental phosphorus, using DMSO as the carrier solvent. The measured concentrations were 6.8, 5.0, 3.6, 1.7, and 1.2 μg/L for channel catfish and 6.8, 2.5, 1.5, 0.7, and 0.6 μg/L for fathead minnows. Solvent controls were exposed to 510 mg/L DMSO. No explanation was given by the authors for this high solvent concentration.

Exposure of channel catfish eggs (50 eggs) began within 96 hr, and exposure of fathead minnow eggs (35 eggs) began within 24 hr of fertilization. Six o seven days after exposure of eggs began, randomly selected channel catfish (25) and fathead minnow (20) fry were transferred to larval growth chambers. Percent hatch and mean percent survival at 30 days were transformed using the arcsine square root transformation and then analyzed using analysis of variance. When treatment effects were significant, means of treatment groups were compared with controls using Dunnet's procedure as described in Steel and Torrie (1960).

At the highest test concentration, 6.8 μg/L, phosphorus exposure did not significantly affect percent hatch for either species (Bentley et al. 1978). For channel catfish, percent survival was significantly lower ($P \leq 0.05$) for fry exposed to a measured concentration of 6.8 μg/L (18 and 20 percent for replicates A and B, respectively, compared with 92-100 percent for all controls). Total length of channel catfish was significantly ($P \leq 0.05$) reduced at 6.8 μg/L (17 and 18 mm for A and B replicates, respectively, compared with 20-21 mm for all controls). In addition, fry exposed to 6.8 and 5.0 μg/L appeared jaundiced by the fifth day of exposure. For fathead minnows, a reduction in length

at concentrations of 1.5 μg/L or greater, was the only significant ($P \leq 0.05$) effect of exposure to elemental phosphorus. Length of control replicates ranged from 17 to 18 mm, and length of fry exposed to 1.5 μg/L or greater ranged from 14 to 16 mm.

3.2.2 Macroinvertebrates

Bentley et al. (1978) exposed Daphnia magna to measured concentrations of white phosphorus ranging from 0 to 8.7 μg/L. The first 3 days of exposure was static, but thereafter a flow rate of 50 mL per 25 min was maintained. Twenty water fleas (< 24 hr old) were initially added to each test aquarium; and on day 21, twenty young from each aquarium were retained for exposure of the second generation. Both negative and solvent controls (50 to 70 mg/L DMSO) were used, but no significant differences were observed between the two. The data were transformed using the arcsine square root transformation and analyzed using analysis of variance. Dunnet's procedure (Steel and Torrie 1960) was used to determine significant ($P \leq 0.05$) differences between treatments and controls.

During exposure of the first generation, 8.7 μg/L of white phosphorus significantly reduced survival. By day 21, 19 percent of the treated water fleas survived exposure to 8.7 μg/L (measured concentration) while 68 percent of the controls survived. The number of young per parthenogenic female was reduced from 6 in the controls to 2 in the 8.7-μg/L treatment group. Concentrations ≤ 6.9 μg/L caused no significant effects on survival or the number of young produced by first- or second-generation water fleas. The number of young produced per female, however, was much smaller than expected for both treatment and control groups. USEPA (1985) guidelines state that a chronic test may be invalid if the average cumulative number of young per female in the controls is < 20 after three broods.

Bentley et al. (1978) found it impossible to complete a two-generation study with Chironomus tentans, due to the failure to obtain fertile egg masses from any of the treatment groups or from the controls. However, continuous exposure of midge eggs and larvae to white phosphorus at concentrations from 0.14 to 2.0 μg/L (measured concentrations) for 8 days significantly reduced survival compared to controls. The authors then exposed midges for one generation (28 days) to a series of lower concentrations, from 0.005 to 0.067 μg/L, and observed no significant effects on survival, emergence, or the number of eggs produced per adult.

Chronic tests using midges exposed to elemental phosphorus through contaminated sediments were also performed by Bentley et al. (1978). Because of errors in preparing samples for quantifying elemental phosphorus and because of poor emergence and survival in both treatment and control groups, few conclusions can be made regarding exposure-response relationships. Bentley et al. (1978) stated that nominal concentrations ranging from 2 to 500 μg of phosphorus per kilogram of sediment appeared to cause a delay in development. By the end of the first generation, however, both percent emergence and percent survival were similar in

treatment and control groups. Midges inhabiting hydrosoil contaminated with 500 μg/kg did not produce eggs. Egg production did not differ significantly among other treatment and control groups.

3.3 TOXICITY TO PLANTS

Bentley et al. (1978) measured the effect of white phosphorus on cell numbers and chlorophyll a content of four phytoplankton species exposed to test concentrations determined from preliminary range-finding tests. Percent effect relative to the controls was calculated.

Response varied among the algae tested. White phosphorus inhibited the growth of Selenastrum capricornutum, a chlorophyte, and Navicula pelliculosa, a chrysophyte, but stimulated the growth of Anabaena flos-aquae and Microcystis aeruginosa, both of which are cyanophytes (blue-green algae). This pattern was indicated by changes in cell density and chlorophyll a content. Ninety-six-hour exposures to nominal concentrations ranging from 200 to 670 μg/L caused reductions in cell density of S. capricornutum ranging from 4 to 63 percent, and reduction in chlorophyll a content ranging from 7 to 68 percent. The reduction in cell density of N. pelliculosa exposed to concentrations ranging from 7 to 670 μg/L was 6 to 63 percent and reduction in chlorophyll a content was 18 to 97 percent. For M. aeruginosa and A. flos-aquae exposed to concentrations ranging from 7 to 670 μg/L, increases in cell densities were 0 to 48 percent and 11 to 41 percent, respectively; and increases in chlorophyll a content were 0 to 45 percent and 32 to 68 percent, respectively. The authors concluded that no clear exposure-response relationship was evident due to the variability of the data and to discrepancies between the real and nominal concentrations, which are more likely when using concentrations near the maximum solubility.

3.4 BIOACCUMULATION

Bioconcentration has been investigated in fishes, invertebrates, and seaweed. Bentley et al. (1978) investigated bioconcentration and elimination of elemental phosphorus by channel catfish (60 fish per treatment and control groups) and fathead minnows (100 fish per treatment and control groups) exposed to 0, 0.2, or 2.0 μg/L of phosphorus (nominal concentrations). Flow-through conditions were maintained, with temperature, dissolved oxygen, pH, hardness, and phosphorus measured weekly. Sampling of phosphorus residues in fish began on days 1 and 2 of exposure and continued weekly through day 47 for channel catfish and one group of fathead minnows. Fish were then transferred to diluent water for a 1-week depuration period, during which phosphorus residues were measured on days 2 and 7 following the transfer. In channel catfish, residues were measured in muscle, liver, and remaining tissues; in fathead minnows, residues were measured only in muscle and remaining tissues (including liver). Another group of fathead minnows remained in the exposure tanks for 77 days; ovaries from mature females were also analyzed in this group.

During bioconcentration studies, nominal concentrations of phosphorus provided a good estimate of measured concentrations. For the channel catfish study, 1.8 μg/L ± 0.37 (S.D.) and 0.18 μg/L ± 0.029 (S.D.) were measured for the nominal concentrations of 2.0 and 0.2 μg/L, respectively. For the fathead minnow tests, 2.2 μg/L ± 0.33 and 0.15 μg/L ± 0.046 were measured for the nominal concentrations of 2.0 and 0.2 μg/L, respectively. Less than 0.017 μg/L phosphorus was measured in the control tanks.

Both species exposed to the lower concentration (0.2 μg/L nominal) appeared healthy throughout the study. All fathead minnows exposed to 2.2 μg/L died between days 11 and 24, and about one-third of the channel catfish exposed to 1.8 μg/L died between days 24 and 47. Fish from both species developed edema, jaundiced livers, and bright green or yellow intestines. Channel catfish exposed to 1.8 μg/L rapidly accumulated phosphorus, with muscle concentrations rising to 106 times the water concentration within 2 days of initiating exposure. Bioconcentration remained in the range of 50 to 100 times ($\bar{X}$ = 67.7 ± 29.9) the water concentration throughout the study (Table 4). Phosphorus concentrations in the liver were slightly lower than those in muscle, with a mean bioconcentration factor (BCF) of 51.5 ± 17.9 following exposure to 1.8 ± 0.37 μg/L (Table 4).

In fathead minnows, a mean BCF in muscle of 35.9 ± 41.3 was calculated for the 47-day exposure to 2.2 ± 0.33 μg/L (Table 4). A peak BCF of 155 (muscle) was observed following 2 days exposure to 2.2 μg/L. After 77 days of exposure to phosphorus, muscle and other tissues in fathead minnows contained 127 and 200 times, respectively, the mean water concentration. Phosphorus was rapidly lost during the depuration period, falling to undetectable levels by day 7.

Bentley et al. (1978) noted many of the fish dying at night during the study. Phosphorus content in dead fish was sometimes substantially lower than that in fish killed at the same time or at other times. The authors suggested that decomposition affected the residues in fish tissues at the time of sampling, which occurred in some cases hours after the fish died.

Dyer et al. (1970) also reported rapid uptake of white phosphorus by Atlantic cod (Gadus morhua) maintained in seawater. However, in contrast to Bentley et al. (1978), Dyer et al. (1970) reported greater bioaccumulation in liver than in muscle. Sixteen-hr exposures to average concentrations of 21 to 83 μg/L resulted in bioconcentration factors of 880-2,000 in liver and 9-26 in white muscle (Table 4). In a preliminary experiment, Dyer et al. (1970) noted that measured concentrations in tanks containing fish were only 1 to 2 ppb, while concentrations of 20 to 30 ppb were reached in tanks without fish; therefore, uptake by the fish was rapid and extensive. Tissue uptake was roughly in proportion to tissue lipid content. The concentration factor was greater at low concentrations of phosphorus, possibly because the gill transport mechanism was damaged at higher concentrations (Dyer et al. 1970).

TABLE 4. BIOCONCENTRATION OF WHITE PHOSPHORUS BY AQUATIC ORGANISMS

Species	Exposure Concentration (µg/L)	Temperature (°C)	Tissue Concentration[a] (µg/kg)	Bioconcentration Factor	References
Fishes					
Ictalurus punctatus	1.8 ± 0.37	20[b]	152.6 ± 106.3[c] (M)	67.7 ± 29.9[d]	Bentley et al. 1978
	1.8 ± 0.37	20[b]	92.7 ± 46.3[e] (L)	51.5 ± 17.9[d]	Bentley et al. 1978
Pimephales promelas	2.2 ± 0.33	20[b]	79.0 ± 90.8[e] (M)	35.9 ± 41.3[f]	Bentley et al. 1978
Gadus morhua	4.4 until death	11	150 µg/g (M)[g]	34.1	Maddock and Taylor 1976
	4.4 until death	11	1,600 µg/g (L)[g]	363.6	Maddock and Taylor 1976
	15.4 until death	11	280 µg/g (M)[g]	18.2	Maddock and Taylor 1976
	15.4 until death	11	3,670 µg/g (L)[g]	238.3	Maddock and Taylor 1976
	21.0 ± 3.0/16 hr	9-10	551 ± 10.5 (WM)	26	Dyer et al. 1970
	21.0 ± 3.0/16 hr	9-10	42,000 ± 13,000 (L)	2,000	Dyer et al. 1970
	24.0 until death	11	280 µg/g (M)[g]	11.7	Maddock and Taylor 1976
	24.0 until death	11	6,000 µg/g (L)[g]	250.0	Maddock and Taylor 1976
	25 ± 6.0/16 hr	9-10	528 ± 22 (WM)	18	Dyer et al. 1970
	25 ± 6.0/16 hr	9-10	35.5 µg/g (L)	1,200	Dyer et al. 1970
	35.4 ± 7.7/16 hr	9-10	320 ± 38 (WM)	9	Dyer et al. 1970
	35.4 ± 7.7/16 hr	9-10	48,700 ± 6,200 (L)	1,400	Dyer et al. 1970
	47.9 ± 9.8/16 hr	9-10	488 ± 41 (WM)	10	Dyer et al. 1970
	47.9 ± 9.8/16 hr	9-10	75,400 ± 13,000 (L)	1,600	Dyer et al. 1970
	76.3 ± 11.6/16 hr	9-10	940 ± 75 (WM)	12	Dyer et al. 1970
	76.3 ± 11.6/16 hr	9-10	76,000 ± 23,000 (L)	1,000	Dyer et al. 1970
	83.2 ± 12/16 hr	9-10	908 ± 94 (WM)	11	Dyer et al. 1970
	83.2 ± 12/16 hr	9-10	73,500 ± 16,000 (L)	880	Dyer et al. 1970
Invertebrates					
Asterias vulgaris	15 ± 9.0/48 hr	9-11	400	26.7	Fletcher 1971
Arctica islandica	15 ± 9.0/48 hr	9-11	262 ± 39	17.5	Fletcher 1971
Homarus americanus	15 ± 9.0/48 hr	9-12	19,000 ± 1,590 (HP)	1,267	Fletcher 1971
	15 ± 9.0/48 hr	9-12	508 ± 14 (TM)	33.9	Fletcher 1971
	15 ± 9.0/48 hr	9-12	388 ± 30 (CM)	25.9	Fletcher 1971
Littorina littorea	15 ± 9.0/48 hr	9-11	637 ± 79	42.5	Fletcher 1971
Mya arenaria	15 ± 9.0/48 hr	9-11	338 ± 35	22.5	Fletcher 1971
Mytilus edulis	15 ± 9.0/48 hr	9-11	157 ± 14	10.5	Fletcher 1971
Seaweed					
Fucus vesiculosis	15 ± 9.0/48 hr	9-11	332 ± 36	22.1	Fletcher 1971
Fucus distichus	15 ± 9.0/48 hr	9-11	342 ± 40	22.8	Fletcher 1971

a. M = Muscle tissue,
L = Liver,
WM = White muscle,
HP = Hepatopancreas,
TM = Tail muscle,
CM = Chela muscle.

b. Temperature not given, but Bentley et al. (1978) conducted other tests with the species at 20°C.

c. Mean ± S.D. of tissue concentrations from days 1, 2, 7, 14, 21, 28, 33, 34, 35, 37, and 47 of exposure. (Calculated by P. Hovatter).

d. Mean ± S.D. of BCF's for tissue concentrations at days 1, 2, 7, 14, 21, 28, 33, 34, 35, 37, and 47 of exposure. (Calculated by P. Hovatter).

e. Mean ± S.D. of tissue concentrations from days 1, 2, 7, 12, 13, 14(2), 15, 16, 19, 20, and 21(2). (Calculated by P. Hovatter).

f. Mean ± S.D. of individual BCF's for tissue concentrations from days 1, 2, 7, 12, 13, 14(2), 15, 16, 19, 20, and 21(2). (Calculated by P. Hovatter).

g. Maddock and Taylor (1976) reported tissue concentrations in µg/g.

Maddock and Taylor (1976), in a study primarily designed to examine the lethality of phosphorus, also investigated accumulation by Atlantic cod. Fish were exposed to concentrations of white phosphorus ranging from 4.4 to 29.0 μg/L until death. Because some animals remained in the treatment tanks several hours after death and the tissues were frozen 1 to 10 days prior to analysis, the authors treated their results with caution. The BCF for liver remained relatively constant over all exposure concentrations, ranging from 115 to 364 (Table 4). The BCF for muscle decreased with increasing concentration, ranging from 34.1 at 4.4 μg/L exposure to 10.3 at 29.0 μg/L exposure (Table 4). For two fish exposed to 24 μg/L phosphorus until death (28 hr), the concentrations in different areas of muscle tissue were 320 and 400 μg/g wet weight (BCF = 13.3 to 16.7) in one fish and 360 and 390 μg/g wet weight (BCF = 15.0 to 16.2) in the other fish. The highest concentrations were observed in the livers of the two fish, 6,600 (BCF = 275) and 6,125 (BCF = 255) μg/g wet weight, respectively. The brain showed the next highest concentration, with 1,500 (BCF = 625) and 1,410 (BCF = 58.8) μg/g, respectively. The BCF for muscle tissue was roughly in agreement with those reported by others (Table 4); however, the concentration factor for liver tissue was an order of magnitude lower, a discrepancy possibly attributable to losses during storage, according to Maddock and Taylor (1976). Dyer et al. (1972) reported a significant decline in phosphorous concentrations during frozen storage of fish tissue containing > 500 ng/g. As in other studies, Maddock and Taylor (1976) observed rapid depuration (half-lives of about 1 hr for both muscle and liver) when Atlantic cod were transferred to clean water.

Fletcher (1971) determined the accumulation of yellow phosphorus by Asterias vulgaris (starfish, N = 7), Homarus americanus (American lobster, N = 5), Littorina littorea (periwinkle, N = 15), Mya arenaria (soft-shell clams, N = 8), Arctica islandica (ocean quahogs, N = 6), Mytilus edulis (blue mussels, N = 30 to 40), Fucus distichus evanescens (seaweed), and Fucus vesiculosis (seaweed). The organisms were acclimated 24 hr prior to adding 2 mg/mL of phosphorus in ethanol to the tank at a rate of 2.4 mL/hr for 48 hr. At the end of 48 hr, some organisms were sampled for phosphorus content, and some were transferred to fresh seawater for a 7-day depuration period. Organisms exposed to a measured (by the method of Addison and Ackman 1970) phosphorus concentration of 15 ± 9.0 (S.D.) μg/L for 48 hr contained roughly 10 to 40 times the concentration of phosphorus in the seawater (Table 4). After 7 days in seawater free of phosphorus, no yellow phosphorus was detected in any species tested (limit of detection 0.002 μg/g).

Fletcher (1971) suggested that the higher BCF in periwinkle may reflect its feeding habits and fat content. Unlike the other invertebrates tested, which are filter feeders, periwinkle graze on seaweed, which may provide a higher phosphorus concentration in the food source. In addition, periwinkle contain a higher percentage lipid, possibly associated with greater accumulation of yellow phosphorus.

In a second experiment, Fletcher (1971) continuously exposed five lobsters to 23.0 ± 7.0 μg/L phosphorus until death. Water concentrations

of phosphorus were determined two to three times daily using the gas-liquid chromatographic method of Addison and Ackman (1970). The distribution of phosphorus in various tissues was similar to that observed in 48-hr experiments with exposure to 15 μg/L. Highest concentrations were reported for the hepatopancreas, with a mean of 19 μg/g in the 48-hr exposure and 45.3 μg/g for the chronic exposure, and for the ovary, with a mean of 4.01 μg/g in the 48-hr exposure and 9.47 μg/g in the chronic exposure. In descending order of concentration were tail muscle, chela muscle, which was significantly lower than that of the tail ($P < 0.05$), gill, and hemolymph, which contained levels only 4 to 5 times greater than that in the exposure water. Fletcher (1971) suggested that concentration was associated with lipid content, because the relative lipid content of various crustacean organs, in descending order, is hepatopancreas ≥ ovary > muscle > hemolymph.

Fletcher (1973), in a study of the toxicity of phosphorus-contaminated fish, contributed to the understanding of the environmental significance of the bioconcentration of phosphorus. He determined that phosphorus-contaminated cod muscle (4-11 μg/g) and liver (194 μg/g) fed to brook trout elicited the same symptoms of phosphorus poisoning as did phosphorus-contaminated water. The approximate lethal dosage ranged from 1.23 to 2.73 mg of white phosphorus per fish. However, only small amounts of phosphorus consumed in the diet were retained in tissue. Fletcher suggested that although contaminated fish are a potential source of lethal phosphorus exposure to predators, bioaccumulation through the food chain is not likely.

3.5 OTHER DATA

3.5.1 Cardiovascular Effects

Several researchers reported cardiovascular changes in fishes associated with phosphorus exposure. These changes, which include excessive hemolysis, yellow or green fluid in the intestine, and abnormal condition of the gills and liver, were first reported in herring in Placentia Bay, Newfoundland. The condition was traced to white phosphorus that was released from a phosphorus reduction plant in the area (Pippy et al. 1972). The symptoms were documented in experimental exposures of fish to white phosphorus. Zitko et al. (1970) noted redness in herring that started at the base of the fins and spread to other parts of the body as exposure times and concentrations increased. Examination after death caused by asphyxiation revealed extensive hemolysis and pale internal organs and blood. The hematocrits, which are normally 37 to 40 percent, approached zero. Although Atlantic salmon were more resistant than Atlantic cod, symptoms similar to those shown by herring occurred.

Fletcher et al. (1970) reported similar symptoms for brook trout exposed to yellow phosphorus concentrations of 0.5 and 7.0 μg/L for 50 to 100 hr. The extent of redness at death was associated with the degree of hemolysis. Lower hematocrits were associated with higher phosphorus concentration. The relationship was defined by the equation

Y = 13.5 + 10.4 log X, where Y is hematocrit and X is phosphorus concentration, and the correlation is r = 0.848. However, fish that died following exposure to low concentrations for longer time periods exhibited low hematocrits. Regression analysis of hematocrit (Y) as a function of time to death (X) gave a correlation (r) of 0.854 for the relationship Y = 60.4 - 21.2 log X, substantiating the inverse relationship between hematocrit and duration of exposure.

Fletcher and Hoyle (1972) reported similar findings for Atlantic salmon. Fish surviving 20 to 70 hr of exposure to concentrations of 40 μg/L or less displayed the greatest degree of redness and hemolysis. The lowest hematocrits were associated with the longest exposures. In contrast, neither Osmerus mordax (smelt) (Fletcher et al. 1970) nor Atlantic cod (Fletcher and Hoyle 1972, Maddock and Taylor 1976) showed evidence of external redness or of hemolysis.

Interspecies variation in sensitivity to phosphorus was evident in the observations in Placentia Bay. Although herring, the most severely affected species, showed extensive external signs of hemolysis and a decline in population of 80 percent from February to March 1969 (Hodder et al. 1972), many other species, such as Tautogolabrus adspersus (cunner), Pseudopleuronectes americanus (winter flounder), and Alosa pseudoharengus (alewife), caught with the affected herring had normal or reasonably high hematocrits (Odense et al. 1972).

Odense et al. (1972) evaluated histological changes in herring experimentally exposed to white phosphorus or exposed to white phosphorus in Placentia Bay, Newfoundland. They reported destruction of the epithelial lining of the gill and disruption of the gill tissue. In the kidney, interstitial tissue was edematous, and the tubular epithelium was shrunken and vacuolated. Tubular necrosis was also observed. Edema and vacuolation of the liver parenchyma and of the spleen were also noted. The authors concluded that white phosphorus is a potent cytotoxin that acts through the disruption of cell and nuclear membranes.

Zitko et al. (1970) reported cardiovascular changes and unusual behavior during the 12 hr prior to death for lobsters exposed to phosphorus. Symptoms included loss of muscle tone, lethargy, jerky movement of the periopods, and, eventually, loss of neuromuscular response. Examination after death revealed various degrees of coagulation of the blood and, in some cases, distention of the heart.

Aiken and Byard (1972) investigated the cause of phosphorus-induced death in their study of histological changes in lobsters associated with phosphorus exposure. Test animals were maintained in 200-L tanks of continuously aerated seawater and phosphorus-contaminated mud. Controls (2) were killed by asphyxiation in oxygen-free seawater. In phosphorus-exposed lobster, only the hepatopancreas and antennal gland showed evidence of degeneration. The blood in the cardiogastric region coagulated in the final hour of phosphorus exposure. Aiken and Byard (1972) suggested that blood coagulation, although likely the direct cause of

death, was not a direct effect of phosphorus exposure but an indirect result of the damage to the antennal gland and hepatopancreas.

3.5.2 Field Studies

The impact of white phosphorus on natural ecological communities provides additional evidence of its extreme toxicity. Although these data cannot be used directly to derive aquatic criteria, they demonstrate the need to adequately protect natural ecosystems and provide evidence of concentrations necessary to protect aquatic biota.

Pearson et al. (1976) studied the benthos of Yellow Lake, Pine Bluff Arsenal, Pine Bluff, Arkansas. As is common with other studies of this type, several factors complicated interpretation of data. First, in addition to white phosphorus, Yellow Lake received dichlorodiphenyltrichloroethane (DDT) from an eroding landfill site, and second, the variables that apparently control the distribution and abundance of some biota in the lake, low pH and low dissolved oxygen, are associated with decaying vegetation. Pearson et al. (1976) measured concentrations of white phosphorus using gas-liquid chromatography and a flame photometer equipped with a detector specific for elemental phosphorus. The authors summarized the distribution and abundance data for benthic fauna using the Shannon diversity index and the Pinkham-Pearson index of biotic similarity and compared the values using cluster analysis. Significant factors associated with the observed differences were identified using principal factor analysis.

Pearson et al. (1976) reported that concentrations of phosphorus and phosphates were the most significant chemical factors governing the distribution and abundance of benthic species. The authors evaluated biotic similarity and diversity indices for different areas of the lake. Many differences were explained by variations in habitat (open water vs. littoral areas). During specific months of the year and in the data set as a whole, the authors noted differences among stations that could not be explained by variations in habitat. The two stations closest to the white phosphorus outfall were generally similar to one another but different from other stations ($P < 0.05$). These stations exhibited the lowest diversity, while other stations of similar habitat but lower phosphorus concentrations showed the highest mean diversities. The authors reported that the occurrence of one species, Limnodrilus hoffmeisteri, was positively correlated with the concentration of elemental phosphorus and phosphates, and species diversity was negatively correlated with these factors. Using these relationships, a critical threshold for community change of 0.25 to 4.0 or 5.0 $\mu g/L$ was derived.

In a study of the creeks entering Yellow Lake, Manuel et al. (1976) examined benthos of White Phosphorus Creek using the same reference area used by Pearson et al. (1976). Because the study area was within White Phosphorus Creek instead of in the backwaters of Yellow Lake, the data from the study of Manuel et al. (1976) are not comparable with those of Pearson et al. (1976). Manuel et al. (1976) described White Phosphorus Creek as a drainage ditch, with portions of the stream containing only

bacterial scum and blue-green algal mats. The study of Manuel et al. (1976) was confounded by unusually high rainfall that resulted in the scouring of invertebrates from the artificial substrate samplers. The authors reported that the reference areas had among the highest mean diversities (2.20) and White Phosphorus Creek had the lowest (0.24). Cluster analysis of similarity indices revealed three groups: stations within a creek that received gross pollution, two stations within White Phosphorus Creek, and all stations that did not receive gross pollution.

