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# Acute Toxicity and Sublethal Effects of White Phosphorus in Mute Swans, *Cygnus olor*

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**Abstract.** Among the waterfowl affected by white phosphorus ($P_4$) at a military base in Alaska are tundra (*Cygnus columbianus*) and trumpeter (*C. buccinator*) swans. To estimate the toxicity of $P_4$ to swans and compare the toxic effects to those of mallards (*Anas platyrhynchos*), we dosed 30 juvenile mute swans (*C. olor*) with 0 to 5.28 mg $P_4$/kg body weight. The calculated $LD_{50}$ was 3.65 mg/kg (95% CI: 1.40 to 4.68 mg/kg). However, many of the swans still had $P_4$ in their gizzards after dying, as determined by ''smoking gizzards'' and characteristic odor, and a lower $LD_{50}$ might be calculated if all of the $P_4$ had passed into the small intestines. We attribute the retention of $P_4$ in swans to the possibility that $P_4$ pellets were mistaken for the similarly sized grit in their gizzards. Most swans took 1 to 4.5 days to die in contrast to the few hours normally required in mallards and death appeared to be related more to liver dysfunction than to hemolysis. White phosphorus affected several plasma constituents, most notably elevated aspartate aminotransferase, blood urea nitrogen, lactate dehydrogenase, and alanine aminotransferase.

White phosphorus (elemental or $P_4$) is used by the military as a smoke obscurant, in incendiary devices, and as a way of marking artillery strikes. When exposed to air, $P_4$ oxidizes to form a thick, white cloud, but when deposited in water or wet sediments, particularly in cold regions, $P_4$ may remain inert for decades (Walsh *et al.* 1996). Because $P_4$ is manufactured as small (approx. 1 mm) pellets, it may be mistaken as a food item by waterbirds that feed from sediments or be accidentally ingested during foraging. Once ingested and absorbed, $P_4$ causes several physiological problems in waterfowl including lethargy, inappetance, severe convulsions, and death (Coburn *et al.* 1950; Sparling *et al.* 1997). White phosphorus–related mortality involving thousands of ducks as well as swans, raptors, and shorebirds has been recorded annually since 1982 from Eagle River Flats, part of Fort Richardson, Anchorage, Alaska (Racine *et al.* 1992; Steele *et al.* 1997). Among the waterfowl species most affected are mallards (*Anas platyrhynchos*), green-winged teal (*A. crecca*), northern pintails (*A. acuta*), and trumpeter and tundra swans (*Cygnus buccinator, C. columbianus*). At least one bald eagle (*Haliaeetus leucocephalus*) and a golden eagle (*Aquila crysaetos*) have been found dead with measurable levels of $P_4$ in their tissues. Currently, the U.S. Army is remediating the tidal marsh through isolating and drying the most contaminated sites, which allows $P_4$ to oxidize, and through dredging and drying the spoils. This study, however, may have bearing on risk assessment at Eagle River Flats and at other sites where $P_4$ has been found in wetlands.

Physiological effects of $P_4$ include lethality, histological changes, and damage to kidneys and liver. Progression of toxicity has been documented in black ducks (*Anas rubripes*) (Coburn *et al.* 1950) and mallards (Sparling *et al.* 1997). Changes in plasma chemistry as possible indicators of exposure to $P_4$ and as additional evidence for physiological effects have been reported for mallards (Sparling *et al.* 1998). Potential effects of $P_4$ in raptors including acute toxicity and accumulation (Nam *et al.* 1994), and secondary poisoning (Roebuck *et al.* 1994; Sparling and Federoff 1997) have also been reported. However, $P_4$ toxicity, including $LD_{50}$s, has been examined in detail only in mallards (Sparling *et al.* 1997). The objective of this paper is to examine $P_4$ toxicity in a second waterfowl species, the mute swan (*Cygnus olor*). Mute swans were selected because they are congeneric with the two species of swans that visit Eagle River Flats and they can serve as a surrogate for these native species, which are difficult to obtain and study under penned conditions.

## Materials and Methods

Mute swans were collected as eggs in 1995 from selected nests along the Chesapeake Bay. This species was introduced onto estates bordering the bay decades ago for aesthetic purposes and has become a nuisance, feral species (Reese 1975). Cygnets were raised by hand within pens, which contained constructed wetlands and grassy borders. They were fed commercial duck chow. In May 1996 they were moved to semi-enclosed outdoor pens under natural photoperiod and temperature conditions and allowed to adjust for 1 week prior to dosing. All swans were juveniles, and both males and females were randomly assigned to treatment without reference to sex.

