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Management: ir. L.G.M.Th. Tuinstra

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STABILITY OF ORGANOCHLORINE PESTICIDES IN A CANDIDATE ANIMAL FEED REFERENCE MATERIAL (CRM 115)

A.H. Roos, ir. L.G.M.Th. Tuinstra and ing. A.M. Matser

The investigation was carried out on behalf of and for account of the Community Bureau of Reference (BCR), Brussels, Belgium (Contract 5322/1/9/358/90/03-BCR-NL(10))

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ABSTRACT

Stability of organochlorine pesticides in a candidate animal feed reference material (CRM 115)

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6 tables, 13 figures, 21 pages, 1 reference

A candidate reference material animal feed enriched with organichlorine pesticides at about 0.02 mg/kg for aldrin, dieldrin, heptachlor, ß-heptachlorepoxide, α-HCH, ß-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 stored at a temperature of -20°C, 20°C and 37°C to check the stability.
A significant decrease in the content of α-HCH, γ-HCH, heptachlor and o,p'-DDT was measured in the animal feed samples stored at 37°C. In the samples stored at -20°C and 20°C no decrease in the pesticide content was found during a period of one year. For p,p'-TDE at a temperature of 37°C a significant increase was measured due to degradation of p,p'-DDT. Due to negative peaks in the gas chromatographic analysis no results for p,p'-DDT, α-endosulfan and ß-heptachlorepoxide are reported. Based on the results of the stability study it should be recommended to store the reference material at a temperature below 20°C.

Keywords: organochlorine pesticides, stability, animal feed, reference material

SAMENVATTING

Een kandidaat referentiemateriaal mengvoeder gecontamineerd met organochloorbestrijdingsmiddelen op het 0,02 mg/kg niveau voor aldrin, dieldrin, heptachloor, ß-heptachloorepoxide, HCB, α-HCH, ß-HCH and γ-HCH resp. 0,05 mg/kg niveau voor γ-chloordaan, α-endosulfan, endrin, p,p'-DDE, p,p'-TDE, o,p'-DDT en p,p'-DDT werd voor controle op de stabiliteit bij -20°C, 20°C en 37°C opgeslagen. Een significante afname van het α-HCH, γ-HCH, heptachloor en o,p'-DDT gehalte werd waargenomen in de monsters bewaard bij 37°C. In de monsters bewaard bij -20°C en 20°C werd gedurende één jaar geen afname in de pesticidegehalte vastgesteld. Voor p,p'-TDE werd bij 37°C een significante toename vastgesteld ten gevolge van ontleding van p,p'-DDT. Voor p,p'-DDT, α-endosulfan en ß-heptachloorepoxide is door gaschromatografische problemen (negatieve pieken) geen uitspraak te doen.

Op basis van de resultaten van de stabiliteitsstudie kan geconcludeerd worden dat het kandidaat referentiemateriaal bij een temperatuur lager dan 20°C opgeslagen moet worden.

CONTENTS page

1 INTRODUCTION

The role 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 for animal feed in the modified EC directive 74/63 are summarized in table 1.

Tabel 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 ß-heptachlorepoxide)	0,01
Hexachlorobenzene (HBC)	0,01
α-Hexachlorocyclohexane (α-HCH)	0,02
ß-Hexachlorocyclohexane (ß-HCH)	0,01
γ-Hexachlorocyclohexane (γ-HCH)	0,20

*) expressed at a moisture content of 12%.

The results of the preparation and homogeneity are reported earlier [Roos]. In this report results of the stability are reported.

2 MATERIALS AND METHOD

2.1 Stability study

After receipt of the samples, at random 60 ampoules should be selected and directly stored at -20°C, 20°C and 37°C. At each time in one replicate in each of five bottles HCB, α-HCH, ß-HCH, γ-HCH, Aldrin, Dieldrin, Heptachlor, ß-Heptachlorepoxide, γ-Chlordane, α-Endosulfan, Endrin, p,p'-DDE, o,p'-DDT, p,p'-TDE and p.p'-DDT should be determined. To assess a good long term reproducibility of the analysis at each occasion of analysis the pesticide content in a raw extract prepared at the beginning of the stability study and stored at -20°C should be determined.
The sampling program and code of the samples used for the stability study is given in table 2.

Table 2. Sampling program and code of samples for the stability study on pesticides in a candidate reference material animal feed (CRM 115).

