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Determination of White Phosphorus Residues in Ducks: An Atomic Emission Detection/ Compound-Independent Calibration-Based Method of Generating Residue Data for Risk Assessment and Environmental Monitoring

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Analysis of phosphorus concentrations in the gizzards of ducks harvested from munitions sites is necessary to ascertain if acute phosphorus toxicity was the cause of death and to estimate potential secondary hazards to predators and scavengers, such as eagles that readily consume the dead ducks. Gas chromatography/atomic emission detection analysis permitted compound-independent quantification of white phosphorus standards following analysis of the stable phosphorus-containing compound triethyl phosphate. The white phosphorus standards were then used to quantify white phosphorus residues in duck gizzard extracts by gas chromatography/flame photometric detection analysis. For gizzards containing less than 0.01 μg of phosphorus, quantification was based on a three-point calibration curve. For gizzards containing 0.01 μg or more of white phosphorus, single-point calibration was used. Mean recoveries for phosphorus-fortified (0.03−3000 μg) gizzards ranged from 73 to 91%. The method limit of detection was 0.013 μg of phosphorus. This method was successfully applied to the quantification of white phosphorus in ducks collected from Eagle River Flats, AK. Potential applications to risk assessment and environmental monitoring are also discussed.

Introduction

During the past 15 years, thousands of dead migratory ducks have been observed in Eagle River Flats (ERF), AK, an estuarine salt marsh that has been used as a U.S. Army artillery impact range (*1*). A study by the U.S. Army Corps of Engineers implicated white phosphorus as the cause of death as high phosphorus residues were noted in the gizzard, intestinal tract, and fat of dead birds collected from this region. High levels of phosphorus were also detected in sediment collected from ERF. Munitions are the likely source of the white phosphorus. During the last 40 years, more than 100 thousand rounds of ordnance have been fired into the artillery range. During 1987−1990, an estimated 975−1630 kg of white phosphorus was fired there. It appears that pieces of white phosphorus, which would normally volatilize on exposure to air and light, were instead immersed in water. As phosphorus has a specific gravity of 1.8, the phosphorus subsequently became lodged in sediment (*2*). White phosphorus-contaminated sediment poses a primary hazard to sediment-feeding organisms such as ducks (*3*). Therefore, methods were developed for the analysis of white phosphorus residues in duck tissues, water, and sediments from ERF (*4*, *5*). These methods were essential for confirming that phosphorus toxicity was the cause of death for the birds collected from ERF.

The National Wildlife Research Center is developing techniques to minimize white phosphorus exposure and subsequent mortality of migratory waterfowl at ERF. To evaluate the effectiveness of this research, assessment of phosphorus exposure in ducks collected from ERF was necessary. To accomplish this, we evaluated the analytical method of Walsh et al. (*4*), which has been used extensively to provide phosphorus residue data for a variety of matrixes collected from ERF and has permitted researchers to identify phosphorus poisoning as the cause of death for the migratory ducks collected there.

To accurately determine residues, it is imperative to formulate a white phosphorus standard solution containing an accurately determined concentration of white phosphorus (*6*). The chromatographic response of this white phosphorus standard is used to quantify phosphorus residues in extracts of biological samples. Accurately weighing pure technical material is nearly impossible as it spontaneously combusts on contact with oxygen at room temperature. White phosphorus also polymerizes upon exposure to light (*2*). Traditionally, standards are weighed using a balance in a nitrogen-purged glovebox. Small pieces of white phosphorus are placed in a preweighed flask containing solvent. The weight of white phosphorus added is determined by weight difference (*4*, *5*, *7*). We found this approach to be cumbersome and suspect with respect to accuracy as it is difficult to determine the precise weight of the standard material. In addition to the difficulties with the technical material itself, the solvents used are volatile, which can contribute to the weight difference estimation of white phosphorus. Further inaccuracy is potentially contributed by impurities including oxidation products that exist within the solid material and by temperature-associated changes in density.

