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Preparation and homogeneity of a candidate animal feed reference material organochlorine pesticides (CRM 115)

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ABSTRACT

Preparation and homogeneity of a candidate animal feed reference material organochlorine pesticides
(CRM 115)

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8 tables, 2 figures, 1 reference, 15 pages.

A candidate reference material animal feed enriched with organochlorine pesticides at about 0.02 mg/kg for aldrin, dieldrin, heptachlor, β - heptachlorepoxide, HCB, α - HCH, β - HCH and γ - HCH and about 0.05 mg/kg for γ - chlordane, α - endosulfan, endrin, p,p'-DDE, p,p'-TDE, o,p'-DDT and p,p'-DDT was prepared, by adding an oil enriched with pesticides to a first mixture of homogenised chicken and swine feed components. After control on homogeneity this premixture was added to the rest of the homogenised chicken and swine feed components. The final mixture was ampouled. All steps in the preparation of the candidate animal feed reference material organochlorine pesticides were checked on homogeneity.
The coefficient of variation in the spiked oil used in the preparation of the premixture was about 2.5% (n=20), in the premixture about 3.7% (n=20) and in the final mixure about 4.3% (n=10).
The coefficient of variation for the standard solution analysed at the same time as the final mixture, was about 3.2% (n=20).
After ampouling of the final mixture comparable results were measured for the within and between homogeneity of the pesticides. The results indicate an excellent homogeneity of the candidate animal feed reference material organochlorine pesticides.

Keywords: organochlorine pesticides, homogeneity, animal feed, reference material.

SAMENVATTING

Een kandidaat referentiemateriaal mengvoeder gecontamineerd met organochloorbestrijdingsmiddelen op het 0,02 mg/kg niveau voor aldrin, dieldrin, heptachloor, β - heptachloorepoxide, HCB, α - HCH, β - HCH and γ - HCH resp. 0,05 mg/kg niveau voor γ - chloordaan, α - endosulfan, endrin, p,p', p,p'-TDE, o,p'-DDT en p,p'-DDT werd bereid. Door aan een olie gecontamineerd met organochloorbestrijdingsmiddelen een hoeveelheid van het gehomogeniseerde basisvoeder, gebaseerd op legpluimvee en varkensvoer, toe te voegen en te homogeniseren werd eerst een premix verkregen. Deze premix werd na controle op homogeniteit met het overige basisvoeder gemengd en gehomogeniseerd. Het verkregen voeder werd geampulleerd.

Tijdens de bereiding is nagegaan of er in de afzonderlijke grondstoffen geen interferenties met de bestrijdingsmiddelen optraden.

Tevens is in alle fasen van de bereiding de homogeniteit gecontroleerd. De variatiecoëfficiënt voor de herhaalbaarheid in de gecontamineerde olie bedroeg ca. 2,5% (n=20) in de premix ca. 3,7% (n=20) en in het uiteindelijke mengvoeder ca. 4,3% (n=10). In de op hetzelfde tijdstip geanalyseerde standaardoplossing bedroeg de variatiecoëfficiënt voor de herhaalbaarheid ca. 3,2% (n=20).

Na het uitvullen van het mengvoeder in de ampullen is opnieuw de homogeniteit bepaald. Deze was vergelijkbaar met de voor ampulleren gemeten variatiecoëfficiënt voor de herhaalbaarheid.

Uit de resultaten blijkt dat de homogeniteit van het referentiemateriaal mengvoeders gecontamineerd met organochloorbestrijdingsmiddelen uitstekend is.

CONTENTS page

1 INTRODUCTION

The role of certified reference materials for verification of the accuracy of methods and for development of new analytical methods is today well established.

The role of the Community Bureau of Reference (BCR) of the European Communities (EC) is to assist in improving the quality and accuracy of the analysis to avoid trade barriers between EC member states.

The reason for the preparation of an animal feed enriched with organochlorine pesticides was necessary, due to the modification of EC directive 74/63 containing maximum residue limits (MRL's) for organochlorine pesticides in animal feed and animal fat. The main considerations for the modification were the danger of the persistent organochlorine pesticides for the health of mankind and animals, and the need of uniform rules in all EC member states to avoid problems in the trade between the countries.

