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TOXICITY OF WHITE PHOSPHORUS TO WATERFOWL: ACUTE EXPOSURE IN MALLARDS

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ABSTRACT: As part of an effort to understand extensive, white phosphorus (P_4)-induced waterfowl mortality at Eagle River Flats, Fort Richardson, Alaska (USA), we conducted a number of acute toxicity tests using penned mallards (*Anas platyrhynchos*) in 1993 and 1994. The 24-hr median lethal dose (LD_{50}) for P_4 dissolved in oil was 6.46 mg/kg in adult males and 6.96 mg/kg in adult females. Although the median lethal doses were not statistically different, the female dose-response curve had a statistically shallower slope than that of males. The LD_{50} for the ecologically more relevant pelletized form of P_4 in adult males was 4.05 mg/kg. In mallards, one mechanism of P_4 toxicity caused rapid (3 to 10 hr) mortality and had signs consistent with anoxia. A second, slower acting mechanism resulted in hepatic and renal pathology including extensive fat deposition in the liver and cellular necrosis. White phosphorus accumulated in adipose tissues, but only for a few days.

Key words: White phosphorus, mallards, acute toxicity, munitions, ordnance, *Anas platyrhynchos*.

INTRODUCTION

White phosphorus (elemental or P_4) is used by the military throughout the world in incendiary devices, as an obscurant, and for range-finding artillery. When exposed to air, P_4 forms a thick, white smoke and is converted to various phosphorus hydroxides, oxides, and acids. When P_4 is fired over wetlands, however, particles can enter the water or saturated sediments and remain inert for years (Agency for Toxic Substances and Disease Registry, 1994).

Persistent epizootics of waterfowl in Eagle River Flats, a 865-ha tidal marsh along the Knik Arm of the Cook Inlet, Alaska, USA (61°19′N, 149°44′W), spurred recent interest in P_4 as an environmental hazard. This site has been used by the U.S. Army as an artillery practice field since the late 1940's. An estimated 1,000 to >2,000 waterfowl, mostly mallards (*Anas platyrhynchos*), green-winged teal (*Anas crecca*) northern pintails (*Anas acuta*), trumpeter swans (*Cygnus buccinator*), and tundra swans (*Cygnus columbianus*), have died annually since 1982 when the first mortalities were reported (Racine et al., 1992). Wetlands in at least eight other U.S. Army installations in the contiguous United States also have tested positive for P_4 and the substance has been found in 71 hazardous waste sites in 29 states on the National Priority List (Agency for Toxic Substances and Disease Registry, 1994).

Eagle River Flats is an important staging area for waterfowl during the spring migration to breeding grounds and before the fall migration. In addition to the species listed above, we have observed northern shovelers (*Anas clypeata*), American wigeon (*Anas americana*), Canada geese (*Branta canadensis*), snow geese (*Chen caerulescens*) and numerous shorebirds, including dowitchers (*Limnodromus scolopaceus, Limnodromus griseus*), red-necked phalaropes (*Phalaropus lobatus*), yellowlegs (*Tringa flavipes, Tringa melanoleuca*), and others feed in the marsh. These birds may mistake the hard, waxy, P_4 pellets as food or simply ingest them incidentally (Racine et al., 1992). Bald eagles (*Haliaeetus leucocephalus*), golden eagles (*Aquila crysaetos*), ravens (*Corvus corax*), and herring gulls (*Larus argentatus*) feed on dead and dying waterfowl in the area and may be exposed indirectly.

White phosphorus toxicity has been studied in humans (Diaz-Rivera et al.,

1950), rats (Goshal et al., 1969) and rabbits (Cameron and Patrick, 1966). Toxic signs in these species include decreased blood pressure, vomiting, intense thirst, restlessness, delirium, muscular weakness, and death.

Coburn et al. (1950) found that mallards and black ducks (*Anas rubripes*) dosed with P_4 dissolved in almond oil had leg weakness, rhythmic swaying of the head, convulsions, and death. Repeated exposures led to weight loss, paralysis of the gullet, loss of coordination, and fatty degeneration of liver and kidneys. Unfortunately, their study was limited by small samples sizes and inadequate analytical methodologies. Roebuck et al. (1994) reported on the possibility that predators may be exposed to P_4 at Eagle River Flats. Nam et al. (1994) and Sparling and Federoff (in press) further examined the potential for secondary toxicity using penreared American kestrels (*Falco sparverius*).

