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Canadian Goose Tissue Analysis:

DETERMINATION OF WHITE PHOSPHORUS RESIDUES

PROJECT NUMBER: 0071/0785

AUTHORS: [redacted]

STUDY SPONSOR:

Rhodia UK Limited
Trinity Street
PO Box 80
Oldbury
West Midlands
B69 4LN
UNITED KINGDOM

TEST FACILITY:

Harlan Laboratories Ltd
Shardlow Business Park
Shardlow
Derbyshire
DE72 2GD
UK

Telephone: +44 (0) 1332 792896

Facsimile: +44 (0) 1332 799018

AUTHENTICATION

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

[redacted] DATE: 14 JAN 2010

STUDY DIRECTOR

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SUMMARY

The quantity of white phosphorus present in the gizzard contents and small intestine contents of the supplied Canadian goose tissues has been determined. The results are shown in the following table:

Sample	Total White Phosphorus Content Present (mg)	Total White Phosphorus Content Present (µg)
Gizzard Contents	4.75×10^{-2}	47.5
Small Intestine Contents	9.7×10^{-4}	0.97

The white phosphorus residue (mg/kg) in a number of Canadian goose tissue samples has also been determined. These results are shown in the following table:

Tissue Sample	White Phosphorus Residue (mg/kg of tissue)
Skin	<0.02
Body Fat	<0.02
Small Intestine	<0.02
Gizzard	0.06
Liver	<0.02

The procedures used were 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.

Canadian Goose Tissue Analysis:

DETERMINATION OF WHITE PHOSPHORUS RESIDUES

1. INTRODUCTION

The objective of this study was to determine the white phosphorus residue in a number of Canadian goose 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 at Harlan Laboratories Ltd between 01 December 2009 and 04 December 2009.

2. ANALYTICAL STANDARD SOLUTION

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	yellow phosphorus standard solution*
Description	:	clear colourless solution with some yellow suspended solids
Concentration	:	2233 mg/l prepared in iso-octane
Date received at Test Facility	:	24 November 2009
Storage conditions	:	room temperature, in the dark

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 a member of Harlan Laboratories Ltd staff with management responsibilities to maintain study integrity.

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* Yellow phosphorus and white phosphorus are both common names for the same phosphorus allotrope, tetraphosphorus (P_4), and therefore this analytical standard solution was completely valid for the analytical work performed.

3. TISSUE SAMPLES

3.1 Identification and Storage Conditions

Sponsor's identification	:	Centre ID26, B 0278, 15/10/09
Tissue Samples Supplied	:	Skin
		Body Fat
		Gizzard
		Small Intestine
		Liver
Date received at Test Facility	:	24 November 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 (0.2233 g) of yellow 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 phosphorus. This yellow phosphorus, which was stored under water, was dried in acetone and then dried further using a tissue prior to weighing and diluting with iso-octane. This procedure was witnessed and documented by a member of staff at Harlan Laboratories Ltd with management responsibilities 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 a minimum of 1 hour.

Gizzard Contents

The gizzard, once defrosted, was dissected longitudinal between the crushing plates to expose the contents. These contents were then transferred to a conical flask and the remaining tissue quantitatively rinsed using 1 x 60 ml and 1 x 15 ml aliquots of degassed

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* Yellow phosphorus and white phosphorus are both common names for the same phosphorus allotrope, tetraphosphorus (P_4), and therefore this analytical standard solution was completely valid for the analytical work performed.

glass distilled water*. These rinsings were added to the contents of the gizzard in the conical flask.

Small Intestine Contents

A peristaltic action was applied to the section of small intestine tissue supplied, once defrosted. The contents of the small intestine were then collected in a conical flask as they were ejected.

Remaining Samples (Including Gizzard and Small Intestine Actual Tissues)

Each tissue sample, once defrosted, was macerated and a sample of each macerated tissue sample (see following table) was then transferred to a conical flask for extraction.

Table 5.1

Tissue Sample	Mass of Tissue Taken (g)
Skin	5.0612
Body Fat	5.0013
Gizzard	5.1019
Small Intestine	5.0677
Liver	5.0229

Extraction of Samples for Analysis

With the exception of the gizzard contents sample (which already contained 75 ml of degassed glass distilled water), each sample was suspended in 30 ml of degassed glass distilled water*). Iso-octane (10 ml, with the exception of the gizzard contents sample where 25 ml was used to maintain the aqueous to organic solvent phase ratio) was then added to each flask, the headspace filled with nitrogen and the samples were 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 iso-octane extract

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

was vialled for analysis in an amber vial. In addition to this, aliquots of the gizzard contents extract and gizzards tissue extract were diluted in duplicate by a factor of 10 and a factor of 100 with iso-octane and again taken in amber vials for analysis.

The remaining volumes of iso-octane extract were placed into storage at approximately -20°C, in the dark.

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.

Standard blank

Iso-octane.