Surveys of Long Harbor, Placentia Bay, Newfoundland, also revealed that white phosphorus released into natural waterways is toxic. Peer (1972) examined sediments and benthic fauna near the effluent pipe of a phosphate reduction plant and reported selective mortality among species. The only live benthic species collected in the vicinity of the outfall was Modiolus modiolus, sea mussel. A more distant station showed reduced biomass and diversity. Scallop mortalities were observed for 1,000 m from the pipe. Five percent of a population of sand dollars (Echinarachnius parma) were surviving in an area where 90 percent would normally be alive. The burrowing sea anemone (Edwardsia sp.) was relatively unaffected.

Hodder et al. (1972), using gill-net surveys, reported an 80-percent decline in abundance of the herring population of Placentia Bay over a 1-month period. Mass mortalities of herring occurred, and, at one point, 80 percent of the population caught in one area were red, a sign of phosphorus exposure. The mortalities resulted in a decreased yield in nearby St. Mary's Bay, where herring migrate to reproduce.

3.6 SUMMARY

Results of toxicity tests for a variety of aquatic organisms suggest that white phosphorus is potentially quite toxic. In static tests with fishes, bluegill sunfish was the most sensitive species, with a 96-hr LC_{50} of 6 $\mu g/L$, and channel catfish was the least sensitive, with a 96-hr LC_{50} of 73 $\mu g/L$. In dynamic bioassays, fish were even more sensitive to white phosphorus. The LC_{50} for bluegill was 2.4 $\mu g/L$, and that for channel catfish was 19 $\mu g/L$. The most sensitive life stages for fathead minnows were 30-day-old (LC_{50} = 21 $\mu g/L$) and 60-day-old (LC_{50} = 18 $\mu g/L$) fry.

Incipient lethal levels, considered by Sprague (1969) to be the most useful single index of toxicity, were difficult to determine. Literature values for incipient lethal levels ranged from 0.6 $\mu g/L$ for bluegill to 18 $\mu g/L$ for Atlantic salmon.

Estimates of LT_{50}'s were made for even the lowest concentrations tested. The LT_{50} for brook trout exposed to 0.5 $\mu g/L$ was 121 hr. The LT_{50} for Atlantic salmon exposed to 0.79 $\mu g/L$ was 195 hr. The toxicity of white phosphorus appears to be cummulative. Fish exposed to white phosphorus and then transferred to clean water show the same LT_{50} as those exposed continuously to white phosphorus.

Several authors suggested that the product of exposure duration times exposure concentration was more important than either individual variable. Regression analysis of continuous versus brief exposure of cod and salmon to white phosphorus indicates that short exposures to high concentrations are lethal within the same time period as continuous exposure to low concentrations.

Studies indicated that water quality has a minimal effect in determining toxicity; temperatures from 15 to 25°C, hardness of 35 or 100 mg/L, and pH of 7 or 8 had little influence on the toxicity of white phosphorus during static conditions. Hard water (250 mg/L) and decreased pH (6.0) decreased toxicity by an order of magnitude.

With the exception of Daphnia magna, macroinvertebrates were substantially more resistant to white phosphorus than were fishes. Estimates of static 48-hr EC50 values (immobilization) for macroinvertebrates ranged from 30 μg/L for Daphnia magna to > 560 μg/L for Asellus militaris. Estimates of LC50's from flow-through tests were not significantly different from estimates from static tests. However, incipient LC50's for macroinvertebrates were substantially lower, ranging from 5 to 24 μg/L.

As with fish, the toxicity of white phosphorus can be expressed as a time-concentration phenomenon. Estimates of LT50's for lobsters were the same for the same total exposure (duration x concentration) whether exposures were intermittent or continuous.

Chronic white phosphorus exposure of the fathead minnow reduced survival in exposures as low as 1.5 μg/L. By day 150, the growth of all the fish surviving exposures to 3.4 and 1.5 μg/L was so stunted that internal and external evidence of sexual maturity was absent. Even at the lowest concentration tested, 0.4 μg/L, hatchability was significantly reduced ($P = 0.05$).

In critical life-stage studies, 30-day exposure of channel catfish and fathead minnow fry did not significantly affect the percent hatch of either species at 6.8 μg/L, the highest test concentration; however, total length and survival of catfish were significantly reduced at this concentration. Length was significantly reduced in fathead minnow fry exposed to 1.5 μg/L or greater concentrations.

In chronic studies with macroinvertebrates, exposure of D. magna to 8.7 μg/L significantly reduced survival. Concentrations < 6.9 μg/L did not significantly affect survival or the number of young produced by first and second generations. The numbers of young produced, however, were lower than expected in both treatment and control groups.

Limited studies of the toxicity of white phosphorus to algae reveal variable results. The growth of two species of blue-green algae, Anabaena flos-aquae and Microcystis aeruginosa, was stimulated, but the growth of Navicula pelliculosa, a diatom, and Selenastrum capricornutum, a green algae, was inhibited.

Bioaccumulation studies indicated rapid and extensive uptake by a variety of aquatic organisms. Uptake appears to be associated with lipid content. Uptake by liver in fishes was greatest, with reported bioconcentration factors of 52 to 2,000. Bioconcentration factors in fish muscle ranged from 9 to 68. Studies with the lobster indicated greatest uptake by the hepatopancreas, with a bioconcentration factor of 1,267. Bioconcentration factors in invertebrates, other than the lobster, ranged from 10 to 43, and in seaweed the bioconcentration factor was approximately 22. Depuration is also rapid, with white phosphorus falling to undetectable levels by 7 days post-exposure.

Although the mechanism of action has not been definitively identified, white phosphorus produced a number of cardiovascular changes. Symptoms included externally visible redness, hemolysis, low hematocrits, and pale internal organs and blood. The extent of redness at death was associated with the degree of hemolysis. The lowest hematocrits were associated with long, low-level exposures. Hemolysis was observed both in fish and in lobsters, the only invertebrate tested. Substantial variation in response existed among species. Herring was the most severely affected aquatic species in the natural environment.

Histological changes, including damage to the gill, kidney, liver parenchyma cells, and spleen, indicated that white phosphorus is a potent cytotoxin that acts through the disruption of cell and nuclear membranes. In lobster, exposure to white phosphorus caused degeneration of the hepatopancreas and antennal gland and coagulation of the blood.

Field studies provided further support for the extreme toxicity of white phosphorus. White phosphorus was the most significant chemical factor governing the distribution of benthic organisms in Yellow Lake, Pine Bluff Arsenal, Arkansas. The distribution of one species, Limnodrilus hoffmeisteri, was positively correlated with white phosphorus concentration. Using these relationships, the critical threshold for community change of 0.25 to 5.0 μg/L was derived. White phosphorus has been associated with reduced biomass and diversity in the benthic community and with mass mortalities of herring in Placentia Bay, Newfoundland.

4. MAMMALIAN TOXICOLOGY AND HUMAN HEALTH EFFECTS

4.1 PHARMACOKINETICS

4.1.1 Animal Data

The uptake, absorption, distribution, accumulation, and elimination of white phosphorus ultimately determine the systemic effects of this agent. Several studies have been carried out to determine if the pharmacokinetics of phosphorus reflects the toxicity with respect to known target organs. Cameron and Patrick (1966) administered radioactive phosphorus (^{32}P) by gastric intubation to mice, rats, and rabbits in order to study the distribution of phosphorus when given in toxic amounts. Mice were given 0.5 mg of [^{32}P]-phosphorus containing 0.1 mCi (millicurie), rats 3.5 mg containing 0.35 mCi, and rabbits 20.0 mg containing 1.0 mCi. Forty-eight hours after intoxication, the animals were killed and the radioactive content in tissue homogenates was analyzed by liquid scintillation spectrometry. The distribution of [^{32}P]-phosphorus was fairly uniform across species. The relative distribution, based on nCi/mg of tissue, was blood >> feces > bowel > liver > kidney ≥ spleen > lung ≥ heart. The distribution was slightly but not significantly different after the organs were perfused to remove blood; liver, kidney, spleen, and lungs contained more radioactivity than other organs.

Liver, kidney, heart, and brain were fractionated into dry residue, water-soluble, and fat-soluble fractions. Almost all of the [^{32}P]-phosphorus was recovered in the dry residue in liver, kidney, and heart, whereas almost all of the radioactivity in the brain was recovered in the fat-soluble fraction. More radioactivity was recovered in the fat-soluble fraction of liver, kidney, and heart than in the water-soluble fraction.

Ghoshal et al. (1971) administered 0.75 mg of phosphorus spiked with 10 μCi of ^{32}P to male Wistar rats by gastric intubation. Within 15 min, less than 5 percent of the radioactivity was detected in blood and liver; within 2 to 3 hr, however, approximately 65-70 percent of the administered dose was recovered in liver, and approximately 40 percent was recovered in liver at 10 hr. The blood levels paralleled but were much lower than the levels in liver. The recovery of radioactivity from other organs at 2 hr was as follows: blood, 12 percent; kidneys, 4 percent; spleen, 0.4 percent; pancreas, 0.4 percent; and brain, 0.39 percent. Therefore, approximately 82 percent of the administered dose was absorbed within 2 hr.

The distribution of radioactivity in hepatic subcellular fractions 2 hr after treatment, expressed as the percentage of total hepatic radioactivity, was as follows: supernatant fraction, 54 percent; microsomal fraction, 18 percent; mitochondrial fraction, 10 percent; and nuclear fraction, 16 percent. Approximately 1.4 to 3 percent of the total administered dose of radioactivity was recovered in hepatic lipids. Almost all of the radioactivity in each fraction, except the

microsomal fraction, was nonprecipitable with 10 percent trichloroacetic acid (TCA). The largest amount of radioactivity in microsomes was recovered in the TCA-precipitable fraction (Ghoshal et al. 1971).

Lee et al. (1975) administered 0.1 percent [^{32}P]-white phosphorus in peanut oil to Charles River rats by gastric intubation. The distribution and excretion from 4 hr to 5 days after treatment are shown in Table 5. Within 4 hr, 41 percent of the administered dose was absorbed; within 24 hr, approximately 68 percent of the dose was absorbed, at which time absorption was essentially complete. A large amount of radioactivity was recovered in the liver, with significant amounts also recovered from blood and skeletal muscle.

TABLE 5. DISTRIBUTION AND EXCRETION OF RADIOACTIVITY IN RATS RECEIVING [^{32}P]-WHITE PHOSPHORUS BY GASTRIC INTUBATION[a]

	% of Administered Dose		
	4 Hours	1 Day	5 Days
Gastrointestinal tract plus contents	57.0 ± 3.4[b]	15.3 ± 4.0	1.7 ± 0.2
Feces	2.0 ± 1.0	16.6 ± 3.8	33.0[c]
Whole blood[d]	6.1 ± 1.1	4.1 ± 0.5	1.7 ± 0.0
Urine	17.1 ± 2.2	34.5 ± 6.1	46.7[c]
Liver	16.1 ± 4.6	16.9 ± 0.7	6.3 ± 0.3
Kidneys	0.7 ± 0.2	0.8 ± 0.1	0.4 ± 0.0
Spleen	0.1 ± 0.0	0.1 ± 0.0	0.1
Brain	0.1 ± 0.0	0.1	0.1
Lungs	0.4 ± 0.0	0.3 ± 0.1	0.2 ± 0.0
Skeletal muscle[e]	4.0 ± 0.0	5.5 ± 0.2	6.0 ± 0.6
Recovery	98.6 ± 5.0	94.0 ± 3.3	96.0

a. Adapted from Lee et al. 1975.
b. Mean ± S.E. of three rats.
c. Pooled samples from three rats.
d. Based on 7.0% of the body weight.
e. Based on 40% of the body weight.

Accumulation of [^{32}P]-white phosphorus was evident by the high ratios of tissue to blood levels of radioactivity; the highest ratios were calculated for liver, bone, and kidney (Table 6). A comparison of the level of radioactivity that accumulated in various organs 24 hr after a single dose and after 5 consecutive daily doses of [^{32}P] white phosphorus is shown in Table 7. Liver, kidney, and bone accumulated the highest levels, whereas brain and lungs had the highest ratios (Lee et al. 1975).

TABLE 6. TISSUE/PLASMA RATIOS OF RADIOACTIVITY IN RATS RECEIVING A SINGLE DOSE OF [^{32}P]-WHITE PHOSPHORUS BY GASTRIC INTUBATION[a]

Tissue	Tissue/Plasma Radioactivity Ratio[b]		
	4 Hours	1 Day	5 Days
Liver	18.7 ± 2.5[c]	51.4 ± 3.9	103.2 ± 10.0
Kidneys	4.2 ± 1.0	14.4 ± 1.2	33.5 ± 4.1
Spleen	1.8 ± 0.4	6.4 ± 2.6	18.6 ± 2.6
Brain	0.3 ± 0.0	0.7 ± 0.0	3.6 ± 0.4
Lungs	2.6 ± 0.1	5.8 ± 0.5	16.5 ± 1.0
Skeletal muscle	0.4 ± 0.0	1.8 ± 0.1	8.7 ± 0.5
Bone	1.7 ± 0.1	12.7 ± 0.1	66.9 ± 17.2

a. Adapted from Lee et al. 1975.
b. Radioactivity in 1 mL or 1 g of wet tissue per radioactivity in 1 mL of plasma.
c. Mean ± S.E. of three rats.

TABLE 7. ACCUMULATION OF RADIOACTIVITY IN RATS RECEIVING [^{32}P]-WHITE PHOSPHORUS BY GASTRIC INTUBATION[a]

	^{32}P 24 Hours After a Single Dose (dpm/g x 10^{-5})	^{32}P 24 Hours After the Last of Five Daily Doses (dpm/g x 10^{-5})	Ratio[b]
Blood	2.91[c]	28.42	9.8
Liver	19.30	79.44	4.1
Kidneys	5.44	39.29	7.2
Spleen	2.38	17.70	7.4
Brain	0.25	2.63	10.5
Lungs	2.18	22.21	10.2
Skeletal muscle	0.68	5.90	8.7
Bone	4.77	36.81	7.7

a. Adapted from Lee et al. 1975.
b. Radioactivity per gram of wet tissue from rats receiving five consecutive daily doses assayed 24 hr after the last dose divided by radioactivity per gram of wet tissue from rats receiving a single dose assayed 24 hours after dosing.
c. Average of three rats.

No information was found on the pharmacokinetics of white phosphorus administered by inhalation. Dalhamn and Holma (1959) studied the retention and systemic distribution of radioactive red phosphorus administered to mice by inhalation of aerosols of red phosphorus dust. Because the animals were exposed to particulates of red phosphorus, these results may also be valid for particulates of white phosphorus. Fifteen mice were exposed for 1 hr and killed immediately after or 20 min, 2 hr, 48 hr, or 10 days after exposure. The distribution of radioactivity was determined by whole-body autoradiography. Radioactivity was detected in lungs throughout the experimental period, whereas radioactivity in the digestive tract was not detected 48 hr or 10 days after exposure. At no time was radioactivity detected in systemic organs.

Whiteley et al. (1953) studied the uptake of radioactive phosphorus by rabbit skin following intravenous administration. Thirty-six Agouti and Chinchilla rabbits of both sexes were injected with 75 μCi/kg of radioactive phosphorus and killed at intervals between 5 min and 72 hr after injection; the skin was analyzed for radioactivity by autoradiography. Within 5 min after injection, radioactivity was taken up by the skin, with more taken up by the areas of active hair growth than by quiescent areas. This difference was maintained throughout the observation period, but the ratios of the density of radioactivity in areas of active and quiescent hair growth increased during this time, 1.65 at 5 min, 2.4 at 9 hr, 2.7 at 24 hr, and 3.4 at 72 hr. The difference between the two areas was attributed to the greater incorporation of ^{32}P in nucleic acids in the areas of active hair growth.

Systemic effects (hemoglobinemia, hemoglobinuria) observed after white phosphorus burns suggest that phosphorus is absorbed through the skin. Walker et al. (1969) burned white phosphorus pellets (25 mg) on the skin of a young pig. The residue on the skin was 24 percent acids of phosphorus, 93 percent of which was orthophosphoric acid. Approximately 2.71 mg of the phosphorus penetrated the skin as orthophosphoric acid. Nevertheless, phosphorus did not penetrate the skin beyond 2.0 mm from the surface, as determined by increases in phosphate levels. Thus, Walker et al. (1947) did not find evidence of systemic absorption of white phosphorus.

Phosphorus is excreted in urine and feces (Cameron and Patrick 1966, Lee et al. 1975). Forty-eight hours after dosing mice, rats, and rabbits by gastric intubation with [^{32}P]-phosphorus, radioactivity appeared in urine of rabbits but not in that of mice and rats. Radioactivity was also found in feces in all three species (Cameron and Patrick 1966). Lee et al. (1975), however, found that 17.1 percent of the administered dose of [^{32}P]-white phosphorus appeared in urine of Charles River rats 4 hr after dosing (Table 5). At 1 and 5 days after dosing, 34.5 percent and 46.7 percent, respectively, appeared in urine. The fecal content of [^{32}P]-white phosphorus was 2.0 percent, 16.6 percent, and 33 percent at 4 hr, 1 day, and 5 days, respectively. Lee et al. (1975) did not determine if the radioactivity in fecal material was due to direct elimination from the gastrointestinal tract or the result of biliary excretion.

Thin-layer chromatography of the urine collected from rats administered [^{32}P]-white phosphorus separated two major radioactive components; one was inorganic phosphate and the other was a more nonpolar component suggestive of organic phosphate (Lee et al. 1975). These results suggest that white phosphorus is incorporated into both inorganic and organic compounds.

4.1.2 Human Data

There is no direct information on the pharmacokinetics of white phosphorus in humans. That white phosphorus is absorbed from the gastrointestinal tract following oral ingestion of substances containing white phosphorus, such as rat and roach paste, can be inferred from the toxic systemic effects (Polson et al. 1983). Diaz-Rivera et al. (1950) suggested that absorption of phosphorus may be facilitated when the element is ingested in a liquid medium, especially alcohol. They further suggested that rapid absorption may be due to the passage of the material to the small bowel, where it is dissolved in bile. The means by which phosphorus was ingested was known in 51 of the 56 cases described by Diaz-Rivera et al. (1950). The mortality rate was 56 percent when phosphorus was ingested with water, 89 percent when it was ingested with rum, 100 percent when it was ingested with wine, and only 25 percent when it was ingested without a vehicle. Although other factors, as discussed by Diaz-Rivera et al. (1950), may have altered the prognosis in these cases, the use of a vehicle correlates very well with mortality.

Although ingestion of white phosphorus causes systemic effects, increases in serum inorganic phosphate are not always demonstrated after acute oral ingestion of elemental phosporus. In most cases, normal inorganic phosphate levels, and sometimes hypophosphatemia, are observed rather than hyperphosphatemia. McCarron et al. (1981) reported that serum inorganic phosphate levels dropped to 1.5 and 1.8 mg/100 mL on the day of and on the fourth day after ingestion of elemental phosphorus, respectively. Normal values were reported by McIntosh (1927), Diaz-Rivera et al. (1950), Caley and Kellock (1955), Simon and Pickering (1976), and Snodgrass and Doull (1982).

Diaz-Rivera et al. (1950) described one case in which an increased serum inorganic phosphate level of 8.60 mg/100 mL (normal 3.0-4.5 mg/100 mL) was not observed until 15 days after intoxication (patient recovered) and another in which the increase occurred 4 or 5 days after intoxication (patient died). McCarron et al. (1981) described a case in which the serum inorganic phosphate level rose to 6.6 mg/100 mL approximately 57 hr after intoxication. The rise was followed by a reduction to 2.3 mg/100 mL on the fourth day after intoxication. This patient also died.

Diaz-Rivera (1950) suggested that the delayed hyperphosphatemia was due to accumulation of phosphorus in tissues, especially bone, that is later mobilized by a change in the acid-base balance. Winek et al. (1973) reported that the phosphorus content in liver was 0.049 mg percent and 0.78 mg percent in patients who died 8 and 22 hr, respectively,

after ingesting elemental phosphorus. In another patient who died within 3.5 hr, the phosphorus content in the kidney was 0.095 mg percent.

There is no convincing evidence that white phosphorus is absorbed in sufficient quantities to cause systemic effects when humans are exposed by acute inhalation or dermal contact (white phosphorus burns) (Walker et al. 1947, Summerlin et al. 1967). Although systemic changes were observed in some cases of chronic exposure to white phosphorus, Hughes et al. (1962) did not find significant differences in mean hematology and blood chemistry values between phosphorus workers and control subjects.

No information was found on the urine levels or excretion of phosphorus following ingestion of elemental phosphorus by humans.

4.2 ACUTE TOXICITY

4.2.1 Animal Data

Table 8 summarizes the lethality data on white phosphorus in laboratory animals. White phosphorus is highly toxic, and systemic effects are observed mainly in the liver. Additional details are discussed in the text.

4.2.1.1 Subcutaneous and oral exposure

Male and female Charles River (CD) rats and albino Swiss mice were given a 0.1 percent solution of white phosphorus in peanut oil by intragastric intubation (Lee et al. 1975). Animals surviving treatment were observed daily for mortality and signs of toxicity. Both mice and rats suffered from depression and anorexia. The livers were enlarged and yellowish in color. The acute LD_{50}'s $\pm$ S.E. in male and female rats were 3.76 $\pm$ 0.22 and 3.03 $\pm$ 0.15 mg/kg, respectively. The acute LD_{50}'s in male and female mice were 4.85 $\pm$ 0.21 and 4.82 $\pm$ 0.38 mg/kg, respectively.

Six rats per dose group were administered 1.48, 1.86, 2.43, and 2.96 g/kg of white phosphorus/felt smoke condensate, containing 65 percent phosphoric acid, by gastric intubation. The acute signs of toxicity were lethargy, gastric distress, prostration, and death within 24 hr (Brown et al. 1980). The mortality data are presented in Table 9. The Bliss probit analysis of the dose-response data showed a 24-hr LD_{50} of 2,346.8 mg/kg and a 14-day LD_{50} of 2,184.5 mg/kg (Brown et al. 1980).

Manthei et al. (1980) also administered white phosphorus/felt smoke residue to 10 Sprague-Dawley rats per dose at doses of 5 or 0.05 mL/kg (volume based on the undiluted residue) by gastric intubation. All 10 animals given 5 mL/kg died within 24 hr, whereas all 10 animals given 0.05 mL/kg survived without displaying signs of toxicity.

TABLE 8. LETHALITY OF WHITE PHOSPHORUS

Species	Route[a]	Dose (mg/kg)	Remarks	Reference
Rat	SC	10	40% mortality, 36 hr, male	Jacqueson et al. 1979b
	SC	10	0% mortality, 36 hr, female	Jacqueson et al. 1979b
	IP	1.0	100% mortality, 48 hr, male	Ganote and Otis 1969
	PO	6	30% mortality, 72 hr, male	Torrielli et al. 1974
	PO	12	80% mortality, 72 hr, male	Torrielli et al. 1974
	Oral (gavage)	3.76	LD_{50}, 14 days, male	Lee et al. 1975
	Oral (gavage)	3.03	LD_{50}, 14 days, female	Lee et al. 1975
	Oral (gavage)	2,346.8[b]	LD_{50}, 24 hr, combined sex	Brown et al. 1980
	Oral (gavage)	2,184.5[b]	LD_{50}, 14 days, combined sex	Brown et al. 1980
	IV	209.6[b]	LD_{50}, 24 hr, combined sex	Brown et al. 1980
	Inhalation	94,126 mg-min/m^3[c]	LCt_{50}, combined sex	Brown et al. 1980
	Oral (gavage)	5.0 ml/kg[b]	100% mortality, 24 hr, combined sex	Manthei et al. 1980
	IV	0.252 ml[b]	LD50, 24 hr and 14 days, combined sex	Manthei et al. 1980
	Inhalation	1,400 mg/m^3[c]	70% mortality, 14 days, combined sex	Manthei et al. 1980
	Inhalation	3,260 mg/m^3[c]	LC_{50}, 5 days, combined sex	White and Armstrong 1935
	Inhalation	4,500 mg/m^3	LC_{100}, combined sex	White and Armstrong 1935
Mouse	Oral(gavage)	4.85	LD_{50}, 14 days, male	Lee et al. 1975
	Oral (gavage)	4.82	LD_{50}, 14 days, female	Lee et al. 1975
	Inhalation	660 mg/m^3[c]	LC_{50}, 5 days, combined sex	White and Armstrong 1935
	Inhalation	1,700 mg/m^3	LC_{100}, combined sex	White and Armstrong 1935
Guinea pig	Inhalation	5,321 mg-min/m^3[c]	LCt_{50}, combined sex	Brown et al. 1980
Goat	Inhalation	7,250 mg/m^3[c]	LC_{50}, 10 days, combined sex	White and Armstrong 1935
	Inhalation	8,000 mg/m^3	LC_{100}, combined sex	White and Armstrong 1935

a. SC = subcutaneous, IP = intraperitoneal, PO = per os, IV = intravenous.
b. White phosphorus/felt smoke condensate.
c. White phosphorus smoke or white phosphorus/felt smoke.

TABLE 9. TOXICITY OF WHITE PHOSPHORUS/FELT SMOKE CONDENSATE ADMINISTERED TO RATS BY GASTRIC INTUBATION[a]

Dose (g/kg)	Mortality[b]	
	24-hr	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

a. Adapted from Brown et al. 1980
b. Number of deaths/total number of animals.

Huruya (1928) injected approximately 140 male rabbits subcutaneously with 5 mg/kg of yellow phosphorus dissolved in olive oil. The animals were killed 8, 16, 24, 32, 40, or 48 hr after injection. Urine, blood, and liver were subjected to chemical analysis in 40 animals, and small specimens of liver and kidney were analyzed microscopically. The data were presented in relation to severity of liver damage.