Thirty swans were weighed to the nearest 10 g and a 3–5-ml blood sample was taken via jugular venipuncture into lithium-heparinized

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syringes 3 days prior to dosing. The birds were then randomly assigned to treatment. Doses were calculated on a mg $P_4$/kg live weight basis and made by extracting molten $P_4$ with a positive displacement pipette and depositing the prescribed mass of pellets into an amber vial containing 3 ml of distilled water. When the $P_4$ cooled it formed pellets that were identical in hardness and composition to those found at Eagle River Flats. Swans were gavaged with $P_4$ and sufficient distilled water to wash the pellets into the esophagus and prevented from regurgitating by gentle but firm clasping of the neck. Control birds were given the same amount of water as dosed swans, but no $P_4$. Swans were subsequently observed every 3 h during daylight for onset of signs. The first birds dosed were fed prior to exposure and were given 4.06 mg/kg $P_4$, which was close to the calculated $LD_{50}$ for mallards (Sparling *et al.* 1997). In subsequent trials, birds were fasted the night before dosing and given only a single dose of $P_4$ (see below). Only those birds given a single dose were used in statistical analyses.

One week postexposure 3–5 ml of whole blood was collected prior to euthanasia with $CO_2$ and frozen at −20°C. We did not collect blood during the critical period of overt clinical signs to avoid increasing risk of mortality through additional stress. Hemoglobin was measured via the cyanomethemoglobin method using Drabkin's reagent (Sigma Chemical, St. Louis, MO). A 20-µl aliquot was centrifuged at 3,000 rpm for 10 min and used for measuring hematocrit. Other blood chemistry components, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), alkaline phosphatase (ALK), glucose, inorganic phosphorus (P), uric acid, and triglycerides, were measured from plasma using a Centrifichem 400 centrifugal analyzer (Union Carbide).

All birds were necropsied for gross pathological changes. Samples of liver, spleen, and kidney were removed for histological examination. These tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm, and routinely stained with hemotoxylin and eosin (H&E). A small subsample was also stained with oil-red O to confirm fatty livers.

Approximately 5 g of fat and skin were sampled from each bird and analyzed for $P_4$ residues. Samples were minced with a scissors, placed in 10 ml of iso-octane in amber vials, and shaken for 30 min. They were then frozen at −20°C until analyzed. White phosphorus concentrations were measured with a Hewlett-Packard Series II model 5890 gas chromatograph equipped with a nitrogen-phosphorus detector following procedures of Addison and Ackerman (1970). Instrument blanks, spikes, and duplicate samples were included with each run.

Statistical analyses were conducted with SAS (SAS 1989). Blood component measurements were log transformed to reduce heteroscedasticity and tested among doses using repeated measures ANOVA over time (pre- and postdose). Because sample sizes were small for some dose by time combinations, we also ran repeated measures ANOVA between control birds and all dosed birds collectively. Total liver weight/initial body weight and proportion of body weight change were transformed with the arcsin transformation and compared across doses with ANOVA. Other measurements were tested using raw scores when normality was met and either ANOVA or regression. Median lethal dose and associated statistics were calculated using probit analysis (Finny 1971). Statistical significance was held at $\alpha = 0.05$.

## Results

### *Lethality*

Few pathological signs were apparent and no deaths occurred within 24 h among the swans dosed first at 4.06 mg/kg. This led us to the mistaken impression that lethal doses would be much higher than expected. Because the number of birds we had to work with was limited, we gave four of these birds a second dose in an attempt to find a lethal dose. After an additional 24 h toxic signs became apparent and included lethargy, ataxia, lack of coordination of the neck, and mild wing extension and flapping as if to maintain balance. All of these birds died within 3 days, and one of the birds given a single 4.06 mg/kg subsequently died 3 days postdose. At death two of the birds given the highest doses had a strong garlic odor and white smoke characteristic of $P_4$ emanating from the cloaca. Upon necropsy we found that all birds given two doses retained $P_4$ in their gizzard as determined by emission of smoke from the gizzard contents ("smoking gizzards") and by the garlic odor. We determined that swans were retaining $P_4$ and modified our technique by fasting the birds the night prior to dosing to facilitate passage of the $P_4$ pellets through the gizzard and allowed up to a week postdose to determine lethal exposure. Whereas in the strict sense, $LD_{50}$ tests are usually measured over a specific period of time (*e.g.*, 24 h) and most often involve toxicants suspended in solution, we realized that because of this retention of pelletized $P_4$, a more meaningful measure of toxicity in swans would be based on single or acute exposure.