Period of analysis/ Code of samples	Storage temperature		
	-20°C	20°C	37°C
July 1991	197-296-585-872-878	275-442-446-513-953	514-559-620-676-811
September 1991	002-178-344-462-712	107-384-444-601-674	078-336-661-710-711
December 1991	141-286-348-494-923	130-396-608-672-797	555-785-874-901-906
June 1992	072-176-204-589-780	009-097-232-505-973	201-382-704-810-975

2.2 Method of analysis

The candidate reference material feed was 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 the above mentioned pesticides was injected direct into the gas chromatographic system.

3 RESULTS AND DISCUSSION

The results for the mean of the organochlorine content of the pesticides and the coefficient of variation for the repeatability of five samples feed, stored at -20°C, analysed during one year and compared to the results obtained in the homogeneity study (2nd column) are given in table 3. The results of the samples stored at 20°C and 37°C are given in table 4 and 5.

To assess a good long term reproducibility of the analysis at each occasion of analysis the pesticide content in the raw extract prepared in June 1991 for the homogeneity study and stored at -20°C was analysed each time. The results are given in table 6.

Figure 1 - 12 shows graphically the results of the pesticide content in the animal feed stored at -20°C, 20°C and 37°C.

Due to negative peaks for ß-Heptachlorepoxide, α-Endosulfan and p,p'-DDT (fig. 13) no results are reported and due to an interference for ß-HCH in July 1991 in the samples stored at -20°C and 20°C and in June 1992 for the raw extract stored at -20°C no result is available.

A significant decrease in the content of α-HCH, γ-HCH, Heptachlor and o,p'-DDT was measured in the animal feed stored at 37°C. In the samples stored at -20°C and 20°C no decrease in the pesticide content was found during a period of one year. For p,p'-TDE at a temperature of 37°C a significant increase was measured due to degradation of p,p'-DDT.

No explanation was found for the degradation of the pesticides. The germ number of aerobic bacteria after nine months of storage at either -20°C or 37°C (code of sample resp. 614 and 201) was respectively 300 and 270 CFU/g (CFU: colony forming units). Similarely the germ number of anaerobic moisture was below 10 CFU/g. This indicates clearly that the moisture content was sufficiently low to prevent these bacteria from growing. A microbial decay of the sample or analytes therefore cannot take place.

The results of the long term reproducibility (table 6) show a good reproducibility, in general better than 5%.

4 CONCLUSION

Based on the results of the stability study it should be recommended to store the reference material at a temperature below 20°C.

LITERATURE

Roos, A.H., L.G.M.Th. Tuinstra and A.M. Matser

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

DLO-State Institute for Quality Control of Agricultural Products, Wageningen, 1991, report 91.59.

Tabel 3. Mean (mg/kg dry mass) and CV(r)(%) of the organochlorine pesticide content in the reference material feed stored at -20°C.

Pesticide	June 1991 (n=20)		July 1991 (n=5)		September 1991 (n=5)		December 1991 (n=5)		June 1992 (n=5)	
	x	CV(r)	x	CV(r)	x	CV(r)	x	CV(r)	x	CV(r)
α-HCH	0.0205	4.4	0.0195	1.7	0.0194	2.4	0.0205	4.0	0.0198	2.3
HCB	0.0193	4.4	0.0191	1.6	0.0194	2.1	0.0197	1.3	0.0187	2.2
ß-HCH	0.0223	4.8	1)		0.0263	5.0	0.0266	4.0	0.0225	4.8
γ-HCH	0.0191	5.3	0.0211	2.9	0.0182	4.9	0.0198	3.4	0.0217	3.0
Heptachlor	0.0196	4.8	0.0189	1.5	0.0181	2.1	0.0194	2.7	0.0198	7.2
Aldrin	0.0210	3.9	0.0192	8.9	0.0176	1.5	0.0194	2.9	0.0205	5.8
ß-Heptachlorepoxide	1)		1)		1)		1)		1)	
γ-Chlordane	0.0585	5.5	0.0513	2.0	0.0538	2.9	0.0578	2.2	0.0558	9.7
α-Endosulfan	1)		1)		1)		1)		1)	
p,p'-DDE	0.0522	5.2	0.0512	2.3	0.0489	3.4	0.0530	3.4	0.0522	4.1
Dieldrin	0.0211	5.6	0.0197	1.9	0.0184	7.2	0.0202	4.3	0.0208	3.8
Endrin	0.0536	7.0	0.0540	3.0	0.0483	7.8	0.0527	3.6	0.0517	4.9
p,p'-TDE	0.0697	6.0	0.0649	3.8	0.0536	9.8	0.0672	3.1	0.0716	3.9
o,p'-DDT	0.0481	6.6	0.0517	1.7	0.0463	5.7	0.0475	3.6	0.0481	2.6
p,p'-DDT	1)		1)		1)		1)			

1) interference, see text

Tabel 4. Mean (mg/kg dry mass) and CV(r)(%) of the organochlorine pesticide content in the reference material feed stored at 20°C.