Our objective was to describe acute toxicity of P_4 in mallards. Specifically, we determined the dose-response characteristics of P_4, examined gross and microscopic pathological changes, measured residues in selected tissues, and estimated the lowest observed adverse effects level (LOAEL). Exposures included both P_4 dissolved in corn oil for comparisons with previously published studies, and solid pellets of P_4 as an ecologically more relevant form of the substance. Most of the work was conducted as discrete projects to address specific questions of the Department of Defense, hence there was some variation in sample sizes and tissues sampled.

The following experiments were conducted on mallards with P_4 dissolved in corn oil: acute toxicity tests in adult (≥18 mo old) males and females; a low-dose test in juvenile males to help estimate the LOAEL; and a test with juvenile males and females to detrmine if there are age-specific differences in response. Experiments with pelletized P_4, which occurred in 1994, focused on comparing the toxicity of pelletized and oil-dissolved P_4. We only used deaths within the first 24 hr post exposure in these experiments to estimate median lethal dose (LD_{50}) levels, but report on clinical signs in survivors as they developed over a 7 to 14 day period post exposure.

METHODS

The studies occurred between May and October of 1993 and 1994 at Patuxent Wildlife Research Center (Laurel, Maryland, USA). Mallards were purchased from a commercial game farm (Whistling Wings, Inc., Hanover, Illinois, USA) and P_4 (certified 99% pure) was obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin, USA). Birds were housed individually in 1-m^2 outdoor pens subject to ambient light and temperature and were provided food and water ad libitum except for fasting 16 hr prior to dosing. Following the experiments, survivors were euthanized by asphyxiation with CO_2. All birds were necropsied and examined for gross pathological changes. Liver, and kidney were preserved in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm; and stained with hematoxylin-eosin (American Histolabs, Gaithersburg, Maryland). A few fresh-frozen liver samples were stained with oil-red-o for confirmation of fatty deposits and deposits of hemoglobin were detected by the Lison stain (McMarus and Mowry, 1960). The severity of the most frequently observed histopathological changes were scored as: mild—involvement of 10 to 19% of the tissue section examined; moderate—pathologic change involving 20 to 60% of section examined; severe—pathologic changes involving >60% of section. These and intermediate conditions were used to rank each slide from 0 (absent) to 6 (severe).

Approximately 5 g of fat, skin, or liver were finely minced with a scissors and mixed with 10 ml of isooctane in 40 ml chemically clean amber vials and gently shaken by hand for 30 sec. They were then shaken for 30 min and frozen at −20 C until analyzed. White phosphorus concentrations were measured with a Hewlett-Packard (Wilmington, Delaware, USA) Series II model 5890 gas chromatograph and nitrogen-phosphorus detector following procedures of Addison and Ackman (1970). Instrument blanks, spikes, and duplicate samples were included in each chromatograph run. Percent recoveries from spiked samples were between 92% and 94%.

Probit analysis (Finney, 1971) was used to determine dose-response curves. Univariate parametric tests (Sokal and Rohlf, 1969) were

used for most comparisons except those relating histopathological findings to dose; the nonparametric Spearman rank correlation was used to compare tissue with dose level, and Fisher's exact test was used to determine if observed mortality differed from an expected frequency (Siegel, 1956). All parametric statistical tests were conducted with SAS Institute, Inc. (1989).

For the tests using P_4 dissolved in corn oil, a stock solution was diluted to the appropriate dosage at a rate of 3 ml corn oil/kg body weight. Doses were administered via oral gavage through a glass tube. Control birds were given 3 ml/kg corn oil without P_4. Birds were watched nearly continuously for the first 8 hr post dose and every 2 to 3 hr after that during daylight.

In July 1993 we conducted an acute toxicity test on adult male and female mallards using four or five birds of each sex at 2, 4, 5.2, 6.1, 7.1, 8.0, and 9.1 mg/kg (all dose concentrations are expressed as mg P_4 per kg live body weight) and eight birds of each sex as controls. Due to low mortality in hens during 1993, we repeated the acute test on females in 1994 at the same time of year and breeding stage using six birds each at 0, 6.0, 7.9, 10.2, 13.5, and 18.0 mg/kg. Survivors were kept for 7 days post exposure to determine if any delayed effects occurred.