Doses, numbers of birds dosed, and (number dying) were: 0 mg/kg, 6 (0); 2.98 mg/kg, 6(1); 3.64 mg/kg, 5(3); 4.06 mg/kg (single dose only), 2(1); 4.40 mg/kg, 5(3); 5.28 mg/kg, 6(5) (Table 1). Signs of toxicity seldom occurred before 8 h postdose, but were often observed upon checking the animals the next morning. They were identical to those seen in birds dosed more than once. Time from dosing to death varied from 1 to 4.5 days with no clear correspondence between dose and time to death. The $LD_{50}$ for a single dose of $P_4$ was calculated as 3.65 mg/kg (95% CI = 1.40 to 4.68 mg/kg) with the dose-response curve defineds:

$$\text{Probit} = -3.822 + 6.787 \log (\text{Dose}) \; (p = 0.033) \text{ and with}$$

$$SE_{slope} = 3.177$$

### *Gross Pathology and Histopathology*

Although no differences were found in initial body weight among dose groups, birds that were dosed experienced greater weight losses than control birds (Table 1). A test among separate dose levels did not result in a significant weight change, but when all dosed birds were combined their mean (±SD) weight loss (−8.6 + 6.9%) was greater than that for controls (−1.5 + 5.2%) ($p = 0.027$). Similarly, an ANOVA across doses for liver/body weight ratio was not significant, but when all dosed birds were combined, their liver/body weight ratio (1.8 + 0.4%) was significantly greater than that for controls (1.4 + 0.2%) ($p = 0.008$).

No significant pathology was found in the tissues of control swans. In contrast, one swan given 2.98 mg/kg had some erosion of the gizzard lining, and a second had petechiations in the pericardial fat. At 3.64 mg/kg, the livers of three male birds contained multiple foci of hemorrhages. The suggestion of fatty changes in the liver, which was determined by the presence of tan or yellow coloration, mottling, and friable textures, was found in three males and both females. Petechial hemorrhages were found in the pancreas of a fourth male, and three birds had smoking gizzards with a garlic odor. The others did not have either evidence of $P_4$ in their gizzard. At 4.06 mg/kg, single dose, only one bird was necropsied, and it appeared normal. All swans given 4.40 mg/kg demonstrated hepatic hemorrhages, hepatomegaly, frank hemorrhage in the intestines; one had a smoking gizzard, whereas the others had neither smoke or odor.

**Table 1.** Mean (±SD) body weights, liver ratios, and white phosphorus concentrations in mute swans dosed with white phosphorus

| | Dose (mg $P_4$/body weight) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2.98 | 3.64 | 4.40 | 5.28 | p |
| n | 6 | 6 | 5 | 5 | 6 | |
| Initial weight (g) | 8,570 | 8,710 | 8,160 | 8,940 | 8,200 | ns |
| SD | 470 | 1,160 | 1,020 | 1,510 | 1,070 | |
| Weight change (%) | −1.5 | −7.3 | −6.4 | −13.7 | −7.8 | 0.073[1] |
| SD | 5.2 | 4.1 | 7.8 | 9.2 | 5.2 | |
| Liver/body weight (%) | 1.4 | 1.7 | 2.0 | 1.8 | 1.8 | 0.067[2] |
| SD | 0.2 | 0.5 | 0.2 | 0.5 | 0.3 | |
| Fat $P_4$ (mg/kg) | 0 | 0.01 | 0.10 | 0.11 | 0.13 | ns[3] |
| SD | 0 | 0.03 | 0.11 | 0.12 | 0.13 | |
| Skin $P_4$ (mg/kg) | 0 | 0.01 | 0.04 | 0.02 | 0.06 | ns[4] |
| SD | 0 | 0.01 | 0.03 | 0.03 | 0.05 | |

Reported p values are for differences across doses, however, different models resulted in significant effects as follows: [1] $p = 0.027$ when all dosed birds are grouped together; [2] $p = 0.008$ when all dosed birds are grouped together; [3] in a model using dose, type of death (on dose or euthanized), and their interaction $p_{dose} = 0.897$, $p_{died} = 0.002$, $p_{interact} = 0.819$; [4] in the same type of model $p_{dose} = 0.881$, $p_{died} = 0.001$, $p_{interact} = 0.778$

The birds at 5.28 mg/kg demonstrated the greatest array of gross pathological changes including hepatic hemorrhage (three birds), pancreatic and pericardial petechiations (one bird each), erosion of gizzard lining (one bird), congested duodenum, frank hemorrhage in intestines (two birds), and smoking gizzards (four birds).