Using this approach, we found the preparation of precise analytical standards to be extremely difficult and impossible to verify. As residues are quantified based on relative chromatographic response for a white phosphorus standard, the preparation of a standard solution is critical to the generation of valid residue data required for risk assessment. To improve the level of accuracy, we developed an analytical method that uses atomic emission detection (AED) to accurately quantify white phosphorus in standard solutions (Figure 1). Gas chromatography (GC)/AED response factors and linearity for phosphorus were determined from the analysis of triethyl phosphate solutions. Triethyl phosphate was selected for two reasons: (i) certified solutions are commercially available and (ii) triethyl phosphate is much more stable than white phosphorus, which facilitates the preparation of precise standard solutions. With AED, 1 mol

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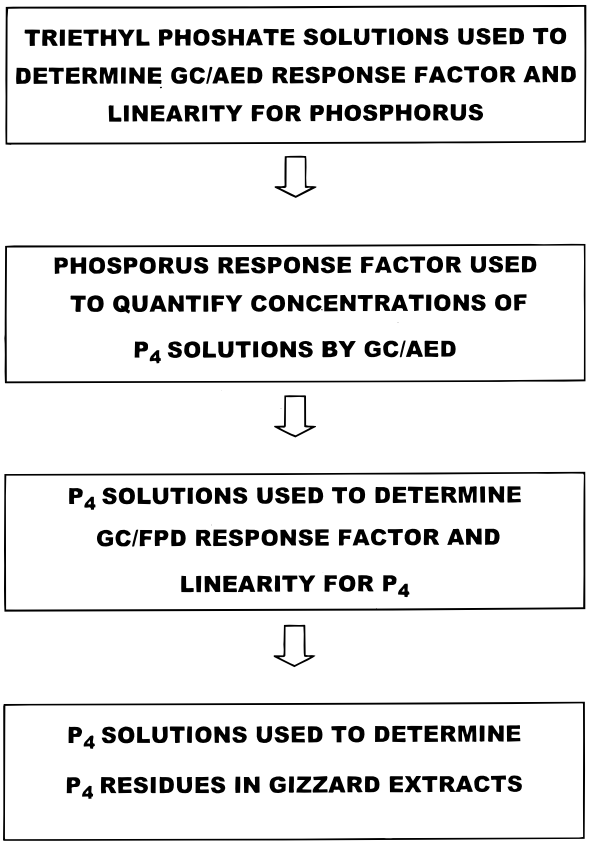

FIGURE 1. Overview of the analytical approach for the quantification of P_4 residues in duck gizzards.

of triethyl phosphate produces the same detection signal as 1 mol of elemental phosphorus or 0.25 mol of P_4. This phosphorus response factor was then used to determine the concentration of phosphorus in the P_4 standard solutions which were then analyzed by GC/AED. As sensitivity and linear range are superior for the flame photometric detector (FPD), sample analyses subsequently were conducted by GC/FPD. We also compared our method to the weight difference approach and discuss potential implications with respect to quantifying phosphorus residues in biological samples.

Materials and Methods

Standard Preparation and Quantification of White Phosphorus Concentrations. Due to the unstable nature of white phosphorus, a compound-independent calibration (CIC) approach was developed to quantify phosphorus in the concentrated white phosphorus standard solution. This was accomplished by comparing the AED response for phosphorus in a certified triethyl phosphate solution with the phosphorus response of a dilution of the concentrated white phosphorus standard solution (white phosphorus verification standard solution). This independently determined phosphorus concentration for the concentrated solution was used to calculate the phosphorus concentration of subsequent dilutions (phosphorus working standards and calibration standards).

Concentrated White Phosphorus Standard Solution. Due to the reactivity of white phosphorus with oxygen, extra precautions in the preparation of the concentrated standard solution were warranted. A glovebox (VWR, Denver, CO) was placed in a fume hood. A plastic tray, tweezers, scalpel, beaker of water, paper towels, 10-mL crimp cap vial, and a bottle containing the white phosphorus technical material (Aldrich Chemical, Milwaukee, WI) immersed in water were then placed inside the glovebox. Air was purged from the box with a gentle flow of nitrogen, and the glovebox was then sealed. The technical material was dried with a paper towel following removal from the water with tweezers. Several small pieces of white phosphorus were cut from the block of technical material, the oxidized layer was removed, and the white phosphorus was placed in the crimp top vial. The crimp top vial was filled with water. The remainder of the technical material was returned to the water-filled bottle and subsequently sealed. The glovebox was opened, and the sealed bottle containing the white phosphorus was removed and placed in the appropriate chemical storage area.