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

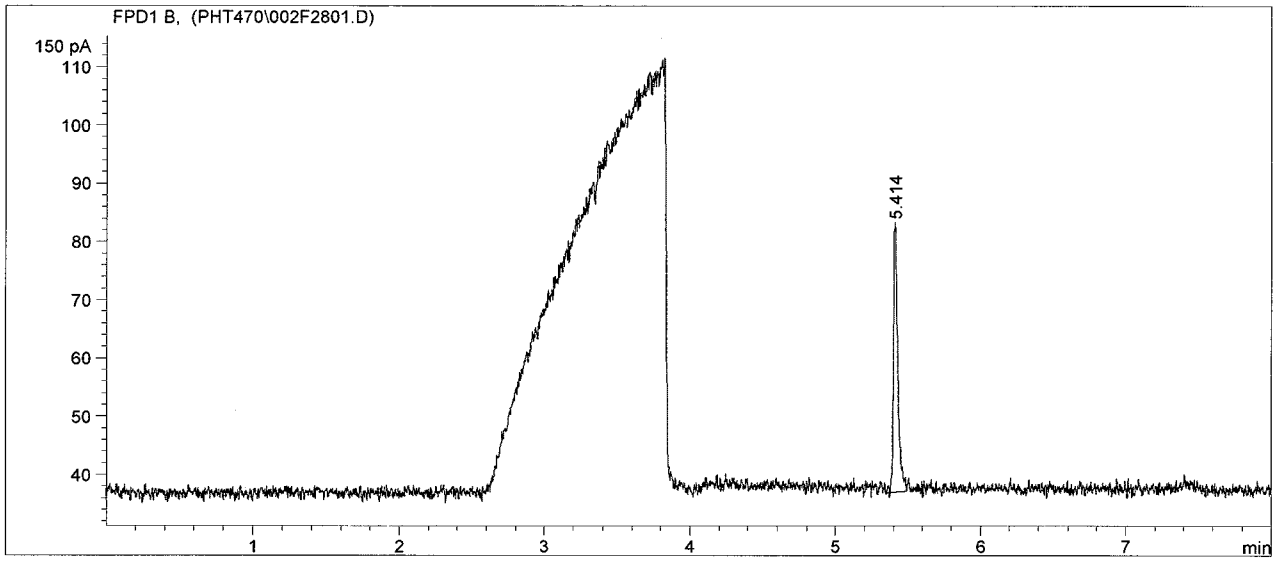
Analysis

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

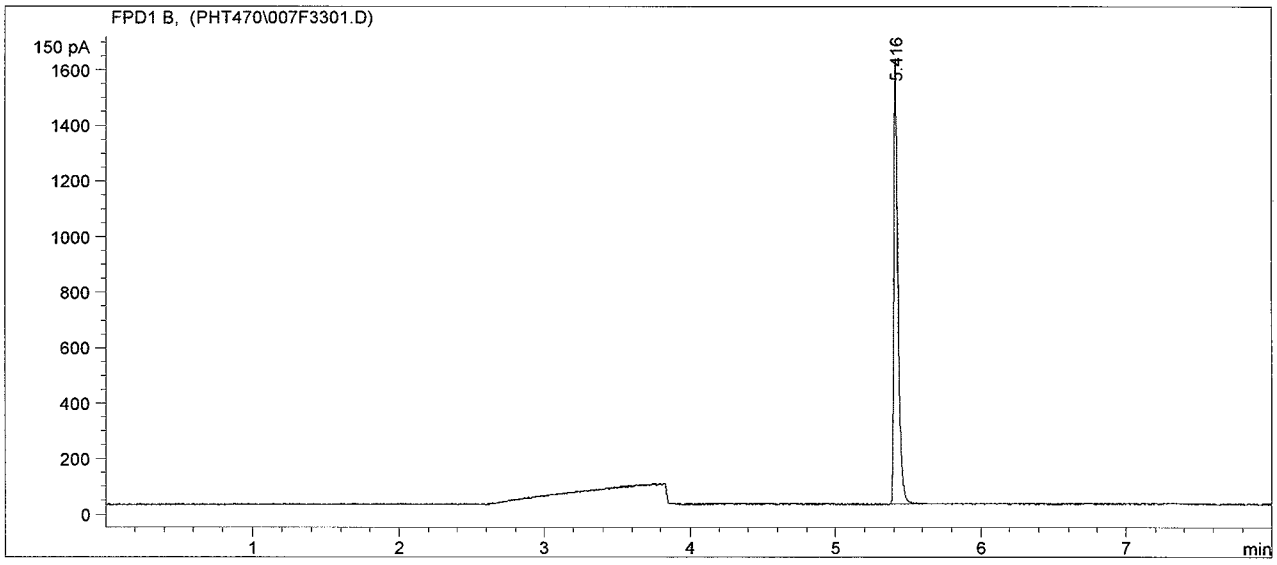
GC System	:	Agilent Technologies 6890, incorporating autosampler and workstation
Column	:	DB-1 (30 m x 0.25 mm id x 0.25 µm film)
Oven temperature program	:	initial 40°C for 0.5 mins rate 20°C/min final 150°C for 2 mins
Injection temperature	:	250°C
Flame photometric detector temperature	:	250°C
Injection volume	:	2 µl
Retention time	:	~ 5.4 mins

