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Swan Tissues:

DETERMINATION OF WHITE PHOSPHORUS RESIDUES

PROJECT NUMBER: 0071/0783

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AUTHENTICATION

This report fully and accurately reflects the procedures used and data generated.

. DATE: 15 SEP 2009

STUDY DIRECTOR

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Swan Tissues:

DETERMINATION OF WHITE PHOSPHORUS RESIDUES

SUMMARY

The white phosphorus residues were determined in a number of swan tissue samples supplied by the Sponsor. The procedure was based on Johnston, JJ, Goldade, DA, Kohler DJ, Cummings, JL (2000). 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. Environ Sci Technol 34(9):1856-1861. The results are shown in the following table:

Tissue Sample	White Phosphorus Residue (mg/kg of tissue)
Skin	<0.02
Body Fat	<0.02
Gizzard	14.8
Liver	<0.02

Swan Tissues:

DETERMINATION OF WHITE PHOSPHORUS RESIDUES

1. INTRODUCTION

The objective of this study was to determine the white phosphorus residue in a number of swan tissue samples supplied by the Sponsor. The procedure was based on Johnston, JJ, Goldade, DA, Kohler DJ, Cummings, JL (2000). 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. Environ Sci Technol 34(9):1856-1861.

Testing was conducted between 16 July 2009 and 31 July 2009.

2. ANALYTICAL STANDARD SOLUTION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	white phosphorus standard solution
Description	:	pale yellow liquid
Concentration	:	1272 mg/l prepared in iso-octane
Date received at Test Facility	:	22 July 2009
Storage conditions	:	approximately 4°C, in the dark, under nitrogen

The integrity of supplied data relating to the identity, purity and stability of the analytical standard solution is the responsibility of the Sponsor. Preparation of the standard solution was performed at the Sponsor's facilities at Oldbury, UK, and this procedure was witnessed and documented by the Study Director to maintain study integrity.

3. TISSUE SAMPLES

3.1 Identification and Storage Conditions

Sponsor's identification	:	skin sample
Reference data/labelling	:	S09-004185 Bird Remains, Skin, Swan Centre ID26 B 0069
Date received at Test Facility	:	22 July 2009
Storage conditions	:	stored frozen at approximately -20°C, in the dark

Sponsor's identification	:	body fat sample
Reference data/labelling	:	S09-004186 Bird Remains, Body Fat, Swan Centre ID26 B 0069
Date received at Test Facility	:	22 July 2009
Storage conditions	:	stored frozen at approximately -20°C, in the dark

Sponsor's identification	:	gizzard sample
Reference data/labelling	:	S09-004183 Bird Remains, Gizzard
Date received at Test Facility	:	22 July 2009
Storage conditions	:	stored frozen at approximately -20°C, in the dark

Sponsor's identification	:	liver sample
Reference data/labelling	:	S09-004184 Bird Remains, Liver
Date received at Test Facility	:	22 July 2009
Storage conditions	:	stored frozen at approximately -20°C, in the dark

The integrity of supplied data relating to the identity and stability of the tissue samples is the responsibility of the Sponsor.

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years, after which instructions will be sought as to further retention or disposal.

5. DETERMINATION OF WHITE PHOSPHORUS RESIDUES

5.1 Method

The determination was carried out using a procedure based on Johnston, JJ, Goldade, DA, Kohler DJ, Cummings, JL (2000). 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. Environ Sci Technol 34(9):1856-1861.

5.2 Procedure

5.2.1 Standard Solution Preparation

An aliquot (0.1272 g) of white phosphorus was diluted to a volume of 100 ml with iso-octane. Preparation of the standard solution was performed at the Sponsor's facilities at Oldbury, UK, due to their experience in handling the potentially reactive white phosphorus. To maximize accuracy of the preparation and to minimize exposure to air, the mass of white phosphorus taken was determined by difference on adding the sample to a vessel containing water. This white phosphorus was then dried in acetone before diluting with iso-octane. This procedure was witnessed and documented by the Study Director to maintain study integrity.

5.2.2 Sample Preparation

Each tissue sample was defrosted by placing the containers in a 20°C nominal temperature waterbath for approximately 1 hour. Each tissue sample was then macerated and returned to its original container. A sample of each macerated tissue sample (see table on following page) was then transferred to a flask and suspended in 30 ml of degassed glass distilled water*.

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* Degassed by boiling vigorously and then purging with nitrogen as it cooled.

Table 5.1

Tissue Sample	Mass of Tissue Taken (g)
Skin	5.0129
Body Fat	5.0128
Gizzard	5.0472
Liver	5.0515

Iso-octane (10 ml) was added to each flask, the headspace filled with nitrogen and the samples were then shaken at approximately 150 rpm on a horizontal flat bed shaker for 18 hours, at ambient temperature, in the dark.