To determine if juvenile birds were equally sensitive to white phosphorous as adults, we followed the recommendations of Link et al. (1996) and dosed 14 male and 14 female juveniles (12 to 18 mo old) with the calculated LD_{50} of 6.5 mg/kg P_4 in corn oil. Survivors were euthanized at the end of 14 days.

To better estimate the LOAEL, with an independent test, we dosed 10 males each at 2.6 and 3.7 mg/kg. Birds were euthanized at 10 days post exposure.

To make pellets, we submerged P_4 under water and melted it in a hot water bath. The required dose per bird was made by extracting molten P_4 with a positive displacement micropipette and exuding it into a 4 ml amber vial filled with 3 ml of chilled distilled water. Doses reported in this study were 1, 2, 4, and 6.4 mg/kg. Control birds were administered 3 ml water without P_4. At dosing, the contents of the vials were emptied into a glass funnel which was inserted into the bird's esophagus and then rinsed with 2 to 3 ml of distilled water. All tests with pelletized P_4 involved adult male mallards. Because we had never observed a bird recover from convulsions during 1993, some birds were euthanized when they were severely convulsing rather than allowing them to suffer. Most birds were found dead in their pens, however. Birds were necropsied and tissues were sampled as per those dosed with P_4 in oil.

RESULTS

During the first (1993) acute test with P_4 dissolved in oil, the percent mortality for adult males at each of the dose levels was: control, 0%; 4 mg/kg, 0%; 5.2 mg/kg, 25%; 6.1 mg/kg, 50%; 7.1 mg/kg, 75%; 8.0 mg/kg, 75%; and 9.1 mg/kg, 100%. The dose-response curve for adult males was:

> Probit mortality = $-7.391 + 9.237 \times$ Log(Dose) (mg/kg) ($P = 0.001$, SE on slope = 3.050) and LD_{50} = 6.46 mg/kg with 95% confidence intervals (C.I.) = 5.19 to 7.69 mg/kg.

The percent mortality for females in this experiment was control, 0%; 4 mg/kg, 25%; 5.2 mg/kg, 0%; 6.1 mg/kg, 25%; 7.1 mg/kg, 25%; 8.0 mg/kg, 25%; and 9.1 mg/kg, 25%. No curve could be generated for adult females from these data.

For the female experiment in 1994 the percent mortality was: 6.0 mg/kg, 50%; 7.9 mg/kg, 67%; 10.2 mg/kg, 83%; 13.5 mg/kg, 83%; and 18.0, mg/kg 100%. The dose-response curve for adult females was:

> Probit mortality = $-3.593 + 4.298 \times$ Log(Dose) (mg/kg) ($P = 0.001$, SE on slope = 1.71) and LD_{50} = 6.96 mg/kg (95% C.I. = 2.66 to 8.96 mg/kg).

The median lethal doses for males and females were not statistically different, but slope of the female dose-response curve was shallower than that of males ($P = 0.045$, Fig. 1).

In the test for LOAEL, none of the birds dosed at 2.6 or 3.7 mg/kg (presumed LD_1 and LD_{10}) died. Mortality at both doses was statistically <50% ($P = 0.017$, Fisher's Exact Test).

In the test for age-specific differences, six of 14 males and nine of 14 females died. There was no difference between sexes in the percent of birds which died in this test (Fisher's Exact Test, $P > 0.10$).

For adult male mallards dosed with pelletized P_4, the percent mortality for each

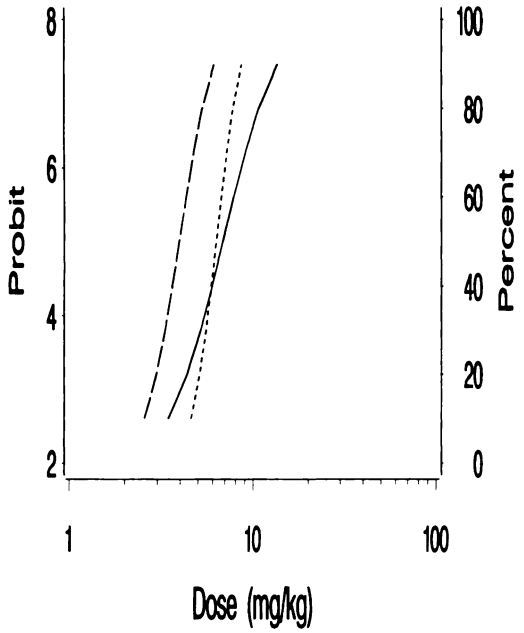

FIGURE 1. Probit and percent mortality expressed as $\log_{10}$ dose for acute toxicity in male (solid line) and female (dashed line) mallards orally gavaged with P_4 dissolved in corn oil, and for males gavaged with pelletized P_4 (dotted line). Dose is expressed as mg P4/kg live body weight.