Tissues from 18 birds systematically selected across dose and sex and dying on dose were evaluated for histopathological changes. All sections of liver tissues from the nine male swans had fatty changes varying in extent from mild multifocal to severe-diffuse (one bird was stained with oil-red O for confirmation). Only four liver samples from females had fatty changes of moderately severe to severe extent. Two of the remaining five samples had moderate to severe hydropic degeneration. Twelve of the 18 liver samples had mild to moderate cholestasis, and 13 had periportal lymphocytic proliferation consistent with extramedullary hematopoiesis (EMH) ranging from mild to moderate. Four of the 18 kidney sections of these birds had mild to severe vacuolation of renal proximal tubular epithelium. However, because one of the six control birds also had mild renal tubular vacuolation, the renal changes seen in affected birds may be considered nonspecific.

Livers of 10 swans (five of each sex) that survived $P_4$ exposure were examined histologically. Seven of these had fatty changes present varying in extent and distribution from mild multifocal to moderately severe-diffuse. Seven had moderate to severe multifocal biliary hyperplasia. This finding was unique to birds that had survived exposure. Mild to moderate EMH was seen in nine of 10 liver sections, consistent with that seen in other experimental groups and controls. Two of the nine kidney sections examined in this group had either a rare focus of lymphocytic infiltrate or a very mild multifocal lymphocytic interstitial infiltrate (chronic interstitial nephritis). This renal change appeared to be incidental and not specific to $P_4$ exposure. No dose-dependent effects in the kidney sections were observed in swans that either died on dose or were euthanized at the end of the study.

### *Changes in Blood Chemistry*

Significant differences in blood chemistry of surviving birds among dose levels were found for ALT, AST, BUN, and LDH (Table 2). In addition, differences due to time (pre- versus postexposure) were found for AST, ALK, hematocrit, and LDH and in interaction terms for AST, BUN, and LDH. The clearest indicator of a response to dosing occurs with significant interaction terms, thus AST, BUN, and LDH showed the strongest responses of the blood constituents to $P_4$. Hematocrit and hemoglobin decreased across most dose levels in the postexposure samples compared to pre exposure, but did not show a differential response to dose.

A regression of post exposure LDH values (log transformed) against percent liver ($100 \times$ (liver weight/body weight)) (PCTLIV) ($p = 0.003$) and percent of body weight lost during exposure ($100 \times$ ((initial weight − final weight) ÷ initial weight)) (PCTBOD) ($p = 0.0012$) accounted for a significant amount of the variance in LDH ($\log(\text{LDH}) = 1.201 + 0.741$ LIVRAT − 0.041 PCTBOD, adjusted $R^2 = 0.645$, $p = 0.0005$) (Figure 1). Partial correlations on each independent variable showed that PCTBOD ($r = 0.752$) and PCTLIV ($r = 0.710$) were equal in accounting for variation in postexposure LDH values. For postexposure AST, $\log(\text{AST}) = 0.919 - 0.004$ PCTBOD + 0.443 LIVRAT, adjusted $R^2 = 0.595$, $p = 0.0011$. Whereas AST varied significantly with loss of body weight ($p = 0.0006$), the p value (0.051) for AST and liver/body weight was slightly higher than the accepted 0.05 (Figure 2) and the partial correlations for the two variables were 0.778 for body weight loss and 0.512 for liver/body weight.

### *Residue Levels*

Percent recovery of $P_4$ from spiked samples was 94%. White phosphorus in fat varied from below detection limits of 0.001 ppm to 0.38 ppm wet weight in a bird dosed with 5.28 mg $P_4$/kg body weight. In skin, $P_4$ varied from below detection limits to 0.16 ppm. Neither tissue demonstrated a significant difference among dose levels when birds that had died on dose and those that survived until euthanized were combined (Table 1). However, there was a significant difference ($p = 0.002$) in fat $P_4$ between birds that died on dose ($0.14 \pm 0.10$ ppm) and those that had been dosed but survived for 1 week ($0.001 \pm 0.003$ ppm). Similarly, there was a difference ($p = 0.001$) in skin

