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(ir L.G.M.Th. Tuinstra)

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Content of pentachlorophenol in liver of cattle, sheep, pigs and chickens

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ABSTRACT

Content of pentachlorophenol in liver of cattle, sheep, pigs and chickens.

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2 Tables 6 References

Pentachlorophenol (PCP) has been used for many years as an all purpose fungicide and pesticide. Its widespread use, especially in the wood preservation industry, has led to its presence in the food chain. It is therefore necessary to detect and quantify levels present in plants and animals to see if the levels are of concern.

Detectable levels of PCP have been found in the liver of pigs in the Netherlands by the Food Inspection Service, Amsterdam in the range 0.01 - 1.2 mg/kg (median 0.06 mg/kg). It was the purpose of this project to measure PCP in the liver of cattle, sheep, pigs and chickens.

The median value for PCP in the liver of chickens was <0.01 mg/kg, range <0.01-0.04 mg/kg (n=10), of pigs was 0.02 mg/kg, range 0.02-0.05 mg/kg (n=10), of cattle was 0.04 mg/kg, range <0.01-0.18 mg/kg (n=11) and of sheep (n=2) respectively 0.06 and 0.14 mg/kg.

5 Procedure

In all series of the samples blank and recovery experiments should be included.

Homogenise the liver in the mixer. Place 4g of liver sample into a 100 ml round bottom flask. Add 5 ml 12M H_2SO_4 and boil the mixture under reflux for 1 hour. After the mixture has cooled to room temperature 10 ml of cyclohexane are added.The mixture is then shaken on a shaking machine for 20 minutes and then the resultant mixture is centrifuged at 1500g for 5 minutes. After centrifugation the organic layer is removed and retained.

Bring 5 ml of the organic extract in a separating funnel containing 80 ml of 0.1M K_2CO_3. The funnel is then inverted to ensure thourough mixing, then 1 ml acetic anhydride is added. This mixture is shaken carefully, and the pressure released frequently until no further gas is evolved. The aqueous layer is removed and discarded. The organic layer is then filtered through anhydrous Na_2SO_4 to dry it and retained in a 25 ml graduated cylinder containing 1 ml of the internal standard (3.6). An additional 10 ml of cyclohexane is added to wash the separating funnel. This washing is then also filtered through the Na_2SO_4 and collected in the graduated cylinder. The volume is then made up to 25 ml with cyclohexane. Inject 2 ul of the extract in the gas chromatograph equipped with ECD.

6 Identification

Compare the retention time of the internal standard and derivatised PCP in the unknown sample with the standard solution. The Relative Retention Time (RRT) of dPCP should not differ more than +/- 5 divided by the absolute retention time of the internal standard in seconds (= A)

$$\text{RRT dPCP(sample)} = \text{RRT dPCP(standard)} \frac{+/- 5}{A}$$

7 Calculation

Calculate the concentration of the PCP in the sample with the following formula:

$$\frac{\text{H sample}}{\text{H standard}} * \frac{1}{a} * V * C \text{ standard} = \ldots\ldots \text{ mg PCP/kg}$$

H sample = Peak Height/Area of derivatised PCP in sample
H standard = " " " " " " " standard
a = Amount of liver present in final extract (= 2g)
V = Volume of the final extract (= 25 ml)
C standard = Concentration of PCP in ug/ml in the underivatised standard used for the derivatisation

8 Reference

M.W. de Kroon, G.J. Distelbrink and R. Hittenhausen-Gelderblom, Pentachloorfenol in varkenslever, Food Inspection Service, Amsterdam, February 1989, Report 88-6

CONTENT

1 INTRODUCTION

Pentachlorophenol (PCP) is a toxic compound that has been found to have various adverse effects on organisms in the environment, both aquatic and terrestrial, including man. Most of the PCP used world wide is in the wood preservation industry and as a method of pest control - it is a highly efficient molluscicide, insecticide, and herbicide. Due to its high volatility it is found in small quantities in most air samples, and also in soil and water samples. The use of PCP in the Netherlands is banned.

Concern about PCP is rising due to the fact that it has entered the food chain and thus, levels of PCP in the diet must be monitored. PCP may be absorbed into the body by several different routes; inhalation, ingestion, and dermal exposure. The NOAEL in a study with (unpurified) PCP in rats was 3 mg/kg body weight. WHO and US-National Academy of Science are using for a person of 60 kg an acceptable daily intake (ADI) of 0.18 mg/day [1].
In the workdocument clorophenols (RIVM) an ADI of 2.4 mg/day is reported based on studies with purified PCP [2].