Fatty deposits (fatty degeneration) appeared in the interstitium and parenchyma of the liver. Damage to the kidney appeared in the renal tubules but rarely in the glomeruli. The following parameters of liver chemistry were shown to increase with severity of fatty degeneration in the liver: weight, nonprotein nitrogen, polypeptide nitrogen, total fatty acid, and cholesterol. Water content, total nitrogen, and lecithin were within normal range or changed very little in relation to fatty degeneration. Chemical analysis of blood revealed that total nitrogen, nonprotein nitrogen, and polypeptide nitrogen increased, but fatty acid content, cholesterol, and lecithin remained within normal range. The volume, total nitrogen content, and urea nitrogen content of urine decreased, whereas the alkalinity and ammonia nitrogen increased (Huruya 1928).

One-year-old female dogs weighing approximately 10 kg were injected subcutaneously with 0.4, 0.2, or 0.1 mg/kg of phosphorus daily (Buchanan et al. 1954). Because the animals receiving the two largest doses died early, the results from those animals will be discussed in this section, but the results from the 0.1-mg dose will be discussed under chronic toxicity (Sect. 4.3).

Two of three animals given 0.4 mg/kg/day died on day 6, and the third animal stopped eating on day 7 and was killed on day 14. Phosphorus-containing cysts or necroses were observed at the site of injection. On day 3 the animals began to vomit a mucus material that became bloody prior to death. All organs were hemorrhagic. The liver parenchyma was necrotic and sometimes hemorrhagic; fatty degeneration of the liver was observed in a narrow zone around the central vein. The

kidney tubules were necrotic, and fatty degeneration was observed in those kidneys less severely damaged (Buchanan et al. 1954).

Only one animal was given 0.2 mg/kg/day. This animal vomited a mucus-like material on day 6, stopped eating on day 7, and was killed on day 11. The liver, intestines, and kidneys were hemorrhagic. Fatty vacuolization was observed in the peripheral areas of the lobules in the liver. Kidney tubules had granular plugs, and the epithelium began to slough off (Buchanan et al. 1954).

Clinical studies showed a significant increase in urine creatine levels in one dog given 0.4 mg/kg/day and killed on day 14 and in the dog given 0.2 mg/kg/day, but creatine levels in the remaining animals were within normal range. Creatinine levels decreased in all animals, causing the creatine/creatinine ratio to increase. In addition, urine choline levels showed a slight increase immediately preceding death (Buchanan et al. 1954).

Thirteen guinea pigs administered 0.5 mg or 1 mg of yellow phosphorus, subcutaneously, at intervals of 3 to 5 days (total dose was 3.5 to 5.5 mg) developed monocytosis. In six animals, the absolute number of monocytes increased by over 700 percent, while the total number of white blood cells and the absolute number of lymphocytes and neutrophils remained within normal range. In three animals, the number of monocytes remained constant but the number of neutrophils increased. There was no response in four animals (Lawrence and Huffman 1929).

Lawrence and Huffman (1929) also administered yellow phosphorus orally to three guinea pigs at a dose of 1 mg/day for 6 to 7 days, to one animal at a dose of 2 mg/day for 6 days, and to two animals at a dose of 1 mg on alternate days for 6 days followed by another dose after an interval of 3 days. One animal showed a marked increase in the number of monocytes accompanied by leukocytosis, but four animals failed to respond. Fatty degeneration was also observed in some of these animals.

In a histological study by Scott (1916) white mice were injected subcutaneously with white phosphorus dissolved in olive oil in order to examine the effect on mitochondria in pancreatic cells. A dose of 0.0125 percent or 0.05 percent in volumes of 0.1 or 0.2 mL was injected at intervals of one or more days in order to induce a mild or severe response. Animals given 0.2 mL of a 0.0125 percent solution became comatose on the fifth day, and those given 0.2 mL of a 0.05 percent solution became comatose on the third day. Mitochondria in poisoned animals showed signs of damage, which increased in severity with the degree of intoxication. The structure of the mitochondria was altered in mildly intoxicated animals, whereas the structure and number of mitochondria were altered in severely intoxicated animals. Other signs of cellular damage in pancreatic cells were ill-defined nuclei, disappearance of the nuclear membrane, the absence of nucleoli, and intense and uneven staining of the cytoplasm. As demonstrated in other experiments, the liver showed signs of very pronounced fatty degeneration. Damage to the kidneys was mild.

Williamson and Mann (1923) demonstrated that in eight male dogs given 1.0 or 0.5 mg/day of phosphorus orally until death, which occurred within 4 days, three animals developed severe hypoglycemia, which became apparent only a few hours prior to death. Blood sugar levels were normal in the remaining animals. Blood urea was increased in seven animals, significantly in six. The severe hypoglycemia suggested that the liver was severely damaged; nevertheless, some animals died without developing hypoglycemia, suggesting that severe damage to the liver was not associated with death.

Cutler (1931) obtained similar results with respect to the induction of hypoglycemia. Dogs were administered phosphorus in olive oil orally in doses of 2 mg/kg on day 1, 1 mg/kg on day 3, and finally 1 mg/kg on day 5, but only if intoxication was not observed earlier. Overt symptoms of phosphorus poisoning included sluggishness, tremors, vomiting, convulsions, and coma. Of the six animals treated, two received a total dose of 3 mg/kg and four received 4 mg/kg. There was a decrease in blood sugar in all animals, in addition to increases in guanidine, nonprotein nitrogen, amino nitrogen, urea, and creatine. These changes were attributed to liver damage induced by phosphorus.

A detailed ultrastructural analysis of hepatic lesions in phosphorus-treated rats was described by Ganote and Otis (1969). Male Sprague-Dawley rats were injected intraperitoneally with yellow phosphorus (dissolved in either olive or mineral oil) in doses of 0.4 to 10.0 mg/kg and killed 6, 12, 24, and 48 hr after treatment. No deaths or hepatic subcellular changes were observed in animals given 0.4 mg/kg; subcellular changes were minimal at 12 hr at higher doses and very obvious at 24 hr. At 24 hr, the rough endoplasmic reticulum (RER) in parenchyma cells was significantly increased, and the cytoplasmic fat was moderately increased. The smooth endoplasmic reticulum (SER) was not increased. Ribosomes were closely associated with membranes, but polysomal aggregates tended to decrease in number (Ganote and Otis 1969). This observation was confirmed biochemically by Pani et al. (1972), who reported that white phosphorus induced disaggregation of hepatic polyribosomes, thereby reducing the number of heavy aggregates and increasing the relative number of monomeric and dimeric ribosomes. The number of dense particles in the Golgi cisternae decreased, but nuclei and mitochondria were not altered.

After 48 hr, there was an increase in the accumulation of fat; the RER, which was arranged in concentric lamellae around nuclei or cytoplasmic components, filled the cytoplasm, but polysomal aggregates were absent. The Golgi cisternae were sometimes extremely dilated but contained no dense particles, and the mitochondria were swollen (Ganote and Otis 1969).

Ganote and Otis (1969) described two cell types in severely damaged livers. The first type, which was prevalent in less severely damaged livers, was characterized by a massive increase in RER with relatively small amounts of SER. These cells represented the most characteristic feature of phosphorus poisoning. The second type, prevalent in the most severely damaged livers, was characterized by a near absence of RER

accompanied by a massive increase in SER, decreased numbers of ribosomes, and swollen mitochondria. Cells that were obviously necrotic resembled the second type, whereas cells in the late stages of necrosis contained only swollen mitochondria and almost no cytoplasmic membranes. Ganote and Otis (1969) suggested that cells with an abundance of SER represented a nonspecific event occurring in irreversibly damaged or prenecrotic cells.

Because white phosphorus intoxication produces a characteristic lesion in the liver, numerous studies have been carried out to analyze various biochemical alterations that may contribute to the mechanism by which white phosphorus induces fatty degeneration in the liver. Huruya (1928) reported that subcutaneous administration of yellow phosphorus to rabbits caused an increase in weight, fatty acids, and cholesterol in the liver. Seakins and Robinson (1964) administered 1.5 mg of white phosphorus in olive oil orally to female albino Wistar rats and observed the following 24 hr after treatment: liver weight was increased by 1.5-fold; total amount and concentration of esterified fatty acids were elevated by 2.6- and 1.8-fold, respectively; total amount and concentration of cholesterol were elevated by 1.7-fold and 1.2-fold, respectively; and total amount of phospholipids was elevated by 1.3-fold, but the concentration was decreased. The mean plasma concentrations of esterified fatty acids, cholesterol, and phospholipids were markedly reduced to 35.5, 12.5, and 33 percent of control levels, respectively.

When the amino acid DL-[1-^{14}C]-leucine was injected into rats 2 hr after administering white phosphorus and analyzed 1.5 hr later, the specific activities (cpm/100 μg of amino nitrogen) of hepatic proteins and plasma low-density lipoproteins, high-density lipoproteins, and residual proteins were significantly reduced. The mean relative specific activities of phospholipids in liver and plasma were not significantly different from controls when [^{32}P]-orthophosphate was injected 2 hr after white phosphorus and analyzed 3 hr later. In addition, the specific activity of cholesterol in white phosphorus-treated animals injected with [1-^{14}C]-acetate was not significantly different from that in control animals (Seakins and Robinson 1964).

Seakins and Robinson (1964) suggested that the induction of fatty degeneration in the liver, accompanied by a reduction in the concentration of plasma lipids, was due to a decrease in the rate of formation of the protein moiety of low-density lipoproteins in the liver. Low-density lipoproteins, which transport triglycerides to the extrahepatic tissues, would be reduced in their ability to carry out this function, thus leading to an accumulation (increase) of triglycerides in the liver and a corresponding decrease in plasma.

Ghoshal et al. (1969) showed that hepatic triglycerides were indeed significantly elevated 6, 12, and 24 hr after administering 7.5 mg/kg of yellow phosphorus (in mineral oil) to male Wistar rats by gastric intubation. The concentrations of hepatic triglycerides in mineral-oil-treated animals ranged from 9.02 to 14.24 mg/100 g body weight. In phosphorus-treated animals the concentrations were increased by 37 percent at 4 hr, 53 percent at 6 hr, and > 400 percent at 12 and 24 hr.

A small, insignificant increase in hepatic triglycerides was observed by Pani et al. (1972) as early as 2 hr after administering 10 mg/kg of white phosphorus by gastric intubation to female Sprague-Dawley rats. After 12 hr, hepatic triglycerides were significantly elevated at 180.71 ± 16.54 mg/100 g body weight (± S.E.) and remained at that level up to 24 hr. The triglyceride level in control animals given mineral oil was 36.34 ± 8.97 mg/100 g body weight (± S.E.).

Jacqueson et al. (1979a) also demonstrated that total hepatic lipids were elevated 2-fold and triglycerides were elevated 5-fold 36 hr after administering 10 mg/kg of white phosphorus subcutaneously to female Wistar rats. Chromatographic analysis of hepatic triglycerides showed increases in the relative amounts of oleic, palmitoleic, and steric acids and a decrease in linoleic acid.

Ghoshal et al. (1969, 1972) demonstrated that an increase in lipid peroxidation of hepatic microsomes (measured by absorption of conjugated dienes), which precedes the elevation in hepatic triglycerides, may be the cause of abnormal fat accumulation in the liver after administering 7.5 mg/kg of yellow phosphorus orally to male Wistar rats. In contrast, Pani et al. (1972) failed to find an increase in lipid peroxidation (conjugated dienes) in female Sprague-Dawley rats administered 10 mg/kg of white phosphorus orally. Lack of detection of conjugated dienes should not rule out the possibility of involvement of lipid peroxidation, because the products may be too rapidly metabolized to permit their accumulation and detection (Kulkarni and Hodgson 1980).

Increased secretion of hepatic enzymes into blood is indicative of hepatotoxicity (Kulkarni and Hodgson 1980). Plasma levels of glutamic-oxalacetic transaminase (GOT) were significantly elevated 24 hr after administering 7.5 mg/kg of yellow phosphorus to male Wistar rats by gastric intubation; the levels of glutamic-pyruvic transaminase (GTP) were not altered (Ghosal et al. 1969). Serum GTP levels in male Swiss Webster mice also remained unaltered 1 and 4 days after administering 5 mg/kg of white phosphorus by gastric intubation (Hurwitz 1972).

Several studies have identified substances that modify the response to white phosphorus intoxication. The antioxidants glutathione and propyl gallate were administered to rats 30 min and 60 min, respectively, prior to white phosphorus. Glutathione alone reduced the hepatic triglyceride levels, and both agents prevented the elevation of hepatic triglycerides induced by white phosphorus, indicating that antioxidants may prevent fatty degeneration of the liver (Pani et al. 1972).

Jacqueson et al. (1979b) showed sex differences in the response of Wistar rats to white phosphorus poisoning. Mortality 36 hr after administering 10 mg/kg of white phosphorus subcutaneously to rats was 40 percent in males and 0 percent in females. Phenobarbitone administered 4 days prior to white phosphorus treatment reduced the mortality in male rats to 0 percent; the mortality rate in females was unaltered. Total hepatic lipids and triglycerides were significantly elevated in both male and female rats after treatment with white phosphorus; the induced

levels, however, were higher in females than in males (Table 10). Pretreatment with phenobarbitone increased the triglyceride level in males to that observed in females. The levels of hepatic phospholipids were unaffected by white phosphorus. Cholesterol levels (range 2.3 ± 0.1 to 3.0 ± 0.3 mg/g of tissue) were unaltered after treatment with white phosphorus and/or phenobarbitone. Increased hepatic triglyceride levels did not reflect the higher mortality rate.

TABLE 10. EFFECTS OF WHITE PHOSPHORUS AND PHENOBARBITONE (PB) ON HEPATIC LIPIDS IN RATS[a,b]

	Hepatic Lipids (mg/g)		
	Total Lipids	Triglycerides	Phospholipids
Male rats			
Controls	49.4 ± 1.4	3.0 ± 0.3	36.8 ± 2.9
White phosphorus	84.2 ± 3.0[c]	34.2 ± 6.2[c]	38.7 ± 2.6
PB	58.0 ± 1.7[c]	6.2 ± 0.7[c]	44.2 ± 1.1[c]
White phosphorus+PB	127.0 ± 17.8[d]	64.0 ± 16.3[d]	48.9 ± 3.8
Female rats			
Controls	44.4 ± 4.8	6.0 ± 2.6	33.1 ± 0.8
White phosphorus	104.1 ± 27.7[c]	60.0 ± 24.7[c]	34.1 ± 3.0
PB	56.1 ± 7.9	11.4 ± 5.3	37.6 ± 0.8[c]
White phosphorus+PB	112.7 ± 26.1	65.1 ± 26.3	38.8 ± 2.4

a. Adapted from Jacqueson et al. 1979b.
b. Dose of PB = 80 mg/kg for 4 days; dose of white phosphorus = 10 mg/kg.
c. $P < 0.02$ for controls vs white phosphorus or PB t-test.
d. $P < 0.01$ for white phosphorus+PB vs PB or white phosphorus t-test.

An earlier study by Jacqueson et al. (1977) showed that sex differences in the effect of white phosphorus on triglycerides may be due to the presence of anabolic steroids in males. The levels of hepatic triglycerides were not significantly affected by pretreating males with testosterone proprionate or 19-nortestosterone proprionate 8 days prior to subcutaneous injections of 10 mg/kg of white phosphorus, whereas pretreating females caused a significant reduction in hepatic triglycerides.

A dose of 6 mg/kg of white phosphorus administered orally to male Wistar rats killed 30 percent of the animals within 72 hr, and a dose of 12 mg/kg killed 80 percent. In contrast to the results presented by Jacqueson (1979b), pretreatment with either phenobarbital or 3-methylcholanthrene did not alter the lethality of white phosphorus (Torrielli et al. 1974). Phenobarbital also had no effect on the lethality of 5, 7.5, or 10 mg/kg of white phosphorus administered to male Swiss Webster mice by gastric intubation (Hurwitz 1972).

4.2.1.2 Intravenous administration

The effects of injecting white phosphorus intravenously are more acute than those of administering white phosphorus orally or subcutaneously. Brown et al. (1980) observed that within 30 min after injecting six Sprague-Dawley rats (three of each sex) per dose intravenously with white phosphorus/felt smoke condensate containing 65 percent phosphoric acid, the animals were affected by ataxia, convulsions, and prostration, with death occurring in some animals. All deaths occurred within 11 min after injection, except for one animal that died 144 min after injection. All animals injected with 148 mg/kg survived, 2 of 6 injected with 186 mg/kg died, 4 of 6 injected with 234 mg/kg died, and all 6 animals injected with 370 mg/kg died. The 24-hr LD_{50} was 209.6 mg/kg (Brown et al. 1980).

Manthei et al. (1980) injected ten Sprague-Dawley rats (five of each sex) with 0.158, 0.200, and 0.250 mL of an undiluted white phosphorus/felt smoke residue containing 65 percent phosphoric acid. The acute effects, which occurred within 30 seconds, included rapid breathing, convulsions, and prostration; death occurred between 1 and 10 min. No deaths occurred after 24 hr, and those that survived appeared normal at that time. The 24-hr LD_{50} was 0.271 mL for males, 0.235 mL for females, and 0.252 mL for males and females combined.

4.2.1.3 Dermal and ocular exposure

Primary skin and eye irritation tests, performed in rabbits (six per test group) and using a 0.1 percent solution in peanut oil applied to intact skin, abraided skin, and eyes, showed that white phosphorus was not an irritant at 24 and 72 hr (Lee et al. 1975). White phosphorus/felt smoke residue (0.5 mL volume of an undiluted solution) containing 65 percent phosphoric acid, placed in contact with intact and abraided skin of rabbits for 24 hr and evaluated 24 and 72 hr after application, was a primary irritant (Manthei et al. 1980). The primary irritation index score was 8, the maximum score possible using their test system. The skin was not tested for toxicity, because the skin irritation test suggested that the residue would be corrosive to skin. A 0.1-mL volume of the undiluted residue applied into the conjuctival sac of six rabbits and evaluated 24 hr, 48 hr, 72 hr, and 7 days later was positively irritating to eyes. It caused severe and irreversible damage to the eyes; the corneas of all six animals were ulcerated, and the conjunctivae were blanched, swollen, and ulcerated (Manthei et al. 1980).

White phosphorus/felt smoke condensate, containing 65 percent phosphoric acid, was diluted in isotonic saline to a final concentration of 0.1 percent, and injected intradermally into ten guinea pigs, according to established procedures. White phosphorus/felt smoke condensate was not a sensitizer in guinea pigs (Brown et al. 1980).

Because white phosphorus ignites spontaneously in air (Van Wazer 1982), its contact with skin can cause serious burns. In order to

provide an explanation for the high mortality rate in humans burned over as little as 10 to 15 percent of the body surface, Bowen et al. (1971) studied the effects of white phosphorus burns in New Zealand white rabbits. They developed a method referred to as the standard white phosphorus burn (SWPB) in which white phosphorus wafers, measuring 2 cm in diameter and weighing 10 g, were placed in a 3-inch-diameter well and ignited on shaved areas of both flanks. The burned areas measured 3 inches in diameter, extended through the full thickness of the skin, and involved 10 to 20 percent of the body surface. A group of 130 animals received burns; 40 of these also received postburn treatment consisting of excision of the burned tissue and closure of the wound 1 hr after treatment; the remaining animals (90) received no postburn treatment. Serum calcium and phosphorus levels were determined 0.5, 1, 3, and 5 days postburn.

The overall mortality rate was 65 percent, with 50 percent of all deaths occurring within 18 hr and 90 percent occurring within 3 days. Symptoms appearing 4 hr postburn included depression, poor response to stimulation, shivering, twitching, and failure to eat, drink, or move about. Histological evaluations of the heart, liver, kidney, and lungs 5 days postburn were all normal (Bowen et al. 1971).

Serum calcium levels were decreased in 80 percent of all animals. In those that died, serum calcium levels were decreased by about 50 percent (from approximately 14 mg percent to 7 mg percent) and serum phosphorus levels were significantly elevated (from approximately 5 mg percent to 8 mg percent). In those that survived, calcium levels were minimally decreased and phosphorus levels were within normal range. No deaths or changes in serum calcium and phosphorus were observed in the control group (Bowen et al. 1971).

In a second group of 24 animals receiving SWPB, serum calcium and phosphorus levels were determined for the first 12 hr postburn. Because of suspected disturbances in cardiac rhythm, electrocardiographic tracings were also made during this time. As in the first group, a high mortality rate was observed, overall 85 percent, with 90 percent of deaths occurring within the first 24 hr. In all animals that died, serum calcium levels were significantly reduced and phosphorus levels were elevated. These changes were observed as early as 1 hr postburn. Electrocardiographic abnormalities, observed in 70 percent of the animals with calcium-phosphorus changes, consisted of prolongation of the Q-T interval, ST segment depression, T-wave changes, bradycardia, and low voltage of the QRS complex. The survivors and controls did not show changes in calcium and phosphorus levels nor in the electrocardiographic tracing (Bowen et al. 1971).

Bowen (1969) also reported that, in rabbits subjected to SWPB's causing 75 percent mortality, serum phosphorus levels increased 1.0 ± 0.34 mg percent ($P < 0.01$) and serum calcium levels decreased 4.4 ± 0.29 mg percent ($P < 0.001$).

Ben-Hur et al. (1972), Ben-Hur and Appelbaum (1973), and Applebaum et al. (1975) used a different model for studying phosphorus burns in

laboratory animals. An incision was made in the inguinal region, and phosphorus was placed in the incision and ignited. By opening and closing the incision, the rate of combustion could be controlled; burning time lasted for 4 min.

In the first study, Hadassah-bred male rats were anesthetized and subjected to burns produced by igniting 10 or 50 mg of phosphorus in the wound (Ben-Hur et al. 1972). Anesthetized controls received 50 mg of phosphorus in a wound that was not ignited, received no treatment, or were burned for 10 seconds with a brass plate heated to 100°C. Ten animals were used in each group. The rats were killed at 2, 6, 12, 24, 36, and 72 hr postburn Serum and urine were collected for biochemical measurements; lungs, liver, and kidneys were examined grossly and histologically.

As in the case with the SWPB, the mortality rate was high, with 50 percent of the animals dying within 3 to 4 days. The wounds were necrotic, yellowish in color, fluorescent, smelled of garlic for 2 days, and showed no signs of healing in 6 days. The results of the serum and urine biochemistry studies are presented in Table 11.

On gross examination the kidneys appeared swollen and pale. Histological examination revealed swelling of kidney cells, desquamation, perinuclear vacuolization and necrosis, and vacuolar degeneration of proximal convoluted tubules. The livers were swollen and yellow-brown in color with hemorrhagic areas on the surface. Histological examination of the livers revealed diffuse areas of necrosis, periportal infiltration with inflammatory cells, areas of ballooning degeneration of hepatic cells, and microthrombi of the portal veins (Ben-Hur et al. 1972). Even in the presence of acute liver damage, death was attributed to renal failure that caused potassium intoxication and subsequently cardiac arrest (Ben-Hur et al. 1972).

Ben-Hur and Appelbaum (1973) conducted a similar study, in which 25 mg of white phosphorus was ignited within an incision made in the inguinal region of male rats. The results of the serum and urine biochemistry and histological studies were similar to those reported previously (Ben-Hur et al. 1972). In addition, Ben-Hur and Appelbaum (1973) reported that the glomeruli in the kidney were ischemic and hypercellular, the capillaries were obliterated, the basement membranes were thicker than normal, and the mesangial area was expanded.

Applebaum et al. (1975) subjected Hebrew University 'sabra' strain male rats to inguinal burns produced by igniting 50 mg of white phosphorus. Results of serum and urine biochemistry and histological examination of the kidney were also similar to those reported previously (Ben-Hur et al. 1972, Ben-Hur and Appelbaum 1973). This study included an ultrastructural analysis of the kidney. In the glomeruli, mesangial cells were proliferating, and their cytoplasmic processes were enmeshed within the thickened basement membrane. The glomerular epithelium was edematous, with projections extending into the urinary spaces. The cells of the proximal convoluted tubules were also edematous; the mitochondria were swollen and rounded in shape and had broken cristae. The

TABLE 11. BLOOD AND URINE BIOCHEMISTRY IN PHOSPHORUS-BURNED RATS[a]

Parameter	Normal	Phosphorus Burn (72 hr)
Water intake (mL/day)	16 (15-18)	45 (40-50)
Urinary output (mL/day)	11 (10-12)	35 (30-40)
Serum PO_4 (mg %)	4.5 (4-5)	10 (10-11)
Serum urea (mg %)	15 (10-20)	100
Serum Na (meq/L)	140 (135-143)	127 (125-130)
Serum K (meq/L)	4.5 (4.5)	8
SGPT (units/mL)[b]	10	100
Serum osmolality (mole/L)	292	350
Urine osmolality (mole/L)	2,100	700
Creatine clearance (mL/min)	1.25	0.75

a. Adapted from Ben-Hur et al. (1972).
b. SGPT = serum glutamic-pyruvic transaminase.

plugs in the proximal convoluted tubules consisted of slightly electron-opaque cellular debris, which probably arose from damaged tubules.

Applebaum et al. (1975) suggested that phosphorus burns interfer with glomerular and/or tubular function. They also suggested that alterations in kidney function, as indicated by biochemical analyses, resulted in polyuria or oliguria, indicating that the nephrons had lost their ability to control urine concentration after the burn.

4.2.2 Human Data

4.2.2.1 Oral exposure

According to Sollmann (1957), the estimated minimal lethal dose of elemental (yellow or white) phosphorus in humans is 50 mg (0.7 mg/kg), most often 100 mg (1.4 mg/kg), but 15 mg (0.2 mg/kg) may cause serious toxic effects. These doses were estimated from patients who did not receive medical treatment after intoxication. Because treatment changes both the prognosis and the lethality of a particular dose of elemental phosphorus (Polson et al. 1983), humans have recovered from larger doses, as will become apparent in this section. Information on the acute oral effects of phosphorus in humans has come primarily from analyzing cases of accidental or intentional ingestion of yellow phosphorus contained in preparations such as pesticide paste, fireworks, and

match tips (McCarron et al. 1981). The major targets of elemental phosphorus are the gastrointestinal tract, brain, liver, kidney, and cardiovascular system (McCarron et al. 1981).