Typical Chromatography

Standard Solution 1.07 x 10^{-2} mg/l

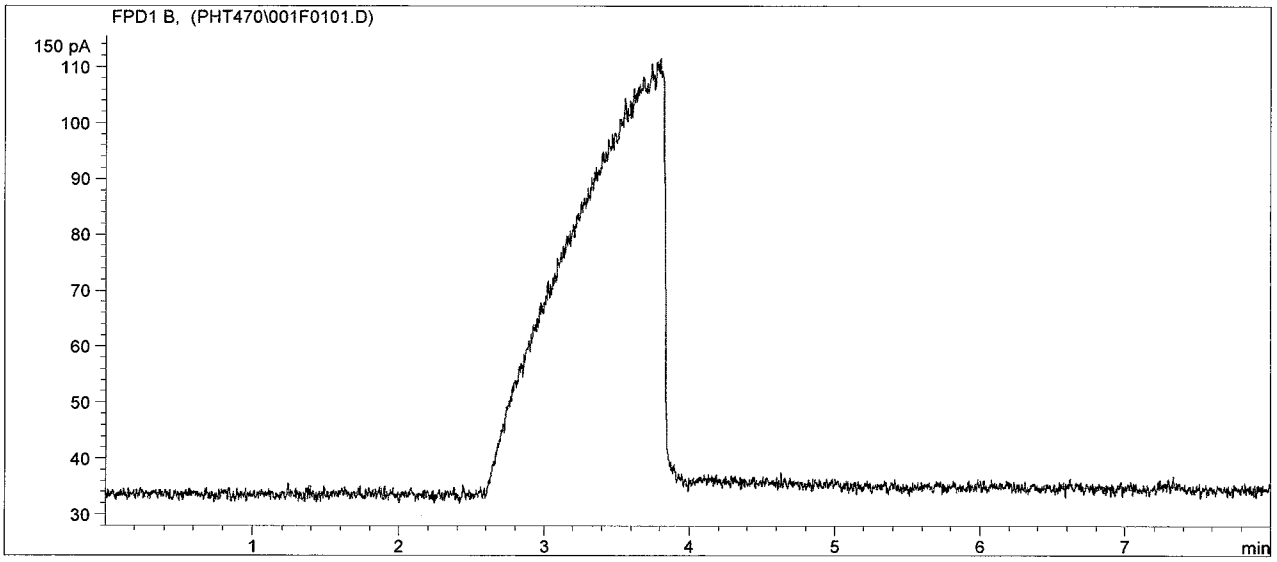


Standard Solution 0.268 mg/l

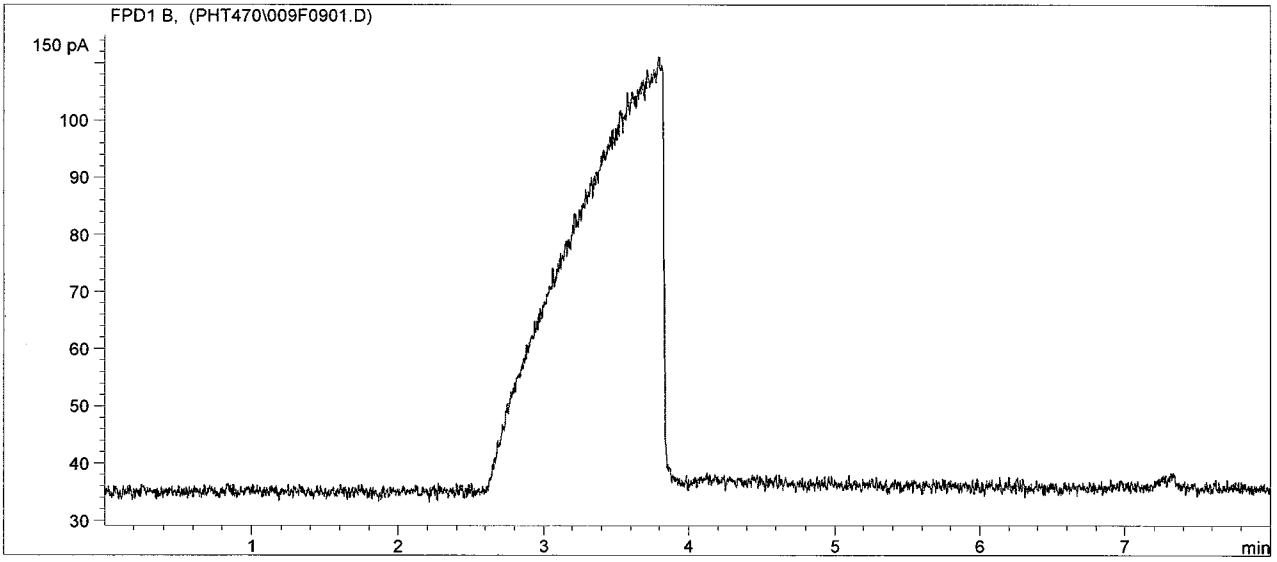


Typical Chromatography