In the Netherlands concentrations of PCP have been found in the liver of pigs, ranging from 0.01-1.2 mg/kg (median value 0.06 mg/kg).[3] The daily intake of PCP measured in a total diet study by CIVO in the period 1984-1986 was 0.001 mg.[4] In duplicate 24 hours-diets a mean intake of 0.004 mg was reported [5].The median found in human liver was 0.013 mg/kg.[6] The intention of this study is to ascertain whether or not it is possible to detect also PCP levels in the liver of cattle, sheep and chicken.
The Maximum Residue Limit (MRL) for mushrooms is 0.05 mg/kg, for other feedstuffs in the Dutch Pesticide Decree the value 0*(0.01) mg/kg is used. This value should be interpreted as the Limit of Detection for PCP. It was not taken into account that PCP could accumulate into the food chain, an adjustment of the Pesticide Decree is therefore desirable.

2 MATERIALS AND METHOD

2.1 Sampling and materials

The liver samples of cattle (n=11), pigs (n=10) and sheep (n=2) were obtained randomly from the National Programme "Overige Stoffen" and the chicken liver samples (n=10) were supplied by COVP "Het Spelderholt". Detailed information on the samples used is given in Appendix A.

2.2 Method of analysis

After homogenisation of the liver sample an aliquot was hydrolysed with H_2SO_4 to release the PCP, which was then extracted into cyclohexane before derivatisation with acetic anhydride. The derivative was then analysed with GC equipped with an electron capture detector. A detailed description is given in Appendix B.
Blank and recovery experiments were included for Good Laboratory Practice (GLP) purposes.

3 RESULTS AND DISCUSSION

3.1 PCP content in the liver samples

The individual results for each liver sample are given in table 1 (mg/kg product). Additional information on the age and origin of the samples is given in Appendix A

Table 1 Content of PCP in the liver of cattle, sheep, pigs and chickens (mg/kg product).

Rikiltnumber	Product	PCP (mg/kg product)
10394	pigs liver	0.03
10407	"	0.02
11453	"	0.02
11456	"	0.02
13002	"	0.03
13014	"	0.02
14088	"	0.02
14095	"	0.02
15214	"	0.03
15217	"	0.05
14077	cattle liver	0.01
14081	"	0.08
14093	"	0.07
51242	"	0.01
51243	"	0.18
51244	"	0.04
51245	"	0.01
51246	"	0.04
51247	"	0.07
51248	"	<0.01
51249	"	0.05
52527	chicken liver	<0.01
52528	"	<0.01
52529	"	<0.01
52530	"	0.01
52531	"	0.01
52532	"	0.02
52533	"	<0.01
52534	chicken liver	<0.01
52535	"	0.04
52536	"	<0.01
14086	sheep liver	0.06
14091	"	0.14

In table 2 a summary of the median value and range of the PCP results in the liver of cattle, sheep, pigs and chickens is given.

Table 2 Mean and range of PCP in animal liver (mg/kg product).

Product	median	range	n
chicken	<0.01	<0.01-0.04	10
pig	0.02	0.02-0.05	10
cattle	0.04	<0.01-0.18	11
sheep	0.10	0.06-0.14	2

The main routes for PCP to enter the animal are; through ingestion, through animal feed and water, inhalation, and dermal exposure e.g. from the wood shavings used in the raising of chickens. Based on the low results obtained in the chicken liver, there would appear to be no effect from the wood shavings. Chicken feed contains the highest fat content compared to the cattle and pig feed, therefore it seems that the animal fat added to the feed does not contribute to the PCP content in the liver.
With exception of one cow (Rikiltnr 14077) all chickens, pigs and calves received as diet only compounded animal feed and not grass or maize. The difference in the PCP contamination between the animals can not be explained from the data obtained in this study.

Further investigation of animal feed, grass, maize and kale should give information about the source of the PCP contamination.

3.2 Method of analysis

Samples of the same liver were spiked at two levels, 0.05 mg/kg and 1.25 mg/kg and then the extraction procedure and the derivatisation procedure carried out. The results were compared to derivatised standards made up at the same time from the same standard used for the spiking.