A 60 °C water bath was prepared by placing a 150-mL beaker that was about half-filled with water on top of a hot plate (Preston Instruments, Niles, IL). The bottle containing the white phosphorus (under water) was placed in the water bath. A 25-μL Eppendorf pipettor (Brinkman Instruments, Westbury, WY) and a preweighed 100-mL volumetric flask containing approximately 50 mL of isooctane (HPLC grade, Burdock & Jackson, Muskegon, MI) were placed in the glovebox. The beaker containing the bottle of white phosphorus was placed in the glovebox, which was then nitrogen-purged and sealed. After the white phosphorus melted, the pipetor was used to transfer 25 μL of the molten white phosphorus along with the pipet tip into the volumetric flask. At this point, the flask was reweighed, and the combined weight of the white phosphorus and the pipet tip was determined by subtraction. Isooctane was added to about 90 mL. The flask was then placed in a sonicator bath (Sonicor Inc., Copiagur, NY) for 15 min followed by a room temperature water bath for another 15 min. The pipet tip was then removed, air-dried, and weighed. The mass of white phosphorus delivered was determined by subtracting the pipet tip mass from the combined weight of the white phosphorus and pipet tip. Isooctane was then added to volume to produce a concentrated stock solution of about 400 μg/mL. The crimp top vial was resealed and permitted to cool to ambient temperature. The white phosphorus solution was then placed in a 100-mL crimp top vial, sealed, and stored at 4 °C for 3 weeks to permit precipitation of any suspended particles. The supernatant was filtered through a Whatman No. 1 filter paper into a new crimp camp vial, which was capped and stored at 4 °C.

White Phosphorus Verification Standard Solution. The white phosphorus concentrated standard solution was removed from the refrigerator and brought to room temperature, and 500 μL was transferred to a 10-mL volumetric flask. Isooctane was added to volume to produce a verification standard solution of approximately 20 μg/mL. The concentration of this solution was confirmed by GC/AED analysis.

Triethyl Phosphate Certified Solution. A certified 100 ppm triethyl phosphate solution was obtained from Chem Services (West Chester, PA).

Triethyl Phosphate Concentrated Standard Solution. Triethyl phosphate technical material (ca. 10.000 mg) (Aldrich Chemical, Milwaukee, WI) was accurately weighed, transferred to a 10-mL volumetric flask, diluted to volume with methanol (HPLC grade, Fisher, Denver, CO), and mixed. The concentration of the final solution was approximately 1000 μg/mL.

Triethyl Phosphate Working Solution. An aliquot (1.00 mL) of the triethyl phosphate concentrated standard solution was transferred to a 10-mL volumetric flask, diluted to volume with methanol, and mixed to yield a solution of approximately 100 μg/mL. This solution was analyzed by GC/AED.

Triethyl Phosphate Calibration Curve Solutions. The triethyl phosphate concentrated standard solution was diluted to yield a series of calibration curve standards. Aliquots of 250 μL, 500 μL, 1 mL, 1.5 mL, and 2 mL were transferred to separate 10-mL volumetric flasks and brought to volume

with methanol to yield standard solutions of approximately 25, 50, 100, 150, and 200 μg/mL, respectively.

GC/AED. A Hewlett-Packard (Palo Alto, CA) model 5890 gas chromatograph equipped with a HP G2350A AED, Hewlett-Packard Chemstation software, and a J&W (Folsom, CA) DB-XLB, 30 m × 0.25 mm, 0.25 μm film thickness capillary column was used for the analysis of white phosphorus and triethyl phosphate solutions. Electronic pressure control maintained the helium carrier gas flow rate at 0.7 mL/min. Injection (splitless) volume and temperature were 1 μL and 250 °C, respectively. For the analysis of phosphorus, the oven temperature program was as follows: 40 °C for 7 min, ramp at 30 °C/min to 120 °C, hold at 120 °C for 6.33 min, ramp at 70 °C/min to 40 °C, hold at 40 °C for 0.1 min. AED parameters were as follows: transfer line temperature, 250 °C; cavity temperature 250 °C; hydrogen pressure, 9.3 psi; oxygen pressure, 24.8 psi; auxiliary pressure, 34 psi; cavity pressure, 1.5 psi; solvent vent on at 0.2. min; split vent off at 12 min; element (wavelength) monitored, carbon (193 nm), phosphorus (178 nm), phosphorus (186 nm). For analysis of triethyl phosphate, the oven temperature program was as follows: 60 °C for 7 min, ramp at 15 °C/min to 190 °C, hold at 190 °C for 0.33 min, ramp at 70 °C/min to 60 °C, hold at 60 °C for 0.5 min. Other parameters were identical except for the following exceptions: solvent vent on at 0.01 min; split vent off at 7.5 min.