After the shaking period, the samples were decanted into centrifuge tubes and centrifuged at 2500 rpm for 15 minutes. Each upper iso-octane phase/extract was then removed and transferred to a clean glass vessel. An aliquot of each extract was vialled for analysis in an amber vial and the remaining volumes placed into storage at approximately -20°C, in the dark, under nitrogen.

Analysis of the gizzard extract was also performed following a dilution step of 100 times with iso-octane; as it exceeded the validated range of the calibration curve on initial undiluted analysis.

A sample blank was prepared by treating a mixture of 30 ml of degassed glass distilled water* and 10 ml of iso-octane as detailed for the samples.

5.2.3 Analysis

The concentration of white phosphorus in the sample solutions was determined by gas chromatography (GC).

Standards

Standard solutions of white phosphorus were prepared in iso-octane to cover a nominal concentration range of 0.01 to 0.25 mg/l.

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* Degassed by boiling vigorously and then purging with nitrogen as it cooled.

Standard blank

Iso-octane.

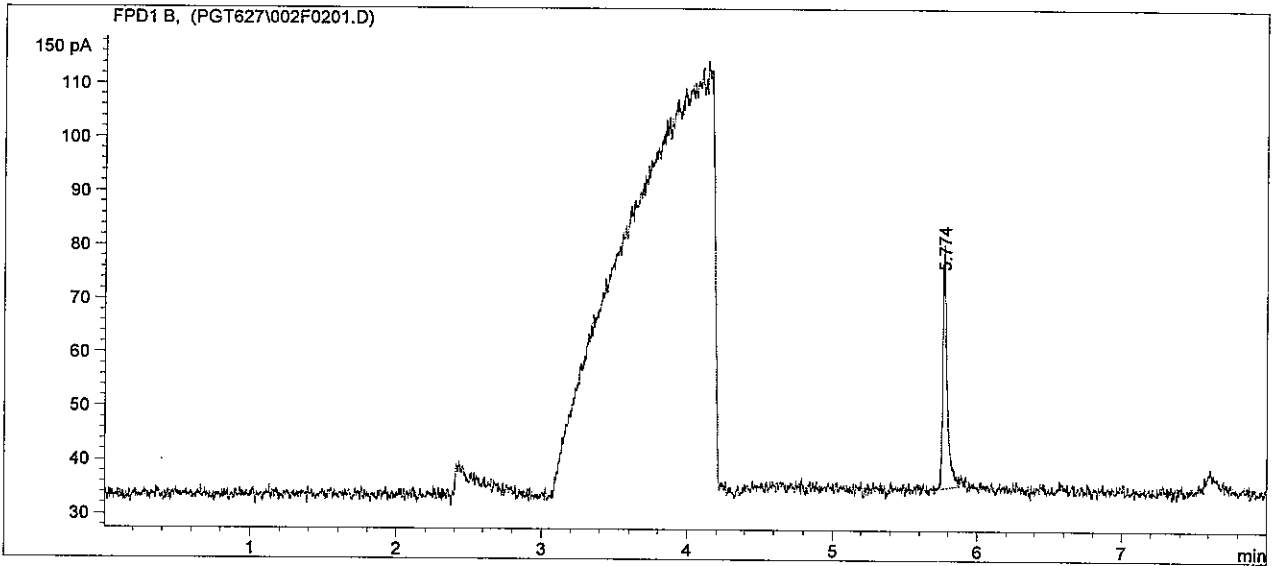
Analysis

The standard and sample solutions were analysed by GC using the following conditions:

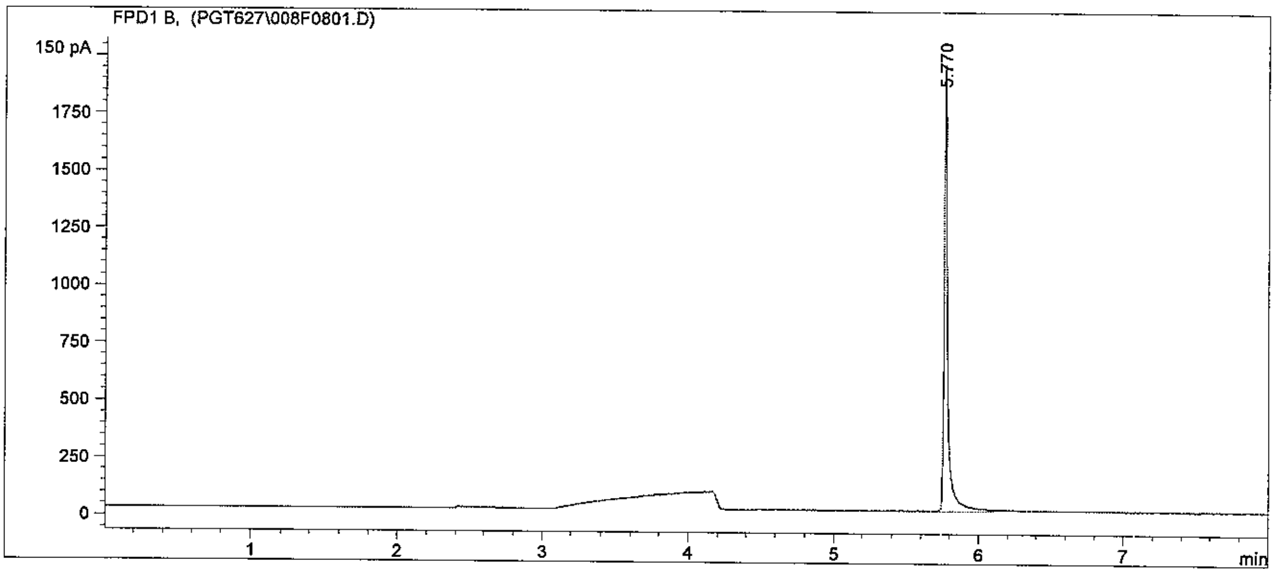
GC System	:	Agilent Technologies 6890, incorporating autosampler and workstation
Column	:	ZB-1 (30 m x 0.32 mm id x 0.25 µm film)
Oven temperature program	:	initial 40°C for 0.5 mins rate 20°C/min final 150°C for 2 mins
Injection temperature	:	250°C
Flame photometric detector temperature	:	250°C
Injection volume	:	2 µl
Retention time	:	~ 5.75 mins