**Table 2.** Geometric means and first and third quartiles (Q1–Q3) for plasma constituents in mute swans before and after dosing with white phosphorus

| Dose (mg/kg) | Time | n | ALT (U/L) | AST (U/L) | ALK (U/L) | BUN (mg/dl) | Hemoglobin (g/dl) | Hematocrit (%) | Inorganic P (mg/dl) | LDH (U/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | Pre | 6 | 24 | 23 | 24 | 3.6 | 15.8 | 47 | 3.7 | 125 |
| | Q1–Q3 | | 24–26 | 22–28 | 23–31 | 2.8–12.6 | 14.5–26.5 | 46–49 | 3.0–4.5 | 94–163 |
| | Post | 6 | 20 | 35 | 33 | 1.3 | 15.1 | 47 | 2.0 | 162 |
| | Q1–Q3 | | 18–24 | 28–38 | 29–35 | 0.5–1.8 | 18.0–24.0 | 46–49 | 1.5–2.4 | 159–222 |
| 2.98 | Pre | 6 | 18 | 30 | 36 | 0.4 | 15.9 | 49 | 3.7 | 111 |
| | Q1–Q3 | | 17–26 | 23–36 | 25–37 | 0.2–1.0 | 17.5–26.0 | 47–53 | 3.4–4.4 | 105–140 |
| | Post | 5 | 14 | 217 | 39 | 0.9 | 14.2 | 42 | 2.6 | 606 |
| | Q1–Q3 | | 12–29 | 57–340 | 33–40 | 0.5–1.1 | 12.0–29.0 | 35–46 | 2.6–3.1 | 224–1,895 |
| 3.64 | Pre | 5 | 23 | 28 | 34 | 1.4 | 14.5 | 48 | 4.4 | 104 |
| | Q1–Q3 | | 14–23 | 23–32 | 30–38 | 0.7–4.2 | 14.0–23.0 | 48–49 | 3.9–5.9 | 90–134 |
| | Post | 2 | 24 | 107 | 43 | 2.1 | 15.2 | 40 | 6.0 | 582 |
| | Q1–Q3 | | 23–26 | 87–127 | 39–48 | 1.0–3.2 | 23–26 | 40–41 | 3.0–9.0 | 410–754 |
| 4.06 | Pre | 5 | 22 | 25 | 29 | 1.4 | 16.3 | 46 | 3.6 | 111 |
| | Q1–Q3 | | 19–25 | 22–30 | 28–35 | 1.2–9.4 | 19–25 | 45–49 | 3.4–4.4 | 85–138 |
| | Post | 2 | 50 | 477 | 29 | 3.8 | 18.0 | 46 | 2.7 | 6,834 |
| | Q1–Q3 | | 28–122 | 262–851 | 25–52 | 2.6–5.2 | 28–122 | 45–48 | 1.8–3.0 | 3,640–10,647 |
| 4.40 | Pre | 5 | 25 | 30 | 31 | 2.4 | 15.8 | 47 | 3.7 | 158 |
| | Q1–Q3 | | 25–29 | 22–39 | 29–39 | 1.4–5.5 | 15–17 | 46–50 | 3.5–4.1 | 114–209 |
| | Post | 2 | 21 | 261 | 46 | 0.6 | 14.0 | 35 | 3.6 | 1,206 |
| | Q1–Q3 | | 14–28 | 71–452 | 26–67 | 0.5–0.7 | 11–16 | 31–40 | 3.1–4.1 | 719–1,694 |
| 5.28 | Pre | 6 | 24 | 35 | 34 | 1.9 | 14.8 | 46 | 3.7 | 121 |
| | Q1–Q3 | | 22–28 | 32–40 | 29–34 | 0.5–3.0 | 13–17 | 43–48 | 3.1–4.2 | 89–155 |
| | Post | 1 | 27 | 414 | 43 | 2.3 | 14.1 | 39 | 5.1 | 1,584 |
| Probabilities[1] | | | | | | | | | | |
| Dose | | | 0.048 | 0.012 | 0.569 | 0.041 | 0.560 | 0.457 | 0.088 | 0.015 |
| Time | | | 0.903 | 0.0001 | 0.036 | 0.089 | 0.554 | 0.017 | 0.329 | 0.0001 |
| Dose × Time | | | 0.314 | 0.008 | 0.619 | 0.014 | 0.738 | 0.491 | 0.137 | 0.004 |

[1] Based on analyses of variance on transformed values for dose, time, and dose by time interaction