Although the use of organochlorine pesticides has been restricted or even banned throughout the EC, they are still present in animal fat. Due to recirculation of animal fat, containing organochlorine pesticides, in animal feed and the use of feed ingredients imported from countries that are still using organochlorine pesticides, animal feed can contain a contamination with these pesticides.

Therefore a control on organochlorine pesticide residues in animal feed is necessary.

The maximum residue limits established for animal feed in the modified EC directive 74/63 are summarized in table 1.

Table 1. Maximum residue limits of organochlorine pesticides in animal feed.

Pesticide	MRL (mg/kg)*
Aldrin and/or Dieldrin	0,01
Chlordane (including isomers)	0,02
DDT (including isomers)	0,05
Endosulfan (including isomers)	0,10
Endrin (including isomers)	0,01
Heptachlor (including β - heptachlorepoxide)	0,01
Hexachlorobenzene (HCB)	0,01
α - Hexachlorocyclohexane (α - HCH)	0,02
β - Hexachlorocyclohexane (β - HCH)	0,01
γ - Hexachlorocyclohexane (γ - HCH)	0,20

*) expressed at a moisture content of 12%.

2 MATERIALS AND METHOD

2.1 Preparation of the animal feed

2.1.1 Preliminary study on irradiation influence on pesticides

A small batch of animal feed enriched with pesticides shall be irradiated using a 2.5 and 5 kGy source, to check if it is possible to preserve the feed for a longer period. The pesticide content shall be determined before and after irradiation. The pesticide content should not decrease by more than 20% (m/m).

2.1.2 Selection of the ingredients

The ingredients used for the animal feed shall resemble a mixture of pig and poultry diets.

Table 2. Final composition of the feed.

Ingredient	%
Wheat	10.0
Corn	15.0
Milocorn	10.0
Peas	8.0
Soybean oilmeal	14.0
Hominy feed	5.0
Tapioca	17.0
Sunflower meal	8.0
Corngluten meal	2.0
Wheat middlings	4.2
Soybean oil	0.875
Animal fat	1.625
Ground limestone	2.0
Monocalcium phosphate	1.0
Salt	0.3
Vitamin-mineral mixture*	1.0

* The vitamin-mineral mixture supplied per 1 kilogramme feed: 5 mg riboflavine, 30 mg niacin amide, 12 mg d-pantothenic acid, 150 mg cholinechloride, 40 mg dl-a-tocopherol acetate, 3 mg menadione, 40 μg cobalamin, 50 mg ascorbic acid, 9000 IU vitamin A, 1800 IU vitamin D3, 40 mg $CuSO_4.5H_2O$, 200 mg $ZnSO_4.H_2O$, 70 mg MnO_2, 400 mg $FeSO_4.7H_2O$, 2.5 mg $CoSO_4.7H_2O$, 0.2 mg Na_2SeO_3, 0.5 mg KI.

The pesticide level shall not exceed 1% (m/m mass fraction) of the final contamination level after enrichment with pesticides. Special attention shall be given to the absence of α - chlordane and β - endosulfan. Depending on the polarity of the capillary column α-chlordane can interfere with α-endosulfan and β-endosulfan with endrin.

2.1.3 Preparation of the basic diet

If the obtained product meets the requirements, its moisture content shall be reduced to less than 6% (m/m) and a γ-irradiation for the stabilisation of the feed shall be carried out. After the sterilization, the feed shall be ground to pass a sieve with apertures of 100 μm and shall be homogenised in a mixing drum. All measures shall be taken to avoid contamination. In total at least 50 kg of homogenous material shall be produced with a final moisture content of less than 6% (m/m). The material shall be stored under dry inert gas in a closed container.

2.1.4 Preparation of the enriched oil.

A batch of 600 g oil enriched with pesticides at the 2 mg/kg level for aldrin, dieldrin, heptachlor, γ - heptachlorepoxide, HCB, α - HCH, β - H and γ - HCH (lindane) and at the 5 mg/kg level for γ - chlordane, α - endosulfan, endrin, p,p'DDE, p,p'-TDE, o,p'-DDT and p,p'-DDT shall be prepared. The homogeneity of the oil spiked with pesticides shall be checked for each pesticide. The coefficient of variation of 20 determinations of each compound shall not exceed 8%,

2.1.5 Preparation of the premixture.

If the homogeneity of the enriched oil is satisfactory a first mixture of 0.5 kg enriched oil with 9.5 kg of homogenised basal diet (2.1.3) shall be prepared. After homogenisation, at least for 48 hours in a mixing drum, the homogeneity shall be checked. The coefficient of variation of 20 determinations of each compound shall not exceed 10%. If the homogeneity is not satisfactory a rehomogenisation shall be carried out and the homogeneity shall be checked again.