Standard Blank

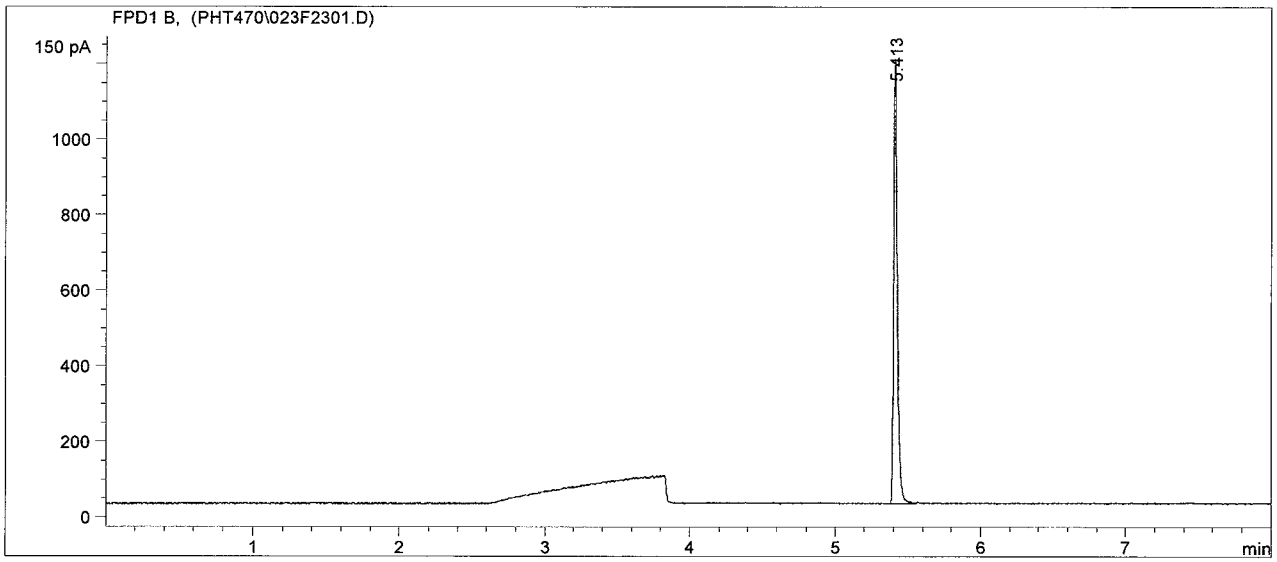


Sample Blank

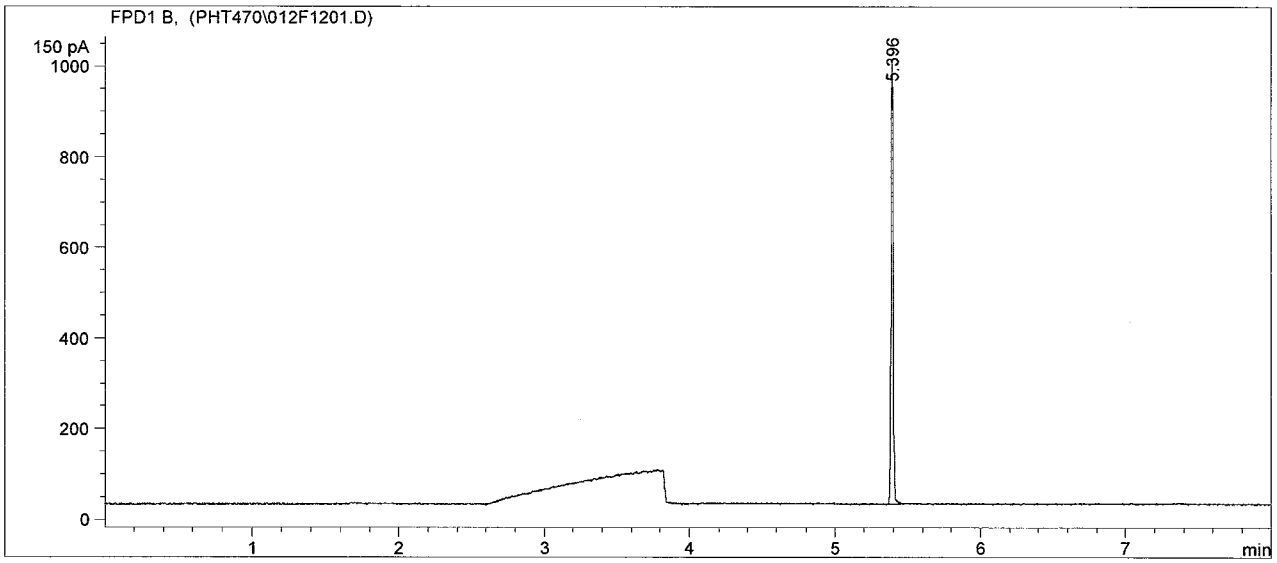


Typical Chromatography

Gizzard Contents Solution (x 10 Dilution)