Pesticide	June 1991 (n=20)		July 1991 (n=5)		September 1991 (n=5)		December 1991 (n=5)		June 1992 (n=5)	
	x	CV(r)	x	CV(r)	x	CV(r)	x	CV(r)	x	CV(r)
α-HCH	0.0205	4.4	0.0202	1.7	0.0193	6.6	0.0191	1.5	0.0197	7.0
HCB	0.0193	4.4	0.0200	1.8	0.0196	4.3	0.0189	2.9	0.0200	1.0
ß-HCH	0.0223	4.8	1)		0.0240	6.3	0.0240	2.1	0.0251	3.3
γ-HCH	0.0191	5.3	0.0210	2.1	0.0181	6.8	0.0186	3.1	0.0194	2.2
Heptachlor	0.0196	4.8	0.0192	6.1	0.0185	6.2	0.0183	2.9	0.0194	9.2
Aldrin	0.0210	3.9	0.0181	3.4	0.0177	5.9	0.0182	2.4	0.0200	7.6
ß-Heptachlorepoxide	1)		1)		1)		1)		1)	
γ-Chlordane	0.0585	5.5	0.0498	3.5	0.0546	2.5	0.0561	1.7	0.0573	5.7
α-Endosulfan	1)		1)		1)		1)		1)	
p,p'-DDE	0.0522	5.2	0.0499	4.5	0.0509	5.6	0.0513	2.0	0.0534	9.0
Dieldrin	0.0211	5.6	0.0189	6.7	0.0196	6.1	0.0197	2.4	0.0212	6.1
Endrin	0.0536	7.0	0.0533	5.1	0.0501	6.3	0.0497	4.1	0.0515	8.1
p,p'-TDE	0.0697	6.0	0.0669	3.5	0.0611	6.2	0.0672	3.2	0.0771	9.9
o,p'-DDT	0.0481	6.6	0.0477	5.1	0.0476	6.5	0.0444	1.5	0.0434	5.0
p,p'-DDT	1)		1)		1)		1)			

1) interference, see text

Tabel 5. Mean (mg/kg dry mass) and CV(r)(%) of the organochlorine pesticide content in the reference material feed stored at 37°C.

Pesticide	June 1991 (n=20)		July 1991 (n=5)		September 1991 (n=5)		December 1991 (n=5)		June 1992 (n=5)	
	x	CV(r)	x	CV(r)	x	CV(r)	x	CV(r)	x	CV(r)
α-HCH	0.0205	4.4	0.0200	0.2	0.0181	5.6	0.0167	2.2	0.0158	6.5
HCB	0.0193	4.4	0.0200	0.4	0.0197	3.7	0.0189	1.7	0.0194	6.8
ß-HCH	0.0223	4.8	0.0259	3.6	0.0229	6.3	0.0232	5.4	0.0245	11.4
γ-HCH	0.0191	5.3	0.0203	2.6	0.0116	2.0	0.0076	14.6	0.0025	46.1
Heptachlor	0.0196	4.8	0.0184	4.2	0.0055	7.3	<0.0010		<0.0010	
Aldrin	0.0210	3.9	0.0184	3.7	0.0166	4.3	0.0170	0.9	0.0207	1.3
ß-Heptachlorepoxide	1)		1)		1)		1)		1)	
γ-Chlordane	0.0585	5.5	0.0506	3.8	0.0547	4.5	0.0538	2.4	0.0606	2.5
α-Endosulfan	1)		1)		1)		1)		1)	
p,p'-DDE	0.0522	5.2	0.0525	5.8	0.0521	3.8	0.0508	1.6	0.0533	3.0
Dieldrin	0.0211	5.6	0.0187	7.6	0.0188	5.9	0.0194	4.0	0.0211	5.1
Endrin	0.0536	7.0	0.0511	5.6	0.0481	5.6	0.0434	6.0	0.0452	12.3
p,p'-TDE	0.0697	6.0	0.0721	3.6	0.0845	6.7	0.0918	2.2	0.1097	3.9
o,p'-DDT	0.0481	6.6	0.0440	4.4	0.0221	3.3	0.0074	24.9	<0.0010	
p,p'-DDT	1)		1)		1)		1)			