2.1.6 Preparation of the final mixture

When the homogeneity of the premixture is satisfactory the premix shall be added to the homogenised basal diet (2.1.3) to obtain the desired final concentration of 0.02 mg/kg for aldrin, dieldrin, heptachlor, β - heptachlorepoxide, HCB, α - HCH, β - HCH and γ - HCH and 0.05 mg/kg for γ - chlordane, α - endosulfan, endrin, p,p'-DDE, p,p'-TDE, o,p'-DDT and p,p'-DDT. The mixture shall be grind and sieved to pass a sieve with apertures of 100 μm and homogenised for at least 48 hours. The moisture content shall not exceed 6% (m/m). The homogeneity shall be verified on 10 samples. The coefficient of variation shall not exceed 12%. The coefficient of varation of the gaschromatographic analysis shall be established by 20 replicate injections of the standard solution. If the results are not satisfactory the material shall be rehomogenised until the homogeneity fulfils the above requirements.

2.1.7 Stabilisation and ampouling of the final mixture

If the bulk homogeneity of the final mixture is satisfactory 20 batches of 50 ampoules shall be prepared with at least 30 g material in each. After each batch the remaining material shall be rehomogenised for 30 minutes. One ampoule out of each batch of 50 shall be taken for the between ampoule homogeneity study.
If the preliminary study (2.1.1) revealed that the pesticides are not affected by the gamma-rays the ampoules shall be irradiated under the most suitable conditions.

2.1.8 Homogeneity study

For the between ampoule homogeneity of the material the pesticide content shall be determined in one replicate and on each of 20 selected ampoules. The within ampoule homogeneity shall be assessed by determining the pesticides by five replicate determinations on each of four ampoules. The coefficient of variation of the within and/or between ampoule homogeneity should not exceed more than 15%.

2.2 Method of analysis

The oil enriched with organochlorine pesticides was dissolved in a mixture of cyclohexane/ethyl acetate. An aliquot is cleaned using gel permeation chromatography. In the pesticide fraction aldrin, dieldrin, heptachlor, β - heptachlorepoxide, HCB, α - HCH, β - HCH, γ - HCH, γ - chlordane, α - endosulfan, endrin, p,p'-DDE, p,p'-TDE, o,p'-DDT and p,p'-DDT were determined on a capillary column using gaschromatography and electron capture detection [Roos].
The ingredients, premixture and final candidate reference material animal feed were analysed according to RIKILT-DLO method of analysis A167. After homogenisation of the sample an extraction of the pesticides from the feed was carried out by shaking in iso-octane for 30 minutes.
The extract of 1 g animal feed was cleaned on a chromatographic column containing basic alumina inactivated with water by eluting with iso-octane. The eluate containing the above mentioned pesticides was injected direct into the gas chromatographic system. Analysis of the final mixture will be carried out on a apolair Supelco 5 column and a polar CL Sil 19 column.

3 RESULTS AND DISCUSSION

3.1 Irradiation damage study

A small batch of feed spiked with pesticides was irradiated under a 2.5 kGy source and under 5.0 kGy. From each level and from the starting material (0 kGy) five samples were analysed.

In table 3 the results are given related to the 0 kGy level which was set at 100% for each pesticide for the mean result of the five determinations. Also the standard deviation is reported.