dose was: control, 0%; 1.0 mg/kg, 0%; 2.0 mg/kg, 0%; 3.4 mg/kg, 10%; 4.0 mg/kg, 83%; and 6.5 mg/kg, 86%. This resulted in a dose-response curve with the characteristics:

> Probit mortality = $-4.023 + 6.725 \times$ Log(Dose) (mg/kg) ($P < 0.023$, SE on slope = 2.967) and LD_{50} = 3.90 mg/kg (95% C.I. = 3.24 to 4.69 mg/kg).

The slope of this curve was similar to that obtained for males dosed with P_4 in oil but greater than that obtained for females ($P = 0.055$) (Fig. 1). In an ancillary test to specifically compare the relative toxicity of P_4 in oil with that of pelletized P_4, 12 of 14 males dosed with pelletized P_4 died at 6.46 mg/kg (the LD_{50} for P_4 in corn oil), which was significantly greater than expected ($P = 0.037$).

Based on the collective data from all tests using corn oil, overt signs of acute P_4 toxicity could be separated into three phases (Table 1). The first phase, which started within 2 hr post dose and lasted for several hours to >1 day, was characterized by lethargy, mild inappetence, increased drinking, and unsteady gait. About a third of the birds observed had some signs of vomiting including a liquid discharge from nares and mouth. Of 23 birds of both sexes that were dosed with P_4 in corn oil and watched carefully, six (26%) recovered, nine (39%) advanced to the next stage, and eight (35%) died without showing other phases.

TABLE 1. Percent of mallards exhibiting specific signs of P_4 toxicity after acute exposures.

Dose (mg/kg)	Sample size (n)	Lethargy (%)	Tremors (%)	Convulsions (%)
4–6	15	33	47	20
6–8	27	41	11	48
≥8	13	31	46	23

The second phase of toxicity was manifested by severe tremors and by a stereotyped head bobbing movement with the bill brought back and forth along the breast as the bird sat. We do not have a reliable estimate of the percent of birds entering this stage because it lasted only a few minutes and could have been missed. All birds seen in this phase subsequently died.

The third phase of acute toxicity consisted of severe and intense convulsions with the neck dorsally flexed and wings extended. The signs of this phase were consistent with convulsions induced by anoxia during euthanasia by CO_2, but were more protracted, lasting for >30 min. Again, convulsions were missed in some birds that died during the night, but most of the birds dying within the first 24 hr after receiving the dose had these signs. All birds observed in this phase died. There were no apparent differences in the behavioral signs of intoxication between birds dosed with P_4 as pellets or in corn oil. Most birds that died from P_4 toxicity, either as pellets

TABLE 2. Mean and range for onset (hr) of signs in mallards orally gavaged with acute doses of P_4 dissolved in corn oil.

Dose[a]	Dose to signs	Signs to death	Dose to death
4 to 6 mg/kg			
Mean	4.9	4.2	5.5
Range	0.3–11.0	0.3–1.0	4.7–6.3
Sample size	15	4	7
6 to 8 mg/kg			
Mean	3.4	1.2	5.9
Range	2.0–7.5	0.2–3.4	2.8–25.9
Sample size	27	20	14
≥8 mg/kg			
Mean	5.1	1.9	8.1
Range	0.7–11.5	0.3–6	2.4–24.2
Sample size	13	4	7

[a] Dose is expressed as mg P_4/kg fresh body weight.

or in oil, developed a very rigid rigor mortis within 5 to 10 min.

The time from dose to onset of signs took from several minutes to >11 hr, but averaged around 5 hr (Table 2). Once signs appeared, mallards took from 10 min to >6 hr to die; three birds were lethargic for >24 hr before succumbing. We were unable to determine time of death for birds that died during the night. For birds given 6.5 mg/kg pelletized P_4, mean (±SD) time from dose to onset of signs was 5.2 ± 2.1 hr (n = 14), and mean time from dose to death was 5.9 ± 2.1 hr (n = 10).