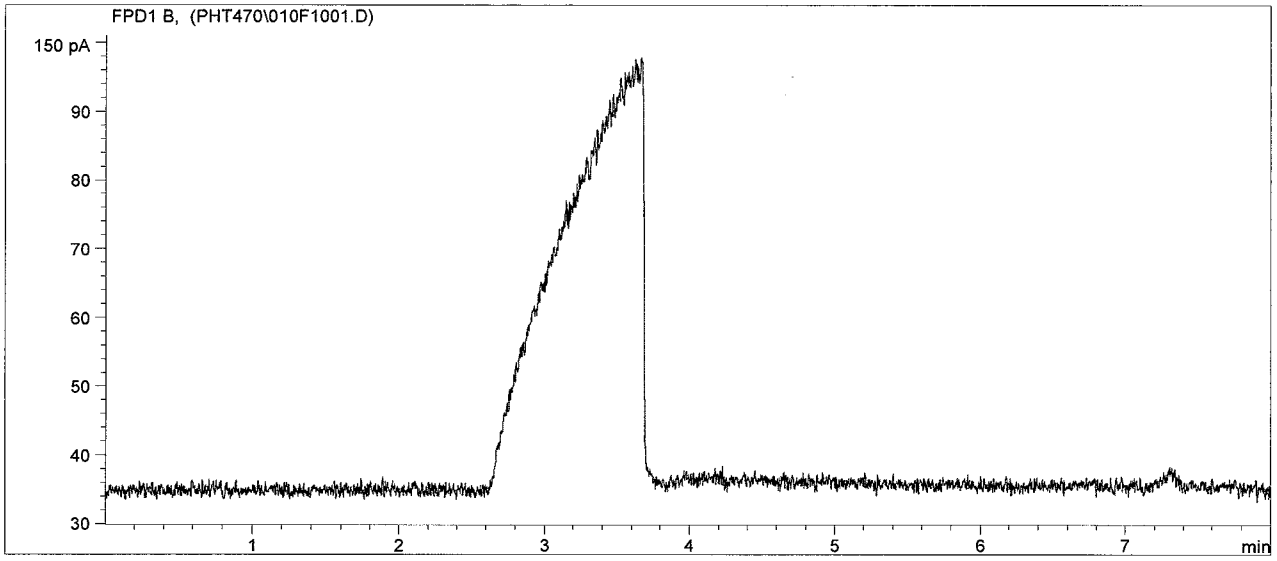


Small Intestine Contents Solution

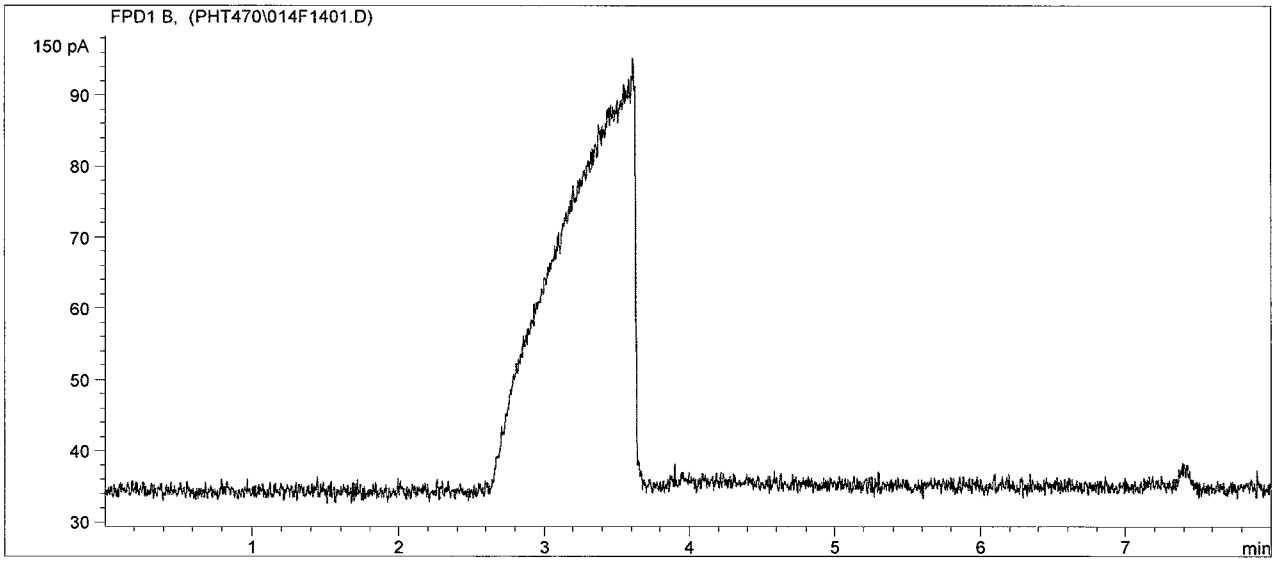


Typical Chromatography

Skin Sample Solution

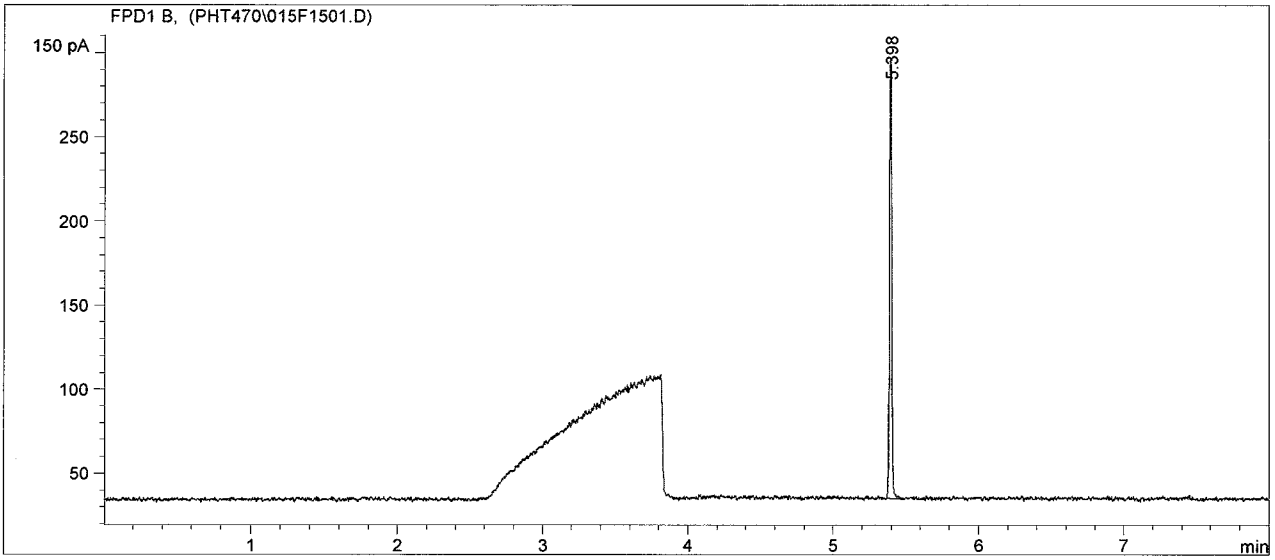


Body Fat Sample Solution

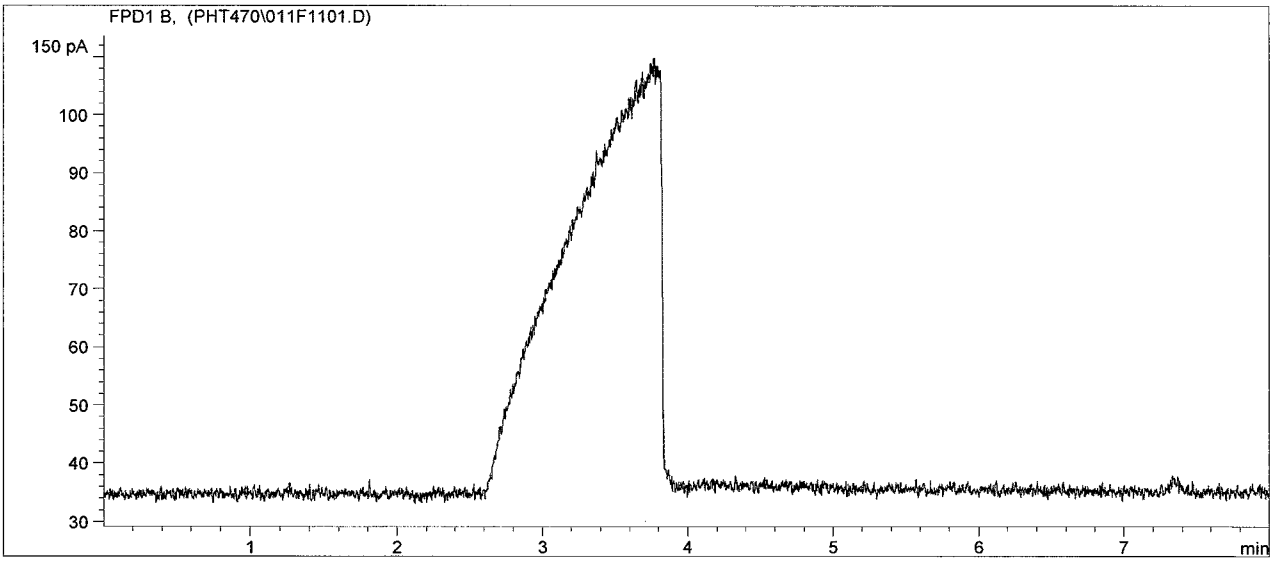


Typical Chromatography

Gizzard (Tissue) Sample Solution

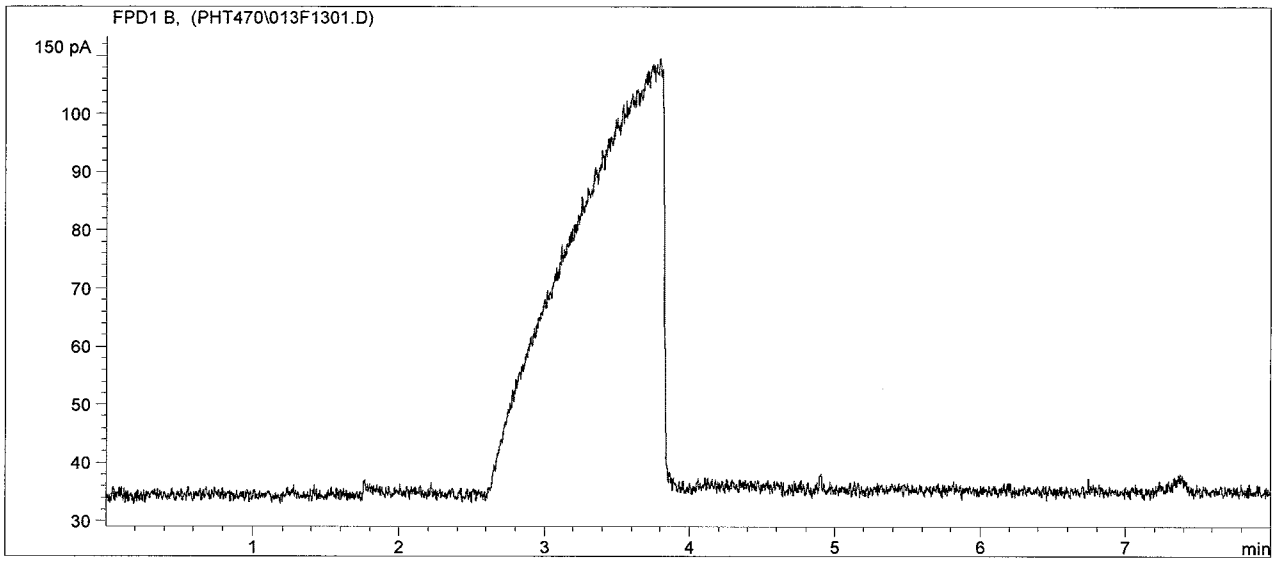


Small Intestine (Tissue) Sample Solution



Typical Chromatography

Liver Sample Solution



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.

The white phosphorus content (mg) present in the gizzard and small intestine contents was calculated using Equation 