Table 3. Results irradiation damage study

Pesticide	0 kGy (n=5)	2.5 kGh (n=5)	5.0 kGh (n=5)
HCB	100 ± 4.4	101.1 ± 5.3	104.4 ± 1.7
α - HCH	100 ± 2.6	99.1 ± 6.5	95.1 ± 2.0
β - HCH	100 ± 7.8	90.1 ± 8.4	101.4 ± 6.2
γ - HCH (Lindane)	100 ± 3.1	54.3 ± 3.6	51.7 ± 3.0
Heptachlor	100 ± 4.6	110.3 ± 2.6	110.8 ± 2.0
Aldrin	100 ± 2.6	107.9 ± 2.8	106.0 ± 2.2
β - Heptachlorepoxide	100 ± 6.5	103.1 ± 2.5	98.6 ± 3.4
γ - Chlordane	100 ± 2.3	97.2 ± 4.3	101.5 ± 3.4
α - Endosulfan	100 ± 3.6	78.5 ± 2.6	75.8 ± 3.2
p,p'-DDE	100 ± 4.2	93.9 ± 4.0	102.6 ± 3.0
Dieldrin	100 ± 1.4	92.8 ± 2.7	95.4 ± 5.0
Endrin	100 ± 2.7	102.2 ± 6.9	90.1 ± 2.2
p,p'-TDE	100 ± 3.4	103.5 ± 4.6	99.0 ± 3.9
o,p'-DDT	100 ± 3.6	97.7 ± 3.5	101.6 ± 2.1
p,p'-DDT	100 ± 2.4	83.7 ± 4.6	85.0 ± 2.2

Based on these results, especially the significant decrease of γ - HCH (lindane) and the lower results for α - endosulfan, endrin and p,p'-DDT it was decided not to use γ - irradiation for the final mixture. The bacterial reduction at 2.5 kGy is only 90%. The reduction of the moisture content to less than 6% (m/m) will be more effective to avoid bacterial grow than irradiation of the feed.

3.2 Ingredients

The feed was composed of commonly used feed ingredients in Dutch practice for formulating pig and poultry diets (Table 2). Before inclusion in the diet all batches of feed ingredients were ground and homogenised. To preserve the feed for a longer period all ingredients were γ - irradiated using a 10 kGy source. The vitamin-mineral mixture was also of a practical composition. This mixture was adequately homogenised.

Next a sample of each ingredient was taken for control on residues of organochlorine before inclusion in the diet. Until mixing all ingredients, exclusive fat and oil, were stored at 15°C. Fat and oil were stored at -20°C.

All individual ingredients were searched for residues of organochlorine pesticides on the apolair Supelco 5 column. No interferences were observed reaching a level of 1% (m/m mass fraction) of the final contamination level in the candidate reference material animal feed.

Special attention was given to the absence of the pesticides α - chlordane and β - endosulfan. The ingredients contained no residues of these components.

3.3 Basic diet

The basic diet was prepared in an amount of 70 kg. Before inclusion, animal fat was brought in a liquid form by heating. The basic diet was homogenised by a Vrieco mixer. The mixing time was 15 minutes. Next the batch basic diet was dried to lengthen the tenability of the feed by using an Almex 15 KW3 dryer, and involved a continuous drying for 40 hrs with air of 50°C. After finishing the drying process, the feed was analysed for dry matter. This analysis was carried out in duplo. A dry matter content of 95.8% was found in the sample. Next the feed was ground by using a pen mill to achieve the desired fineness of $\leq$ 100 μm. The latter was controlled by passing the feed through a Retch vibaration sieve of 100 μm. Then the feed was homogenised by using a Vrieco mixer. The mixing time was 5 minutes. Finally the feed was packed in plastic bags and sterilized by gamma irradiation under a 10 kGy source as a possible way to preserve the feed for a longer period.

3.4 Enriched oil

The standards used for the preparation of the enriched oil, obtained from Ehrenstorfer, Augsburg (Germany) and Analabs, North Haven, Connecticut (USA), had a purity higher than 99%. A solution was made of each pesticide in iso-octane. Suitable volumes were combined to obtain a mixture of desired concentrations for the spike of the oil. A portion of 600 g oil was spiked with aldrin, dieldrin, heptachlor, β - heptachlorepoxide, HCB, α - HCH, β - HCH and γ - HCH to a level of 2 mg/kg each and with γ - chlordane, α - endosulfan, endrin, p,p'-DDE, p,p'-TDE, o,p'-DDT and p,p'-DDT to a level of 5 mg/kg each. The oil was homogenised with a magnetic stirrer using a stirring bar in the oil at a temperature of 50 - 60°C.

The statistical evaluation of the results of 20 samples oil was carried out according to ISO 5725. In two samples results for all pesticides were outlier and eliminated.

The coefficient of variation (CV) for the homogeneity in the enriched oil (n=18) summarized in table 4 (column 2) ranged from 2.1 to 3.8% and met the requirement for the CV of 8% for each pesticide.