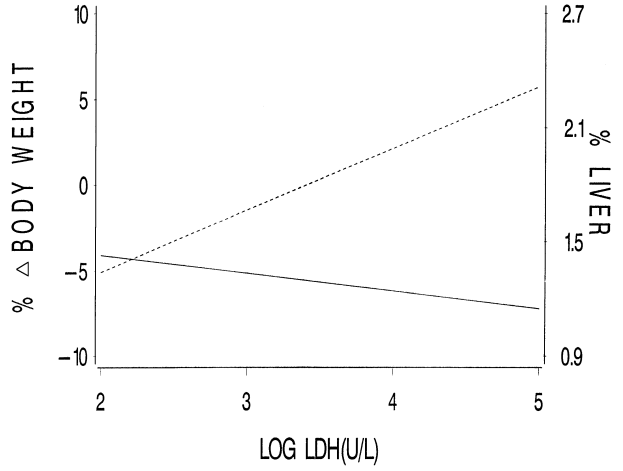


**Fig. 1.** Relationships between postexposure, log-transformed values of LDH in plasma versus percent body weight change (solid line) and percent liver weight (dotted line) for mute swans dosed with white phosphorus

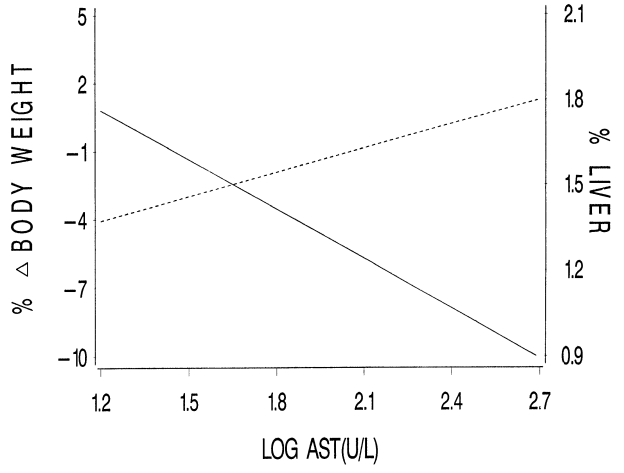


**Fig. 2.** Relationships between postexposure, log-transformed values of AST in plasma versus percent body weight change (solid line) and percent liver weight (dotted line) for mute swans dosed with white phosphorus

concentrations of $P_4$ for dead swans (0.06 ± 0.04 ppm) compared to survivors (p = 0.002 ± 0.002 ppm). White phosphorus concentrations in fat and skin tended to follow dose concentrations ($r^2$ = 0.221, p = 0.010 for fat and $r^2$ = 0.260, p = 0.005 for skin). Fat and skin concentrations of $P_4$ were correlated (r = 0.895, p = 0.0001).

## Discussion

### *Lethality*

The median lethal dose of $P_4$ calculated for mute swans acutely exposed to a single pellet was 3.65 mg/kg body weight. This is

very comparable to the 4.05 mg/kg found with pelletized $P_4$ in mallards (Sparling *et al.* 1997) and confidence intervals for the two species completely overlap. Note that all but a very few of the dosed mallards died within 24 h and we could safely limit our calculations to 24 h in this species. However, mortality within 4.5 days was allowed in the calculation of the $LD_{50}$ for swans. We believe that this allowance is justified because the difference between species could be more assuredly ascribed to the way they mechanically handled the pellets rather than to physiological differences in response. Mute swans retained the $P_4$ pellets in their gizzards far more frequently than did mallards as determined by the occurrence of smoking gizzards, garlic odor, and the frequent absence of $P_4$ in the gizzards of mallards a week postdose as determined through these signs and confirmed with gas chromatography (Sparling *et al.* 1997). In fact, because of the retention by swans even after a week, the amount of $P_4$ actually assimilated by the swans was lower than that administered and the calculated $LD_{50}$ for mute swans is higher than would be estimated if all of the $P_4$ had passed through the gizzard.

Based on the calculated $LD_{50}$, however, for an average male mute swan weighing $9.34 \pm 0.7$ kg at the start of the study, a lethal dose would equate to 36 mg of $P_4$. For females, which weighed significantly less than males ($7.73 \pm 0.51$ kg, $p < 0.0001$), 30 mg of $P_4$ would kill an average bird. Trumpeter swan male and female body weights average 12.7 and 10.3 kg, respectively whereas the smaller tundra swans average 7.3 and 6.3 kg (Bellrose 1976). If their $LD_{50}$s are similar to those of mute swans, trumpeters would require 37.6 to 46.3 mg and tundras 23 to 26.7 mg, or less. White phosphorus concentrations at Eagle River Flats are spatially very clumped, but the mean concentration among 11 highly contaminated sample sites was 0.8 mg/g soil dry weight (high 3.1 mg/g) (Racine 1994). Thus trumpeter swans would need to process 47 to 58 g of highly contaminated soil and tundra swans 29 to 33 g to ingest a lethal dose of $P_4$. Because diets of migrating tundra swans are approximately 9% soil (Beyer personal communication) a lethal amount of $P_4$ could be ingested with 522 to 644 g of food for trumpeter swans and 322 to 367 g, perhaps within two or three feeding bouts (assuming that $P_4$ ingestion is incidental with soil). If $P_4$ particles are mistaken as food items and actively selected or if all of the $P_4$ is passed into the intestines, the probability of ingesting a lethal amount could be greatly increased. Of course, these calculations are crude estimates, but could be refined through additional research on feeding habits of swans, depending on the needs of risk assessment.