Gross internal pathology included petechia in coronary cardiac band and pancreas, ecchymoses and focal areas of hemorrhage in the liver, mucosal congestion in the duodenum, and meningeal hemorrhaging. With exposure to P_4 in oil, necrotic and hemorrhagic foci in liver first appeared among survivors given 4.0 mg/kg P_4; the frequency of occurrence of hepatic problems increased with dosage except for the highest dosages where mortality was greatest (Table 3). Petechiations in the cardiac coronary band and pancreas became apparent at doses ≥4 mg/kg.

Birds given >5.2 mg/kg that survived for several hours after dosing had enlarged, pale, friable livers indicative of fatty liver degeneration. The ratio of liver weight to body weight in these birds correlated positively with dose in both females (r = 0.447, P = 0.006, n = 36) and males (r = 0.349, P = 0.022, n = 43). Birds also lost

TABLE 3. Gross pathological signs of mallards orally gavaged with varying concentrations of P_4 dissolved in oil. Data are pooled from several experiments and include both birds dying from dose and survivors.

	Dose (mg/kg)					
	Control	2–4.0	4.3–6.1	6.2–8.0	9.1–10.2	13.5–22
Sample size	38	40	22	50	16	18
Dead (%)	0	2.5	27.2	44.0	62.5	94.4
Liver foci (%)	0	7.5	36.4	12.0	43.7	5.5
Liver necrosis (%)	0	2.5	0	10.0	12.5	0
Coronary petechiae (%)	0	0	4.5	2.0	31.2	0
Pancreatic petechiae (%)	0	0	9.1	0	12.5	0
Congested duodena (%)	0	0	9.1	4.0	37.5	16.7
Weight change[a]						
Mean	2.2^{c}	-1.0^{cd}	-2.2^{cd}	-3.2^{d}	-5.0^{d}	0.0^{cd}
SD	7.8	9.1	5.6	4.8	4.0	1.7
Liver/body weight[b]						
Mean	2.0^{c}	2.0^{c}	2.5^{de}	2.1^{cd}	2.9^{ef}	2.8^{f}
SD	0.3	0.4	0.7	0.5	0.7	0.5

[a] Expressed as percent change from pretreatment weight; ANOVA P = 0.046; groups with the same letter designations could not be distinguished at P = 0.05.

[b] Expressed as fresh liver weight/pretreatment body weight; ANOVA P = 0.0034; groups with the same letter designations could not be distinguished at P = 0.05.

TABLE 4. Gross pathological signs in mallards orally gavaged with pelletized P_4 at varying dose levels. Data are pooled from three experiments and include birds dying from dose and survivors.

	Dose (mg/kg)			
	Control	1 to 2	3 to 4	6.5
Sample size	14	34	13	14
Dead (%)	0	0	83.3	85.7
Liver foci (%)	0	5.9	7.7	14.3
Liver necrosis (%)	0	5.9	7.7	0
Coronary petechiae (%)	0	0	7.7	0
Pancreatic petechiae (%)	0	0	0	0
Congested duodena (%)	0	8.8	15.4	64.3
Enlarged spleen (%)	7.1	14.7	7.7	50
Pale kidney (%)	0	5.9	7.7	0
Weight change[a]				
Mean	−5.8	−6.2	−6.3	−4.4
SD	0.3	5.2	2.2	4.8
Liver/body weight[b]				
Mean	1.8	2.2	2.5	2.5
SD	0.2	0.5	0.7	0.6

[a] Expressed as percent change from pretreatment weight.
[b] Expressed as a ratio of fresh liver weight/pretreatment body weight.

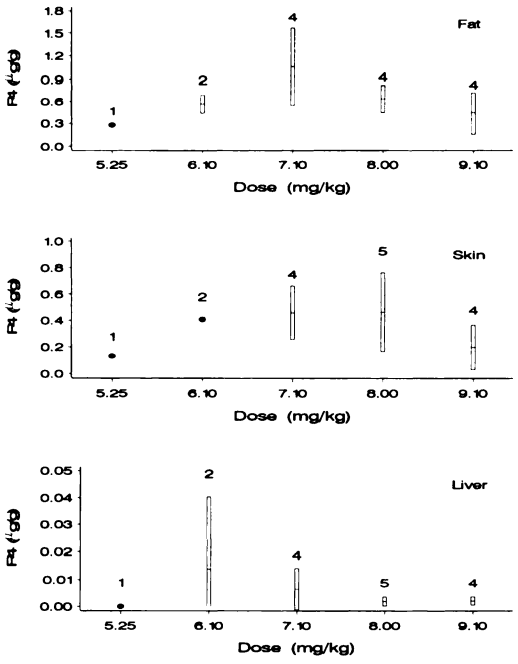

FIGURE 2. Mean (±SD) P_4 residues in fat, skin, and liver of male and female mallards orally gavaged with P_4 dissolved in oil in birds that died from dosing. Concentrations are expressed as μg P_4/g fresh weight of tissue. Numbers above each bar represent sample size.