3.5 Premixture

From the sterilized basic diet, a representative sample of 9.88 kg was taken. To this sample about 520 g of the oil spiked with pesticides was added. By weighing it was shown to be 513.4 g oil. Before addition, the oil was made liquid by placing the bottle in a water bath of 60°C. Feed with the spiked oil was premixed in a Stephan cutter. Next it was further homogenised by using a planet mixer (Colette MP 900). The mixing time of the premixture was 48 hours.

The statistical evaluation of the results of 20 samples premixture was carried out according to ISO 5725. In one samplle an outlier was found for α-endosulfan and this value was eliminated.

The CV for the homogeneity in the premixture (n=20) summarized in table 4 (column 3) ranged from 2.6 to 6.1% and met the requirement for the CV of 10% for each pesticide.

3.6 Final mixture

From the sterilized basic diet, a representative amount of 32 kg was taken. To this amount of feed 8 kg of the premixture was added and homogenised. This was done by using a planet mixer (Colette MP 90). The mixing time was 48 hours. After termination of the mixing procedure, samples were collected for the determination of the homogeneity. The remaining feed was packed in a plastic bag, and put into a container. The container with feed was kept at 5°C until the time it was transported.

The statistical evaluation of the results of 10 samples final mixture was carried out according to ISO 5725. An outlier was found for two data of dieldrin in the final mixture and for one data of endrin in the standard solution and these data were eliminated.

The CV for the homogeneity in the final mixture (n=10) summarized in table 4 (column) ranged from 2.8 to 6.2% and met the requirement for the CV of 12% for each pesticide.

The CV measured in the standard solution (n=20) at the same time as the final mixture ranged from 2.1 to 4.3%. From these results it is clear that the CV in the gaschromatographic analysis of the pesticides is in the same order as in the samples.

3.7 Ampouling

A perspex drum was, after filling with animal feed, rotated during 2 hours to homogenise the feed. During this the drum was flushed with dry nitrogen gas. This flushing with nitrogen continued during and after the opening of the drum.

By means of a glass spoon, approximately 30 g of the animal feed was introduced into each of the ampoules. For this process a long stemmed funnel was used in order to prevent contamination of the sealing tube.

After dry nitrogen was introduced into the ampoules they were sealed by melting and sealing the sealing tube. The sealing was done in the flame of a natural gas/oxygen burner.

In this way 20 series of 50 ampoules each were filled. After completion of each series the feed in the rotating drum was again homogenised during 30 minutes.

3.8 Homogeneity of ampouled feed

The CV of the within and between homogeneity of the pesticides in the ampouled animal feed analysed on the apolair Supelco 5 column respectively the polar CP Sil 19 column is summarized in table 5 and 6.

The statistical evaluation was carried out according to ISO 5725. In the between homogeneity check an outlier was found for α-HCH on the Supelco 5 column and these data was eliminated. For HCB and α-endosulfan an outlier was found on the CP Sil 19 column and these data were eliminated. The results for β - heptachlorepoxide, α - endosulfan and p,p'-DDT on the Supelco 5 column were influenced by negative peaks. The repeatabilty of eight standard injections carried out during the homogeneity test, especially for late eluting components, was at that time higher (up to 7%) than normal obtained (below 5%).

The results on the CP Sil 19 column of the same extracts show also that the homogeneity for all components in the final mixture met the requirements (below 12%). The response for o,p'-DDT and p,p'-DDT was below the limit of quantification. The results for β-HCH and p,p'-TDE on the CP Sil 19 column were influenced by negative peaks. For the stability study gaschromatographic conditions were changed to eliminate this problem. Taking into account the repeatability of the injection of standard solutions an excellent homogeneity in the animal feed is measured for the within and between homogeneity.

The homogeneity was also tested with the F-test. The test confirmed the homogeneity of the candidate reference material animal feed.

The mean content of the pesticides measured on the Supelco 5 column and the CP Sil 19 column is summarized in table 7 and 8. All results are corrected for recovery.

The theoretical content should be, depending on the pesticide 0.02 mg/kg for aldrin, dieldrin, heptachlor, β - heptachlorepoxide, HCB, α - HCH, β - HCH and γ - HCH respectively 0.05 mg/kg for γ - chlordane, α - endosulfan, endrin, p,p'-DDE, p,p'-TDE, o,p'-DDT and p,p'-DDT.