Mute swans took from 1 to 4.5 days to die from $P_4$ intoxication, which is substantially longer than the few hours observed for the vast majority of mallards (Sparling *et al.* 1997). We attribute the difference in part to the type of grit used by the two species in our studies. Mallards had pebble-sized (approximately 3–7 mm) grit in their gizzards, whereas the gizzards of the swans were filled with coarse sand-sized ($<$0.5–2 mm) particles. The grit within the swans was similar to the size of the $P_4$ pellets, and it is likely that the swans retained the pellets as grit. Passage and assimilation of the $P_4$ pellets, therefore, was spread over a substantially longer period of time than for mallards. Field studies would have to verify if free-ranging swans retained pellets in a similar manner, but tundra and mute swans were frequently found with grit $< 1.18$ mm in diameter in a study of lead-contaminated soil ingestion in Idaho (Scott Hansen, personal communication). If swans preferentially seek small grit particles at Eagle River Flats as well, many birds could ingest a lethal dose of $P_4$ during fall migration and die elsewhere, thus greatly increasing risk.

### *Gross Pathology and Histopathology*

Swans dosed with $P_4$ lost from 2–15% of their body weight within a week whereas control birds demonstrated no net loss of weight. Weight loss is a frequent sign of $P_4$ toxicity (Sparling *et al.* 1997; Sparling and Federoff 1997) and is probably due to malaise and inappetance during the period of actual exposure in that birds quickly regained body weight after dosing in repeated dose experiments (Sparling, unpublished data). Clearly in such situations, the longer birds are exposed to toxins that reduce appetite, the greater the degree of weight loss. Because swans were acutely dosed their weight loss was relatively small.

Hepatic foci, fatty liver changes, and hepatomegaly, as expressed by increased liver/body weight ratios, are also common and are directly related to the retention of fat by the liver (Lombardi and Recknagel 1962; Goshal *et al.* 1969; Sparling *et al.* 1997). The gross lesions in the liver, pancreas, and heart are consistent with those found in mallards (Coburn *et al.* 1950; Sparling *et al.* 1997) and kestrels (Sparling and Federoff 1997). Fatty liver changes, which are extremely common in $P_4$ toxicity in birds, are due to inhibition of lipoprotein production which facilitates lipid removal from the liver (Lombardi and Recknagel 1962). The lesions and foci are probably due to localized responses to $P_4$ or its degraded products. Swan sample sizes were limited, but the frequency of lesions appeared to be similar to or slightly above that of mallards at comparable dose levels and seemed to generally increase in severity as dose level increased. There appeared to be greater sloughing of the lining of the gizzard in swans than in mallards, and this may have accounted for a brownish fluid consistent with digested blood seen in the lumen of the intestines.

We believe that smoking gizzards and garlic odor are sensitive, qualitative tests for the presence of $P_4$ in the gizzard. Even when smoke is not emitted from a freshly dissected gizzard, there is often a distinctive odor in freshly dead birds. These signs were observed two or three times among scores of mallards, as late as 6 days postdose (Sparling *et al.* 1997, unpublished data). However, the frequency of smoke or odor in gizzards of swans given 3.64 mg/kg $P_4$ or more was appreciably greater than that seen in mallards and supports our premise that swans were retaining the pellets.

Three histological trends were apparent in the mute swans. First, a gender predisposition was apparent in that males appeared to be more affected than females in the type and degree of pathologic changes present in the liver. Most significant was the presence and extent of fatty change seen in livers of male swans that died on dose. Sparling *et al.* (1997, 1998) found some differences in dose response curves and in blood chemistry between mallard males and females given $P_4$. In this study, a more striking trend was a temporal difference between those birds that died on dose versus those that were euthanized at the termination of the study. Only those that survived dosing developed hepatic biliary hyperplasia. This change appears to reflect injury or a toxic insult to the hepatic biliary parenchyma such that the bile ducts were induced to proliferate. Enlarged

gallbladders may be related to inappetance and supported the presence of cholestasis. Birds that died acutely would not have sufficient time to produce the tissue response. The third notable trend was an absence of specific renal effects. Mallards demonstrated hyaline droplet nephrosis in the renal convoluted tubules (Sparling *et al.* 1997) and Coburn *et al.* (1950) observed renal damage in black ducks and mallards, but this was not seen in swans.