1) interference, see text

Tabel 6. Pesticide content in the raw extract, stored at -20°C, analysed during the stability study (mg/kg dry mass)

Pesticide	June 1991 (n=20)	July 1991 (n=5)	September 1991 (n=5)	December 1991 (n=5)	June 1992 (n=5)
α-HCH	0.0205	0.0212	0.0216	0.0212	0.0218
HCB	0.0193	0.0203	0.0207	0.0202	0.0218
ß-HCH	0.0223	0.0214	0.0229	0.0232	1)
γ-HCH	0.0191	0.0192	0.0191	0.0192	0.0207
Heptachlor	0.0196	0.0205	0.0196	0.0199	0.0211
Aldrin	0.0210	0.0191	0.0188	0.0192	0.0216
ß-Heptachlorepoxide	1)	1)	1)	1)	1)
γ-Chlordane	0.0585	0.0489	0.0582	0.0599	0.0644
α-Endosulfan	1)	1)	1)	1)	1)
p,p'-DDE	0.0522	0.0527	0.0557	0.0548	0.0577
Dieldrin	0.0211	0.0180	0.0203	0.0205	0.0222
Endrin	0.0536	0.0558	0.0565	0.0539	0.0557
p,p'-TDE	0.0697	0.0669	0.0747	0.0701	0.0773
o,p'-DDT	0.0481	0.0574	0.0489	0.0498	0.0550
p,p'-DDT	1)	1)	1)	1)	1)