In practice these values should be, due to a correction of the moisture content (4,2%) and a lower amount of the spiked fat (513,4 g instead of 520 g), respectively 0.0206 and 0.0516 mg/kg on dry mass. The results of β - heptachlorepoxide, α - endosulfan and p,p'-DDT on the Supelco 5 column and β - HCH and p,p'-TDE on the CP Sil 19 column are not reported as these components are interfered by negative peaks (zie figure 1 and 2).

The obtained results are in general within the expected range (0.02 $\pm$ 0.002 mg/kg and 0.05 $\pm$ 0.005 mg/kg). Only for γ - chlordane, p,p'-TDE and p,p'-DDT a higher deviation from the theoretical value is measured. There is no explanation for the higher results of γ - chlordane. The higher value for p,p'-TDE may be caused by degradation of p,p'-DDT.

4 CONCLUSION

A candidate reference material animal feed enriched with organochlorine pesticides at about 0,02 mg/kg for aldrin, dieldrin, heptachlor, β - heptachlorepoxide, HCB, α - HCH, β - HCH and γ - HCH and about 0,05 mg/kg for γ - chlordane, α - endosulfan, endrin, p,p'-DDE, p,p'-TDE, o,p'-DDT and p,p'-DDT was prepared, by adding an oil enriched with pesticides to a first mixture of homogenized

chicken and swine feed components. After control on homogeneity this premixture was added to the rest of the homogenized chicken and swine feed components. The final mixture was ampouled. All steps in the preparation of the candidate animal feed reference material organochlorine pesticides were checked on homogeneity.

The coefficient of variation in the spiked oil used in the preparation of the premixture was about 2.5% (n=20), in the premixture about 3.7% (n=20) and in the final mixure about 4.3% (n=10).

The coefficient of variation for the standard solution analysed at the same time as the final mixture, was about 3.2% (n=20). After ampouling comparable results were measured for the within and between homogeneity of the pesticides. The results indicate an excellent homogeneity of the candidate animal feed reference material organochlorine pesticides.

LITERATURE

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Table 4: Coefficient of variation for the homogeneity of the pesticides content during the preparation of the animal feed (%)

Pesticide	Enriched oil (n=18)[1)]	Premixture (n=20)	Final mixture (n=10)	Standard solution (n=20)
α-HCH	2.1	3.6	3.7	2.8
HCB	2.3	3.3	3.3	2.4
ß-HCH	2.1	4.1	5.5	3.2
γ-HCH	2.5	3.6	3.8	2.1
Heptachlor	2.2	4.3	4.4	3.7
Aldrin	2.3	6.1	3.6	2.7
ß-Heptachlorepoxide	2.3	3.4	6.2	4.2
γ-Chlordane	2.6	3.2	4.4	2.9
α-Endosulfan	2.3	4.6[1)]	5.6	2.9
p,p´-DDE	2.6	3.8	4.1	3.2
Dieldrin	2.1	2.9	2.8[1)]	4.0
Endrin	2.4	2.6	3.6	3.5[1)]
p,p´-TDE	3.4	4.3	4.0	2.4
o,p´-DDT	3.1	3.7	3.6	2.8
p,p´-DDT	3.8	3.6	3.7	4.3

1) After elimination of outliers, according to ISO 5725 (see text).

Table 5: Within and between homogeneity of the pesticides in the ampouled animal feed - Supelco 5 column - (%).

Pesticide	CV(R) between ampoules (n=20)	CV(r) within ampoules 1(n=5)	2(n=5)	3(n=5)	4(n=5)	CV Standard (n=8)
α-HCH	4.4[2)]	4.4	2.8	5.6	6.8	4.0
HCB	4.4	5.0	3.5	6.3	3.6	4.2
ß-HCH	4.8	4.3	5.8	5.3	4.8	4.0
γ-HCH	5.3	4.7	8.9	10.3	4.5	4.4
Heptachlor	4.8	3.7	4.8	5.7	3.0	4.0
Aldrin	3.9	3.5	3.4	6.6	5.7	4.7
ß-Heptachlorepoxide[1)]						
γ-Chlordane	5.5	4.6	3.4	5.1	5.8	4.7
α-Endosulfan[1)]						
p,p´-DDE	5.2	4.4	3.8	5.7	6.9	5.4
Dieldrin	5.6	4.5	3.3	4.2	6.2	3.0
Endrin	7.0	4.5	6.8	6.4	8.2	5.8
p,p´-TDE	6.0	5.4	3.1	4.1	8.3	6.2
o,p´-DDT	6.6	5.4	5.3	4.3	6.8	7.0
p,p´-DDT[1)]						