2 to 5% of their body weight at dose levels >4.0 mg/kg.

Two birds in the LOAEL test, one each at 2.6 and 3.7 mg/kg dose, had small necrotic areas on their livers. However, there was no apparent hepatomegaly or weight loss at these low dose levels.

Internal pathological changes were less frequent among birds given pelletized P_4 (Table 4) than those given P_4 in oil. Among survivors, fat deposition in the liver was common, and liver to body weight ratio increased, especially in males where the relationship between dose and liver ratio was significant ($r = 0.420$, $P = 0.037$, $n = 25$) (Fig. 2).

On histologic examination, there were four common pathological changes in captive mallards challenged with P_4. The most common of these was ground glass vacuolations in the liver which were associated with glycogen storage. The frequency of occurrence and severity of these vacuolations decreased with dosage in birds given P_4 in oil (Spearman rank correlation coefficient, $r_s = -0.441$, $P < 0.0001$, $n = 69$) (Table 5).

Hepatocellular vacuolations, indicative

TABLE 5. Major histopathological abnormalities in adult mallards given a single dose of P_4 dissolved in oil by prevalence and mean severity (0 = absent, 6 = severe). Data are pooled from several studies and include birds dying on dose and survivors.

	Dose (mg/kg)							
	0	2	4	5.25	6.1	7.1	8	9.1
Sample size	12	10	10	7	7	8	7	8
Heptacellular vacuolations								
Percent	8	20	30	14	14	50	71	62
Severity	0.5	0.7	1.5	0.9	0.6	1.7	2.6	3.5
Liver foci								
Percent	0	10	0	0	43	25	57	50
Severity	0	0.2	0	0	0.4	0.4	2.1	1.4
Hyaline droplet nephrosis								
Percent	0	0	10	14	14	37	29	38
Severity	0	0	0.2	0.7	0.7	1.0	1.1	2.1
Ground glass vacuolations								
Percent	75	60	50	71	71	25	0	13
Severity	4.1	3.4	1.9	3.4	3.7	0.9	2.1	0.7

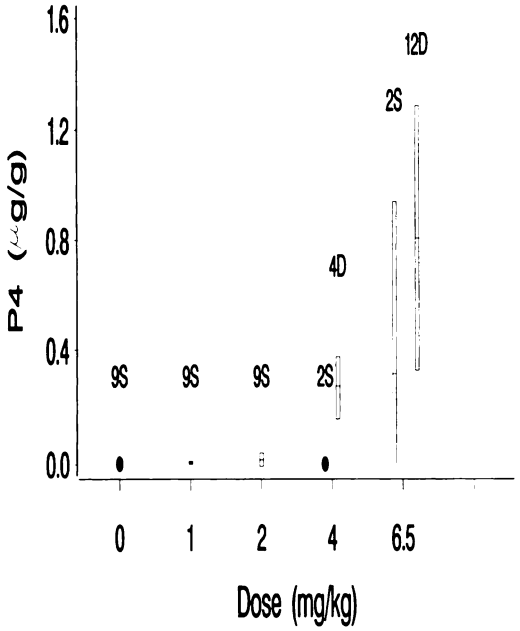

FIGURE 3. Mean (±SD) P_4 residues in fat of male mallards orally gavaged with P_4 pellets by fate of bird. Concentrations are expressed as µg P_4/g fresh weight of tissue. Values above each bar are sample sizes and fate (S = survived; D = died from dose).

of fat deposition, were the second most prevalent histopathological finding, and their severity increased with dosage (r_s = 0.391, P = 0.0009). Cellular necrosis in the liver was less commonly seen, but increased in frequency and severity with P_4 dosage (r_s = 0.462, P = 0.0001).

The only consistent histopathological finding in the kidneys was hyaline droplet nephrosis in renal convoluted tubules. The accumulated deposits within the tubular cytoplasm were identified as hemoglobin by the Lison's stain method. The severity and frequency of hyaline droplet nephrosis increased with dosage (r_s = 0.382, P = 0.0012).