The classical description of acute oral phosphorus poisoning in humans divides the symptoms into 3 stages: initial (stage 1), latent (stage 2), and systemic (stage 3) (McCarron et al. 1981, Hayes 1982). Stage 1 symptoms, attributed to local irritation of the gastrointestinal tract, include nausea, vomiting, abdominal pain, thirst, garlic breath, hematemesis, and slight diarrhea. Stage 2 symptoms are described as a period of well-being, during which there is an abatement of symptoms. Stage 3 symptoms include the reappearance of more severe nausea, vomiting, and abdominal pain, and the appearance of hepatomegaly, jaundice, central nervous system (CNS) injury, hemorrhage, oliguria, peripheral vascular collapse, coma, and death (Cameron and Rentoul 1963, McCarron et al. 1981, Hayes 1982). The length of each stage is variable: stage 1 lasts from 24 to 48 hr, stage 2 from a few hours to as long as 10 days, and stage 3 may begin within the first 4 to 5 days and last for a variable length of time, depending upon the degree of intoxication (Cameron and Rentoul 1963, Hayes 1982).

Table 12 summarizes the gross symptoms in several case studies of patients who either died or recovered after ingesting elemental phosphorus.

In a review of 91 cases of phosphorus poisoning, McCarron et al. (1981) described three groups based on the initial manifestation of symptoms (gastrointestinal tract, CNS or both) If the initial symptoms were related to the gastrointestinal tract, the mortality rate was 23 percent; if the initial symptoms were related to CNS toxicity, the mortality rate was 73 percent; and if the initial symptoms were related to both the gastrointestinal tract and CNS toxicity, then the mortality rate was 47 percent. Because of overlaps in the doses of phosphorus ingested by patients in each group, it was not possible to relate dose to initial manifestation of symptoms.

The relationship between dose of phosphorus and mortality rates in the 56 suicide cases studied by Diaz-Rivera et al. (1950) is presented in Table 13. The patients ingested pesticides containing known amounts of phosphorus, and the dose of phosphorus ingested was estimated by measuring the amount remaining in the tubes or by questioning the patient on the amount of pesticide ingested. For the most part, doses of 1.57 g or more were fatal, with only 2 out of 21 patients surviving ingestion of 1.57 g of phosphorus. Doses of 0.78 g or less were associated with a high survival rate, with 27 of 33 patients surviving. The patient who ingested 0.19 g of phosphorus did not survive because she refused medical treatment. Following a detailed analysis of the 56 cases, Diaz-Rivera et al. (1950) concluded that:

(1) the gross mortality rate was 48.2 percent;
(2) the average dose ingested by males was 1.27 g, and that ingested by females was 0.84 g;

TABLE 12. GROSS SYMPTOMS OF PATIENTS WHO INGESTED ELEMENTAL PHOSPHORUS

Age	Sex	Approx. dose of P (g)	Vomiting and/or Hematemesis	Abdominal Pain	CNS Toxicity	Shock or Low BP	Liver Toxicity	Renal Toxicity	Cardiac Damage	Other Effects	Time of Death or Recovery	Reference
Died												
65 yr	F	0.10[a]	-	+	+	+	-	+	-	-	52 hr	LaDue et al. 1944
19 yr	F	0.156	+	+	+	-	+	+	-	-	3-1/2 days	Hann and Veale 1910
31 yr[b]	F	0.19	+	+	+	+	+	+	-	-	8 days	Diaz-Rivera et al. 1950
19 yr	F	0.23	+	-	+	+	-	-	+	-	8 hr	Rubitsky and Myerson 1949
69 yr	F	0.70	+	-	+	+	+	+	+	-	5 days	McCarron et al. 1981
16 yr	F	1.10	-	-	-	+	-	+	+	Vascular damage	22 hr	Talley et al. 1972
43 yr	M	1.134	+	+	+	-	+	+	+	-	Day 5	Cameron and Rentoul 1963
28 yr	M	1.57	+	+	+	-	+	-	+	Hyperphosphatemia	Day 6	Diaz-Rivera et al. 1950
Recovered												
10 mo	F	0.12	-	-	+	+	+	-	-	-	Day 5	McCarron et al. 1981
23 yr	F	0.13	+	+	+	-	+	+	-	Leukocytosis	Day 29	McCarron et al. 1981
71 yr	F	0.5	+	-	+	+	+	-	-	Anemia	Day 42	Caley and Kellock 1955
22 yr	M	0.78	-	+	+	-	+	+	+	Hyperphosphatemia	Day 30	Diaz-Rivera et al. 1950
30 yr	M	0.78[a]	+	+	+	+	+	+	+	Anemia	Day 30	Pietras et al. 1968
61 yr	M	1.20[a]	+	+	-	-	+	+	-	-	Day 56	LaDue et al. 1944
24 yr	M	1.57	+	+	+	-	+	+	-	-	Day 21	Diaz-Rivera et al. 1950

a. Dose estimated by McCarron et al. 1981.

b. Patient refused medical treatment.

TABLE 13. ORAL TOXICITY OF ELEMENTAL PHOSPHORUS IN HUMANS[a]

Dose of P (g)	No. Cases	Mortality (%)
6.30	1	100
4.62	1	100
1.57	21	90
0.78	18	16.3
0.39	14	14.3
0.19[b]	1	100

a. Adapted from Diaz-Rivera et al. 1950.
b. Patient refused medical treatment.

(3) the average dose ingested by females who died was 1.37 g, and that ingested by males who died was 1.72 g;
(4) the average dose ingested by males who recovered was 1.13 g;
(5) liquid vehicles, especially alcohol, facilitated absorption of phosphorus and increased mortality (at least 89 percent mortality with an alcohol vehicle, compared with 25 percent with no vehicle);
(6) early gastric lavage or vomiting reduced the mortality rate in those who ingested 0.78 g or less; for those who injested larger doses the outcome was usually death;
(7) signs of impending death or increased mortality rate included shock, or early onset of hepatomegaly, clinical jaundice, hypoglycemia (signs of liver damage), azothemia (evidence of kidney damage), severe restlessness, delirium, and toxic insanity (evidence of CNS toxicity) in combination with shock and coma;
(8) other symptoms of phosphorus poisoning were hyperphosphatemia, myocardial damage, leukopenia, leukocytosis, lymphocytosis, and erythrocytosis.

Myocardial damage was shown to increase proportionally with the dose of white phosphorus. The prevalence of abnormalities in EKG's in relation to dose was 33 percent of 6 patients ingesting less than 0.38 g, 45 percent of 11 patients ingesting 0.39-0.74 g, 56 percent of 23 patients ingesting 0.75 to 1.49 g, and 67 percent of 15 patients ingesting 1.57 g or more (Diaz-Rivera et al. 1961). Myocardial damage was also observed in a 16-year-old female who ingested 1.11 g of phosphorus and died within 33 hr (Talley et al. 1972), in a 30-year-old male who ingested approximately 1.18 g (Pietras et al. 1968), and in a 21-year-old male who ingested 1.5 g and recovered (Newburger et al. 1948).

Myocardial damage induced by acute phosphorus poisoning consisted of abnormalities in the electrocardiograms (EKG) characterized by prolongation of QT interval, ST and T wave changes, abnormalities in rhythm, and low voltage of QRS complexes (Diaz-Rivera et al. 1961).

Pietras et al. (1968) observed that abnormalities in ST segment and T waves as well as pathological Q waves and QRS changes were reversible. They suggested that phosphorus renders a portion of the myocardium electrically inert by means of a direct or indirect reversible biochemical effect. This condition would simulate a transmural myocardial infarction (Pietras et al. 1968). Microscopic examination of the heart showed fatty degeneration, interstitial edema without cellular infiltrates, and cells with vacuolated cytoplasm (Diaz-Rivera et al. 1961, Cameron and Rentoul 1963). Wechsler and Wechsler (1951) also found evidence of myocardial necrosis.

The mortality rate in patients showing signs of CNS toxicity was also very high (Diaz-Rivera et al. 1950). In those showing signs of restlessness, the mortality rate was 56.6 percent (30 cases), delirium 80.5 percent (20 cases), toxic psychosis 100 percent (16 cases), and early coma 87.5 percent (8 cases). These results were confirmed by McCarron et al. (1981), who reported a mortality rate of 73 percent in patients who presented early signs of CNS toxicity.

Hepatomegaly is one of the characteristic symptoms of phosphorus poisoning. Of the 56 cases reported by Diaz-Rivera et al. (1950), 41 (71 percent) developed hepatomegaly. Impending death was associated with patients who developed this symptom within the first 48 hr (52 percent mortality), whereas all of those who developed the symptom after 48 hr survived. Grossly the livers of patients who ingested phosphorus was yellow, with areas of necrosis. Microscopically the livers had slight to moderate leukocytic infiltration, fibrosis, extensive fatty degeneration with vacuolization, and sometimes a loss of the lobular structure (Dwyer 1925, LaDue et al. 1944, Wechsler and Wechsler 1951, Cameron and Rentoul 1963).

LaDue et al. (1944) took serial liver biopsies, between day 33 and 191 of illness, from a patient who survived after ingesting 0.567 g of phosphorus contained in roach paste. During the early stages of illness, parenchymatous degeneration and fatty metamorphosis of liver cells associated with small areas of focal necrosis and moderate infiltration of polymorphonuclear leukocytes into the periportal connective tissue were noted. Focal necrosis was no longer apparent by day 45, and there was a decrease in cytoplasmic degeneration. Lymphocytes replaced polymorphonuclear leukocytes as the predominant inflammatory cell type. There was a progressive increase in periportal connective tissue (fibrosis) such that by day 86 the general architecture was distorted due to the extention of periportal connective tissue into the parenchyma. Fibrosis did not increase between day 86 and 191, but the collagen content did.

4.2.2.2 Inhalation exposure

Information on human exposure to white phosphorus by means of inhalation is very scanty. Exposing 108 men to 87 to 1,770 mg/m^3 of white phosphorus smoke (length of exposure not given) caused throat irritation and coughing. This experiment led Cullumbine (1944, as

reported by Wasti et al. 1978) to establish a minimum harassing concentration of 700 mg/m^3 in working men, and 1,000 mg/m^3 in resting men.

White and Armstrong (1935) conducted a very limited experiment in which male human subjects (ages not given) were exposed to white phosphorus smoke in a gassing chamber. The individuals entered the chamber prior to smoke production (average concentration) or after the target concentration was established (actual concentration). Because each person was asked to describe the effects upon themselves, the results were subjective. The concentrations of smoke and length of exposure were as follows: 0.185 mg/L for 5 min, 0.187 mg/L for 5 min, 0.408 mg/L for 10 min, 0.453 mg/L for 10 min, 0.425 mg/L for 15 min, 0.514 mg/L for 15 min (average concentrations), 0.588 mg/L for 2 min, and 0.592 mg/L for 3.5 min (actual concentrations). The effects of white phosphorus smoke during and after exposure were related to respiratory tract irritation. Irritation of the throat, especially while talking, was the most common effect even at the lowest concentration of 0.185 mg/L. Coughing was frequently reported, in addition to congestion, tightness in the chest, and nasal discharge. White and Armstrong (1935) suggested that exposure to 0.514 mg/L (average concentration) approached the maximum dose at which humans can be exposed for 15 min without encountering serious effects. This concentration is near the minimum harassing concentration of 700 mg/m^3 reported by Cullumbine (1944, as reported by Wasti et al. 1978).

Walker et al. (1947) described the effects in four females, 29 to 45 years old, exposed to white phosphorus smoke during an accident in a plant processing white phosphorus munitions at Edgewood Arsenal, Maryland. Other substances, in addition to those produced by the burning of white phosphorus, may have been present in the air, but no data on these substances were available. The women were exposed for 15 to 20 min in a closed room that rapidly filled with a dense, white, gray, and black smoke. None of the women lost consciousness, but all suffered from respiratory symptoms including choking sensations, feelings of suffocation, sense of tightness in the chest, coughing, expectorating tenacious sputum, and soreness of the throat. Hoarseness was also reported in two women. Physical examination revealed restlessness, anxiousness, coughing, nonfrothy expectoration, and shortness of breath. Sibilant and sonorous rales were heard throughout the lungs. The patients who were hoarse also showed erythema and edema of the larynx and vocal cords. The patients expectorated bronchial casts composed of a necrotic superficial layer of the bronchial epithelium, which was evidence of injury extending down into the bronchial tubes. Chest X-rays revealed patchy areas of infiltration that cleared within 5 to 10 days. Coughing and expectoration subsided within several days, but the hoarseness persisted in one patient for 8 months. Walker et al. (1947) concluded that exposure to white phosphorus smoke for 15 to 20 min causes respiratory injury ranging from tracheobronchitis to laryngo-tracheo-bronchopneumonitis.

Five males were exposed to white phosphorus vapors composed of 0.035 mg/L of phosphorus and 0.22 mg/L of phosphorus pentoxide at an industrial site (Aizenshatdt et al. 1971, as reported by Wasti et al.

1978). They were exposed for 2 to 6 hr at 7-hr intervals (total exposure time not given) while cleaning a tank of "Cottrell Milk" (an aqueous suspension of phosphorite, quartzite, and coke dust) by hand and without the aid of protective equipment. Within 6 to 20 hr of completing the work, all developed symptoms of malaise, weakness, dry cough, and slight hyperthermia. The next day, dyspnea, cough with thick discharge, high fever (5/5), headache, vertigo, chest pains (2/5), rhinitis, and epitaxis (1/5) were noted. Further examination revealed hyperemia of the face and pharynx (2/5), multiple diffuse rales (5/5), bubbling rales (3/5), tender liver upon palpation (4/5), and hepatomegaly (1/5). Laboratory tests showed evidence of leukocytosis with relative lymphocytopenia, increased erythrocyte sedimentation rate, normal bilirubin and residual nitrogen, reduced cholesterol, and dysproteinemia. Erythrocyte acetylcholinesterase was reduced by 17 percent, and plasma acetylcholinesterase was reduced by 35 percent.

4.2.2.3 Dermal exposure: white phosphorus burns

White phosphorus burns are sustained in industrial accidents and on the battlefield (Walker et al. 1947, Summerlin et al. 1967, Berkowitz et al. 1981). Walker et al. (1947) evaluated 27 casualties resulting from four accidents in plants processing white phosphorus munitions at Edgewood Arsenal, Maryland. A summary of the mortality data is presented in Table 14. Of the 27 casualties, 9 with third degree burns over 90 percent or more of the body surface died almost immediately, 3 with third degree burns over 35 percent to 65 percent of the body surface died within 19 hr, 15 with third degree burns over 0 percent to 19 percent and different amounts of second degree burns survived. Two patients suffered from massive hemolysis and both survived.

Two types of burns were encountered, one due to burning of clothing (second degree) and the other due to burning of white phosphorus directly on the skin (third degree). The skin of third degree burns was pale, avascular, and destroyed down to and including the superficial parts of the dermis. Near the end of the first week, the plaque of necrotic tissue of third degree burns began to undergo marginal and basal autolysis; it separated cleanly within 10 to 12 days, leaving a moderately thick layer of granulation tissue. Both second and third degree burns were similar to thermal burns. Systemic effects (except massive hemolysis) due to white phosphorus burns were not noted; liver damage, as indicated by serum bilirubin levels and bromsulfalein retention studies, was not observed; blood sugar and serum calcium were normal; phosphorus excretion was reduced rather than elevated (Walker et al. 1947).

Summerlin et al. (1967) described three cases of white phosphorus burns accompanied by massive hemolysis. Case 1 was a 25-year-old male who had sustained burns over 29 percent of his body surface, Case 2 was a 46-year-old male who had burns over 12.5 percent of his body surface, and Case 3 was a 24-year-old male who had burns over 7.5 percent of his body surface. In each case, hemoglobinemia, hemoglobinuria, hematuria, bilirubinemia, mild (Cases 2 and 3) to severe (Case 1) hypocalcemia,

TABLE 14. SUMMARY OF CASUALTIES FROM WHITE PHOSPHORUS BURNS IN FOUR ACCIDENTS AT EDGEWOOD ARSENAL[a]

Body Surface Burned (%)		Outcome	Comments[b]
Total	Third Degree		
≥ 90	≥ 90	Death	Almost immediate death[c]
65	60	Death	Deep shock, died within 3 hr
50	45	Death	Restlessness, shock within 1 hr, coma, died within 11 hr
40	35	Death	Drowsy, mentally oriented, coma, NPN = 370 mg %, died within 19 hr
20	19	Survived	Little systemic reaction, leukocytosis, infection, NPN = 95 mg %
25	15	Survived	Massive hemolysis, hemoglobinemia, hemoglobinuria, sickle cell trait, leukocytosis, NPN = 290 mg %
20	12	Survived	Semistupor, delirium, low plasma Cl, infection, NPN = 65 mg %
15	8	Survived	Massive hemolysis, hemoglobinemia, hemoglobinuria, sickle cell trait, leukocytosis, NPN = 328 mg %
5-10	0-5	Survived	No marked systemic reaction except edema of face and neck in 1, fever in 2, and leukocytosis in 1[d]

a. Adapted from Walker et al. 1947.
b. NPN = nonprotein nitrogen.
c. Total of 8 patients.
d. Total of 11 patients.

oliguria, and renal failure were observed. Case 2 showed evidence of hyperphosphatemia. Case 1 also showed evidence of myocardial ischemia, which disappeared on the fifth hospital day. Malarial rings or parasites (Cases 1 and 3) were detected in two patients.

Massive hemolysis was not definitely attributed to systemic effects of white phosphorus burns (Walker et al. 1947, Summerlin et al. 1967). A copper sulfate solution was used during the initial emergency treatment of white phosphorus burn patients (Walker et al. 1947, Summerlin et al. 1967), because it inactivates the phosphorus particles embedded in the skin and makes them more visible by producing a black coating on the phosphorus particles (Blackwell 1967). Prolonged contact with or use of concentrated solution of copper sulfate may cause massive hemolysis (Pruitt 1970). Berkowitz et al. (1981) reported that malarial parasites can destroy red blood cells, causing hemolysis. Thus, hemolysis could be attributed to systemic effects of copper or malarial parasites as well as to phosphorus.

4.3 SUBCHRONIC AND CHRONIC TOXICITY

4.3.1 Animal Data

4.3.1.1 Subcutaneous and oral exposure

Studies on subchronic and chronic toxicity in laboratory animals are very limited and are not adequate models for chronic toxic effects in human. Mallory (1933) administered 0.25 to 1 mg/kg phosphorus in oil of sweet almonds, per os, to 85 rabbits and guinea pigs daily until the animals were sacrificed. Phosphorus-induced cirrhosis of the liver was observed at 8 weeks in animals administered 1 mg/kg. Animals given an average daily dose of 0.25 or 0.33 to 1 mg/kg required 4-1/3 months to develop cirrhosis of the liver. Phosphorus caused damage to fibroblasts of the stroma and to hepatic cells throughout the liver. Damage to fibroblasts was followed by regeneration, as indicated by mitotic activity and periportal fibrosis that extended irregularly into the lobule. Damage to hepatic cells, which was extensive after administering 1 mg/kg/day, was also followed by regeneration, as indicated by mitotic activity and the appearance of acidophilically stained hyaline reticulum in the cytoplasm. Mallory (1933) did not indicate which animals, rabbits or guinea pigs, were described in the figures presented in his manuscript; thus it is not possible to attribute the effect to a definite species.

Ashburn et al. (1948) were not able to induce cirrhosis in guinea pigs administered phosphorus for 35 weeks. The animals (51) were administered 0.75 mg/kg for 4 days each week or 1.5 mg/kg twice weekly in a 0.1 percent solution of olive oil, per os. Dosing, however, was not continued on a regular basis because single doses were occasionally omitted or reduced. Two to four animals were killed at irregular intervals, and the livers were processed for histological evaluation.

The lesions appearing after dosing four times per week were identical in incidence and type to those appearing after dosing twice weekly. Nine weeks after initiating treatment, hypatic lesions, with clear outlines and variable sizes and shapes, appeared in the hilar portion of most lobes and extended for short distances toward the free surface of the liver. As treatment progressed, the lesions increased in size and frequency of appearance. Extreme atrophy was observed in lobes containing lesions, whereas hypertrophy was observed in the uninvolved lobes. In lesions that appeared early, microscopic evaluation showed moderate to almost complete destruction of parenchyma cells; the surviving cells showed hydropic, fatty, or other degenerative changes but not hyperplasia. Moderate to mild bile duct proliferation was also observed. An inflammatory response characterized by moderate to mild infiltration of lymphocytes and large mononuclear cells was observed more frequently during the early period of treatment (Ashburn et al. 1948).

In late lesions, very few parenchyma cells, moderate to extensive fibrous tissue, a few normal bile ducts, and collapsed sinusoids were observed. Extensive necrosis was very rare, and frank necrosis was limited to small groups of cells or to a few isolated cells distributed irregularly throughout the liver. Fatty degeneration of the parenchyma cells was mild and occasionally absent. A slight increase in the amount of periportal collagen was regularly observed after 16 weeks. In four animals thin, fibrous trabeculae composed partially of collagen connected the portal areas and lobes but did not disorganize the architecture of the lobes; therefore, Ashburn et al. (1948) did not describe this condition as cirrhosis.

One-year-old dogs weighing 10 kg were injected subcutaneously with 0.1 mg/kg/day of phosphorus for 55 to 115 days (Buchanan et al. 1954). The exact times of death or sacrifice of all animals were not indicated. One animal lost its appetite and developed diarrhea prior to death on day 55, and the remaining animals became ill prior to the time of sacrifice. All animals lost between 2.5 and 3.0 kg of weight between days 25 and 51 of treatment, then gained 1.85 to 2.85 kg prior to sacrifice. Some animals developed fatty degeneration of the liver, hydropic degeneration of the kidney, and accumulated large amounts of hemosiderin in the spleen. Liver weights expressed as percentage of body weight were similar to paired controls. Urine creatine, creatinine, and the creatine/creatinine ratio varied considerably but were in the range of pretreatment and control values.

Groups of 6 or 10 young female or old male rats were fed a diet containing phosphorus such that the median daily doses were 0.072, 0.018, 0.0033, or 0.0027 mg/kg (Sollmann 1925). The young females were fed the poisoned diet for 22 weeks and the old males were fed the diet for 25 weeks. One-half of the animals were removed from the poisoned diet at various times after initiating treatment. A pronounced depression of growth was observed in the females placed on the diet containing 0.075 mg/kg/day. The loss of weight was so severe that the final weight was less than the starting weight. Animals removed from the poisoned diet at 10 weeks did not gain weight but ceased to lose weight. Growth

fluctuated considerably in female rats fed 0.018 mg/kg/day of phosphorus. The final weight was 15 percent below normal at 22 weeks. Animals removed from the diet containing phosphorus at 16 weeks resumed normal growth. Growth of young females placed on 0.0033 mg/kg/day was unaffected by phosphorus until the 15th week, at which time growth ceased. Animals removed from the diet containing phosphorus at 18 weeks experienced a rapid increase in weight gain such that, at 22 weeks, treated animals weighed more than controls. Old male rats placed on 0.0027 mg/kg/day of phosphorus showed considerable fluctuations in growth prior to the 15th week. After the 15th week, growth was rapid and was 13 percent above that of controls by the 25th week of treatment.

A recent study by Monsanto (1985) did not confirm the extreme loss of weight in rats given 0.075 mg/kg/day of white phosphorus (in corn oil) by gavage. The doses in the Sollmann (1925) study were median daily doses with the upper range of the 0.072-mg/kg dose equal to 0.189 mg/kg. The differences in the doses in the two studies may have accounted for the differences in the results.

Lhota and Hannon (1979) also observed that rats, injected subcutaneously with 0.5, 1.0, and 2.0 mg/kg/day yellow phosphorus in corn oil for 30 days or less, lost weight. Young adult rats injected with 0.5 mg/kg/day lost less weight than fully mature or young rapidly growing rats. Whatever the age or weight at the beginning of treatment, the period of weight loss (between the third and eleventh dose) was followed by a period of cyclic weight loss and gain with an overall net weight gain. Animals injected with 1.0 mg/kg/day suffered a more severe weight loss than those injected with a lower dose; almost all the animals died prior to the recovery period. All animals injected with 2.0 mg/kg/day died during the period of weight loss.

Fleming et al. (1942) administered white phosphorus dissolved in peanut oil and mixed with stock diet to groups of six rats for their entire lifetime, up to 512 days in some animals. Control animals were given the same diet containing peanut oil only. The equivalent daily doses and mortality data are presented in Table 15.

Except for the group receiving 0.8 mg/kg/day, mortality decreased with decreasing doses of phosphorus; the average survival of all treated animals, however, was greater than or equal to that of controls. Fleming et al. (1942) suggested that growth was retarded probably due to inanition rather than systemic effects (weight data was not presented in the manuscript). Inanition may also have contributed to the weight loss in the study described by Sollmann (1925). Histopathological evaluation revealed changes in the bones consisting of thickening of the epiphyseal line and extension of the trabeculae into the shaft in all animals exposed to white phosphorus but in none of the controls. Changes in the lungs, consisting of bronchopneumonia, pneumonitis, congestion, and edema, were present in both treated animals and controls; therefore, these changes were not related to ingestion of white phosphorus (Fleming et al. 1942).

TABLE 15. ORAL AND SUBCUTANEOUS TOXICITY OF WHITE PHOSPHORUS IN RATS[a]

Dose (mg/kg/day)	Total Dose (mg)	Avg. Survival (days)	Deaths/100 Animal-Days
Oral			
1.6	718	449	0.25
0.8	265	332	0.30
0.4	181	454	0.22
0.2	96	479	0.21
Controls	0	348	0.33
Subcutaneous			
3.2	10	3.2	31.6
1.6	15	0.3	10.7
1.2	13	11.0	9.1
0.8	112	140	0.72
0.4	136	340	0.30
0.2	89	442	0.23
0.1	53	530	0.19
0.05	31	610	0.17
Controls	0	480	0.24

a. Adapted from Fleming et al. 1942.