1) interfered by a negative peak
2) after elimination of outliers according to ISO 5725 (see text)

Table 6: Within and between homogeneity of the pesticides in the ampouled animal feed. - CP Sil 19 column - (%)

Pesticide	CV(R) between ampoules (n=20)	CV(r) within ampoules 1(n=5)	2(n=5)	3(n=5)	4(n=5)	CV Standard (n=8)
α-HCH	3.6	2.9	4.8	5.3	2.9	3.0
HCB	3.4[2)]	2.4	5.6	3.8	3.1	2.4
ß-HCH[1)]						
γ-HCH	3.3	3.0	5.7	4.4	2.7	3.9
Heptachlor	2.8	4.1	3.4	5.2	3.0	4.4
Aldrin	3.3	3.3	5.2	5.0	3.0	2.7
ß-Heptachlorepoxide	3.8	2.2	4.0	4.2	3.5	2.7
γ-Chlordane	3.3	5.3	6.7	7.3	4.1	2.5
α-Endosulfan	3.1[3)]	3.5	5.9	3.8	5.3	2.4
p,p´-DDE	3.4	3.7	6.4	5.1	4.2	3.4
Dieldrin	3.9	5.4	6.4	5.0	5.9	2.8
Endrin	3.6	3.1	6.6	4.7	4.3	1.3
p,p´-TDE [1)]						
o,p´-DDT[2)]						
p,p´-DDT[2)]						

1) interfered by a negative peak
2) gaschromatographic response below limit of quantification
3) after elimination of outliers according to ISO 5725 (see text)

Table 7: Mean concent of organochlorine pesticides determined in the ampouled feed in the between and within homogeneity check on the Supelco 5 column (mg/kg on a dry mass basis)

Pesticide	Between ampoules (n=20)	Within ampoules 1(n=5)	2(n=5)	3(n=5)	4(n=5)
α-HCH	0.0205	0.0189	0.0185	0.0186	0.0193
HCB	0.0193	0.0179	0.0175	0.0166	0.0187
ß-HCH	0.0223	0.0212	0.0202	0.0196	0.0216
γ-HCH	0.0191	0.0177	0.0172	0.0172	0.0187
Heptachlor	0.0196	0.0181	0.0180	0.0169	0.0193
Aldrin	0.0210	0.0195	0.0189	0.0183	0.0201
ß-Heptachlorepoxide[1)]					
γ-Chlordane	0.0585	0.0520	0.0521	0.0500	0.0567
α-Endosulfan[1)]					
p,p´-DDE	0.0522	0.0465	0.0450	0.0438	0.0498
Dieldrin	0.0211	0.0191	0.0188	0.0179	0.0206
Endrin	0.0536	0.0470	0.0460	0.0430	0.0499
p,p´-TDE	0.0697	0.0618	0.0598	0.0578	0.0680
o,p´-DDT	0.0481	0.0425	0.0146	0.0404	0.0467
p,p´-DDT[1)]					

1) interfered by a negative peak

Table 8: Mean concent of organochlorine pesticides determined in the ampouled feed in the between and within homogeneity check on the CP Sil 19 column (mg/kg on a dry mass basis)

Pesticide	Between ampoules (n=20)	Within ampoules 1(n=5)	2(n=5)	3(n=5)	4(n=5)
α-HCH	0.0208	0.0194	0.0191	0.0200	0.0194
HCB	0.0203	0.0191	0.0190	0.0199	0.0194
ß-HCH[1)]					
γ-HCH	0.0197	0.0185	0.0183	0.0189	0.0186
Heptachlor	0.0193	0.0182	0.0184	0.0185	0.0189
Aldrin	0.0208	0.0199	0.0196	0.0204	0.0200
ß-Heptachlorepoxide	0.0198	0.0181	0.0184	0.0193	0.0187
γ-Chlordane	0.0608	0.0581	0.0584	0.0618	0.0584
α-Endosulfan	0.0498	0.0470	0.0472	0.0492	0.0480
p,p´-DDE	0.0524	0.0484	0.0491	0.0519	0.0504
Dieldrin	0.0204	0.0188	0.0197	0.0192	0.0195
Endrin	0.0570	0.0527	0.0512	0.0521	0.0521
p,p´-TDE[1)]					
o,p´-DDT[2)]					
p,p´-DDT[2)]					