5.1. These results have also been multiplied by 1 x 10^{-3} to present the values in µg within the report.

Equation 5.1

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

where:

M_{wp} = mass of white phosphorus present in contents (mg)
C_{wp} = concentration of white phosphorus determined in the sample solution (mg/l)
V_{iso} = volume of iso-octane used for extraction (ml)

The white phosphorus residue (mg/kg) in the original tissue sample was calculated using Equation 5.2.

Equation 5.2

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

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 table:

Table 5.2

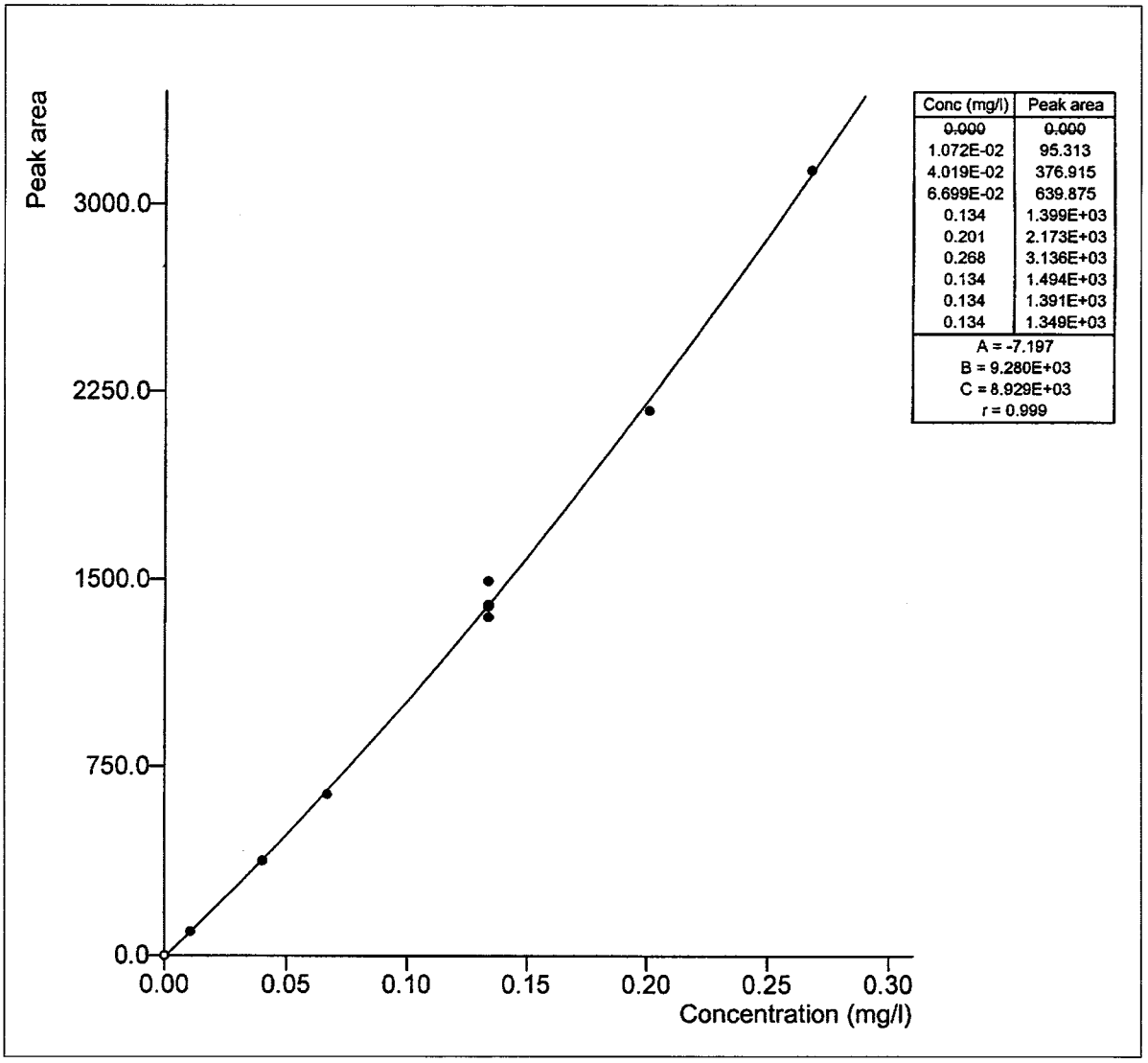
Solution	Mean Peak Area
Standard blank	none detected
Standard 1.07 x 10^{-2} mg/l	95.313
Standard 4.02 x 10^{-2} mg/l	376.915
Standard 6.70 x 10^{-2} mg/l	639.875
Standard 0.134 mg/l	1.399 x 10^{3}
Standard 0.134 mg/l (duplicate)	1.494 x 10^{3}
Standard 0.201 mg/l	2.173 x 10^{3}
Standard 0.268 mg/l	3.136 x 10^{3}
Sample blank	none detected
Gizzard contents	2.176 x 10^{4}
Gizzard contents (x 10 dilution, A)	2.051 x 10^{3}
Gizzard contents (x 10 dilution, B)	2.096 x 10^{3}
Gizzard contents (x 100 dilution, A)	180.986
Gizzard contents (x 100 dilution, B)	178.172
Small intestine contents	976.366
Skin sample solution	none detected