1) interference, see text

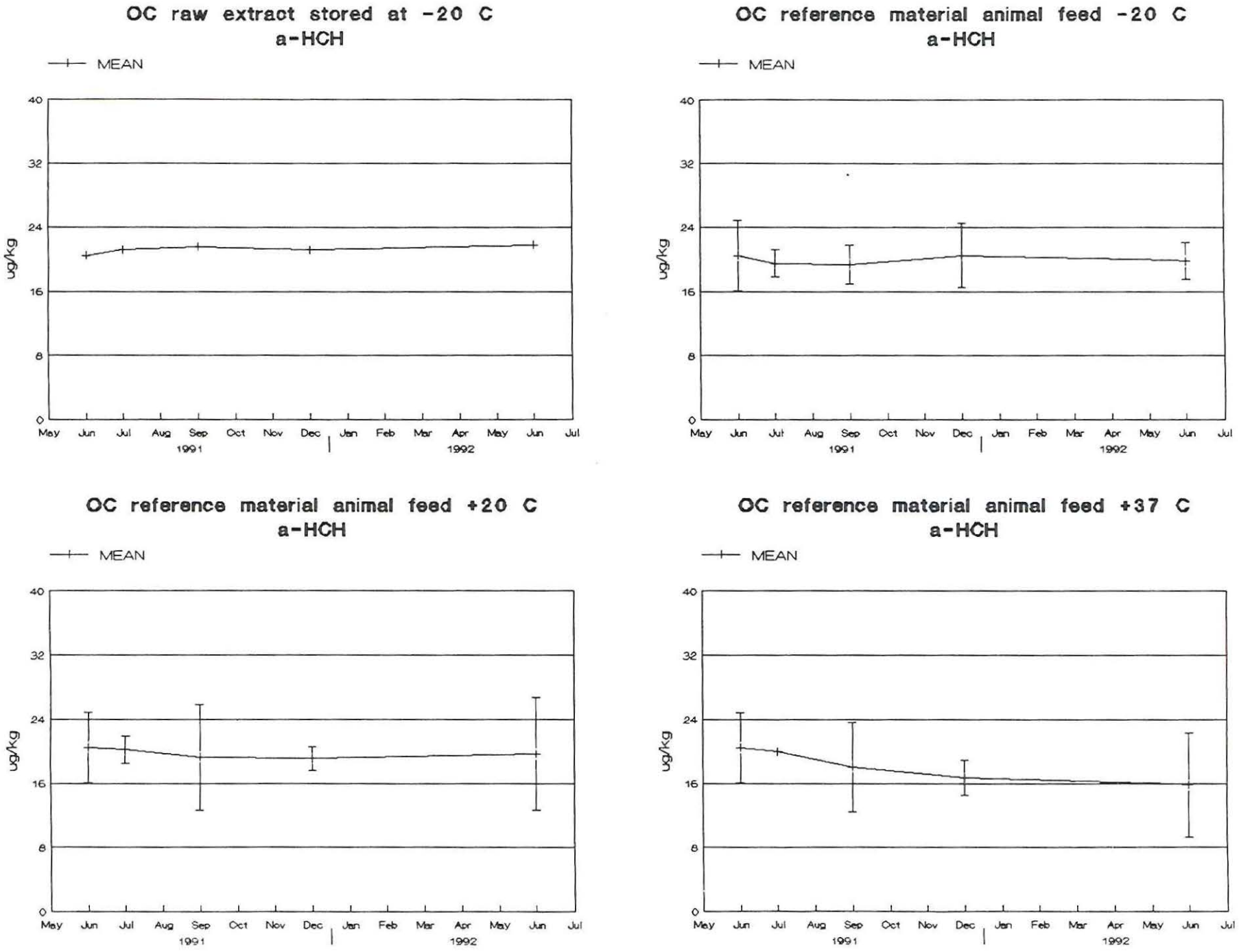
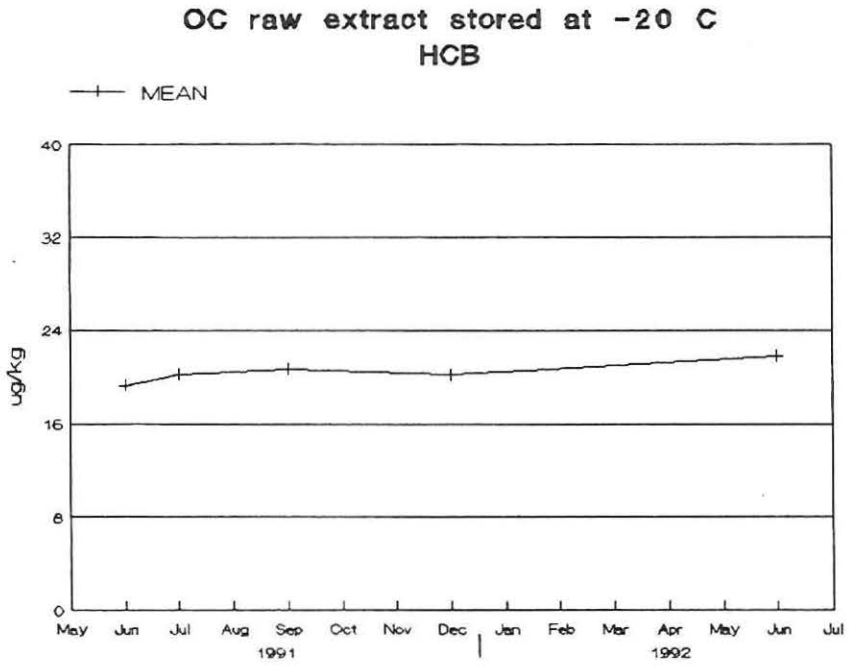
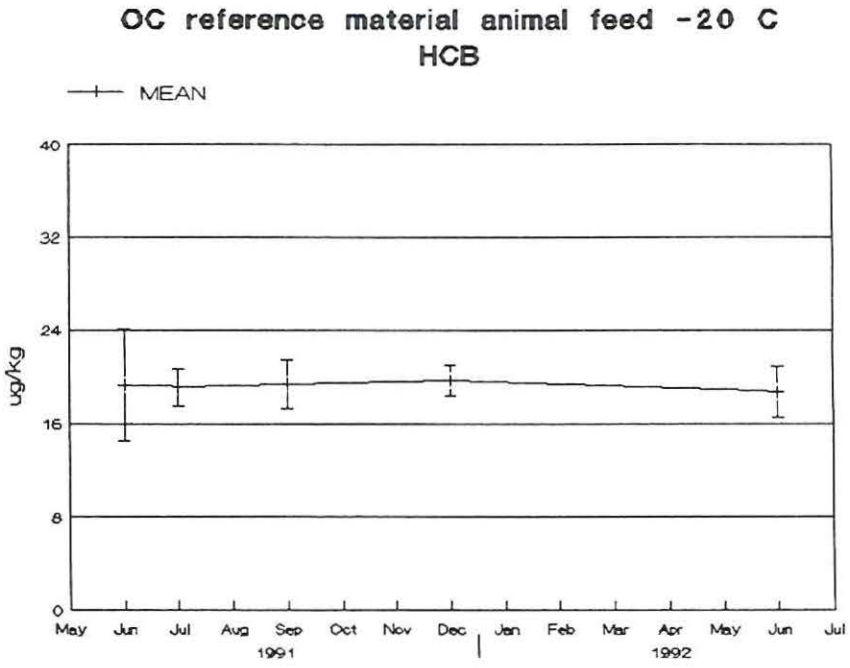
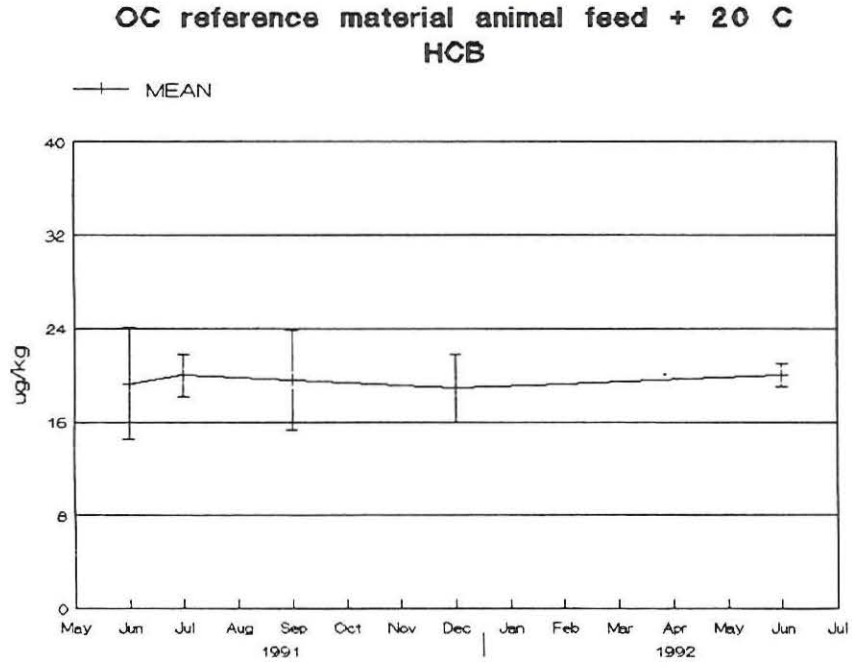