### *Changes in Blood Chemistry*

Several plasma constituents including AST, ALT, BUN, and LDH increased with $P_4$ exposure. Elevations of each of these constituents is consistent with findings in mallards (Sparling *et al.* 1998). Intoxicated American kestrels (*Falco sparverius*) also demonstrated elevated LDH, glucose, and ALT (Sparling and Federoff 1997). We did not see significant changes in uric acid, glucose, hemoglobin, hematocrit, or inorganic P in swans. In mallards, however, both hematocrit and hemoglobin declined significantly with $P_4$ intoxication whereas uric acid and inorganic P increased, but these increases were only loosely associated with $P_4$ exposure.

LDH is found in several tissues, including muscle, red blood cells, and liver (Hochleitner 1994). In mallards we suggested that elevated LDH activity in plasma was more related to liver damage than to hemolysis (Sparling *et al.* 1998). In that study, birds were either euthanized too quickly to observe substantial changes in body weight or were allowed to continue laying eggs weeks after the last dose and were able to regain their body weights. In this study, however, the absence of notable hemolysis would support the notion that something other than destruction of red blood cells was the primary source of the elevated LDH. The significant relationships between postexposure LDH levels and both the decline in body weight and the increase in the liver weight relative to initial body weight suggested that increased LDH came from both liver and muscle damage. Elevated LDH values may be mutually dependent on damage to both tissues as the two factors had nearly equal partial correlations with LDH. AST is also found in kidney as well as liver and muscle in waterfowl (Franson 1982). The relationship between postexposure AST and liver/body weight ratio was only marginally significant whereas percent change in body weight accounted for a significant amount of variance in AST. Therefore, inappetance and the resulting loss of mass may have been the principal causes for elevated AST. Elevated ALT and BUN could be indicative of mild kidney damage, which could not be identified during histological examination.

In mallards we observed an acute phase of $P_4$ toxicity characterized by intense convulsions and a subacute phase of liver and renal damage (Sparling *et al.* 1997). The apparent slow release of $P_4$ in this study may have circumvented the acute form of toxicity in swans, thus making the subacute damage to liver more apparent. Sparling *et al.* (1998) proposed a biomarker of $P_4$ exposure for mallards consisting of the hemoglobin concentration divided by LDH values. This biomarker would not be applicable to swans based on this study but elevated LDH and AST are strongly related to $P_4$ and may indicate exposure.

### *Residue Values*

White phosphorus concentrations in fat and skin were related to dose level and to mode of death. Fat levels in birds dying on dose were three to four times higher than those in skin, and both were much higher than the respective tissues in birds that survived until euthanasia. These findings are consistent with data from fish (Dyer *et al.* 1972), American kestrels (Nam *et al.* 1994) and mallards (Sparling *et al.* 1997) which showed that the lipophilic $P_4$ tends to concentrate in adipose tissue more than other tissues and that it is metabolized or excreted within a few days postexposure. The relationships between dose level and residue concentration in fat or skin, although statistically significant, had small regression coefficients, which indicates that other factors besides dose play a role in deposition of $P_4$. Until more information on the fate of $P_4$ is known, any explanations of these low coefficients would be speculative.

## Conclusions

Although the calculated $LD_{50}$ for acutely dosed mute swans was approximately the same as the 24-h $LD_{50}$ for mallards, the sensitivity of mute swans to $P_4$ may be lower due to the retention of pellets in the gizzards. The longer duration between dosing and death in swans compared to mallards demonstrates that unexpected factors such as grit size may have important effects on hazard assessment, especially if swans leave Eagle River Flats or other contaminated sites after ingesting a lethal dose of $P_4$. Although most acute toxicity studies that employ oral gavage dissolve the target substance in a liquid carrier, our study suggests that the relation between pellet and grit size may be an important variable when dealing with particulate contaminants such as metals or some pesticide formulations. Whereas most of the mallards tested in earlier studies died from acute toxicity, most or all of the swans in this study appear to have died from subacute liver dysfunction. Changes in AST, ALT, and LDH were consistent between both mallards and mute swans, but swans appeared to be less susceptible to hemolysis and reduction of hemoglobin. Thus a plasma biomarker developed for mallards would not be appropriate for swans, but elevated LDH and AST may be good but not specific indicators of $P_4$ exposure at contaminated sites for swans.

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