Typical Chromatography

Standard Solution 9.16 x 10^{-3} mg/l

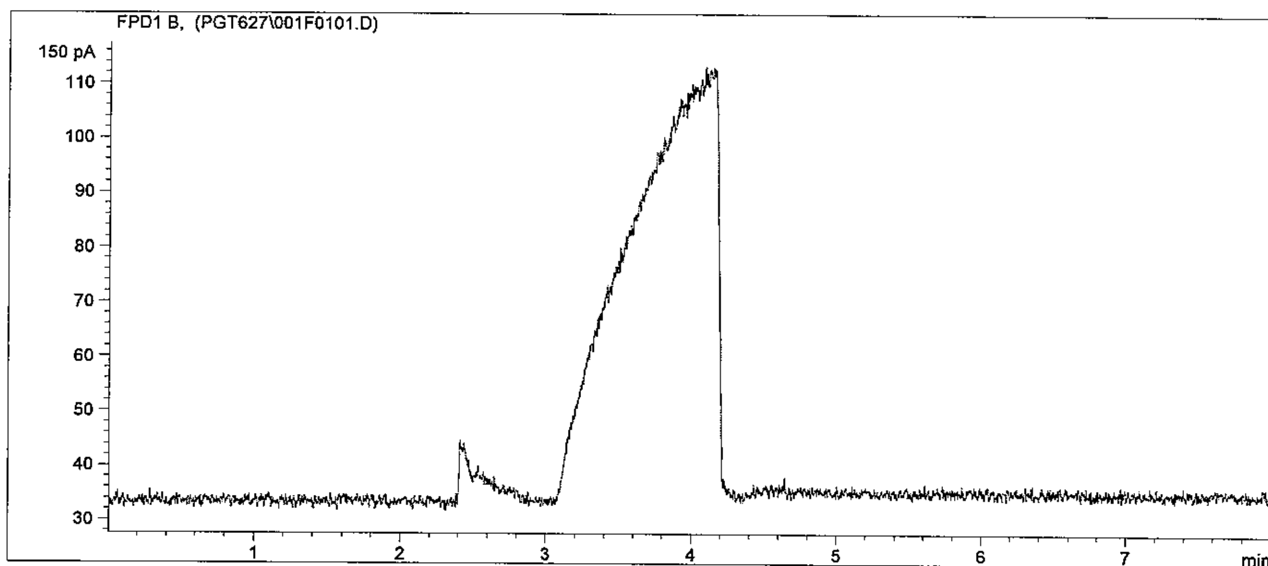


Standard Solution 0.229 mg/l

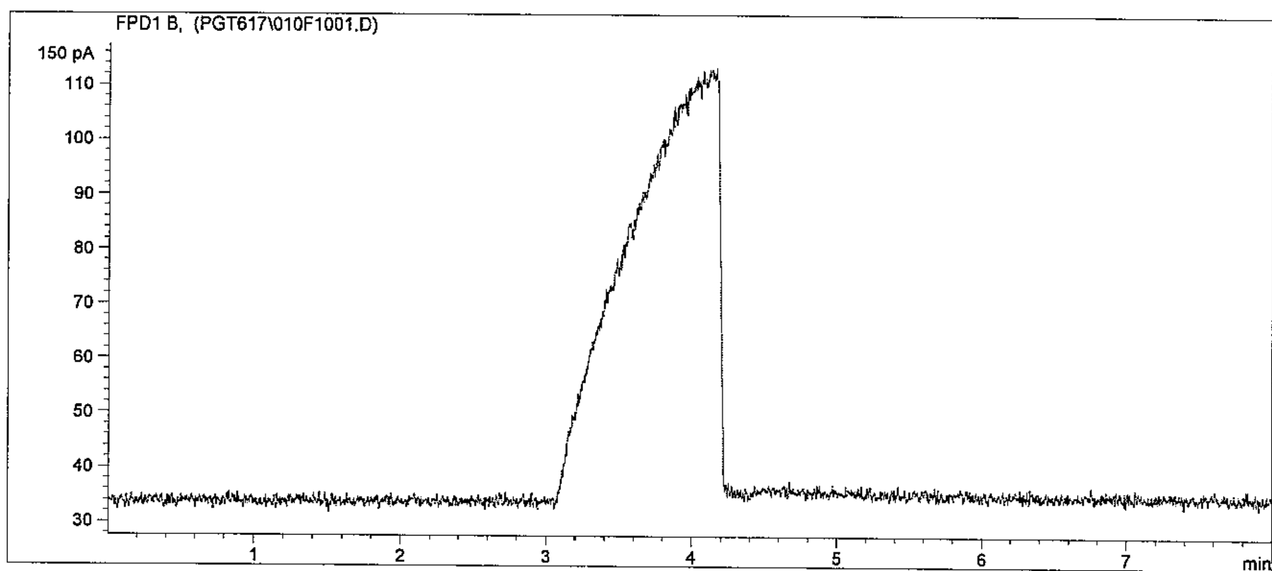


Typical Chromatography

Standard Blank

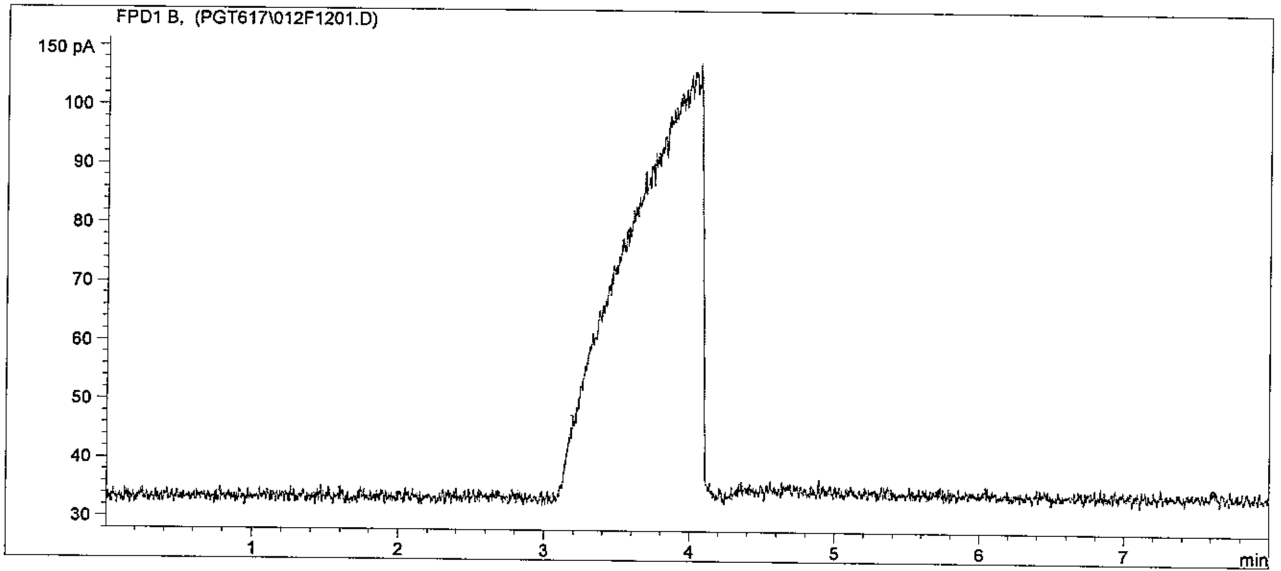


Sample Blank

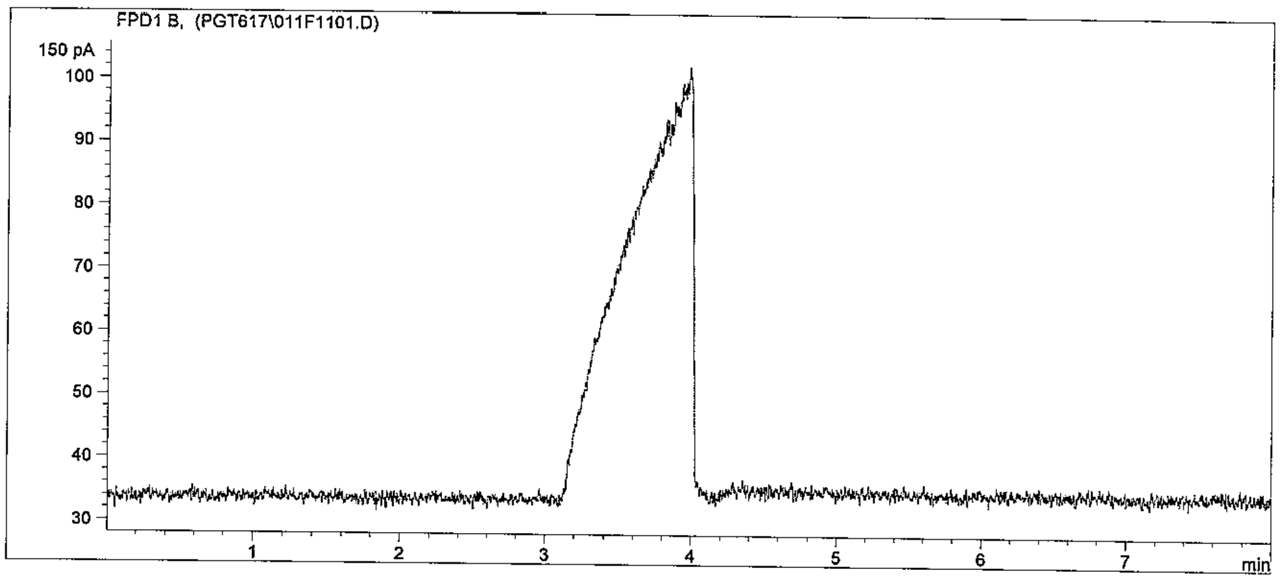


Typical Chromatography

Skin Sample Solution

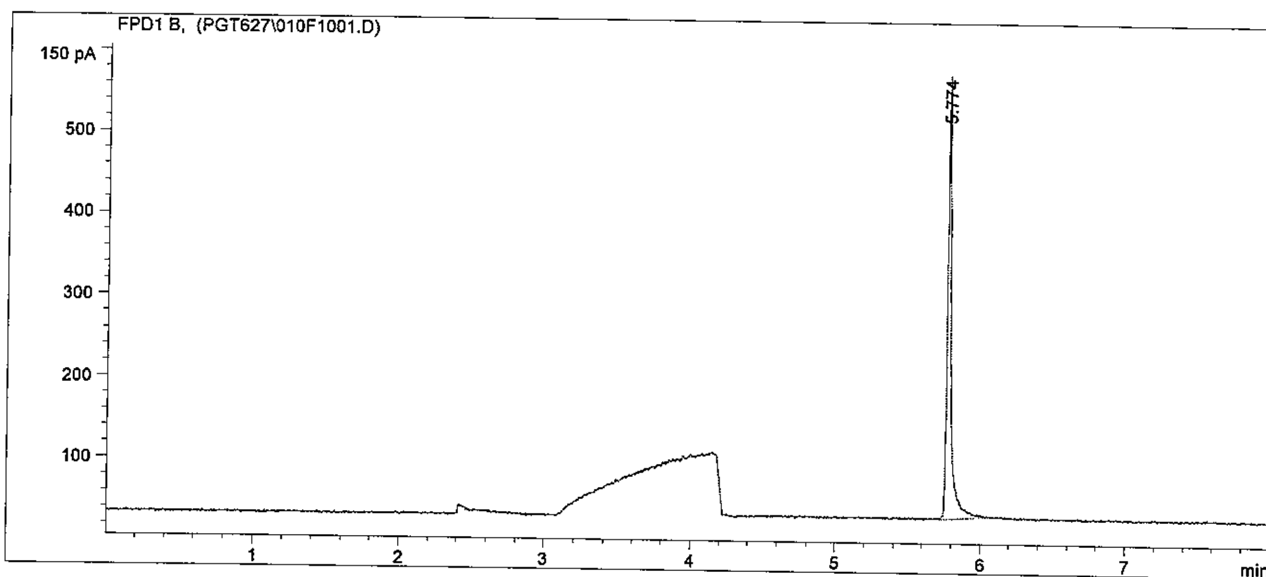


Body Fat Sample Solution

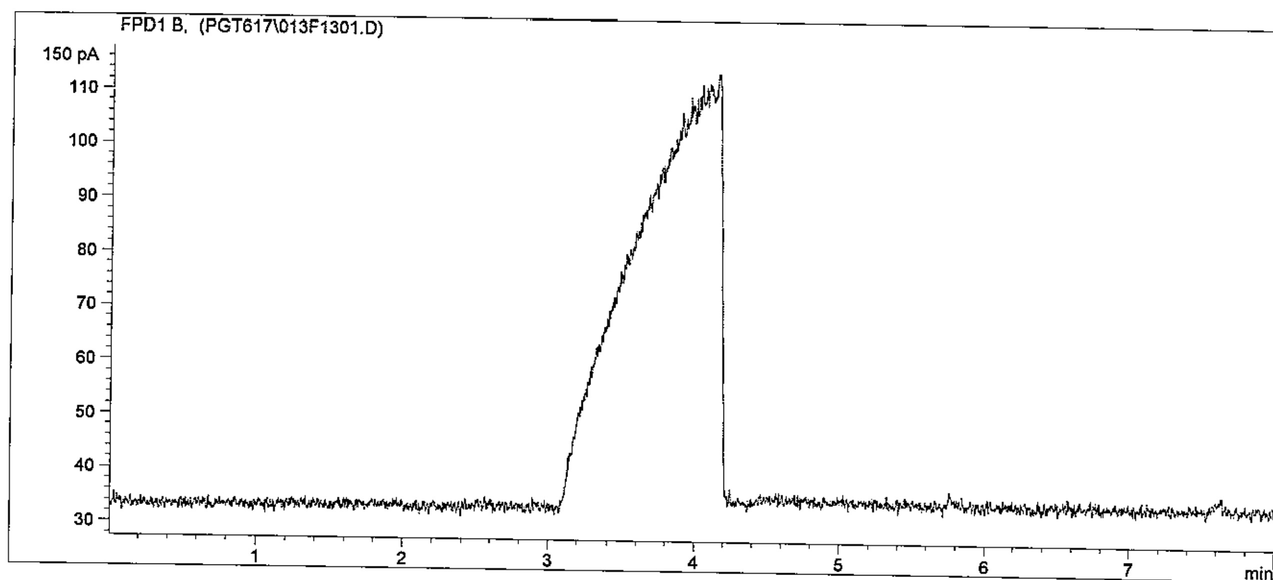


Typical Chromatography

Gizzard Sample Solution (x 100 Dilution Factor)



Liver Sample Solution*



* See discussion, Section 5.5.

5.3 Calculation

The mean peak area and concentration of each standard were plotted on a calibration curve and the sample concentration (mg/l) interpolated from the curve. The concentration was then corrected for dilution factor if relevant (x 100, applied to the reanalysed gizzard extract sample only).

The white phosphorus residue (mg/kg) in the original tissue sample was calculated using Equation 5.1.

Equation 5.1

$$R_{wp} = C_{wp} \times \frac{V_{iso}}{1000} \times \frac{1}{M_{tis}} \times 1000$$

where:

R_{wp} = residue of white phosphorus (mg/kg)
C_{wp} = concentration of white phosphorus determined in the sample solution (mg/l)
V_{iso} = volume of iso-octane used for extraction (ml)
M_{tis} = mass of tissue taken for extraction (g)

5.4 Results

The mean peak areas relating to the standard and sample solutions are shown in the following tables:

Table 5.2 – Initial Analysis, Undiluted Samples

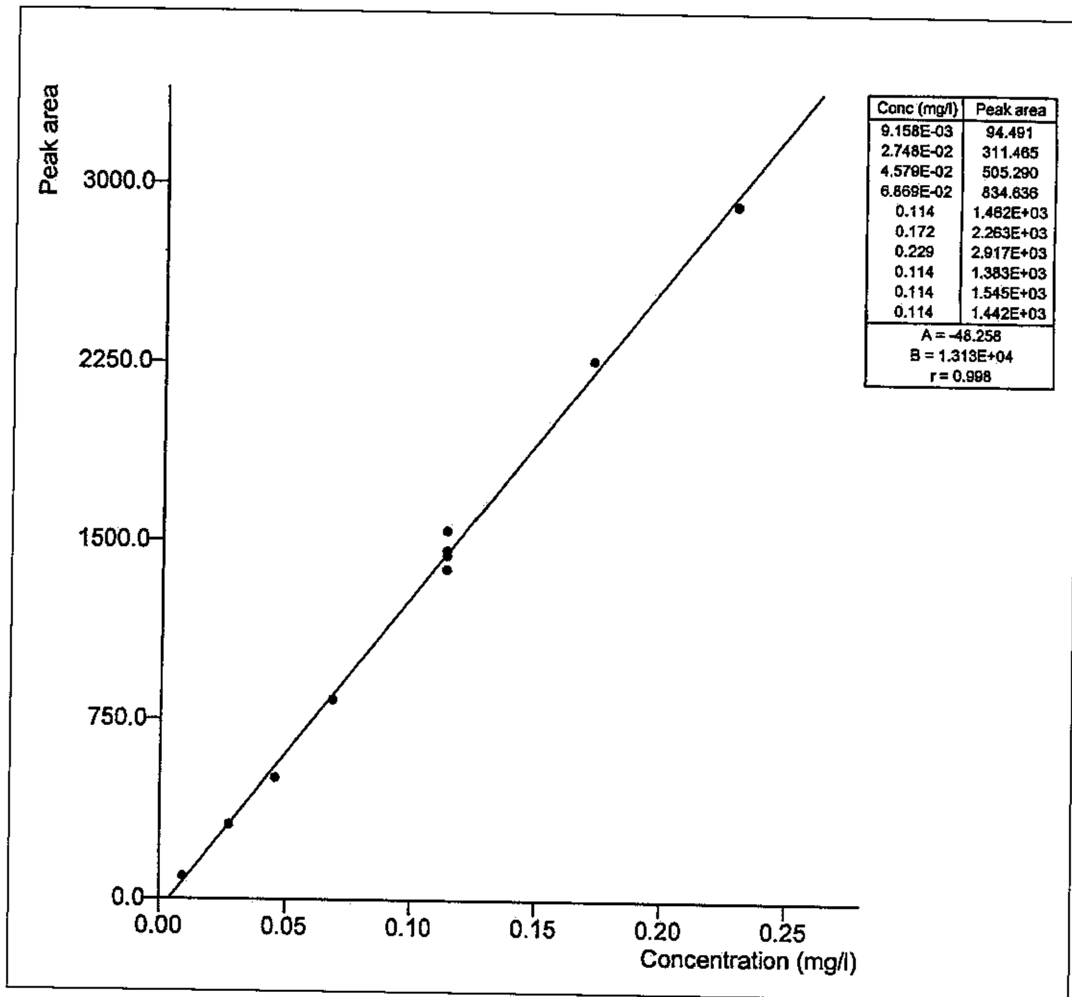
Solution	Mean Peak Area
Standard blank	none detected
Standard 9.16×10^{-3} mg/l	94.491
Standard 2.75×10^{-2} mg/l	311.465
Standard 4.80×10^{-2} mg/l	505.290
Standard 6.87×10^{-2} mg/l	834.636
Standard 0.114 mg/l	1.462×10^{3}
Standard 0.114 mg/l (duplicate)	1.383×10^{3}
Standard 0.172 mg/l	2.263×10^{3}
Standard 0.229 mg/l	2.917×10^{3}
Sample blank	none detected
Skin sample solution	none detected
Body fat sample solution	none detected
Gizzard sample solution	exceeded calibrated range
Liver sample solution	none detected
Standard 0.114 mg/l (post-sample)	1.545×10^{3}
Standard 0.114 mg/l (post-sample)	1.442×10^{3}

Table 5.3 – Repeat Analysis, Diluted Gizzard Sample

Solution	Mean Peak Area
Standard blank	none detected
Standard 9.16×10^{-3} mg/l	93.501
Standard 2.75×10^{-2} mg/l	317.990
Standard 4.80×10^{-2} mg/l	607.249
Standard 6.87×10^{-2} mg/l	942.328
Standard 0.114 mg/l	1.611×10^{3}
Standard 0.114 mg/l (duplicate)	1.626×10^{3}
Standard 0.172 mg/l	2.471×10^{3}
Standard 0.229 mg/l	3.442×10^{3}
Gizzard sample solution	1.041×10^{3}
Standard 0.114 mg/l (post-sample)	1.666×10^{3}
Standard 0.114 mg/l (post-sample)	1.630×10^{3}

The calibration curve from the initial undiluted sample analysis is shown in Figure 5.1.