Histological changes were less frequent in birds dosed with pelletized P_4 than with P_4 in oil. Of 14 birds given 6.5 mg/kg, five had liver changes but none of these were specific to P_4 toxicity.

Among the 18 birds that died from P_4 in oil in the acute toxicity test, P_4 residues ranged from 0.12 to 1.78 µg/g fresh weight in fat, 0.05 to 0.96 µg/g in skin, and from below detection limits (0.001 µg/g) to 0.02 µg/g in liver (Fig. 3). There was a significant difference in white phosphorous concentrations in fat due to dose (P = 0.003)

with birds given 7.1 mg/kg having higher concentrations than those given 9.1 or 5.25 mg/kg. No differences due to sex were observed for any of the tissues ($P > 0.14$). White phosphorus concentrations generally fell below detection limits within the 7 to 10 days between dose and when survivors ($n = 67$) were euthanized, hence there was a highly significant difference in concentrations between survivors and those that died at the same dose levels ($P < 0.0001$ for fat and skin and $P = 0.019$ for liver).

With pelletized P_4, P_4 levels in fat of dead males ranged from 0.12 to 0.36 μg/g at 4.0 mg/kg and 0.13 to 3.30 μg/g at 6.5 mg/kg. One survivor at 6.5 mg/kg had a P_4 concentration of 0.63 μg/g in its fat. Liver levels seldom exceeded detection limits.

DISCUSSION

The median lethal dose for mallards receiving P_4 dissolved in oil was 6.46 mg/kg for adult males and 6.96 mg/kg for adult females. These estimates are higher than the 1.5 to 3.0 mg/kg estimated by Coburn et al. (1950) as a lethal exposure to ducks. However, Coburn et al. (1950) used black ducks as well as pen-reared and wild mallards, which could affect their results; also, their lethal dose was the lowest level at which they observed mortality, not the median lethal dose. Sex-specific mortality did not differ ($P > 0.15$) from the expected 50% determined from adult males dosed with P_4 in oil (Link et al., 1996).

The slope of the dose-response curve was steeper for male mallards than for females although the median lethal doses did not differ significantly between sexes. This implies that the response to P_4 was more variable in females than in males. Part of this variability may be due to variation in body condition. In both years, females had just completed reproduction and had ovaries which were regressing. Many had depleted abdominal fat stores but varying levels of yolk. Males, however, were in prime condition with ample fat reserves. Little is known about the assimilation of P_4, but because it is highly lipophilic, larger amounts of fat could serve as repositories, altering the exposure of other tissues such as blood, liver, and kidney, to the toxic influence of P_4.

If the differences in sensitivity to P_4 is consistent with free-ranging waterfowl that ingest pelletized P_4, seasonal variation in fat reserves associated with migratory hyperphagia, egg laying, and brood rearing may influence sensitivity. When mallards and other waterfowl arrive at Eagle River Flats in the spring, they presumably are in poorer body condition than when they leave because some fat reserves that were used during migration are restored during staging. Therefore, vulnerability as well as risk of exposure to P_4 may increase with duration on Eagle River Flats. Females may reduce risk by partitioning some of the P_4 into developing ova because measurable levels of P_4 have been found in herring gull (Racine et al., 1992) and chicken egg yolks (Nam, 1995).

The median lethal dose of 4.05 mg/kg for pelletized P_4 in males was significantly lower than that for adult males or females dosed with P_4 in oil. The higher acute toxicity may be due to differences in assimilation of the two forms of P_4 in the body. An oil carrier may reduce the rate of P_4 assimilation and allow more of it to be excreted. At the highest doses of P_4 in oil, we observed smoke and a strong garlic odor emanating from cloacae which demonstrate that not all of the P_4 was absorbed. Alternatively, because P_4 volatizes at room temperature under aerobic conditions (Agency for Toxic Substances and Disease Registry, 1994), pelletized P_4 could evolve vapors at a greater rate than P_4 in oil and these vapors may be absorbed more rapidly than liquid or solid P_4 by the digestive system.