Calculation of White Phosphorus Concentrated Solution Concentration. Prior to the quantification of any standards by GC/AED, system stability was assessed by multiple injections of the 100 μg/mL triethyl phosphate working standard. After achieving a relative standard deviation of less than 2% for five consecutive injections, triplicate 1-μL aliquots of the triethyl phosphate certified standard solution were analyzed. The phosphorus concentration of the triethyl phosphate certified solution (Chem Service, West Chester, PA) was calculated as:

$$\text{P concentration } (\mu\text{g/mL}) = \text{triethyl phosphate concentration } (\mu\text{g/mL}) \times \frac{\text{MW phosphorus}}{\text{MW triethyl phosphate}}$$

The AED response from the 186-nm phosphorus emission line was used to quantify the phosphorus concentrations in the triethyl phosphate working standards and the triethyl phosphate calibration standards based on the response for the triethyl phosphate certified solution. Linearity was assessed by analysis of duplicate 1-μL injections of the triethyl phosphate calibration curve standards. Linear regression analysis was performed on the log AED response vs log phosphorus concentration (*8*). The antilog of the AED response from the analysis of white phosphorus isooctane verification standard was then used to determine the phosphorus concentration of this solution. The concentration of the white phosphorus concentrated standard solution was then calculated by correcting for dilution:

$$\mu\text{g of P/mL of white phosphorus concentrated standard} = \mu\text{g of P/mL of verification standard} \times \frac{\text{dilution volume (mL)}}{\text{aliquot volume (mL)}}$$

Method Validation and Sample Analysis. As sensitivity and linear range were superior for the FPD as compared to the AED, sample analysis was conducted via GC/FPD. Fortification solutions for the preparation of validation and quality control samples were prepared in tricaprylin as the solubility of white phosphorus in tricaprylin is significantly greater than for isooctane. To confirm the concentration of phosphorus in the concentrated tricaprylin fortification solution, dilution in isooctane was required.

TABLE 1. Preparation of White Phosphorus Calibration Standards[a]

concn (μg/mL)	aliquot vol (μL)	source (μg/mL)
10	250	concn stock solutionn
4	100	concn stock solution
1	500	verification std solution
0.5	250	verification std solution
0.1	100	10
0.05	50	10
0.01	100	1
0.003	30	1
0.0015	150	0.1

[a] final volume = 10 mL; diluent = isooctane.

Concentrated Sample Fortification Solution. The procedure for the preparation of the white phosphorus concentrated standard solution was also used to prepare sample fortification solutions by transferring about 30 μL of molten white phosphorus technical material into 20 mL of tricaprylin in a 25-mL volumetric flask. The solution was brought to volume with tricaprylin (Sigma, St. Louis, MO) to yield a solution of approximately 2000 μg/mL.

White Phosphorus Sample Fortification Verification Standard Solution. An aliquot (100 μL) of the white phosphorus sample fortification solution was transferred to a 10-mL volumetric flask. Isooctane was added to volume to give a concentration of approximately 20 μg/mL. GC/AED analysis of this solution was used to determine the concentration of the concentrated sample fortification solution.

White Phosphorus Fortification Solutions. The intermediate (5 μg/mL) and low level (0.3 μg/mL) fortification solutions were prepared by diluting 25 μL of the concentrated sample fortification or 10 μL of the intermediate fortification solution, respectively, with tricaprylin in 10-mL volumetric flasks. The concentrated sample fortification solution was used for high level fortification. All three solutions were used to fortify gizzard contents for method validation. The intermediate solution was used to prepare QC standards for sample analysis.

White Phosphorus Calibration Curve Standards. To assess FPD response linearity for white phosphorus, a series of white phosphorus solutions were prepared as outlined in Table 1. Aliquots were diluted to 10 mL final volume with isooctane. Linear regression analysis of peak response vs concentration was used to determine the linearity of detector response. Linear regression analysis of the log peak response vs log concentration was used to assess the proportionality of the detector response.