Fleming et al. (1942) also administered white phosphorus dissolved in olive or peanut oil twice weekly to groups of 6 to 10 male and female rats and to groups of 7 to 8 guinea pigs by subcutaneous injections. White phosphorus was administered up to 720 days to rats and 1,160 days to guinea pigs. Controls were injected with an equal amount of oil.

The mortality rate in treated rats decreased with decreasing doses of phosphorus (Table 15). The mortality rate in control animals was greater than that of rats administered 0.1 or 0.05 mg/kg/day. Thus, as in the previous experiment, low doses of phosphorus were associated with improved survival in rats (Fleming et al. 1942).

As in the oral studies, histopathological evaluation revealed that almost all animals exposed to phosphorus by subcutaneous injections developed changes in the bones consisting of thickening of the epiphyseal line and extension of trabeculae into the shaft. These changes were more conspicuous than those observed in rats administered white phosphorus in their diets. The livers of a few animals showed mild fatty degeneration, and those of two animals showed periportal fibrosis. Thus, liver damage was insignificant considering the long period of

treatment. Interstitial nephritis and fibrosis were observed in two animals but did not appear to be agent related. Mild hemosiderosis of the spleen was observed in rats receiving 0.05 mg/kg/day of white phosphorus; this condition, however, was pronounced and equally severe in all other treatment groups, including controls. Changes in the lungs, consisting of bronchopneumonia, pneumonitis, and bronchitis, were observed in 20 of 39 treated animals and 9 of 10 controls. Other organs were examined but showed no changes (Fleming et al. 1942).

Guinea pigs administered twice weekly subcutaneous injections of white phosphorus in oil at doses equivalent to 0.05 to 0.4 mg/kg/day showed the same skeletal changes, although less severe, as those observed in rats. None of the controls were affected. Fat droplets were found in liver cells of both treated animals and controls; thus this effect was not agent related. No changes were observed in other organs (Fleming et al. 1942).

Fleming et al. (1942) did not present weight data nor comment on the overall condition of the animals that were fed white phosphorus in their diet. Because all groups of rats fed white phosphorus in their diets survived as long as the controls, it is not possible to assign a response level for oral exposure. Rats that were administered white phosphorus by subcutaneous injection showed an increased mortality at the highest doses, increased survival at the lowest doses, and bone pathology at all doses; thus it appears that the bone pathology did not affect survival. These studies using rats and guinea pigs, administered white phosphorus orally or subcutaneously, did not establish a no observed effect level (NOEL), a NOAEL, or a lowest observed adverse effect level (LOAEL), because all doses elicited pathological changes in bones (Fleming et al. 1942). The relationship between bone pathology in laboratory animals and the effects of chronic occupational exposure to elemental phosphorus in humans (necrosis of the jawbone, Section 4.3.2) is unknown.

Adams and Sarnat (1940) confirmed that phosphorus has an effect on the bones of rats and rabbits. They administered one pill per day containing 0.6 mg of yellow phosphorus orally to approximately 17 rabbits (weights not given) for 13 to 117 days and phosphorized cod liver oil (0.01 percent phosphorus) to 14 rats for 22 to 57 days. Changes in bones and teeth were determined by X-rays taken at intervals and just prior to death and by histological examination of tibias and teeth. General growth and longitudinal bone growth in both rabbits and rats were adversely affected by yellow phosphorus. The average daily increase in tibial diaphysis was 0.36 mm in control rabbits and only 0.27 mm in phosphorus-treated littermates. In animals administered phosphorus, histological evaluation revealed dense bands called "phosphorus bands" in the metaphysis of long bones during the period of exposure and increased numbers of trabeculae due to reduced resorption of the intercellular calcified cartilage matrix. Zones of abnormally calcified dentin were also found in molars and incisors during the period of ingestion.

Because phosphorus causes changes indicating that fat metabolism may be disturbed, such as fatty degeneration of liver and other organs, Fleming and Collings (1951) carried out studies to determine if the fat content in the blood (as measured by the chylomicron count) may also be altered by phosphorus. Groups of 15 rats were administered yellow phosphorus 3 times a week at an equivalent dose of 1.1 mg/kg/day by subcutaneous injections. The 12 control rats were administered the vehicle, peanut oil. The animals were placed on a fat-free diet for 8 to 12 hr in order to determine the base chylomicron count in control and phosphorus-treated animals. Phosphorus had no effect on the base count. When control animals were returned to normal food, the chylomicron count peaked at 4 hr (4-hr counts). The 4-hr counts in phosphorus-treated animals, which were initiated on the 5th day of treatment, were markedly reduced, with a nadir at 12 days. Thereafter, the chylomicron count continued to rise even in the presence of continued phosphorus treatment until day 50, at which time the 4-hr count was not very different from that of controls. When the dose of yellow phosphorus was reduced to an equivalent of 0.8 mg/kg/day and administered for 80 days, the 4-hr chylomicron count was again reduced, but not to extent of the larger dose. The nadir occurred around day 17, and recovery was again evident at the end of the cycle of treatment. The animals were then withdrawn from yellow phosphorus for one month. When treatment resumed, the response was similar to the first cycle, but the nadir occurred around the 10th day. A third cycle of treatments was initiated; by the 14th day, the reduction in the 4-hr chylomicron count was greater than that observed in the previous cycles. If the recovery observed in the first two cycles indicated that the rats developed a tolerance for yellow phosphorus, then the tolerance was quickly lost after withdrawal of phosphorus.

4.3.1.2 Inhalation exposure

Brown et al. (1981) carried out a systematic study to examine the effects of chronic exposure to white phosphorus smoke on Sprague-Dawley rats. The four concentrations were 1,161 mg/m^3 (high), 589 mg/m^3 (intermediate), 192.5 mg/m^3 (low), and 0 (control). The high and intermediate dose groups were composed of 36 males and 36 females; the low dose group was composed of 18 males and 18 females. The control groups were composed of a total of 90 animals equally divided beween the two sexes.

The rats were exposed to smoke, generated by burning white phosphorus/felt, for 15 min/day, 5 days/week, for 13 weeks. After 6 weeks of exposure one-third of the animals were withdrawn: one-half of this group were bled for hematology and blood chemistry, and the tissues were submitted for histopathological evaluation; the remaining half were examined for physiological and behavioral changes. At the end of 13 weeks, one-half of the remaining animals were submitted to the same studies as those animals killed after 6 weeks of exposure. The other half was allowed to recover for 4 weeks; the animals were bled and tissues were submitted for histopathological evaluation.

The mortality rate was high in the high-dose group, but no animals in the intermediate and low exposure groups died prior to scheduled sacrifice. Of the 72 rats exposed to the high dose, 23 (32 percent) died within 6 weeks, and by the end of the experiment a total of 29 (40 percent) had died. Immediate effects of the high dose were dyspnea and wheezing, which cleared up within 2 hr (Brown et al. 1981)

There were no agent-related changes in body and organ weights during the course of this experiment. Blood chemistry and hematology, likewise, showed no agent-related changes, suggesting that inhalation of white phosphorus/felt smoke does not cause systemic effects under the conditions of this study. Pulmonary physiology tests revealed moist rales in 3 of 12 rats in the high-dose group but none in the intermediate-dose, low-dose, or control groups. There were indications that the tidal volume was reduced and breathing rate was increased in males exposed to the high dose for 13 weeks. The tendency toward pulmonary effects prompted a close histopathological examination of the organs and tissues of the respiratory system.

Histopathological evaluations revealed that almost all (> 70 percent) of the animals that died spontaneously after exposure to the high dose developed laryngitis, tracheitis, and congestion. Bronchitis was observed in 20 percent and interstitial pneumonia was observed in 53 percent. Laryngitis, tracheitis, and congestion were severe to moderate, bronchitis was slight, and interstitial pneumonia was slight to minimal. With the exception of interstitial pneumonia, these lesions were agent related.

The incidence of agent-related lesions observed in rats exposed for 6 weeks, 13 weeks, and 13 weeks plus a 4-week recovery period is presented in Table 16. Agent-related systemic lesions were not observed in any dose group.

Microscopically, the lesions in the larynx and trachea consisted of thickening of the lamina propria and submucosa by amphophilic to basophilic collagen, endothelial cell proliferation, and macrophage infiltration. Lesions in the bronchi and bronchioles, similar to but less extensive than those in the larynx and trachea, consisted of focal areas of altered collagen within the lamina propria (Brown et al. 1981).

According to Brown et al. (1981), the rats may have developed a tolerance to repeated exposures to white phosphorus/felt smoke, especially to the low dose. After exposing the animals to the low dose for 6 weeks, respiratory lesions consisted of only one case of tracheitis and no cases of laryngitis in a total of 30 organs examined. The small number of tracheas examined in the remaining low-dose groups may have limited detection of additional cases. Therefore, the absence of tracheitis in animals exposed to the low dose and killed after 13 weeks of exposure with or without 4 weeks of recovery is not convincing evidence that these animals developed a tolerance for white phosphorus smoke. Also, no evidence for tolerance was noted in the intermediate-dose group. From the data presented by Brown et al. (1981), an LOAEL was observed at 193 mg/m^3.

TABLE 16. INCIDENCE OF PATHOLOGICAL LESIONS IN RATS AFTER EXPOSURE TO WHITE PHOSPHORUS/FELT SMOKE[a]

Lesions	Doses[b]							
	Control		High		Intermediate		Low	
	M	F	M	F	M	F	M	F
6-week exposure								
Laryngitis	0/7	0/4	0	2/2	0	1/3	0	0/2
Tracheitis	0/3	0/7	0	4/4	3/6	2/4	1/6	0/3
Congestion	0/9	0/9	0	1/6	2/6	0/6	0/6	0/6
Bronchitis	0/9	0/9	0	3/6	0/6	0/6	0/6	0/6
13-week exposure								
Laryngitis	0/5	0/3	2/2	1/2	0	0	0/1	0/4
Tracheitis	0/7	0/8	0	2/3	3/5	3/6	0/5	0/4
Congestion	0/9	0/9	0/4	0/4	0/6	0/6	0/6	0/6
Bronchitis	0/9	0/9	0/4	0/4	0/6	0/6	0/6	0/6
13-week exposure + 4-week recovery								
Laryngitis/ tracheitis	0/15	0/14	9/9	6/7	10/12	10/12	0/6	0/6
Bronchitis/ bronchiolitis	0/15	0/14	4/9	2/7	4/12	1/12	0/6	0/6
Granulomatous bronchiolitis/ pneumonia	0/15	0/14	3/9	2/7	0/12	1/12	0/6	0/6

a. Brown et al. 1981.
b. 1,161 mg/m^3 (high), 589 mg/m^3 (intermediate), 192.5 mg/m^3 (low).

4.3.2 Human Data

Chronic exposure of humans to white (yellow) phosphorus causes only one-very characteristic-lesion, necrosis of the jaw, sometimes referred to as phosphorus necrosis or "phossy jaw." According to Oliver (1938), the first lucifer matches containing white phosphorus were made in 1827, and the first case of phosphorus necrosis was diagnosed in 1838 by Dr. Lorinser in Vienna. As the match-making industry flourished, more cases of phosphorus necrosis appeared, resulting eventually in phosphorus being the first industrial poison subjected to international legislation. The international treaty of Berne (drafted in 1906) prohibiting the manufacture, importation, and sale of matches containing white phosphorus was signed by almost all the countries of the world by 1925 (Ward 1926). Although Ward (1926) and Oliver (1938) believed that phosphorus necrosis due to industrial sources of phosphorus was a disease of the past, subsequent cases were reported by Kennon and Hallam (1944), Heimann (1946), and Hughes et al. (1962). A very recent case, although not of industrial origin, was described by Jakhi et al. (1983).

Phosphorus necrosis of the jaw was also associated with the manufacture of fireworks that contained white (yellow) phosphorus. Phosphorus fireworks were introduced into the United States in 1904. In 1926 the U.S. Fireworks Manufacturers' Association passed a resolution to support the efforts of the Department of Labor in eliminating white phosphorus from the fireworks industry (Ward 1926).

The incidence of phosphorus necrosis was less than 5 percent of those exposed to phosphorus (Sollmann 1957), and the estimated mortality rate from phosphorus necrosis was 20 percent (Hunter 1969, as reported by Miles 1972). Although the number of people affected was small, the disease was the most disfiguring and horrible of all occupational diseases in the last century and the early part of this century (Ward 1926).

According to Ward (1926), 37 cases of phosphorus necrosis were reported from 1893 to 1898 among 1,700 workers in Great Britain and Ireland, 7 cases among 570 workers in Holland (date not given), more than 34 cases among 2,600 workers from 1860 to 1895 in Belgium, 11 cases among 256 workers from 1874 to 1879 in Denmark, 140 (with 47 in one year) (number of workers not given) from 1881 to 1897 in Austria-Hungary, 5 to 10 cases per year among 300 workers in Switzerland (date not given), 28 cases among 400 workers from 1880 to 1893 in Norway, and 69 cases (number of employees not given) from 1860 to 1870 in Sweden. In a match-making factory in France, there were 32 cases of phosphorus poisoning in 1894, 125 cases in 1895, and 223 cases (1/3 of the work force) in 1896 (Oliver 1938).

In a survey of 15 match-making factories in the United States, from 1908 to 1909, 2,334 (65 percent) of 3,591 workers were exposed to phosphorus. More than 150 cases of phosphorus necrosis were discovered (four were fatal); the majority were women and children less than 16 years old. In three fireworks factories employing 71 workers, 14 cases of phosphorus necrosis (two fatal) were discovered (Ward 1926).

For several years after 1906, there was a decrease in the number of cases of phosphorus necrosis until a resurgence occurred in the years from 1914 to 1918 (Legge 1920). Eleven cases were discovered in a factory involved in the production of phosphorus in Great Britain (Legge 1920). Kennon and Hallam (1944) reported eight cases in a phosphorus production plant, and Hughes et al. (1962) reported 10 cases of phosphorus necrosis and six cases of delayed healing following extraction of teeth, which occurred after 1947, in workers exposed to phosphorus.

A description of the clinical course of phosphorus necrosis of the jaw as described by Bristowe (no date given) and reported by Hughes et al. (1962) is as follows:

> The disease, it was noticed, began usually with aching in one of the teeth. At first, this was probably mistaken for ordinary toothache, and would, indeed, at times intermit. Sooner or later, however, recurrence of pain necessitated the extraction of the tooth, and the pain and annoyance for a time probably ceased. The wound in the gum, however, was found not to heal; offensive matter began to ooze from it, and ere long a portion of the alveolus became exposed. Occasionally, the portion of bone thus denuded came away, bringing with it, perhaps one or two of the neighboring teeth, and the disease made no further progress. More frequently, however, the disease continued to spread; and, sometimes slowly, sometimes rapidly, more and more of the jaw-bones became denuded, the gums grew spongy and retreated from the alveoli, the teeth got loose and fell out, the fetid suppuration became more and more copious, the soft parts around grew swollen, tender, and infiltrated, and often the seat of sinuses. And thus, the disease continued to progress, till in the course of six months, a year, two years-it might be even five or six years-the patient sank from debility, or from phthisis, or from some other consequence of the local affection; or, having lost piecemeal, or in the mass, large portions-one half, or even the whole-of the upper or of the lower jaw, returned to his original state of good health, but the victim of shocking and permanent deformity. During the earlier, and more acute, stages of the disease, constitutional disturbance, as might be expected, generally showed itself, indicated by febrile symptoms, loss of appetite, thirst, constipation, a sallow, pasty condition of the skin; and these were often associated with intense pain in the affected parts, and consequent sleeplessness. After a while, however, (especially in cases that were tending to a favourable issue), pain and constitutional symptoms diminished, and the patient sometimes recovered the aspect of health, even while necrosis of the jaw was still progressing.

Recent cases of phosphorus necrosis were very mild compared with the disease described by Bristowe (as reported by Hughes et al. 1962). The progress of the disease, in earlier cases, resulted in the loss of large portions of the jawbone as large sequestra were formed; consequently, the facial structures became grossly disfigured. Sequestra are pieces of dead bone that become separated from sound bone during the process of necrosis. Sequestra from phosporus necrosis are light in weight, yellow to brown in color and have a worm-eaten appearance similar to that of pumice stone; they are osteoporotic and also decalcified (Hughes et al. 1962). Kennon and Hallam (1944) referred to recent cases as the "era of small sequestra," because the severity of this occupational disease has been reduced such that it should be regarded as one of caries and not of necrosis.

Although the cause of phosphorus necrosis is still questionable, phosphorus itself (Oliver 1938; Hughes et al. 1962), combustion products (fumes, vapors, or smoke) made up of oxides of phosphorus (Hughes et al. 1962; Miles 1972), phosphoric acid, and phosphorous acid (Oliver 1938) have each been implicated as the causative agent. Hughes et al. (1962) suggested that these agents entered the jawbones through a breach in the integrity of the jaw covering produced by caries, an extracted tooth, gingival inflammation, or inflammation of the overlying mucoperiosteum. These agents, in combination with the bacterial flora of the mouth, could then give rise to infections and subsequently cause necrosis and the formation of sequestra. In contrast, the fragility of long bones manifested by spontaneous multiple fractures of the femur in phosphorus workers is suggestive of a systemic route (Dearden 1901, as reported by Miles 1972). In addition, the delayed onset of the disease, which may extend for at least 2 years after workers are removed from the source of exposure (Hunter 1955), is also suggestive of a systemic route (Hughes et al. 1962). Nevertheless, the evidence for a systemic route for induction of phosphorus necrosis of the jawbone is still not convincing.

Hughes et al. (1962) studied 48 healthy men working in a phosphorus plant in order to evaluate the systemic effects of phosphorus exposure. The duration of exposure ranged from 1 to 17 years. The 28 control subjects were not perfectly age matched. Statistical differences were not found in the hematological evaluation or in plasma levels of inorganic phosphorus, alkaline phosphatase, calcium, and magnesium. Radiographs did not reveal differences in density of bones.

The concentration of phosphorus in the work environment was not estimated in any of the cases presented in this report. Therefore, the degree of exposure cannot be correlated with the disease process. The duration of exposure (length of exployment in phosphorus environment) prior to onset of phosphorus necrosis was known in most cases but varied considerably. Ward (1926) observed that exposure ranged from less than 3 months to 12 years prior to the onset of the disease. In two fatal cases, one worker was exposed for 6 years and the other for 2 years. The duration of illness, time from onset to recovery, ranged from about 5 months in one worker employed for 10 years to 6 years in another worker employed for 12 years (Ward 1926). In the 11 cases reported by Legge (1920), the duration of exposure ranged from 5 months to 23 years.

The duration of illness ranged from 2 months to 5 years. The duration of exposure in the cases described by Kennon and Hallam (1944) ranged from 13 months to 10 years. In three cases the disease did not reveal itself until after the worker had left the phosphorus process.

The evidence suggests that some individuals are more susceptible to phosphorus necrosis than others, particularly those with poor oral hygiene, caries, a tooth extracted during exposure to phosphorus, or other dental diseases (Ward 1926, Kennon and Hallam 1944, Miles 1972, Jahki et al. 1983).

4.4 GENOTOXICITY

4.4.1. Animal data

White phosphorus was tested for mutagenicity in the Ames test. Salmonella typhimurium strains TA-100 and TA-1535 were used to test for base-pair substitution, and strains TA-98, TA-1537, and TA-1538 were used to test for frame-shift mutations. An undiluted saturated solution of white phosphorus in distilled water (phossy water) used at a concentration of 100 μL/plate was not mutagenic either in the presence or the absence of the S-9 fraction of liver (Ellis et al. 1978).

Manthei et al. (1980) tested the same strains of Salmonella typhimurium using white phosphorus/felt smoke residue containing 65 percent phosphoric acid. White phosphorus/felt smoke residue in concentrations of 0.001 to 10.0 μL/plate was not mutagenic in any of the five strains tested. A concentration of 10 μL/plate was cytotoxic to all five strains, and 1.0 μL/plate was cytotoxic to strain TA 1535.

White phosphorus/felt smoke condensate was diluted with distilled water, mixed with dry food, and tested for its ability to induce mutations in fruit flies. Oregon-K strain Drosophila melanogaster red-eyed male fruit flies, 1 to 2 days old, were exposed to the mixture for 42 hr. The Muller-5 technique (sex-linked recessive lethal test) was used to test for mutations in the survivors. In this test, a recessive lethal mutation on the X-chromosome is indicated by the absence of the wild-type eye shape and color in males (Brown et al. 1980).

White phosphorus/felt smoke condensate did not induce mutations at concentrations ranging from 0.01 to 10 percent. Nevertheless, a dose-dependent increase in toxicity was observed; a concentration of 10 percent produced 100 percent mortality within 72 hr, 1 percent concentration produced 11 percent mortality, 0.1 percent concentration produced 2 percent mortality, and 0.01 percent caused no mortality (Brown et al. 1980).

Starke et al. (1982) conducted studies to determine if white phosphorus/felt smoke produced dominant lethal mutations in sustained-barrier, pathogen-free random-bred colony rats, AMRI:(SDxWI) (Sprague-Dawley). The concentrations of smoke, monitored during exposure, were 500 mg/m^3 (low dose), 1,000 mg/m^3 (high dose), and 0 (control).

Fertile male rats were exposed at 13 weeks of age for 15 min/day, 5 days/week for 10 weeks. This exposure period covered a whole spermatogenic cycle. Each of ten males from each group was mated with two virgin females for 5 days during the first and second week after exposure. The pregnant females were killed 11 days after separation from the males. The number of viable fetuses, dead fetuses or resorptions, and corpora lutea were counted.

Four animals died during exposure to the high dose. All males impregnated at least one female during both mating periods. For the most part, there were no significant differences between controls and animals exposed to smoke. Resorptions were observed in more females mated to males exposed to the low dose than in females mated to control or high-dose males. Because this paramater was not dose dependent, it was not significantly related to exposure. Therefore, white phosphorus smoke at doses of 500 mg/m^3 or 1,000 mg/m^3 did not induce dominant lethal mutations in rats.

4.4.2 Human Data

No data were found on the genotoxicity of white phosphorus in humans.

4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY

4.5.1 Animal Data

Sustained-barrier, pathogen-free, random-bred colony rats, AMRI:(SDxWI) (Sprague Dawley) were used to determine the effect of white phosphorus/felt smoke on development and reproduction (Starke et al. 1982). In a teratogenicity test, each 12-week-old male was mated with two 12-week-old virgin females. After confirmation of mating, 24 females were assigned to the control group, 24 to the low-dose group (500 mg/m^3), and 36 to the high-dose group (1,000 mg/m^3). The animals were exposed daily for 15 min/day, starting with day 6 of pregnancy and continuing to day 15. On day 20 of pregnancy, the fetuses were delivered by Caesarean section. The results are shown in Table 17. Major variations were unilateral anophthalmia, narrow atria, short tongue, brachygnathia, and thin-walled heart. Minor variations were ectopic kidneys, ectopic testicles, and reversed ductus arteriosus.

In a single-generation study, 12-week-old male rats were exposed to the same doses of white phosphorus/felt smoke 15 min/day, 5 days/week for 10 weeks, in order to cover a complete spermatogenic cycle, and 12-week-old female rats were exposed for 3 weeks, in order to cover 4 to 5 estrous cycles (Starke et al. 1982). Exposure to the females continued throughout mating, gestation, and lactation. Litters were exposed up to

TABLE 17. WHITE PHOSPHORUS/FELT SMOKE: VISCERAL VARIATIONS AND ABNORMALITIES[a]

Variations and Abnormalities	Air Control	Low Dose (500 mg/m^3)	High Dose (1,000 mg/m^3)
Visceral			
Prominent renal pelvis	4	3	5
Ectopic kidney(s)	1	4	
Narrow atrium	1	1	1
Thin-walled heart			1
Reversed ductus arteriosus			9
Underdeveloped testicles	3	1	
Ectopic testicles			3
Hemorrhagic eyes		1	
Anophthalmia, unilateral[b]	1		
Short tongue[b]		1	
Brachygnathia[b]			1
Skeletal			
Fourteenth rib extra (rudimentary)	16	39	25
Cleft sternebrae	2	0	2
Dumbbell-shapped sternebrae	16	7	6
General hypoplasia of the sternebrae	35	46	33
Dumbbell-shaped vertebra - thoracic	9	11	2
Hypoplasia of xyphoid process	2	11	19

a. Adapted from Starke et al. 1982.
b. Abnormalities.

21 days of age. Pups were examined and weighed at 1, 4, 7, and 21 days of age. There were no abnormalities in any of the pups delivered nor significant differences in the litter sizes. The mean body weights of the pups in the high-dose group were lower at all ages than those in the low-dose and control groups. The survival, viability, and lactation indices of pups in the high-dose group were significantly lower than those in the low-dose and control groups. Because the mothers did not resume nursing for 2 to 3 hr after exposure, this difference was attributed to the weakened condition of mothers exposed to 1,000 mg/m^3. Because the results of the single generation study did not confirm the teratogenic effects (brachygnathia, thin-walled heart, reversed ductus arteriosus in the high dose group) of white phosphorus/felt smoke, the results of the teratology study were not agent related.

Elemental yellow phosphorus in corn oil was administered by gavage to 8-week-old male and female Sprague-Dawley rats (Monsanto 1985). Groups of 15 males and 30 females were given 0.075 (high dose), 0.015 (intermediate dose), and 0.005 (low dose) mg/kg/day for 80 days prior to mating. Treatment continued throughout mating, gestation, and lactation. Because of the low fertility rate in all groups including controls, treatment was continued for a second cycle from mating through lactation. Controls, consisting of 15 males and 30 females, were exposed to corn oil only.