Quantity added	mg/kg	Recovery (%)	CV(%) (n=3)
0.2 ug	0.05	46.7	7.8
5.0 ug	1.25	92.3	2.5

The low recovery for the low level spiking (0.05 mg/kg) may be explained due to the amount of PCP in the liver used, which was 0.04 mg/kg, almost the same as the spike, therefore the errors involved were very great.

The coefficient of variation for the repeatability in a liver at the 0.05 mg/kg level was 18.8% (n=6) and at the 0.01 mg/kg level 55.3% (n=4). Based on this a limit of quantification of 0.01 mg/kg was used. The results reported are not corrected for recovery.

4 CONCLUSION

Detectable levels of PCP have been found in all the livers examined. The median value for PCP in the liver of chickens was <0.01 mg/kg, range <0.01-0.04 mg/kg (n=10), of pigs was 0.02 mg/kg, range 0.02-0.05 mg/kg (n=10), of cattle was 0.04 mg/kg, range <0.01-0.18 mg/kg (n=11) and of sheep (n=2) respectively 0.06 and 0.14 mg/kg.

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APPENDIX A

INFORMATION ON THE ORIGIN OF THE LIVER SAMPLES

Rikilt number	Product	Age	Origin
10394	pig	5 months	*
10407	"	5 "	Chaam
11453	"	6 "	*
11456	"	6 "	*
13002	"	*	*
13014	"	6 months	*
14088	"	4 "	*
14095	"	9 "	*
15214	"	*	*
15217	"	*	*
14077	cattle	2 years	Enkhuizen
14081	"	6 months	Nieuwkerk a/d IJssel
14093	"	*	*
51242	"	5 months	*
51243	"	5 "	*
51244	"	5 "	*
51245	"	5 "	*
51246	"	5 "	*
51247	"	5 "	*
51248	"	5 "	*
51249	"	5 "	*
52527	chicken	6 weeks	Achtmaal
52528	"	6 "	Holten
52529	"	6 "	Bergentheim
52530	"	6 "	Hoeve
52531	"	6 "	Balkbrug
52532	"	6 "	Beltrum
52533	"	6 "	Loenen
52534	"	6 "	Didam
52535	"	6 "	Sinderen
52536	"	6 "	Broekland
14086	sheep	2 years	Herveld
14091	"	1 "	*

* Information not provided.

APPENDIX B

State Institute for Quality Control of Agricultural Products,
Wageningen, The Netherlands.

1990-09-07

METHOD OF ANALYSIS FOR PENTACHLOROPHENOL IN LIVER

1 Scope

The determination of pentachlorophenol (PCP) in the liver of chickens, pigs, sheep and cattle. The limit of detection is 0.005 mg/kg on a product basis. Recovery experiments at the 1 mg/kg level show results better than 80 %.

2 Principle

After homogenisation of the liver an extraction is carried out with sulphuric acid. PCP is extracted in cyclohexane and derivatised with acetic anhydride before gas chromatographic detection using an electron capture detector.

3 Reagents

The chemicals used should not give interferences at the retention time of the derivatised PCP.

3.1 Cyclohexane, distilled

3.2 Pentachlorophenol, 99 % (Analabs, North Haven, U.S.A.), standard solutions of 1, 10, 100 ug/ml were made up in distilled cyclohexane

3.3 Potassium carbonate 0.1M solution
Dissolve 13.8g in 1000 ml of distilled water

3.4 Acetic anhydride, p.a.

3.5 Sodium sulphate, p.a.
Dried at 150°C in an oven for at least 16 hours

3.6 CB 198 (2,3,4,5,6,-2'3'5' octachlorobiphenol) 2 ug/ml in cyclohexane, is used as the internal standard

3.7 Sulphuric acid 12M solution
Add 2 volumes sulphuric acid (18M) to 1 volume distilled water keeping the flask cool with running water

4 Apparatus

4.1 Waterbath

4.2 Centrifuge capable of at least 1500g

4.3 Mixer

4.4 Gas Chromatograph (Perkin Elmer 8700)
Equipped with an electron capture detector, CP Sil 8 CB column
Length 25 m, i.d. 0.25 mm, film thickness 0.41 um.
Temp. program: 80°C(2 min) - rate 10°C/min - 240°C(10 min)
Injector 220°C, Detector 300°C
Carrier gas: Helium about 35 cm/s
Make up gas: Argon:Methane (95:5) about 30 ml/min