Linearity Determination. The calibration curve standards were prepared in duplicate and analyzed by GC/FPD. Mean responses for each standard vs concentration were analyzed by linear regression (*8*).

White Phosphorus Working Standard Solution. The white phosphorus concentrated standard solution was removed from the refrigerator and brought to room temperature, and 100 μL was transferred to a 10-mL volumetric flask. Isooctane was added to volume to produce a working standard solution of approximately 4 μg/mL. The exact concentration of this solution was based on the GC/AED analysis of the white phosphorus concentrated standard solution. This working standard was analyzed by GC/FPD and used to calculate the phosphorus concentrations of the gizzard content extracts with responses greater than or equal to those of the 0.01 μg standard. A three-point calibration curve was used to quantify extracts containing lower concentrations of phosphorus.

Gizzard Preparation. To minimize the likelihood of contamination, samples were prepared in a fume hood that had not been exposed to concentrated white phosphorus solutions. Individually bagged, frozen gizzards were removed from the freezer and placed in an ambient temperature circulating water bath for approximately 1 h to thaw. Gizzards were then removed from the plastic bag, placed in a plastic weigh boat, and split in half with a scalpel by making an incision around the perimeter of the gizzard.

Fortification of Control Gizzards. For method validation, control gizzards contents were fortified with 0.03 or 0.5 μg of phosphorus by adding 100 μL of the low or intermediate level sample fortification solution, respectively. Control gizzards were also fortified with 3000 μg of phosphorus by adding 1.5 mL of the concentrated sample fortification solution to the contents of each gizzard. A fortification check standard was also prepared by fortification of a 50-mL test tube containing 10 mL of isooctane and approximately 5 g of glass beads. Helium-sparged deionized water (30 mL) was added to each tube, which was then processed and analyzed with the samples. To ascertain the probability of matrix interference and to calculate a method limit of detection (MLOD), five unfortified control gizzards were also analyzed. Control gizzards were obtained from nusiance ducks removed from the Denver metropolitan area. These ducks had not been exposed to white phosphorus. For the preparation of daily QC samples, three control gizzards were fortified with 0.5 μg of phosphorus. One unfortified control gizzard was also analyzed on each analysis day.

Sample Preparation. A spatula and glass funnel were used to transfer the gizzard contents into a preweighed 50-mL glass tube containing exactly 10.00 mL of isooctane. The tube was reweighed to determine the mass of the gizzard contents. The gizzard and weigh boat were rinsed with six 5-mL aliquots of helium-sparged deionized water into the test tube containing the isooctane. The test tubes were capped.

Sample Extraction. The samples were shaken horizontally on a mechanical shaker (Eberbach, Ann Arbor, MI) at low speed for 18 h. The shaker was covered to prevent exposure of the samples to light. The tubes were centrifuged (Fisher Centrific, Pittsburgh, PA) at (1100*g*) for 5 min. Approximately 1−1.5 mL of the supernatant was transferred to an amber GC vial and capped. The remaining supernatant was transferred to a 10-mL glass test tube, capped, and stored at approximately 4 °C. Phosphorus content of the extracts was determined by GC/FPD analysis.

Gas Chromatography/Flame Photometric Detection. A Hewlett-Packard model 5890 gas chromatograph equipped with a HP 19256A FPD, Hewlett-Packard Chemstation Software, and a J&W DB-1, 15 m × 0.32 mm, 1.0 μm film thickness capillary column was used for the analysis of white phosphorus standard and sample extract solutions. Electronic pressure control maintained the helium carrier gas flow rate at 0.3 mL/min. Injection (splitless) volume and temperature were 1 μL and 250 °C, respectively. The oven temperature program was as follows: 40 °C for 4 min, ramp at 60 °C/min to 150 °C, hold at 150 °C for 0.3 min, ramp at 70 °C/min to 40 °C, hold at 40 °C for 1.5 min. FPD parameters were as follows: detector temperature, 200 °C; auxiliary (nitrogen) flow rate, 120 mL/min; oxygen flow rate, 26 mL/min; hydrogen flow rate, 75 mL/min.