The mortality rates in males were very low: 0, 0, 2, and 1 per 15 animals in the high, intermediate, low, and control groups, respectively. The mortality rates in females were 16 (53 percent), 1, 1, and 4 per 30 animals in the high, intermediate, low, and control groups, respectively. In the high-dose group, seven females died on gestation day 21 or 22 of the first cycle and six died during the last two days of gestation of the second cycle. Because of the timing of almost all of these deaths, the cause of death was attributed to difficulty in parturition. No other clinical signs of toxicity were observed in treated animals, with the exception of hair loss on both forelimbs in the high-dose group. Mean body weights of adults and pups, mean number of viable pups, mean number of dead pups, survival from birth to weaning, and histopathological evaluation revealed no statistically significant dose- or agent-related changes. These results contrast those by Sollmann (1925), who observed a pronounced weight loss in rats given median daily doses of 0.072 mg/kg in their diets. Thus, the adverse effect of 0.075 mg/kg/day on parturition was the only agent-related change observed in this study. The NOAEL was 0.015 mg/kg/day (Monsanto 1985).

4.5.2 Human Data

No data were found on the teratogenicity or reproductive toxicity of white phosphorus in humans.

4.6 ONCOGENICITY

4.6.1 Animal Data

Specific data on oncogenicity of white phosphorus were not found. Neoplastic lesions were not reported in studies of rats administered white phosphorus orally (0.2 to 1.6 mg/kg/day) or subcutaneously (0.05 to 3.2 mg/kg/day) nor in guinea pigs administered white phosphorus subcutaneously (0.05 to 0.4 mg/kg/day) for their entire life time (Fleming et al. 1942).

4.6.2 Human Data

No data were found on the oncogenicity of white phosphorus in humans.

4.7 SUMMARY

White phosphorus is absorbed from the gastrointestinal tract in laboratory animals, such as mice, rats, and rabbits. Although the rate of absorption may vary depending on the dose, absorption is evident within a few minutes and is essentially complete by 24 hr. The major tissues that accumulate white phosphorus are liver, kidney, lung, bone, and skeletal muscle. For the most part, the blood level is lower than that of the liver, indicating that white phosphorus is accumulated in tissues. White phosphorus is eliminated from the body by urinary excretion as inorganic and organic phosphates. It also appears that white phosphorus is eliminated in feces by direct passage through the gastrointestinal tract or by means of biliary excretion into the small intestine. There is no convincing evidence that white phosphorus enters the systemic circulation following inhalation or dermal exposure of laboratory animals.

There is no direct information on the pharmacokinetics of white phosphorus in humans. The systemic effects of ingested white phosphorus indicate that white phosphorus is absorbed from the gastrointestinal tract and that a liquid vehicle, especially alcohol, facilitates the absorption. The early onset of severe effects leading to death suggests that the dose of phosphorus ingested may affect the rate at which white phosphorus is absorbed; death has occurred as early as 3.5 hr and as late as 8 days following ingestion. Following ingestion, phosphorus blood levels may be reduced, elevated, or, as in most cases, remain normal. There is no convincing evidence that white phosphorus is absorbed from the lungs or skin in humans.

The effects of acute exposure to white phosphorus in laboratory animals and in humans are similar. After ingestion the major target organs are the gastrointestinal tract, liver, kidney, brain, and cardiovascular system. The effects on the gastrointestinal tract are due to local irritation, whereas the effects on the other organs are probably due to systemic absorption. A characteristic lesion due to white

phosphorus intoxication, in both laboratory animals and humans, is fatty degeneration of the liver.

The acute oral LD50's for white phosphorus in peanut oil are 3.76 and 3.03 mg/kg for male and female rats, respectively, and 4.85 and 4.82 mg/kg in male and female mice, respectively. The acute oral LD50 for white phosphorus/felt condensate in rats is 2,346.8 mg/kg at 24 hr and 2,184.5 mg/kg at 14 days. The acute intravenous LD50 for white phosphorus/felt condensate, containing 65 percent phosphoric acid, in rats is 209.6 mg/kg or 0.252 mL of the undiluted condensate at 24 hr. Therefore, white phosphorus given orally is more toxic than white phosphorus/felt smoke condensate given either orally or intravenously.

The minimum lethal dose of white phosphorus in humans who do not receive medical treatment has been estimated as 100 mg (1.4 mg/kg) but could be as low as 50 mg (0.7 mg/kg) for a 70-kg person. Toxicity may be observed at doses of 15 mg (0.2 mg/kg). In one of the cases presented in this report, 190 mg was lethal in the absence of medical treatment. With medical treatment, the mortality rate in those who ingested doses of 1.57 g (22.4 mg/kg) or more was 90 percent, whereas in those who ingested 0.78 g (11.1 mg/kg) or less the mortality rate was only 18 percent.

The effects of inhaling white phosphorus smoke are limited to the respiratory tract, especially the upper respiratory tract. The minimum harassing concentration is 700 mg/m^3 in working humans and 1,000 mg/m^3 in resting humans. Humans inhaling as little as 185 mg/m^3 for 5 min suffer from sore throat, coughing, nasal discharge, tightness in the chest, and congestion. In one study, inhaling an unknown amount for 15 to 20 min also caused laryngitis, which persisted for 8 months.

Because white phosphorus ignites spontaneously in air, it causes severe burns if it comes in contact with the skin. Third degree burns covering 35 percent or more of the body surface cause death in humans, and burns covering 20 percent of the body surface of rabbits cause at least 65 percent mortality within 3 days. White phosphorus ignited within an inguinal incision in rats causes an increase in serum inorganic phosphate and clinical changes indicative of renal damage.

White phosphorus in concentrations of 0.1 percent in peanut oil is not irritating to rabbit skin or eyes. A 0.1 percent concentration of white phosphorus/felt smoke condensate, containing 65 percent phosphoric acid, does not sensitize guinea pig skin. Undiluted white phosphorus/felt smoke condensate applied to rabbit skin causes severe irritation, and 0.1 mL produces irreversible damage to the eyes.

Unlike acute exposure, which causes similar effects in laboratory animals and humans, subchronic/chronic exposure causes very different effects in laboratory animals and humans. In laboratory animals, oral or subcutaneous administration causes reduced growth, reduced survival at high doses, and increased survival at low doses. Liver damage is usually insignificant. Bone pathology, observed at doses as low as 0.05 mg/kg/day in rats and guinea pigs, is characterized by a thickening of

the epiphyseal line and extension of the trabeculae into the shaft. This lesion is very different from those produced in humans by chronic occupational exposure to white phosphorus.

In humans, chronic occupational exposure to white phosphorus produces a suppurative lesion in which the jawbones become necrotic, leading, in some instances, to total loss of the upper or lower, or sometimes both jawbones. The disease called phosphorus necrosis of the jaw, or phossy jaw, had an estimated incidence rate of less than 5 percent of those exposed and a mortality rate of 20 percent in the last century. The duration of exposure prior to diagnosis of phosphorus necrosis ranged from 3 months to 23 years. Susceptible individuals are those with poor oral hygiene, caries, tooth extraction during exposure, or other dental diseases. In recent years, strict medical and dental surveillance, early diagnosis, and treatment have led to reductions in both the incidence and severity of this occupational disease.

Subchronic inhalation exposure to white phosphorus/felt smoke in laboratory animals causes lesions in the respiratory tract similar to those observed in humans after acute inhalation. The mortality rate in rats exposed to 1151 mg/m^3 was 40 percent. Histopathological examination revealed that subchronic inhalation of white phosphorus/felt smoke causes laryngitis, tracheitis, congestion, and bronchitis.

A saturated solution of white phosphorus in distilled water (phossy water) is not mutagenic in five strains of Salmonella typhimurium in the presence or absence of metabolic activation. White phosphorus/felt smoke condensate is not mutagenic in the Ames test, but it is toxic to all five strains at a concentration of 10 μL/plate and toxic to one strain at 1.0 μL/plate. White phosphorus/felt smoke condensate does not induce sex-linked recessive mutations on the x-chromosome in Oregon-K strain Drosophila melanogaster but causes 100 percent mortality at a concentration of 10 percent. White phosphorus/felt smoke at concentrations of 1,000 and 500 mg/m^3 does not induce dominant lethal mutations in rats.

There are no data on the mutagenic, teratogenic, or carcinogenic effects of white phosphorus in humans.

In the only teratology test available, white phosphorus/felt smoke at 1,000 mg/m^3 induced a few major malformations consisting of brachygnathia, thin-walled heart, and reversed ductus arteriosus. Because these malformations were not confirmed by the single-generation study, white phosphorus/felt smoke is not considered to be teratogenic. It does, however, reduce survival, viability, and lactation indices in pups at 1,000 mg/m^3.

In one report, female rats administered 0.75 mg/kg of yellow phosphorus orally for 80 days prior to mating, and through two gestation periods, experienced a high mortality rate. A total of 13 females (43 percent) died within 2 days of parturition; therefore, death was attributed to difficulty in parturition. Because doses of 0.015 and 0.005 mg/kg had no effects, an NOAEL of 0.015 mg/kg was established.

5. CRITERION FORMULATION

5.1 EXISTING GUIDELINES AND STANDARDS

The USEPA (1976, as reported in Poston et al. 1986) recommends a limit of 0.1 μg/L of total phosphorus in marine or estuarine waters. Lai (1979a) reports a recommended discharge standard of 0.01 μg/L.

The 8-hr time-weighted average (TWA) for exposure to yellow phosphorus established by the Occupational Safety and Health Administration (OSHA) is 0.1 mg/m^3 (OSHA 1974). According to the International Labor Office, in all countries listed in their survey, the standards range from 0.03 to 0.15 mg/m^3 (ILO 1980).

The threshold limit value (TLV) adopted by the American Conference of Govermental Industrial Hygienists (ACGIH) is the same as the OSHA standard, 0.1 mg/m^3 (TWA); the 15-min time-weighted average (STEL) is 0.3 mg/m^3 (ACGIH 1985).

OSHA/NIOSH (National Institute for Occupational Safety and Health) recommended that medical surveillance of employees exposed to white phosphorus include a complete medical history and physical examination prior to employment, a semiannual medical examination, which would include liver function test and hematology, and a dental examination with X-rays (OSHA/NIOSH 1978). According to Hughes et al. (1962), if adequate dental standards are attained in all prospective employees and regular dental examinations are conducted during employment, then the incidence and severity of phosphorus necrosis would be reduced. They suggested that employees working in areas where exposure is heavy (production workers) should be examined every two months, those regularly exposed should be examined every four months, and those casually exposed should be examined semiannually.

5.2 OCCUPATIONAL EXPOSURE

Occupational exposure to elemental phosphorus causes one very characteristic disease, phosphorus necrosis of the jawbone, or "phossy jaw." Because white phosphorus was used in the match-making industry, numerous cases of this occupational disease appeared during the 19th century and the early part of the 20th century. The countries that signed the international treaty of Berne in 1906 agreed to prohibit the manufacture, importation, and sale of matches containing white phosphorus. Thereafter, the incidence and severity of the disease decreased (Ward 1926, Oliver 1938, Kennon and Hallam 1944), with a brief resurgence during World War I (Legge 1920). The United States did not sign the treaty of Berne, but laws imposing a tax of 2 cents per 100 matches in 1913 and inhibiting the importation of matches containing white phosphorus in 1914 effectively ended the use of white phosphorus in matches in the United States (Ward 1926).

White phosphorus was first used in making fireworks in the United States in 1904. In 1926 the U.S. Fireworks Manufacturers' Association passed a resolution to support the efforts of the Department of Labor in eliminating the use of white phosphorus in the fireworks industry (Ward 1926). Between 1908 and the present, more than 195 cases of phosphorus necrosis of the jawbone were found in workers involved in phosphorus production, fireworks manufacture, and the match-making industry (Ward 1926, Kennon and Hallam 1944, Heimann 1946, Hughes et al. 1962). Surprisingly, no cases were reported in the rat and roach paste production industry that use white phosphorus as a pesticide.

The available literature did not provide information on the recent incidence of phosphorus necrosis in workers involved in the manufacture of smoke materials containing white phosphorus. Although white or yellow phosphorus was used for military purposes during World War I, no cases of phosphorus necrosis were reported in the United States or Great Britain (Ward 1926).

The airborne levels of white phosphorus were not known in the case histories presented in the literature; therefore, the incidence of phosphorus necrosis could not be correlated with concentrations of white phosphorus in the air. In a 1975 survey, the levels of white phosphorus in a facility involved in the production of smoke materials on a wet fill production line were 0.224 and 0.450 mg/m^3. In 1977 the levels on a dry fill line were 0.7 to 1.2 $\mu g/m^3$. According to Berkowitz et al. (1981), the 1975 levels, which exceeded the OSHA standards, were probably underestimated due to problems with quality assurance; the 1977 levels were below the OSHA standard, but the samples were not taken during full production.

5.3 PREVIOUSLY CALCULATED CRITERIA

Bentley et al. (1978) estimated a water quality criterion for the protection of aquatic life and its uses for elemental phosphorus by applying a safety factor to the lowest observed effect level during chronic exposure. The authors concluded from their studies and from data in the literature that the two species most sensitive to phosphorus are the fathead minnow and the midge. Exposure of parental fathead minnows to 0.40 $\mu g/L$ (lowest test concentration) resulted in significant mortality of second-generation embryos (Bentley et al. 1978). Therefore, by applying a safety factor of 0.1 to the chronic exposure level of 0.40 $\mu g/L$, the authors recommend an ambient water quality criteria of 0.04 $\mu g/L$ for the protection of aquatic organisms.

Sullivan et al. (1979) derived a safe concentration level for white phosphorus using a conservative general application factor of 0.01. This is based on the ability of white phosphorus to persist in sediments in aquatic systems and its cumulative toxicity (National Academy of Sciences 1973, as reported in Sullivan et al. 1979; EPA 1976, as reported in Poston et al. 1986). This factor is applied to the lowest acute toxicity value of 2 $\mu g/L$ for bluegill to give a safe concentration of 0.02 $\mu g/L$.

The U.S. Army (1982, 1983), in assessing a review by Sullivan et al. (1979) of the current aquatic toxicity data for white phosphorus, determined that the lack of a tested "no-effect" level prevents the use of proposed EPA methodology for calculating a water quality criterion. However, an interim criterion for the protection of aquatic organisms of ≤ 0.01 µg/L was determined by extrapolation of the existing data.

A previous attempt to calculate a criterion for the protection of human health failed because of insufficient data to derive the criterion. A subchronic toxicity study by Lhota and Hannon (1979) failed to yield a NOEL, and data from chronic toxicity studies were not available (U.S. Army 1982, 1983).

5.4 AQUATIC CRITERIA

A brief description of the methodology proposed by the USEPA for the estimation of water quality criteria for the protection of aquatic life and its uses is presented in Appendix A. The aquatic criteria, as proposed by USEPA, consists of two values, a Criterion Maximum Concentration (CMC) and a Criterion Continuous Concentration (CCC) (Stephan et al. 1985). The CMC is equal to one-half the Final Acute Value (FAV), whereas the CCC is equal to the lowest of the Final Chronic Value, the Final Plant Value, or the Final Residue Value.

Although many data on toxicity to aquatic organisms are available, few fit the characteristics needed to include them in the calculation of an aquatic criterion. For example, LT_{50} values, although they were derived from flow-through tests with measured phosphorus concentrations, cannot be used, because they are difficult to compare with LC_{50} values. These data, however, are supportive evidence that white phosphorus is highly toxic.

Concentrations of white phosphorus may decline during static testing; therefore, the use of nominal concentrations can result in the overestimation of total exposure. However, Stephan et al. (1985) state that results from static tests using nominal concentrations are acceptable. Therefore, valid acute toxicity data are available for eight families of aquatic organisms. These families do not represent the specified eight families required by the USEPA guidelines (Stephan et al. 1985); data for two of the required eight families are missing. However, a tentative FAV of 2.3060 µg/L is estimated, with N equal to 8; calculations are given in Table 18.

Acute and chronic flow-through tests using measured concentrations are required for three species in order to calculate an acute/chronic ratio (Stephan et al. 1985). Results from such acute tests are not available, and the chronic studies are inadequate. The study for Pimephales promelas conforms to the guidelines; however, the data lack a no-observable-effects level (Bentley et al. 1978). The study with Chironomus tentans by Bentley et al. (1978) failed to produce viable eggs in control or treatment groups. For multivoltine species, such as the midge, large numbers of larvae are required to assure mating. In

TABLE 18. CALCULATIONS FOR FINAL ACUTE VALUE (FAV) OF WHITE PHOSPHORUS[a]

Rank (R)	GMAV[b]	ln GMAV[c]	(ln GMAV)^2	P=R/(N+1)[d,e]	$\sqrt{P}$
4	30.0	3.4012	11.5682	0.4444	0.6666
3	22.0	3.0910	9.5543	0.3333	0.5773
2	20.0	2.9957	8.9742	0.2222	0.4714
1	3.8	1.3335	1.7782	0.1111	0.3333
Sum:		10.8214	31.8749	1.1111	2.0486

a. Based on calculation methods discussed in Stephan et al. 1985.
b. GMAV = genus mean acute value in μg/L.
c. ln GMAV = natural log of GMAV.
d. N = 8.
e. P = probability for each GMAV; R = rank of four lowest GMAVs.

$$S^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

$$S^2 = \frac{31.8749 - (10.8214)^2/4}{1.1111 - (2.0486)^2/4} = 41.9903; \quad S = 6.4800$$

$$L = [10.8214 - (6.4800)(2.0486)]/4 = -0.6134$$

$$A = (6.4800)(\sqrt{0.05}) + (-0.6134) = 0.8355$$

$$FAV = e^{0.8355} = 2.3060$$

the study with Daphnia magna (Bentley et al. 1978), insufficient numbers of offspring were produced in both treatment and control groups. In addition, data for D. magna lacked a no-observable-effects level. Consequently, a Final Chronic Value could not be calculated.

Bentley et al. (1978) studied the changes in cell density and chlorophyll a content in four species of phytoplankton following exposure to nominal concentrations ranging from 7 to 670 µg/L. No clear exposure-response relationship was established for either test. Selenastrum capricornutum and Navicula pelliculosa showed reductions in cell density and chlorophyll a content, while Anabaena flos-aquae and Microcystis aeruginosa exhibited increases in both parameters. A Final Plant Value could not be derived, due to the inconclusiveness of the data and the use of nominal concentrations. USEPA guidelines (Stephan et al. 1985) require the use of measured concentrations in aquatic plant toxicity tests.

Bioconcentration factors were calculated for the uptake of white phosphorus by various species of fish and invertebrates and two species of seaweed. Values range from 9 to 2,000 in fishes, 10 to 1,267 in invertebrates, and 22 for seaweed. Uptake is rapid and extensive, occurring primarily in the liver and muscle in fish and the hepatopancreas in lobster. Depuration is also rapid, with white phosphorus undetected within 7 days post-exposure. According to the guidelines (Stephan et al. 1985), a Final Residue Value is the maximum permissible tissue concentration divided by appropriate bioconcentration factors. However, no information is available to determine the maximum permissible tissue concentration; consequently, a Final Residue Value cannot be calculated.

In conclusion, a tentative CMC of 1.1530 µg/L is calculated from the available data; however, it should be stressed that data for two of the required eight families are missing. It is important to note that the lowest acute 96-hr LC50 value obtained from a flow-through test was 2.4 µg/L (nominal concentrations) for bluegill. Because the final chronic, plant, and residue values could not be calculated, a CCC cannot be determined for white phosphorus. However, chronic studies with fathead minnows indicate that growth is stunted at 1.5 µg/L, and survival and hatchability are reduced at 0.4 µg/L.

5.5 HUMAN HEALTH CRITERIA

There is no evidence that white phosphorus is carcinogenic in laboratory animals or humans. There are also no acceptable data for estimating nonthreshold or threshold chronic toxicity in humans. Therefore, the data presented by Monsanto (1985) on the reproductive toxicity in rats administered 0.075, 0.015, or 0.005 mg/kg/day of yellow phosphorus by gavage for approximately 164 days were used to calculate a human health criterion. Because only the pregnant females were sensitive to yellow phosphorus in the Monsanto study, the criterion would also protect males and nonpregnant females. At a dose of 0.075 mg/kg/day of yellow phosphorus, 16 of 30 females (53 percent) died

during treatment, 13 (43 percent) of which died during the last 2 days of gestation. The suggested cause of death was difficulty in parturition. Deaths in the other groups (3.3 percent at 0.015 and 0.005 mg/kg/day, 13 percent in vehicle control) were not associated with parturition. One other toxic effect, loss of hair, was also observed at 0.075 mg/kg/day. Observations on weights and a complete histopathological evaluation, including bone and liver, did not reveal significant effects of yellow phosphorus in exposed males, females, or pups. This study suggested that pregnant females are a sensitive subgroup. That bone pathology was not observed in this study is significant, because Fleming et al. (1942) observed bone pathology in rats given twice-weekly subcutaneous injections of white phosphorus at a dose rate of 0.05 mg/kg/day for their entire lifetime. Therefore, bone appears to be a less sensitive target than the pregnant female, or the duration of exposure in the Monsanto study was insufficient for the development of bone pathology.

A "frank effect level" (FEL) in females was observed at 0.075 mg/kg/day and a NOAEL was established at 0.015 mg/kg/day. Applying the USEPA guidelines (summarized in Appendix B) for deriving a water quality criterion for the protection of human health, an uncertainty factor of 100 was used to calculate an acceptable daily intake (ADI) for a 70-kg person (USEPA 1980). An uncertainty factor of 100 was chosen because the experimental procedure and the data were judged to be satisfactory for a reproductive toxicity study. In addition, the terms "subchronic" and "chronic" do not apply to reproductive toxicity studies. Consequently, the uncertainty factor should be based not on the duration of treatment but on the validity of the data. The calculated ADI using the equation shown below is 0.0105 mg/day. The acute lethal dose in untreated humans (100 mg) is about 9.5×10^3 times higher, and the acute toxic dose (15 mg) is 1.4×10^3 times higher than the ADI.

$$\text{ADI (mg/day)} = \frac{70 \text{ kg} \times \text{NOAEL (mg/kg/day)}}{\text{uncertainty factor}}$$

The following equation was used to calculate the human health criterion for white phosphorus:

$$C = \frac{\text{ADI} - (\text{DT} + \text{IN})}{2 \text{ L/day} + (0.0065 \text{ kg/day} \times \text{BCF})}$$

where

C = water quality criterion
ADI = acceptable daily intake
DT = daily non-fish consumption
IN = daily intake by inhalation
2L = daily water intake
0.0065 kg/day = daily fish and shellfish intake
BCF = bioconcentration factor = 67.7 (see Table 4)

$$C = \frac{0.0105 \text{ mg/day} - (0 + 0)}{2 \text{ L/day} + (0.0065 \text{ kg/day} \times 67.7)}$$

$$C = 4.303 \ \mu g/L$$

This criterion for the protection of human health should be considered an interim criterion until the data presented by Monsanto (1985) have been confirmed and a 2-year feeding study has been conducted. A criterion of 4.303 μg/L is 275 times higher than the interim criterion of 0.01 μg/L for the protection of aquatic life (U.S. Army 1982, 1983) and 3.7 times the aquatic criterion of 1.1530 μg/L (CMC) reported in this document. From the available data on the toxicity of white phosphorus in humans, the interim criterion for the protection of aquatic life would also be adequate for the protection of human health, including pregnant women.

5.6 RESEARCH RECOMMENDATIONS

In order to meet the requirements established by the USEPA for deriving water quality criteria, the following research studies are recommended to fill gaps in the existing data.

1. Acute toxicity tests, which follow ASTM procedures, should be conducted for a family in a phylum other than Arthropoda or Chordata (e.g., an annelid) and for a family in any insect order or phylum not represented.

2. Chronic flow-through tests should be conducted using measured concentrations for a fish species (e.g. Pimephales promelas), an invertebrate species (e.g., Chironomus tentans), and a sensitive freshwater species (e.g., Lepomis macrochirus or Daphnia magna) in order to calculate a Final Chronic Value.

3. As a part of the chronic study explained in (2) above, acute flow-through tests using measured concentrations should be conducted on the same three species in order to calculate acute-chronic ratios.

4. A conclusive toxicity test with an alga or aquatic vascular plant should be conducted using measured concentrations and a biologically important end point in order to calculate a Final Plant Value.

5. A chronic wildlife feeding study or a long-term wildlife field study should be conducted in order to determine a maximum permissible tissue concentration for the calculation of a Final Residue Value.

6. A 2-yr oral toxicity study to determine the long-term effects of white phosphorus administered by gavage should be conducted in rats or mice. The dose levels should be in small increments, starting at 0.2 mg/kg as used by Fleming et al. (1942), in order to establish no- and low-effect levels. A complete histopathological evaluation should be conducted with special emphasis placed on the liver, kidney, teeth, and long bones (femur).

7. The implications of the Monsanto (1985) report suggest that high priority should be given to repeating their reproductive toxicity study. Doses between 0.015 and 0.075 mg/kg/day should be included in order to adequately define the lowest adverse effect level. These experiments should determine if chronic pretreatment is a prerequisite for eliciting the lethal effects of white phosphorus in pregnant animals or if treatment during the gestation period only is sufficient.

6. REFERENCES

ACGIH. 1985. American Conference of Governmental Industrial Hygienists. Threshold Limit Values and Biological Exposure Indices for 1985-86. ACGIH Publication Office, Cincinnati.

Ackmar, R.G., R.F. Addison, and J. Hingley. 1970. An assessment of the assimilation of elemental phosphorus by Newfoundland marine organisms in the 1969 pollution problem and in the 1970 monitoring operation. Fish. Res. Board Can. Tech. Rep. 208.

Ackman, R.G., R.F. Addison, P.J. Ke, and J.C. Sipos. 1971. Examination of bottom deposits, Long Harbour, Newfoundland, for elemental phosphorus and for fluorides. Fish. Res. Board Can. Tech. Rep. 233.

Adams, C.O. and B.G. Sarnat. 1940. Effects of yellow phosphorus and arsenic trioxide on growing bones and growing teeth. Arch. Pathol. 30:1192-1201.

Addison, R.F. 1971. Analysis of elemental phosphorus and some of its compounds by gas chromatography. Proc. Int. Symp. Identification Measurement Environmental Pollutants, Ottawa, Ont., June 14-17, 1971. pp. 386-390.