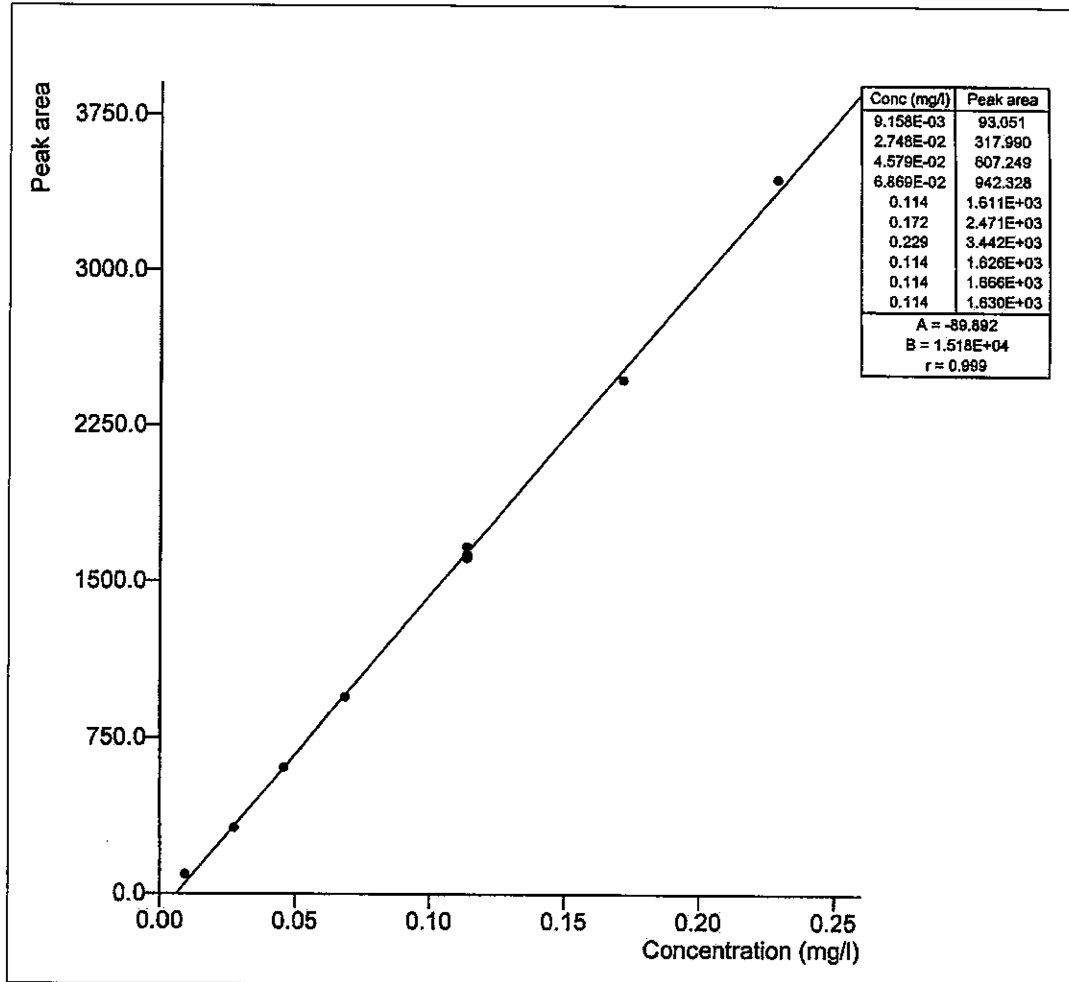
Figure 5.1



Conc (mg/l)	Peak area
9.158E-03	94.491
2.748E-02	311.465
4.579E-02	505.290
6.869E-02	834.636
0.114	1.462E+03
0.172	2.263E+03
0.229	2.917E+03
0.114	1.383E+03
0.114	1.545E+03
0.114	1.442E+03
A = -48.258	
B = 1.313E+04	
r = 0.998	

The calibration curve from which the diluted gizzard sample concentration was interpolated is shown in Figure 5.2.

Figure 5.2



Conc (mg/l)	Peak area
9.158E-03	93.051
2.748E-02	317.990
4.579E-02	607.249
6.869E-02	942.328
0.114	1.611E+03
0.172	2.471E+03
0.229	3.442E+03
0.114	1.626E+03
0.114	1.666E+03
0.114	1.630E+03
A = -89.892	
B = 1.518E+04	
r = 0.999	

The white phosphorus concentration determined in each of the analysed tissue extracts and the resulting calculated white phosphorus residue (mg/kg) in the original tissue samples is shown in the following table:

Table 5.4

Tissue Sample	Analysed Concentration of White Phosphorus (mg/l)	White Phosphorus Residue (mg/kg of tissue)
Skin	<0.01	<0.02
Body Fat	<0.01	<0.02
Gizzard	7.45	14.8
Liver	<0.01	<0.02

5.5 Discussion

No detectable residue of white phosphorus was present in the skin or body fat tissue sample extracts on analysis. Therefore a limit value has been reported for the white phosphorus residue present in each of these samples, calculated from the lowest nominal calibration standard concentration (0.01 mg/l) and the nominal tissue sample mass of 5 g.

On initial analysis of the gizzard tissue sample extract it exceeded the validated range of the calibration curve. It was therefore necessary to dilute the sample by a factor of 100 with iso-octane and repeat the analysis procedure.

Although there was evidence of possible phosphorus content in the liver sample extract; it remained insufficient to quantify. Typically three times baseline noise would be applied as an estimate of the limit of detection (LOD) and five times baseline noise as a limit of quantification (LOQ) for an analytical method. In the case of the liver sample, the minor deviation in the baseline at the approximate retention time of white phosphorus remained below three time baseline noise. However, the application of LOD and LOQ was further complicated for this method as the intercepts of the calibration graphs were slightly displaced from the origin. Therefore, to retain complete confidence in the limit value reported the use of a confirmed quantifiable standard concentration, as used for the skin and body fat samples, has been applied to the liver sample also.

5.6 Conclusion

The white phosphorus residue (mg/kg) in a number of swan tissue samples has been performed. The results are shown in the following table:

Table 5.5

Tissue Sample	White Phosphorus Residue (mg/kg of tissue)
Skin	<0.02
Body Fat	<0.02
Gizzard	14.8
Liver	<0.02