Calculation of White Phosphorus Concentration. The quantity of phosphorus in the gizzard contents was determined from the analysis of the gizzard content extracts and the white phosphorus working standard solution:

$$\text{phosphorus mass} = (R_s/R_{std})C_{std} \times \text{sample volume}$$

where phosphorus mass = μg of white phosphorus in gizzard contents, R_s = peak response from sample solution, R_{std} = peak response from working standard, C_{std} = phosphorus concentration in working standard (approximately 4 μg/mL), and sample volume = volume (mL) of isooctane extraction solvent plus volume of fortification solutions.

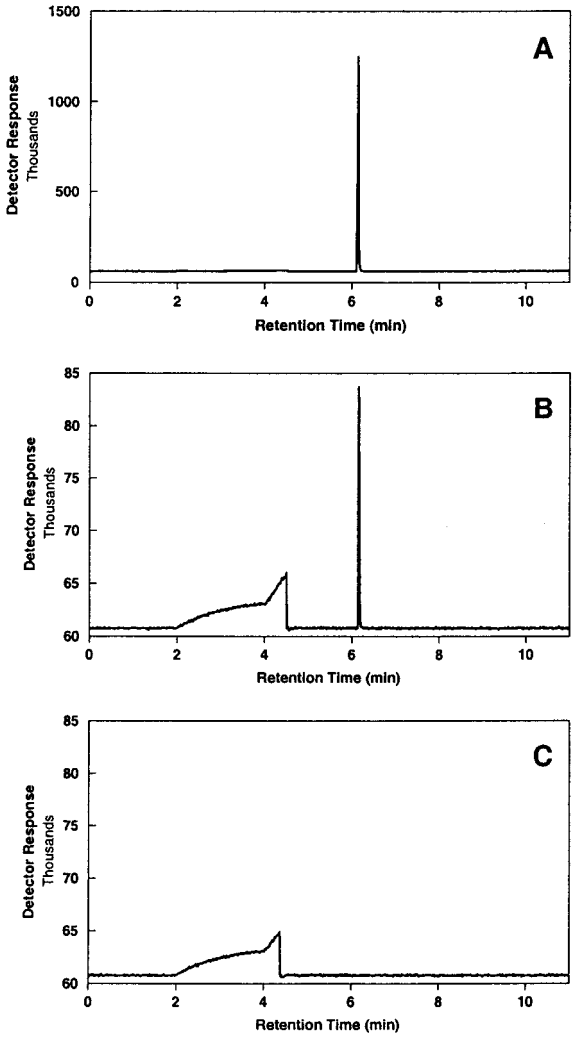

FIGURE 2. GC/FPD chromatograms of (a) standard solution, (b) fortified gizzard extract, and (c) control duck gizzard extract. The retention time of phosphorus was approximately 6.1 min.

Results and Discussion

AED. AED is a powerful technique that employs a plasma source to heat samples to extremely high temperatures. These temperatures are sufficiently high to break all chemical bonds and to reduce compounds to their elemental components. In addition, the energy (15−24 eV) is sufficient to promote these atoms to an excited energy state in which they emit photons at discrete wavelengths. By comparing the magnitude of responses for individual elements from a standard containing the same elements as the unknown compound, one can obtain quantitative and qualitative data for the compound. This technique permits compound-independent calibration (CIC), the quantification of compounds for which a true standard does not exist (*9*). In the case of white phosphorus, identification was not necessary as the structure and elemental formula were already known. CIC permitted the quantification of the volatile and difficult-to-handle white phosphorus against a more inert and easily handled phosphorus-containing compound, triethyl phosphate.

Chromatography. As illustrated in Figure 2, the GC/FPD retention time of white phosphorus in both standard solution and fortified gizzard extract was approximately 6.1 min. The

TABLE 2. Percent Recoveries from White Phosphorus Fortified Gizzards

	target fortification levels		
replicate	0.03 μg	0.5 μg	3000 μg
1	68.5[a]	85.3	86.6
2	82.7	85.3	87.1
3	63.3	84.0	82.3
4	66.7	80.5	101
5	84.7	84.2	98.2
mean	73.(2)	84.(0)	91.(0)
SD	9.8	2.0	8.1

[a] Percent recovery.

chromatogram of the control duck gizzard extract indicated a lack of any chromatographic interference at the retention time of white phosphorus.