Addison, R.F. and R.G. Ackman. 1970. Direct determination of elemental phosphorus by gas-liquid chromatography. J. Chromatogr. 47:421-426.

Aiken, D.E. and E.H. Byard. 1972. Histological changes in lobsters (Homarus americanus) exposed to yellow phosphorus. Science 176:1434-1435.

Aizenshtadt, V.S., S.M. Nerubai, and I.I. Voronin. 1971. [Clinical aspects of acute poisoning by vapors of phosphorus and its oxides under industrial conditions]. Gig. Tr. Prof. Zabol. 15(10):48-49 (Russian). (As reported in Wasti et al. 1978).

Appelbaum, J., N. Ben-Hur, and J. Shani. 1975. Subcellular morphological changes in the rat kidney after phosphorus burn. Pathol. Eur. 10(2):145-154.

Ashburn, L.L., A.J. McQueeney, and R.R. Faulkner. 1948. Scarring and precirrhosis of the liver in chronic phosphorus poisoning of guinea pigs. Proc. Soc. Exp. Biol. Med. 67:351-358.

Ben-Hur, N. and J. Appelbaum. 1973. Biochemistry, histopathology and treatment of phosphorus burns: An experimental study. Isr. J. Med. Sci. 9(1):40-48.

Ben-Hur, N., A. Giladi, Z. Neuman, B Shugerman, and J. Applebaum. 1972. Phosphorus burns-A pathophysiological study. Br. J. Plast. Surg. 25:238-244.

Bentley, R.E., J.W. Dean, T.A. Hollister, G.A. LeBlanc, S. Sauter, B.H. Sleight III, and W.G. Wilson. 1978. Laboratory Evaluation of the Toxicity of Elemental Phosphorus (P_4) to Aquatic Organisms. AD A061785. EG&G, Bionomics, Wareham, MA. DAMD17-74-C-4101.

Berkowitz, J.B., G.S. Young, R.C. Anderson, A.J. Colella, W.J. Lyman, A.L. Preston, W.D. Steber, R.G. Thomas, and R.G. Vranka. 1981. Research and Development for Health and Environmental Hazard Assessment Task Order 5. Occupational and Environmental Hazards Associated with the Formulation and Use of White Phosphorus-Felt and Red Phosphorus-Butyl Rubber Screening Smokes. AD A116956. Arthur D. Little, Inc., Cambridge, MA. DAMD17-79-C-9139.

Blackwell, R.Q. 1967. Recommendation for a Safer Treatment of White Phosphorus Burns. Technical Report NAMRU-2-TR-300, AD 668020. U.S. Naval Medical Research Unit No. 2, Taipei, Taiwan, Republic of China.

Bohn, H.L., G.V. Johnson, and J.H. Cliff. 1970. Detoxification of white phosphorus in soil. J. Agric. Food Chem. 18:1172-1173.

Bowen, T.E. 1969. Production of a standard white phosphorus burn and its effect upon serum calcium and phosphorus. Clin. Res. 17(2):379 (abstr.)

Bowen, T.E., T.J. Whelan, Jr., and T.G. Nelson. 1971. Sudden death after phosphorus burns: Experimental observations of hypocalcemia, hyperphosphatemia and electrocardiographic abnormalities following production of a standard white phosphorus burn. Ann. Surg. 174(5):779-784.

Brown, B.J., G.E. Affleck, R.L. Farrand, et al. 1980. The Acute Effects of Single Exposures to White Phosphorus Smoke in Rats and Guinea Pigs. Technical Report 80013, AD B051836L. Chemical Systems Laboratory, Aberdeen Proving Ground, MD

Brown, B.J., R.L. Farrand, G.E. Affleck, et al. 1981. The Subchronic Effects of Repeated Exposure to White Phosphorus/Felt Screening Smokes in Rats. Technical Report ARCSL-TR-80068, AD B058048. Chemical Systems Laboratory, Aberdeen Proving Ground, MD.

Buchanan, D.J., M.V. Sigal, Jr., C.S. Robinson, et al. 1954. Studies of phosphorus intoxication. I. Changes in blood, urine, and tissues of dogs poisoned with phosphorus. Arch. Ind. Hyg. Occup. Med. 9:1-8.

Bullock, E. and M.J. Newlands. 1969. Decomposition of phosphorus in water. Proc. Conf. Poll. Chem. Inst. Can., Halifax, N.S. Aug 24-26. pp. 23-24. As reprinted in 1972. Fish. Res. Board Can. Circ. No. 2.

Caley, J.P. and I.A. Kellock. 1955. Acute yellow phosphorus poisoning with recovery. Lancet 1:539-541.

Cameron, J.M. and R.S. Patrick. 1966. Acute phosphorus poisoning - The distribution of toxic doses of yellow phosphorus in the tissues of experimental animals. Med. Sci. Law 8:209-214.

Cameron, J.M. and E. Rentoul. 1963. Acute phosphorus poisoning. Med. Sci. Law 3:71-76.

Chemical & Engineering News. 1952. Oldbury Electro-Chemical's phosphorus plant solves problem of waste disposal by installing unit for treatment of effluent with chlorine. Chem. Eng. News 30:466.

Corbridge, D.C.E., M.S. Pearson, and C. Walling. 1966. The structural chemistry of phosphorus compounds. In E.J. Griffith and M. Grayson, eds. Topics in Phosphorus Chemistry. Vol. 3, pp. 57-80. John Wiley-Interscience, New York.

Cullumbine, H. 1944. The Burning Power and Harassing Effects of White Phosphorus. Military Intelligence Division, Great Britain, Porton Report No. 2604. (As reported in Wasti et al. 1978).

Cutler, J.T. 1931. The accumulation of guanidine in the blood following acute liver injury by carbon tetrachloride, chloroform, arsenic or phosphorus. J. Pharmacol. Exp. Ther. 41:337-345.

Dalhamn, T. and B. Holma. 1959. Animal experiments on the spread of inhaled radioactive red phosphorus in the body. AMA Arch. Ind. Health. 20:429-431.

Dearden, W.F. 1901. (No Title). Br. Med. J. 2:408. (As reported in Miles 1972).

Diaz-Rivera, R.S., P.J. Collazo, E.R. Pons, and M.V. Torregrosa. 1950. Acute phosphorus poisoning in man: A study of 56 cases. Medicine 29:269-298.

Diaz-Rivera, R.S., F. Ramos-Morales, M.R. Garcia-Palmieri, and E.A. Ramirez. 1961. The electrocardiographic changes in acute phosphorus poisoning in man. Am. J. Med. Sci. 241:758-765.

Dwyer, H.L. and F.C. Helwig. 1925. Phosphorus poisoning in a child, from the ingestion of fireworks. J. Am. Med. Assoc. 84:1254-1256.

Dyer, W.J., D.F. Hiltz, R.G. Ackman, J. Hingley, and G.L. Fletcher. 1970. In vivo assimilation by cod muscle and liver tissue of elemental phosphorus from polluted sea water. J. Fish. Res. Board Can. 27:1131-1139.

Dyer, W.J., D.F. Hiltz, R.G. Ackman, J. Hingley, G.L. Fletcher, and R.F. Addison. 1972. Stability of elemental phosphorus in edible muscle tissue of cod during processing including icing, freezing and thawing, frozen storage, salting, and cooking. J. Fish. Res. Board Can. 29:1053-1060.

Edzwald, J.D. 1977. Phosphorus in aquatic systems: The role of the sediments. In I.H. Suffet, ed. Fate of Pollutants in the Air and Water Environments, Part I. p. 183. John Wiley and Sons, New York. (As reported in Berkowitz et al. 1981).

Ellis, H.V., J.R. Hodgson, S.W. Hwang, L.M. Halfpap, D.O. Helton, B.S. Andersen, D.L. VanGoethem, and C.-C. Lee. 1978. Mammalian Toxicity of Munitions Compounds. Phase I: Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization, Disposition and Metabolism, and Ames Tests of Additional Compounds. Report, AD A069333. Midwest Research Institute, Kansas City, MO. DAMD17-74-C-4073.

Fleming, R.B.L. and G.H. Collings, Jr. 1951. The chylomicron count as an indicator of phosphorus poisoning. AMA Arch. Ind. Hyg. Occup. Med. 4:567-572.

Fleming, R.B.L, J.W. Miller, and V.R. Swayne, Jr. 1942. Some recent observations on phosphorus toxicology. J. Ind. Hyg. Toxicol. 24:154-158.

Fletcher, G.L. 1971. Accumulation of yellow phosphorus by several marine invertebrates and seaweed. J. Fish. Res. Board Can. 28:793-796.

Fletcher, G.L. 1973. The acute toxicity of a yellow phosphorus contaminated diet to brook trout, (Salvelinus fontinalis). Bull. Environ. Contam. Toxicol. 10(2):123-128.

Fletcher, G.L. and R.J. Hoyle. 1972. Acute toxicity of yellow phosphorus to Atlantic cod (Gadus morhua) and Atlantic salmon (Salmo salar) smolts. J. Fish. Res. Board Can. 29:1295-1301.

Fletcher, G.L., R.J. Hoyle, and D.A. Horne. 1970. Yellow phosphorus pollution: its toxicity to seawater-maintained brook trout (Salvelinus fontinalis) and smelt (Osmerus mordax). J. Fish. Res. Board Can. 27:1379-1384.

Fletcher, G.L., R.J. Hoyle, and D.A. Horne. 1971. Relative toxicities of yellow phosphorus production wastes to seawater-maintained fish. Fish. Res. Board Can. Tech. Rep. 255.

Ganote, C.E. and J.B. Otis. 1969. Characteristic lesions of yellow phosphorus-induced liver damage. Lab. Invest. 21(3):207-213.

Ghoshal, A.K., E.A. Porta, and W.S. Hartroft. 1969. The role of lipoperoxidation in the pathogenesis of fatty livers induced by phosphorus poisoning in rats. Am. J. Pathol. 54:275-291.

Ghoshal, A.K., E.A. Porta, and W.S. Hartroft. 1971. Isotopic studies on the absorption and tissue distribution of white phosphorus in rats. Exp. Mol. Pathol. 14(2):212-219.

Ghoshal, A.K., E.A. Porta, and W.S. Hartroft. 1972. Comparative studies on the hepatotoxicity of CCl4 and white phosphorus in rats. Gastroenterology 62(1):193.

Gorzny, K. 1972. Analysis of elemental phosphorus. In M. Halmann, ed. Analytical Chemistry of Phosphorus Compounds. pp. 361-370. John Wiley-Interscience, New York.

Hann, R.G. and R.A. Veale. 1910. A fatal case of poisoning by phosphorus, with unusual subcutaneous hemorrhages. Lancet 1:163-164.

Hawley, G.G. 1981. The Condensed Chemical Dictionary, 10th ed., p. 810. Van Nostrand Reinhold, New York.

Hayes, W.J., Jr. 1982. Pesticides Studied in Man. pp. 50-53. Williams and Wilkins, Baltimore.

Heimann, H. 1946. Chronic phosphorus poisoning. J. Ind. Hyg. Toxicol. 28:142-150.

Hodder, V.M., L.S. Parsons, and J.H.C. Pippy. 1972. The occurrence and distribution of "red" herring in Placentia Bay, February-April 1969. Fish. Res. Board Can. Circ. No. 2. pp. 45-52.

Hughes, J.P.W., R. Baron, D.H. Buckland, et al. 1962. Phosphorus necrosis of the jaw: A present day study. Br. J. Ind. Med. 19:83-99.

Hunter, D. 1955. Diseases of Occupations. pp. 331-339. Little, Brown and Co., Boston.

Hunter, D. 1969. Diseases of Occupations. 5th ed., pp. 127, 374-383. English Univ. Press, London. (As reported in Miles 1972).

Huruya, A. 1928. Studies on the phosphorus intoxication of rabbits. J. Biochem. (Japan) 10:63-95.

Hurwitz, A. 1972. Effects of microsomal enzyme inducers on animals poisoned with hepatotoxins. Toxicol. Appl. Pharmacol. 22:339-346.

Idler, D.R. 1969. Coexistence of a fishery and a major industry in Placentia Bay. Chem. Can. 21:16-21.

ILO. 1980. International Labor Office. Occupational Exposure Limits for Airborne Toxic Substances, a Tabular Compilation of Values from Selected Countries, 2nd ed. pp. 174-175. ILO, Geneva.

Isom, B.G. 1960. Stream pollution: toxicity of elemental phosphorus. J. Water Pollut. Control Fed. 32(12):1312-1316.

Jaqueson, A., M. Thevenin, J.M. Warnet, J.-R. Claude and R. Truhaut. 1977. Sex influence on the experimental fatty liver induced by white phosphorus and amanita-phalloides in the rat: Consequences on the protective effect of some steroids. Acta Pharmacol. Toxicol. Suppl. 41:322-329 (abstr.)

Jacqueson, A., M. Thevenin, J.M. Warnet, et al. 1979a. Analysis of tissue triglycerides by thin layer (TLC) and gas-liquid (GC) chromatography in some experimental toxic fatty livers. Chromatogr. Symp. Ser. 1 (Recent Dev. Chromatogr. Electrophor.):169-173.

Jacqueson, A, M. Thevenin, J.-M. Warnet, J.-R. Claude, and R. Truhaut 1979b. Accentuation of white phosphorus induced fatty liver by phenobarbitone in male rats compared to female rats. Arch. Toxicol. (Suppl 2):327-331.

Jakhi, S.A., B.K. Parekh, and S. Gupta. 1983. Phosphorus necrosis of the maxilla. J. Oral Med. 38(4):174-176.

Jangaard, P.M. 1970. The role played by the Fisheries Research Board of Canada in the "red" herring phosphorus pollution crisis in Placentia Bay, Newfoundland. Fish. Res. Board Can. Circ. No. 1.

Katz S., A. Snelson, R. Butler, W. Bock, N. Rajendran, and S. Relwani. 1981. Physical and Chemical Characterization of Military Smokes. Part III. White Phosphorus-Felt Smokes. Final Report, AD A115657. IIT Research Institute, Chicago, IL. DAMD17-78-C-8085.

Kennon, R. and J.W. Hallam. 1944. Modern phosphorus caries and necrosis. Br. Dent. J. 76(12):321-330.

Krasnov, S.K. 1970. Effect of elementary phosphorus on fish (pathophysiological studies). Vop. Vod. Toksikol. 125-128. (Chem. Abstr. 122039t).

Krishnan, S.S. and R.C. Gupta. 1970. Determination of phosphides and white phosphorus in biological materials by neutron activation analysis. Anal. Chem. 42(6):557-560.

Kulkarni, A.P. and E. Hodgson. 1980. Hepatoxicity. In E. Hodgson and F. E. Guthrie, eds. Introduction to Biochemical Toxicology. pp. 341-356. Elsevier, New York.

LaDue, J.S., J.R. Schenken, and L.H. Kuker. 1944. Phosphorus poisoning. A report of 16 cases with repeated liver biopsies in a recovered case. Am. J. Med. Sci. 208:223-234.

Lai, M.G. 1979a. Characterization of White Phosphorus in Water. I. Determination of White Phosphorus in Environmental Waters at the Nanogram Level. Final Report NSWC TR 79-3, AD B042884. Naval Surface Weapons Center, Silver Spring, MD.

Lai, M.G. 1979b. Characterization of White Phosphorus in Water. II. Degradation of White Phosphorus in Aqueous Systems. Final Report NSWC TR 79-5. Naval Surface Weapons Center, Silver Spring, MD.

Lai, M.G. 1981. Characterization of White Phosphorus in Water: III. Behavior of White Phosphorus in the Water-Sediment Interface. Technical Report NSWC TR 81-87, AD B059442L. Naval Surface Weapons Center, Silver Spring, MD.

Lai, M.G. and D.H. Rosenblatt. 1977a. Determination of Ultramicro Quantities of Elemental Phosphorus in Water by Neutron Activation Analysis. Final Technical Report NSWC/WOL TR 77-49, AD A091773. Naval Surface Weapons Center, Silver Spring, MD.

Lai, M.G. and D.H. Rosenblatt. 1977b. Identification of Transformation Products of White Phosphorus in Water. Final Technical Report NSWC/WOL TR 76-103, AD A041068. Naval Surface Weapons Center, Silver Spring, MD.

Lawrence, J.S. and M.M. Huffman. 1929. An increase in the number of monocytes in the blood following subcutaneous administration of yellow phosphorus in oil. Arch. Pathol. 7:813-819.

Lee, C.-C., J.V. Dilley, J.R. Hodgson, et al. 1975. Mammalian Toxicity of Munition Compounds. Phase I: Acute Oral Toxicity, Primary Skin and Eye Irritation, Dermal Sensitization, and Disposition and Metabolism. Report No. 1, AD B011150. Midwest Research Institute, Kansas City, MO.

Legge, T.M. 1920. Cases of phosphorus necrosis. J. Ind. Hyg. 2:50-52.

Lhota, J. and J.P. Hannon. 1979. Yellow Phosphorus Toxicity. Body Weight Changes of Young and Mature Rats During the Early Stages of Chronic Exposure. Technical Note No. 79-11. Letterman Army Institute of Research, Presidio, San Francisco.

Litchfield, J.T., Jr. 1949. A method for rapid graphic solution of time-percent effect curves. J. Pharmacol. Exp. Ther. 97:399-408. (As reported in Fletcher et al. 1970).

Litchfield, J.T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 98:99-113. (As reported in Fletcher et al. 1970).

Maddock, B.G. and D. Taylor. 1976. The acute toxicity of dissolved elemental phosphorus to cod (Gadus morhua). Water Res. 10:289-294.

Mallory, F.B. 1933. Phosphorus and alcoholic cirrhosis. Am. J. Pathol. 9:557-567.

Manuel, K.L., E.S. Bender, and J.G. Pearson. 1976. Results of Aquatic Surveys at Pine Bluff Arsenal, Arkansas, September 1973-October 1974. Report EB-TR-76038, AD A024382. Department of the Army Headquarters, Edgewood Arsenal, Aberdeen Proving Ground, MD.

Manthei, J.H., F.K. Lee, Jr., M. Donnelly, and J.T. Weimer. 1980. Preliminary Toxicity Screening Studies of 11 Smoke Candidate Compounds. Technical Report ARCSL-TR-79056, AD C021764L. Chemical Systems Laboratory, Aberdeen Proving Ground, MD.

McCarron, M.M., G.P. Gaddis, and A.T. Trotter. 1981. Acute yellow phosphorus poisoning from pesticide pastes. Clin. Toxicol. 18(6):693-711.

McIntosh, R. 1927. Acute phosphorus poisoning. A report of a case with recovery. Am. J. Dis. Child. 34:595-602.

Miles, A.E.W. 1972. Phosphorus necrosis of the jaw: "Phossy jaw". Br. Dent. J. 133(5):203-206.

Monsanto. 1972. Personal communication with R. A. Kimerle and G. Brautigaon. (As reported in Sullivan et al. 1979).

Monsanto. 1985. FYI-OTS-0785-0423, Initial Sequence A. Elemental Yellow Phosphorus: One Generation Reproduction Study in Rats. Office of Toxic Substances, U.S. Environmental Protection Agency, Washington, DC.

National Academy of Sciences. 1973. Water Quality Criteria: 1972. Environmental Protection Agency publication. EPA-R3-73-033. (As reported in Sullivan et al. 1979).

Newburger, R.A., S.B. Beaser, and H. Shwachman. 1948. Phosphorus poisoning with recovery accompanied by electrocardiographic changes. Am. J. Med. 4:927-930.

Odense, P.H., Y.M. MacDougall, T.C. Leung, and V.H. Logan. 1972. Hematological and histological studies resulting from the pollution problem at Long Harbour, Placentia Bay, Newfoundland. Fish. Res. Board Can. Circ. No. 2. pp. 195-207.

Oliver, T. 1938. The conquest of an occupational disease: Phosphorus poisoning in lucifer match making. J. R. Inst. Pub. Health Hyg. 1:660-666.

OSHA. 1974. Occupational Safety and Health Administration. Occupational Health and Environmental Control. Fed. Regist. 39:23540-23542.

OSHA/NIOSH. 1978. Occupational Safety and Health Administration/ National Institute of Occupational Safety and Health. Occupational health guideline for phosphorus (yellow).

Pani, P., G. Enrico, C. Mazzarino, and E. Burdino. 1972. On the mechanism of fatty liver in white phosphorus poisoned rats. Exp. Mol. Pathol. 16:201-209.

Pearson J.G., E.S. Bender, D.H. Taormina, K.L. Manuel, P.F. Robinson, and A.E. Asaki. 1976. Effects of Elemental Phosphorus on the Biota of Yellow Lake, Pine Bluff Arsenal, Arkansas, March 1974 - January 1975. Edgewood Arsenal Technical Report EO-TR-76077, AD A035925. Department of the Army Headquarters, Edgewood Arsenal, Aberdeen Proving Ground, MD.

Pearson, J.G., P.F. Robinson, and E.S. Bender. 1978. The Toxicity of Phossy Water to Selected Freshwater Organisms. Technical Report ARCSL-TR-7824, AD A054[illegible]. Chemical Systems Laboratory, Aberdeen Proving Ground, MD.

Peer, D.L. 1972. Observations on mortalities of benthic organisms after contamination of the bottom of Long Harbour, Placentia Bay, Newfoundland, with elemental phosphorus. Fish. Res. Board Can. Circ. No. 2. pp. 181-186.

Pietras, R.J., C. Staurakos, R.M. Gunnar, and J.R. Tobin. 1968. Phosphorus poisoning simulating acute myocardial infaction. Arch. Intern. Med. 122:430-434.

Pippy, J.H.C., V.M. Hodder, and L.S. Parsons. 1972. Symptoms of "red" herring in relation to the mass mortalities in Placentia Bay, February-April 1969. Fish. Res. Board Can. Circ. No. 2. pp. 187-191.

Polson, C.J., M.A. Green, and M.R. Lee. 1983. Phosphorus. In Clinical Toxicology, 3rd ed. pp. 522-539. J.B. Lippincott, Philadelphia.

Poston, T.M., K.M. McFadden, R.M. Bean, M.L. Clark, B.L. Thomas, B.W. Killard, L.A. Prohammer, and D.R. Kalkwarf. 1986. Acute Toxicity of Smoke Screen Materials to Aquatic Organisms, White Phosphorus-Felt, Red Phosphorus-Butyl Rubber and SGF No. 2 Fog Oil. Final Report PNL-5584. Pacific Northwest Laboratory, Richland, WA.

Pruitt, B.A., Jr. 1970. Management of Burns in the Multiple Injury Patient. AD 715523. Brooke Army Medical Center, Fort Sam Houston, TX.

Rubitsky, H.J. and R.M. Myerson. 1949. Acute phosphorus poisoning. Arch. Intern. Med. 83:164-178.

Rushing, D.E. 1962. A tentative method for the determination of elemental phosphorus in air. Am. Ind. Hyg. Assoc. J. 23:383-387.

Scott, W.J.M. 1916. Experimental mitochondrial changes in the pancreas in phosphorus poisoning. Am. J. Anat. 20:237-253.

Seakins, A. and D.S. Robinson. 1964. Changes associated with the production of fatty livers by white phosphorus and by ethanol in the rat. Biochem. J. 92:308-312.

Simon, F.A. and L.K. Pickering. 1976. Acute yellow phosphorus poisoning "smoking stool syndrome." J. Am. Med. Assoc. 235(13):1343-1344.

Snodgrass, W.R. and J. Doull. 1982. Early aggressive activated charcoal treatment of elemental yellow phosphorus poisoning. Vet. Hum. Toxicol. (Suppl 24):96-98.

Sollmann, T. 1925. Studies of chronic intoxications on albino rats. VII. Yellow phosphorus. J. Pharmacol. Exp. Ther. 24:119-122.

Sollmann, T. 1957. Elementary Phosphorus. In A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology, 8th ed. pp. 1068-1073. W. B. Saunders, Philadelphia. (As reported in Hayes 1982).

Spanggord, R.J., R. Rewick, T.-W. Chou, R. Wilson, R.T. Podoll, T. Mill, R. Parnas, R. Platz, and D. Roberts. 1985. Environmental Fate of White Phosphorus/Felt and Red Phosphorus/Butyl Rubber Military Screening Smokes. Final Report PYU-4937. SRI International, Menlo Park, CA. DAMD17-82-C-2320.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3:793-821.

Starke, W.C., R.J. Pellerin, and D.C. Burnett. 1982. White Phosphorus-Felt Smoke: Effects on Reproduction in the Rat. Technical Report ARCSL-TR-82002, AD A118682. U.S. Army Armament Research and Development Command, Aberdeen Proving Ground, MD.

Steel, R.G.D. and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York. (As reported in Bentley et al. 1978).

Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. 1985. Guidelines for Deriving Numerical Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. Final Report PB85-227049. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

Sullivan, J.H., Jr., H.D. Putnam, M.A. Keirn, B.C. Pruitt, Jr., and J.C. Nichols. 1979. A Summary and Evaluation of Aquatic Environmental Data in Relation to Establishing Water Quality Criteria for Munitions-Unique Compounds. Part 3: White Phosphorus. Final Report, AD A083625. Water and Air Research, Inc., Gainesville, FL. DAMD17-77-C-7027.

Summerlin, W.T., A.I. Walder, and J.A. Moncrief. 1967. White phosphorus burns and massive hemolysis. J. Trauma 7(3):476-484.

Talley, R.C., J.W. Linhart, A.J. Trevino, L. Moore, and B.M. Beller. 1972. Acute elemental phosphorus poisoning in man: Cardiovascular toxicity. Am. Heart J. 84:139-140.

Torrielli, M.V., P. Pani, L. Gabriel, and E. Gravela. 1974. The pathophysiological significance of the hepatic DMES in the liver damage induced by CCl_4 and white phosphorus. In W.A.M. Duncan, ed. Experimental Model Systems in Toxicology and Their Significance in Man, Proc. Eur. Soc. for the Study of Drug Toxicity. Vol. XV, pp. 294-300. American Elsevier, New York.