White phosphorus appears to produce two phases of toxicity, each through different mechanisms. Rapid onset of mortality (5 to 10 hr post dose) was accompanied by rhythmic head bobbing and intense convulsions. The signs of this phase are con-

sistent with prolonged anoxia and may be aggravated by severe trauma to the cardiovascular system. In humans acute, lethal exposure to P_4 leads to vascular collapse, cyanosis, deep pallor, abnormal electrocardiogram, and heart failure (Diaz-Rivera et al., 1950, 1961). Our observations of frequent petechiations in various organs are consistent with observations of Winek et al., 1973 and are evidence for vascular trauma. Phosphine gas can be produced by the oxidation of P_4 (Nam, 1995) and this may contribute to the mortality by oxidizing hemoglobin (Potter et al., 1991).

The second phase of toxicity is delayed and involves cumulative damage to liver, kidneys, and other organs. Fat deposition in livers and hepatomegaly have been attributed to an inhibition of hepatic protein synthesis (Lombardi and Recknagel. 1962). Based on our observations, this fat deposition may start within a few hours after exposure and continue for several days. The liver is also the primary site of detoxification of P_4 (Goshal et al., 1969) which probably results in the high incidence of hepatic lesions and necrosis.

The presence of hyaline droplet nephrosis in renal tubules is related to the hemolytic properties of P_4 and to fatty degeneration of the kidney (Appelbaum et al., 1975). Coburn et al. (1950) also found extensive necrosis and hemorrhaging in kidneys of mallards.

The incidence of gross and microscopic hepatic fatty degeneration, hemorrhagic and necrotic hepatic foci, and renal pathology were also dose-dependent. These lesions occurred at a low rate and severity at the lowest doses and were most pronounced at intermediate doses. Fatty degeneration of the liver and kidneys was less common at the highest dose levels, probably because birds died before these lesions could develop. The reduction in the frequency and severity of ground glass vacuolations in dosed birds may be related to glycogen depletion or might simply be background noise that is masked by the fatty changes. Hypoglycemia has been observed in humans (Diaz-Rivera et al., 1950) and this could deplete glycogen stores.

Lowest observed adverse effects levels are dependent on the end point of interest and are lower for sublethal effects than for mortality. Based on the dose-response curves for mortality and on the low dose test, the LOAEL for single doses of P_4 in oil was probably between 2.5 and 3.0 mg/kg for male mallards and slightly lower for females. The lowest dose in which liver or kidney damage was observed was 2.0 mg/kg. Mortality-based LOAEL for pelletized P_4 in male mallards was around 3.0 mg/kg. Hemorrhagic and necrotic foci appeared in birds given a single 2 mg/kg P_4 pellet and the acute LOAEL for this end point may be below 1 mg/kg.

White phosphorus is rapidly excreted or metabolized by living organisms. Its breakdown products include P_4O_{10}, P_4O_6, phosphoric acids, and orthophosphate (Agency for Toxic Substances and Disease Registry, 1994). Under alkaline conditions, typical of small intestines, P_4 may be converted to hypophosphite, phosphite, and phosphine (Agency for Toxic Substances and Disease Registry, 1994). Within a week post dose, residue levels dropped below detection limits in most of our survivors. This is similar to the rapid loss of P_4 from American kestrel tissues (Nam et al., 1994) and from fish (Fletcher, 1974).

Goshal et al. (1971) proposed that the liver was the principal organ of storage for P_4 but they used ^{32}P, which reflected both the parent compound and its metabolites, and they did not examine fat. Coburn et al. (1950) found that total phosphorus content of the liver dropped after administration of P_4 in ducks but that the phosphorus levels in heart, kidney, and muscle increased. We found little hepatic P_4, thus the substance may be rapidly metabolized in the liver. Instead, our data support those of Nam et al. (1994) that the principal tissue for the unaltered compound is fat.

The short duration of P_4 in living organisms limits its use in determining expo-

sure. Dyer et al. (1972) showed that P_4 is more persistent in dead tissues, however, and the presence of P_4 in fat of dead animals may be a reliable indicator of cause of mortality.

Occasionally, birds seemingly may provide exceptions to the short duration of P_4 in living tissues. One of our surviving birds that had been given 6.5 mg/kg P_4 in pellet form had measurable levels in both fat and liver 10 days post dose. Three other birds given pelletized P_4 (out of approximately 50) in other unpublished studies that we conducted at Patuxent had smoking gizzards when necropsied seven days post dose. We propose that birds can retain P_4 pellets in the gizzard for an indeterminate time before actually passing them to lower portions of the digestive system, perhaps to become intoxicated days after initial exposure. Depending on the incidence of P_4 retention and resulting intoxication, estimates of avian mortality at Eagle River Flats could be slightly to moderately conservative.

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