Linearity. For phosphorus concentrations from 0.01 to 10 μg/mL, the linear regression analysis indicated a linear response via a r^2 of 0.999 and an intercept that was not significantly different from 0 ($P = 0.2129$) for peak response (y) vs concentration (x). The resulting linear regression equation was $y = 520\,600x + 18442$. The plot of log peak response vs log concentration resulted in a r^2 of 0.999 and a slope of 1.00, which is indicative of a proportional response. Thus, for this range of concentrations, these data indicate that the detector response was both linear and proportional. For phosphorus concentrations from 0.0015 to 0.01 μg/mL, the plot of peak response vs concentration resulted in a r^2 of 0.989 and a regression equation of $y = 550\,006x + 527$ with an intercept that was significantly different than 0 ($P = 0.0005$). For the same log−log plot, the r^2 was 0.984 and the slope was 0.811. These results indicated that detector response was linear but was not proportional for this range of concentrations.

On the basis of these results, 0.0015, 0.003, and 0.01 μg/mL calibration standards were analyzed with every batch of samples. Phosphorus concentration for sample responses less than the 0.01 μg/mL standard were calculated from the 0.0015−0.01 μg/mL three-point standard curve. Phosphorus concentration for sample responses equal to or greater than the 0.01 μg/mL standard were calculated from the single-point response of the 4.0 μg/mL standard. Sample extracts with responses greater than the 10 μg/mL standard were diluted to produce a response calculated to fall within the range of the 0.01 and 10 μg standards and reanalyzed.

MLOD. No chromatographic interferences were observed in the chromatograms of the extracts from the untreated gizzards (controls). The MLOD was calculated as the quantity of white phosphorus expected to generate a response equal to 3 times the mean baseline noise in the chromatogram of the control extracts at the retention time of white phosphorus. This calculation was based on the mean response from the gizzards fortified with 0.03 μg of white phosphorus. These calculations resulted in a MLOD of 0.013 μg/gizzard. Daily MLODs were similarly calculated from the QC samples analyzed with each batch of samples.

Recoveries. The recovery data presented in Table 2 indicate that white phosphorus recoveries increased with increasing fortification level. At the low fortification level of 0.03 μg, the mean recovery was 73%. At the highest level, 3000 μg, the mean recovery was 91%. Standard deviations ranged from 2% at the 0.5-μg fortification level to 9.8% at the 0.03-μg fortification level.

Residues. This method was then used to analyze the white phosphorus content of gizzards from 43 ducks collected from a U.S. Army impact artillery range in ERF, AK. Mean recovery of the 0.05-μg QC samples was 78.6%. No matrix interference was observed in the control QC samples. Up to 27 samples were analyzed on a single day. Phosphorus content of the actual samples ranged from <MLOD to 6.15 mg. Nearly all of the gizzards analyzed were recovered intact from birds equipped with radiotransmitters. In most cases, the collected birds were dead for less than 24 h and frozen within hours of collection. It is unlikely that the P_4 in the gizzards decomposed during this time period. The residue levels determined during the laboratory analysis of these samples are likely accurate indicators of field residues.

TABLE 3. Standard Concentrations Calculated by Weight Difference vs Compound-Independent Calibration

standard	wt difference (μg/mL)	compound-independent calibration (μg/mL)	%
working std A	696	916	76.0
working std B	600	492	122
QC std A	795	1000	79.5
QC std B	478	370	129
fortification std A	8240	2670	309
fortification std B	1259	1010	125

Compound-Independent Calibration vs Weight Difference. This method used the CIC technique to quantify the phosphorus concentration of the standard solutions. We also calculated the concentrations of phosphorus by the weight difference approach. The concentrations calculated by both approaches were significantly different (Table 3). Linear regression analyses of FPD response versus concentration were calculated for both methods of concentration determination. For CIC estimated concentrations, r^2 was 0.99. For the weight difference estimated concentrations, r^2 was 0.57. As the FPD detector is linear over the range of concentrations assayed, these results indicate that the precision of the CIC approach is superior to the weight difference approach for estimating phosphorus concentrations. On average, the weight difference approach resulted in estimated concentrations that were 156% of the CIC estimates (Table 3). The greatest difference observed was more than 300%. We feel that residue data calculated from the CIC are likely more accurate as the CIC estimate is less effected by impurities in the phosphorus and/or solvent evaporation. In four out of the six standards prepared by this method, the weight difference based calculation resulted in a higher estimated concentration for the standard. The net result would be an overestimation of phosphorus residues and subsequent erroneous risk assessments based on these residue data.