U.S. Army. 1982. Office of the Surgeon General, Letter dated 17 November 1982. "Environmental Criteria for Explosives in Drinking Water."

U.S. Army. 1983. Office of the Surgeon General, Letter dated 17 February 1983. "Environmental Criteria for Explosives in Drinking Water."

USEPA. 1975. U.S. Environmental Protection Agency. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series 600/3-76/046. (As reported in Bentley et al. 1978).

USEPA. 1976. U.S. Environmental Protection Agency. Quality Criteria for Water. U.S. Environmental Protection Agency, Washington, DC. (As reported in Poston et al. 1986).

USEPA. 1980. U.S. Environmental Protection Agency. Guidelines and Methodology Used in the Preparation of Health Effects Assessment Chapters of the Consent Decree Water Quality Criteria Documents. Fed. Regist. 45:79347-79357.

USEPA. 1985. U.S. Environmental Protection Agency. Toxic Substances Control Act Test Guidelines; Final Rules. U.S. Environmental Protection Agency. Fed. Regist. 50:39336-39342.

Van Wazer, J.R. 1982. Phosphorus and the phosphides. In M. Grayson and D. Eckroth, eds. Kirk-Othmer Encyclopedia of Chemical Technology. pp. 473-490. John Wiley and Sons, New York.

Walker, J., Jr., M. Galdston, J. Wexler, M.L. Hill, and G. Midgely. 1947. WP Casualties at Edgewood Asenal, Maryland, 1945. Report 103, AD 824420. Edgewood Arsenal, MD.

Walker, J., Jr., J. Wexler, and M.L. Hill. 1969. Quantitative Analysis of Phosphorus-Containing Compounds Formed in WP Burns. Technical Report EASP 100-49, AD 687240. Research Laboratories, Edgewood Arsenal, MD.

Wang, L.K., M.H. Wang, V.J. Ciccone, and R.G. Ross. 1982. Sources of Pollution from Explosives and Propellants Production Installations. Final Technical Report LIR-11-81/1, PB82-173915. Lenox Institute for Research Inc., Lenox, MA. DAAG-53-75-C-0252 and DAAG-02-73-C-0206.

Ward, E.F. 1926. Phosphorus necrosis in the manufacture of fireworks and in the preparation of phosphorus. Bull. U.S. Bur. Labor Statistics 405:1-44.

Warnock, R.E. 1972. Escape of elemental phosphorus from soil when applied as white phosphorus or the phosphorus-sulfur eutectic solution. J. Environ. Qual. 1(3):311-314.

Wasti, K., P.N. Craig, K.J.R. Abaidoo, and J.E. Villaume. 1978. A Literature Review - Problem Definition Studies on Selected Toxic Chemicals. Vol. 2. Occupational Health and Safety Aspects of Phosphorus Smoke Compounds. AD A056019. Franklin Institute Research Laboratories, Philadelphia, PA. DAMD-17-77-C-7020.

Weast, R.C. and M. J. Astle, eds. 1985. CRC Handbook of Chemistry and Physics. Vols. I and II, p. B-122. CRC Press, Boca Raton, FL.

Wechsler, L. and R.L. Wechsler. 1951. Phosphorus poisoning: The latent period and unusual gastrointestinal lesions. Gastroenterology 17:279-283.

White, S.A. and G.C. Armstrong. 1935. White Phosphorus Smoke: Its Irritating Concentration for Man and Its Toxicity for Small Animals for One-Hour Exposures. Report E.A.T.R. 190, AD B956983. War Department, Chemical Warfare Service, Edgewood Arsenal, MD.

Whiteley, H.J., H.B. Stoner, and C.J. Threlfall. 1953. The uptake of radioactive phosphorus by the skin of the rabbit. Br. J. Exp. Pathol. 34:73-80.

Williamson, C.S. and F.C. Mann. 1923. Studies on the physiology of the liver. V. The hepatic factor in chloroform and phosphorus poisoning. Am. J. Physiol. 65:267-276.

Windholz, M., S. Budavari, R.F. Blumetti, and E.S. Otterhein, eds. 1983. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. 10th ed. Merck and Company, Rahway, NJ.

Winek, C.L., W.D. Collom, and E.P. Fusia. 1973. Yellow phosphorus ingestion - three fatal poisonings. Clin. Toxicol. 6(4):541-545.

Zitko, V., D.F. Aiken, S.N. Tibbo, K.W.T. Besch, and J.M. Anderson. 1970. Toxicity of yellow phosphorus to herring (Clupea harengus), Atlantic Salmon (Salmo salar), lobster (Homarus americanus), and beach flea (Gammarus oceanicus). J. Fish. Res. Board Can. 27:21-29.

7. GLOSSARY

ACGIH	American Conference of Governmental Industrial Hygienists
ADI	Acceptable daily intake
allotrope	Different forms, usually in the same phase, in which an element can exist; e.g., white phosphorous can be amorphous, cubic crystals, or hexagonal crystals
ASTM	The American Society for Testing and Materials
BCF	Bioconcentration factor
benthos	Aquatic organisms living on or in the bottom substrate
CCC	Criterion Continuous Concentration
CMC	Criterion Maximum Concentration
CNS	Central nervous system
Ct	Concentration x time
DDT	Dichlorodiphenyltrichloroethane
DMSO	Dimethyl sulfoxide
DO	Dissolved oxygen
EC_{50}	Effective concentration causing 50 percent death (based on immobilization)
EKG	Electrocardiogram
FAV	Final Acute Value
FEL	Frank effect level
FPD	Flame photometric detector
GOT	Glutamic-oxalacetic transaminase
GTP	Glutamic-pyruvic transaminase
Incipient LC_{50}	Lethal concentration for 50 percent mortality from long exposure
LC_{50}	Lethal concentration causing 50 percent mortality
LCt_{50}	Lethal concentration x time causing 50 percent mortality
LD_{50}	Lethal dose causing 50 percent mortality
littoral	Area of a body of water that is on or near the shore
LOAEL	Lowest observed adverse effect level
LT_{50}	Median time to death for 50 percent of the test population
multivoltine	Producing several generations per year
NIOSH	National Institute of Occupational Safety and Health

NOAEL	No observed adverse effect level
NOEL	No observed effect level
OSHA	Occupational Safety and Health Administration
RER	Rough endoplasmic reticulum
SER	Smooth endoplasmic reticulum
STEL	Short-term exposure limit
SWPB	Standard white phosphorus burn
TCA	Trichloroacetic acid
TL_m	Median tolerance limit
TLV	Threshold limit value
TWA	Time-weighted average
USEPA	United States Environmental Protection Agency

APPENDIX A

SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect aquatic life with emphasis on the specific regulatory needs of the U.S. Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedures and were written by the following researchers from the USEPA's regional research laboratories: C. E. Stephan, D. I. Mount, D. J. Hansen, J. H. Gentile, G. A. Chapman, and W. A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species at all times was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species is very sensitive. The small fraction is set at 0.05, because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from

acute and chronic toxicity to animals, toxicity to plants, and bioaccumulation by aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors, such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived; however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative; much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.

3. Definition of the material should include an operational analytical component. It is also necessary to reference or describe analytical methods that the term is intended to denote. Primary

requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

NOTE: Analytical chemistry of the material may have to be considered when defining the material or when judging acceptability of some toxicity tests, but a criterion should not be based on sensitivity of an analytical method. When aquatic organisms are more sensitive than analytical techniques, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants; (b) FDA action levels (FDA Guidelines Manual); and (c) chronic feeding studies and long-term field studies with wildlife that regularly consume aquatic organisms.

2. All data used should be available in typed, dated, and signed hard-copy with enough supporting information to indicate that acceptable test procedures were used and the results should be reliable.

3. Questionable data, whether published or not, should not be used.

4. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the test material should not be used.

5. For some highly volatile, hydrolyzable, or degradable materials it may be appropriate to only use results of flow-through tests in which concentration of test material in test solutions were measured using acceptable analytical methods.

6. Do not use data obtained using brine shrimp, species that do not have reproducing wild populations in North America, or organisms that were previously exposed to significant concentrations of the test material or other contaminants.

4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater animals in at least eight different families such that all of the following are included:

a. the family Salmonidae in the class Osteichthyes;

b. a second family (preferably an important warmwater species) in the class Osteichthyes (e.g., bluegill, fathead minnow, or channel catfish);

c. a third family in the phylum Chordata (e.g, fish or amphibian);

d. a planktonic crustacean (e.g, cladoceran or copepod);

e. a benthic crustacean (e.g, ostracod, isopod, or amphipod);

f. an insect (e.g., mayfly, midge, stonefly);

g. a family in a phylum other than Arthropoda or Chordata (e.g, Annelida or Mollusca); and

h. a family in any order of insect or any phylum not represented.

2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.

3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.

4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly, if all required data are not available a numerical criterion should not be derived except in special cases. For example, even if sufficient acute and chronic data are available, it may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.

2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).

3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.

4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L), should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.

5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:

   a. Tests with daphnids and other cladocerans should be started with organisms <24 hr old, and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC_{50} based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} is not available from a test, the 48-hr LC_{50} should be used in place of the desired 48-hr EC_{50}. An EC_{50} or LC_{50} of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.

   b. The result of tests with all other aquatic animal species should be the 96-hr EC_{50} value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC_{50} value is not available from a test, the 96-hr LC_{50} should be used in place of the desired EC_{50}.

   c. Tests with single-cell organisms are not considered acute tests, even if the duration was $\leq$96 hr.

d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.

6. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.

7. If the data indicate that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV, because a species can only be considered protected from acute toxicity if all life stages are protected.

8. Consider the agreement of the data within and between species. Questionable results in comparison with other acute and chronic data for the species and other species in the same genus probably should not be used.

9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentrations of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.

   NOTE: Data reported by original investigators should not be rounded off, and at least four significant digits should be retained in intermediate calculations.

10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.

11. Order the GMAVs from high to low, and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.

12. Calculate the cumulative probability (P) for each GMAV as $R/(N+1)$.

13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are <59 GMAVs, these will always be the four lowest GMAVs).

14. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\Sigma((\ln GMAV)^2) - ((\Sigma(\ln GMAV))^2/4)}{\Sigma(P) - ((\Sigma(\sqrt{P}))^2/4)}$$

$$L = (\Sigma(\ln GMAV) - S(\Sigma(\sqrt{P})))/4$$

$$A = S(\sqrt{0.05}) + L$$

$$FAV = e^A$$

15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV.

16. Go to Section 7.

6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.

3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.

4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.

5. Similarly normalize the values of the water quality characteristic for each species individually.

6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).

9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation: $Y = \ln W - V(\ln X - \ln Z)$.

10. For each species calculate the SMAV using: $SMAV = e^{Y}$.

11. Obtain the FAV at Z by using the procedure described in Section 5 (Nos. 10-14).

12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.

13. The Final Acute Equation is written as: $FAV = e^{(V[\ln(\text{water quality characteristic}) + \ln A - V[\ln Z])}$, where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

   NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one; this should avoid confusion as

to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.

3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.

4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic does not affect toxicity.

5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:

   a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young <48 hr old, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young <24 hr old and last for not <21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.

   b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not <24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

NOTE: Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used, because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.

7. If chronic toxicity of the material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.

8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species, and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (Nos. 10-14). Then, go to Section 7 (No. 13).

9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same

laboratory and dilution water may be used or acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.

10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.

11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.

    a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be calculated as the geometric mean of all species whose SMAVs are close to the FAV.

    b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and saltwater species.

    c. If the most appropriate species mean acute-chronic ratios are <2.0, and especially if they are <1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

    If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and an FCV probably cannot be calculated.

12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.

13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.

14. Go to Section 9.

8. FINAL CHRONIC EQUATION

1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.

a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.

b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z (see Section 6, No. 13) by the Final Acute-Chronic Ratio.

c. Use V = pooled acute slope (see Section 6, No. 13) as L = pooled chronic slope.

d. Go to Section 8, No. 2, item m.

2. The procedure described in this section will usually result in the chronic slope being different from the acute slope.

a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.

b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.

c. Decide whether data for each species are useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 8), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.

d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.

e. Similarly normalize the values of the water quality characteristic for each species individually.

f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related values of the water quality characteristic.

i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation: $Q = \ln M - L(\ln P - \ln Z)$.

j. For each species calculate a SMCV at Z as the antilog of Q ($SMCV = e^{Q}$).

k. Obtain the FCV at Z by using the procedure described in Section 5 (Nos. 10-14).

l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.

m. The Final Chronic Equation is written as: $FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$, where L = mean chronic slope and S = FCV at Z.

9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.

2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.

3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which concentrations of test material were measured and the endpoint is biologically important.

10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) an FDA action level (FDA administrative guidelines) for fish oil or for the edible portion of fish or shellfish or (b) a maximum acceptable dietary intake (ADI) based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section 11, because a Final Residue Value cannot be derived.

2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs) are the quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution to which the organism has been exposed. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. A BAF is intended to account for net uptake from both food and water in a real-world situation, and almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey. Because so few acceptable BAFs are available, only BCFs will be discussed further, but an acceptable BAF can be used in place of a BCF.

3. If a maximum permissible tissue concentration is available for a substance (e.g, parent material or parent material plus metabolite), the tissue concentration used in BCF calculations should be for the same substance. Otherwise the tissue concentration used in the BCF calculation should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.

   a. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of test material in tissue and in the test solution, and exposure continued at least until either apparent steady-state (BCF does

not change significantly over a period of time, such as two days or 16 percent of exposure duration, whichever is longer) or 28 days was reached. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached; (b) highest BCF obtained, if apparent steady-state was not reached; and (c) projected steady-state BCF, if calculated.

b. Whenever a BCF is determined for a lipophilic material, percentage of lipids should also be determined in the tissue(s) for which the BCF is calculated.

c. A BCF obtained from an exposure that adversely affected the test organisms may be used only if it is similar to that obtained with unaffected individuals at lower concentrations that did cause effects.

d. Because maximum permissible tissue concentrations are rarely based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.

e. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, in which case the BCF for the longest exposure should be used.

4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:

   a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.

   b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.

5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.

a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.

b. Calculate the geometric mean normalized BCF.

c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.

- For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.
- For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).
- For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.

6. The FRV is obtained by selecting the lowest of available residue values.

11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.

3. The Criterion Continuous Concentration (CCC) is equal to the lowest of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.

4. Round both the CCC and CMC to two significant figures.

5. The criterion is stated as:

   The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that (except possibly where a locally important species is very sensitive) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) μg/L more than once every three years on the average and if the one-hour average concentration does not exceed (4) μg/L more than once every three years on the average.

   Here,

   (1) = insert freshwater or saltwater,

   (2) = insert name of material,

   (3) = insert the Criterion Continuous Concentration, and

   (4) = insert the Criterion Maximum Concentration.

13. REFERENCES

ASTM Standards E 729. Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.

ASTM Standards E 724. Practice for Conducting Static Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs.

Dixon, W. J. and M. B. Brown, eds. 1979. BMDP Biomedical Computer Programs, P-Series. University of California, Berkeley. pp. 521-539.

FDA Administrative Guidelines Manual. Food and Drug Administration.

Hamelink, J. L., et al. 1971. A Proposal: Exchange Equilibria Control the Degree Chlorinated Hydrocarbons are Biologically Magnified in Lentic Environments. Trans. Am. Fish Soc. 100:207-214.

Huth, E. J., et al. 1978. Council of Biological Editors Style Manual, 4th ed., p. 117.

Lunsford, C. A. and C. R. Blem. 1982. Annual Cycle of Kepone Residue in Lipid Content of the Estuarine Clam, Rangia cuneata. Estuaries 5:121-130.

Nexer, J. and W. Wasserman. 1974. Applied Linear Statistical Models. Irwin, Inc., Homewood, Illinois.

Schnoor, J. L. 1982. Field Validation of Water Quality Criteria for Hydrophobic Pollutants. In: Aquatic Toxicology and Hazard Assessment: Fifth Conference, ASTM STP 766, J.G. Pearson, R.B. Foster, and W.E. Bishop, eds., American Society for Testing and Materials, pp. 302-315.

Sidwell, V. D. 1981. Chemical and Nutritional Composition of Finfishes, Whales, Crustaceans, Mollusks, and Their Products. NOAA Technical Memorandum NMFS F/SEC-11, National Marine Fisheries Service.

Stephan, C. E., D. I. Mount, D. J. Hansen, J. H. Gentile, G. A. Chapman, and W. A. Brungs. 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. Final Report, PB85-227049. US Environmental Protection Agency, Office of Research and Development, Washington, DC. 97 pp.

US Environmental Protection Agency (USEPA). 1982. Fed. Reg. 47:49234-49252, October 29, 1982.

APPENDIX B

SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final US Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect human health and is slanted towards the specific regulatory needs of the U.S. Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

1 INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

a) Carcinogenicity,
b) Toxicity, and
c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the values represent estimates that would prevent adverse health effects, or for suspect and proven carcinogens, estimates of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

2. DATA NEEDED FOR HUMAH HEALTH CRITERIA

Criteria documentation requires information on: (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes, including ingestion of contaminated water and edible aquatic and nonaquatic organisms as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish.

Generally, exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to be insignificant; however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions, which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).

2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.

3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a substance in all or part of an organism divided by the concentration in ambient water to which the organism has been exposed. The BCF is a function of lipid solubility of the substance and relative amount of lipids in edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used, depending upon lipid solubility and availability of bioconcentration data:

1. For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980).

   Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCF_{avg} = BCF_{sp} \times \frac{3.0\%}{PL_{sp}},$$

   where BCF_{sp} is the bioconcentration factor for an aquatic species and PL_{sp} is the percent lipids in the edible portions of that species.

2. Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF = (0.85 \log P) - 0.70$$

   for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent

lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

3. For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity; synergistic and antagonistic effects; and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations.

From an assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects, is selected for criteria formulation.

3. HUMAN HEALTH CRITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of 10^{-7} to 10^{-5} (one additional case of cancer in populations ranging from ten

million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

3.1 METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTHRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific lifetime carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065\ BCF)}$$

where

- C = ambient water concentration;
- PR = the probable risk (e.g., 10^{-5}; equivalent to one case in 100,000);
- BCF = the bioconcentration factor; and
- q_1^* = a coefficient, the cancer potency index (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = \frac{q_1^* \times C\ (2 + 0.0065\ BCF)}{70}$$

or

$$PR = \frac{q_1^* \times 2C + (0.0065\ BCF \times C)}{70}$$

where 2C is the daily exposure resulting from drinking 2 liters of water per day and (0.0065 BCF x C) is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

In simplified terms the equation can be rewritten

$$PR = q_1^* X\ ,$$

where X is the total average daily exposure in mg/kg/day or

$$q_1^* = \frac{PR}{X}\ ,$$

showing that the coefficient q_1^* is the ratio of risk to dose, an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/response curve). At low doses then, the ratio of risk to dose does not change appreciably and q_1^* is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of 10^{-7} to 10^{-5}, which correspond to very low doses, the q_1^* value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

3.2 CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk [RR(X)] of a cohort of individuals at exposure X compared with the risk in the control group [PR(control)] (e.g., if the cancer risk in group A is five times greater than that of the control group, then RR(X) = 5). In such cases the "excess" relative cancer risk is expressed as RR(X) − 1, and the actual numeric, or proportional, excess risk level [PR(X)] can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(control) \ .$$

Using the standard risk/dose equation

$$PR(X) = b \times X$$

and substituting for PR(X):

$$[RR(X) - 1] \times PR(control) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(control)}{X} \ ,$$

where b is equal to the carcinogenic potency or q_1^*.

3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.

2. The data set giving the highest index of carcinogenic potency (q_1^*) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.

3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of q_1^* from all data sets is used in the risk assessment.

4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the 2/3 power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}$$

2. If the dose (s) is given as mg per kg of body weight:

$$S = \frac{m}{W}$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times w)}{W^{2/3}}$$

or

$$X = s \times W^{1/3} .$$

3. The dose must also be normalized to a lifetime average exposure. For a carcinogenic assay in which the average dose per day (in mg) is m, and the length of exposure is l_e, and the total length of the experiment is L_e, then the lifetime average exposure (X_m) is

$$X_m = \frac{l_e \times m}{L_e \times W^{2/3}} .$$

4. If the duration of the experiment (L_e) is less than the natural life span (L) of the test animal, the value of q_1^* is increased by a factor of $(L/L_e)^3$ to adjust for an age-specific increase in the cancer rate.

5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = ppm \times F \times r ,$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to differences in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man; 0.05 for a 0.35-kg rat; and 0.13 for a 0.03-kg mouse).

Substituting (ppm x F) for m and fW for F, the daily exposure (dose/surface area/day or $m/W^{2/3}$) can be expressed as

$$X = \frac{ppm \times F}{W^{2/3}} = \frac{ppm \times f \times W}{W^{2/3}} = ppm \times f \times W^{1/3} .$$

6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol and is absorbed proportionally to to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption, which, in turn, is a function of total body surface area.

3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t;d) = 1 - \exp\{-g(d)H(t)\},$$

where P(t;d) is the probable response for dose d and time t, g(d) is the polynomial function defining the effect of dose level, and H(t) the effect of time:

$$g(d) = \sum_{i=0}^{a} \alpha_i d^i$$

$$H(t) = \sum_{i=0}^{b} \beta_i t^i$$

(with α and $\beta \geq 0$, and $\Sigma \beta_i = 1$).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each α as a multiplicative constant (Crump 1980):

$$p(d/t) = 1 - \exp\left(- \sum_{i=0}^{a} \alpha d^i \right) ,$$

or as given in the EPA guidelines (USEPA 1980):

$$P(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)],$$

where P(d) is the lifetime risk (probability) of cancer at dose d.

For a given dose the excess cancer risk A(d) above the background rate P(o) is given by the equation:

$$A(d) = \frac{P(d) - P(o)}{1 - P(o)}$$

where,

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \ldots + q_k d^k)] .$$

Point estimates of the coefficients $q_1 \ldots q_k$ and consequently the extra risk function A(d) at any given dose are calculated by using the statistical method of maximum likelihood. Whenever q_1 is not equal to 0, at low doses the extra risk function A(d) has approximately the form:

$$A(d) = q_1 \times d .$$

Consequently, $q_1 \times d$ represents a 95 percent upper confidence limit on the excess risk, and R/q_1 represents a 95 percent lower confidence limit on the dose producing an excess risk of R. Thus, A(d) and R will be a function of the maximum possible value of q_1 which can be determined from the 95 percent upper confidence limits on q_1. This is accomplished by using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure q_1^*, the 95 percent upper confidence limit, is calculated by increasing q_1 to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_0 - L_1) = 2.70554 ,$$

where L_0 is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$\chi^2 = \sum_{i=1}^{h} \frac{(X_i - N_i P_i)^2}{N_i P_i (1 - P_i)}$$

where N_i is the number of animals in the ith dose group, X_i is the number of animals in the ith dose group with a tumor response, P_i is the probability of a response in the ith dose group estimated by fitting the multistage model to the data, and h is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square (χ^2) is larger than the cumulative 99 percent point of the chi-square distribution with f degrees of freedom, where f equals the number of dose groups minus the number of nonzero multistage coefficients.

4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at and below which the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065 \text{ kg} \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criterion.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

(1) NOEL = No-Observed-Effect-Level,
(2) LOEL = Lowest-Observed-Effect-Level,
(3) LOAEL = Lowest-Observed-Adverse-Effect-Level,
(4) FEL = Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.

2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.

3. If an LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to an NOAEL or an LOAEL.

4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.

5. If for reasonably closely spaced doses only an NOEL and an LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, an NOAEL, an LOAEL, and a clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.

2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.

3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals, with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported, but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criterion (using the uncertainty factor approach). Also, the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to

those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = \frac{TLV \times BR \times DE \times d \times A_A}{(A_O \times SF)}$$

where

BR = daily air intake (assume 10 m^3),
DE = duration of exposure in hours per day,
d = 5 days/7 days,
A_A = efficiency of absorption from air,
A_O = efficiency of absorption from oral exposure, and
SF = safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = \frac{CA \times D_E \times d \times A_A \times BR \times 70\ kg}{BW_A \times A_O \times SF)}$$

where

C_A = concentration in air (mg/m^3),
D_E = duration of exposure (hr/day),
d = number of days exposed/number of days observed,
A_A = efficiency of absorption from air,
BR = volume of air breathed (m^3/day),
70 kg = standard human body weight,
BW_A = body weight of experimental animals (kg),
A_O = efficiency of absorption from oral exposure, and
SF = safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may, under some circumstances, result in depressed fluid intake which, in

turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant; however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

6. REFERENCES

Crump, K. S. 1979. Dose-response problems in carcinogenesis. Biometrics 35:157.

Crump, K. S., and W. W. Watson. 1979. GLOBAL,79. A FORTRAN program to extrapolate dichotomous animal carcinogenicity data to low dose. National Institute of Health Science Contract No. 1-ES-2123.

International Commission on Radiological Protection. 1977. Recommendation of the ICRP, Publication No. 26. Pergammon Press, Oxford, England.

Krewski, D., K. S. Crump, J. Farmer, D. W. Gaylor, R. Howe, C. Portier, D. Salsburg, R. L. Sielken, and J. Vanryzin. 1983. A comparison of statistical methods for low-dose extrapolation utilizing time-to-tumor data. Fund. Appl. Toxicol. 3:140-160.

NAS. 1977. Drinking water and health. Safe Drinking Water Committee, Advisory Center on Toxicology, National Research Council, National Academy of Sciences, Washington, DC. 939 pp.

Stephan, C. E. 1980. July 3 memorandum to J. Stara, U.S. Environmental Protection Agency. (As cited in USEPA 1980).

Stokinger, M. E. and R. L. Woodward. 1958. Toxicological methods for establishing drinking water standards. J. Am. Water Works Assoc. 50:517.

USEPA. 1980. Water Quality Criteria Documents, Environmental Protection Agency. Fed. Regist. 45:79318-79357.

Veith, G. D., et al. 1980. Measuring and estimating the bioconcentration factors of chemicals in fish. J. Fish Res. Bd. Can. 36:1040. (As cited in USEPA 1980).

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