Table 5.2 – Continued

Solution	Mean Peak Area
Body fat sample solution	none detected
Gizzard tissue sample solution	267.844
Gizzard tissue sample solution (x 10 dilution, A)	30.036
Gizzard tissue sample solution (x 10 dilution, B)	29.113
Gizzard tissue sample solution (x 100 dilution, A)	none detected
Gizzard tissue sample solution (x 100 dilution, B)	none detected
Small intestine tissue sample solution	none detected
Liver sample solution	none detected
Standard 0.134 mg/l (duplicate)	1.391×10^3
Standard 0.134 mg/l (duplicate)	1.349×10^3

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

Figure 5.1


Peak area
3000.0
2250.0
1500.0
750.0
0.0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Concentration (mg/l)
Conc (mg/l) | Peak area
0.000 | 0.000
1.072E-02 | 95.313
4.019E-02 | 376.915
6.699E-02 | 639.875
0.134 | 1.399E+03
0.201 | 2.173E+03
0.268 | 3.136E+03
0.134 | 1.494E+03
0.134 | 1.391E+03
0.134 | 1.349E+03
A = -7.197
B = 9.280E+03
C = 8.929E+03
r = 0.999

The white phosphorus concentration determined in each of the analysed gizzard content and small intestine content extracts and the resulting calculated white phosphorus content (mg and µg) present is shown in the following table:

Table 5.3

Tissue Sample	Analysed Concentration of White Phosphorus (mg/l)*	Total White Phosphorus Content Present (mg)	Total White Phosphorus Content Present (µg)
Gizzard Contents	1.90	4.75×10^{-2}	47.5
Small Intestine Contents	9.7×10^{-2}	9.7×10^{-4}	0.97

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	2.88×10^{-2}	0.06
Small Intestine	<0.01	<0.02
Liver	<0.01	<0.02

5.5 Discussion

No detectable residue of white phosphorus was present in the skin, body fat, small intestine and liver 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.

For the gizzard content extract, the undiluted extract exceeded the valid range of the calibration standards analysed with the samples. Of the remaining x 10 and x 100 factor diluted extracts, the x 10 dilution factor extract sample responses were most appropriate

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* See discussion, Section 5.5.

with respect to the calibration standards and therefore these mean peak areas were used for calculation of the definitive reported values. For the gizzard tissue samples, the undiluted sample extract gave the most appropriate instrument response for calculation of the definitive result.

5.6 Conclusion

The quantity of white phosphorus present in the gizzard contents and small intestine contents of the supplied Canadian goose tissues has been determined. The results are shown in the following table:

Table 5.5

Sample	Total White Phosphorus Content Present (mg)	Total White Phosphorus Content Present (µg)
Gizzard Contents	4.75×10^{-2}	47.5
Small Intestine Contents	9.7×10^{-4}	0.97

The white phosphorus residue (mg/kg) in a number of Canadian goose tissue samples has also been determined. These results are shown in the following table:

Table 5.6

Tissue Sample	White Phosphorus Residue (mg/kg of tissue)
Skin	<0.02
Body Fat	<0.02
Small Intestine	<0.02
Gizzard	0.06
Liver	<0.02