Currently our laboratory is investigating a safer procedure for the preparation of the white phosphorus standards based on suggestions from the U.S. Army Cold Regions Research and Engineering Laboratory. For this procedure, the bottle containing the white phosphorus was submerged in a 60° C water bath. After the white phosphorus melted, a 25-μL positive displacement pipet (Labsystems, Helsinki, Finland) was used to transfer molten white phosphorus to a test tube submerged in the same water bath. The entire transfer procedure was conducted under water to ensure that the white phosphorus was not exposed to air. The test tube was then capped and refrigerated overnight. The following day, the refrigerated white phosphorus pellet, a pair of tweezers, a 100-mL beaker, and a preweighed 100-mL volumetric flask containing approximately 50 mL of toluene (HPLC grade, Burdock & Jackson, Muskegon, MI) were placed in a glovebox. The box was then nitrogen-purged and sealed. The water was poured out of the test tube into the beaker, and using the tweezers, the solid pellet of white phosphorus was removed from the tube and dried on a paper towel before being placed in the volumetric flask containing toluene. The flask was removed from the glovebox and reweighed to

determine the mass of white phosphorus added. Our preliminary data indicate that this approach improves the precision of the weight difference determined standards. However, there still a significant difference in the absolute concentrations as calculated by CIC versus weight difference.

Risk Assessment. Bald eagles (*Haliaeetus leucocephalus*), herring gulls (*Larus argentatus*), and common ravens (*Corvus corax*) have been observed consuming sick and/or dead ducks at ERF (*10*). For estimating of secondary phosphorus exposure to predators, determination of the phosphorus content of the ducks' digestive tract is required. As the gizzard generally contains the majority of phosphorus residues in the digestive tract, gizzard phosphorus content is frequently used for secondary hazard exposure estimates (*11*).

On the basis of the work of Coburn et al. (*12*), Roebuck et al.(*11*) estimated the lowest acutely toxic phosphorus dose to bald eagles to be between 1 and 3 mg/kg. Using the CIC quantified phosphorus standards, the highest phosphorus gizzard content observed was 6.15 mg. Using this value for worst case secondary exposure estimates, the worst case dose for a 5-kg eagle is 0.70 mg/kg, which is less than the minimum estimated toxic dose of 1 mg/kg. However, if we had used the standards prepared by the weight difference approach, the highest gizzard residue would have been estimated to be 156% greater or 9.59 mg. This would result in an estimated worst case dose of 1.1 mg/kg, which is greater than the estimated minimum toxic dose. This comparison illustrates that accurately quantified residues are essential for generating valid risk assessments.

Environmental Monitoring. Contamination of aquatic and marine environments with phosphorus results in increased risk for terrestrial, aquatic, and marine wildlife. For example, in 1968, a new phosphorus production facility in New Foundland released effluent containing suspended white phosphorus into the Placentia Bay. Within 2 days, an extensive fish kill including eels, flounder, cod, lobster, and crab was noted (*13*, *14*). The potential for phosphorus to present secondary hazards in this environment was documented as phosphorus-contaminated cod tissue was shown to be lethal to trout (*15*). An extensive cleanup operation followed in which most of the contaminated sediment was removed by dredging (*16*). After 1.5 year, sampling of both dredged and nondredged areas indicated that the phosphorus levels did not decrease where the sediment was not mechanically moved. A similar effect was noted at the Pine Bluff Arsenal in Arkansas in 1970. Heavy rains resuspended sediment in a lake into which white phosphorus munitions had been previously discharged. Water containing the resuspended phosphorus washed into a neighboring lake, resulting in an extensive fish kill. Phosphorus appears to persistent in aquatic environments. To minimize the undesirable effects of phosphorus contamination, contaminated areas must be identified and cleaned up. As initial studies in our laboratory suggest that the CIC approach is also applicable to phosphorus determinations in a variety of matrixes such as fat and sediment, this analytical method could be used to identify phosphorus-contaminated sediment, water, and wildlife. The resulting accurate residue data are vital for conducting valid primary and secondary risk assessments and cost−benefit analyses regarding potential cleanup efforts.

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