1) interfered by a negative peak
2) gaschromatographic response below limit of quantification

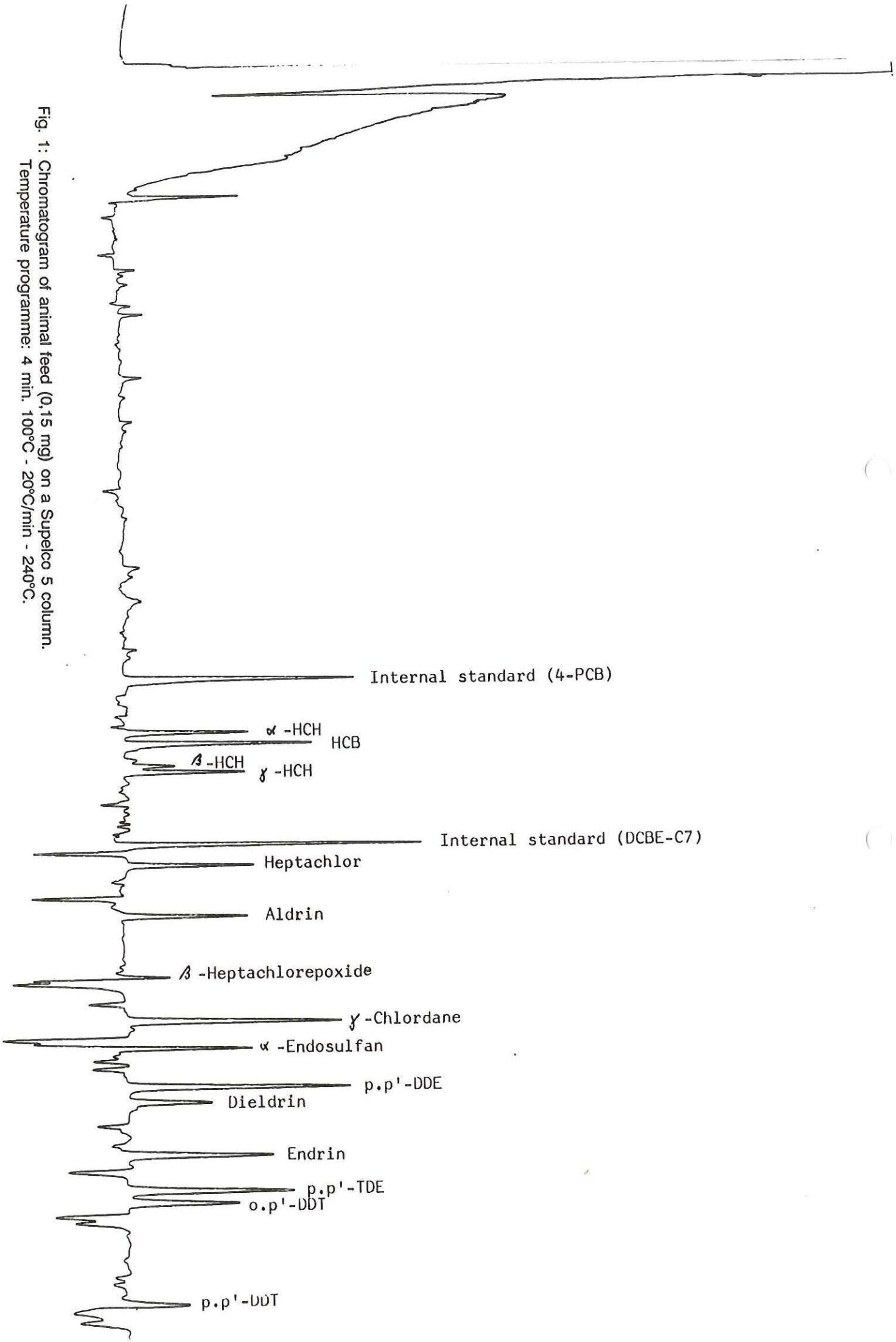

Fig. 1: Chromatogram of animal feed (0,15 mg) on a Supelco 5 column.
Temperature programme: 4 min. 100°C - 20°C/min - 240°C.