Fig. 1. Results of α-HCH in the raw extract and samples (μg/kg dry mass).







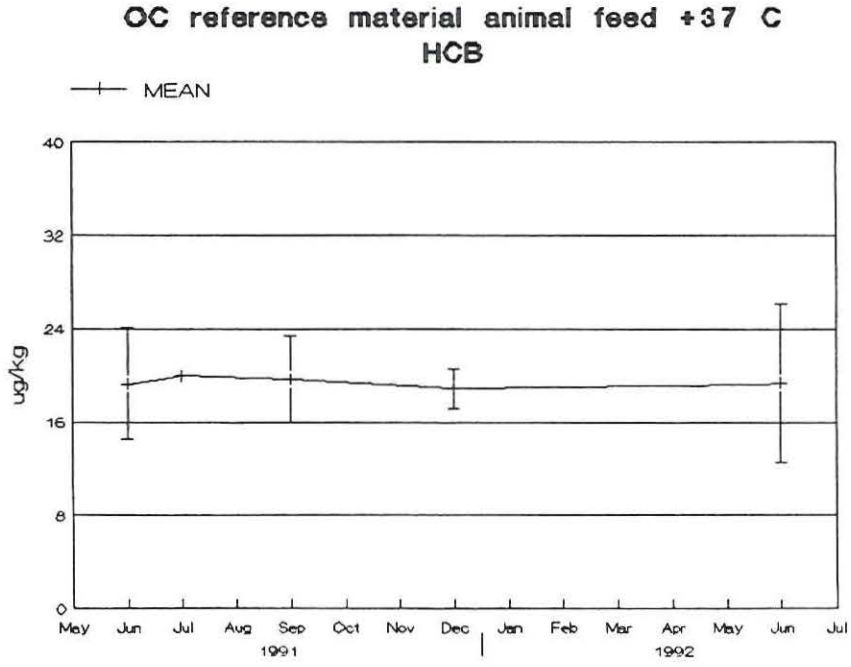


Fig. 2. Results of HCB in the raw extract and samples (µg/kg dry mass).

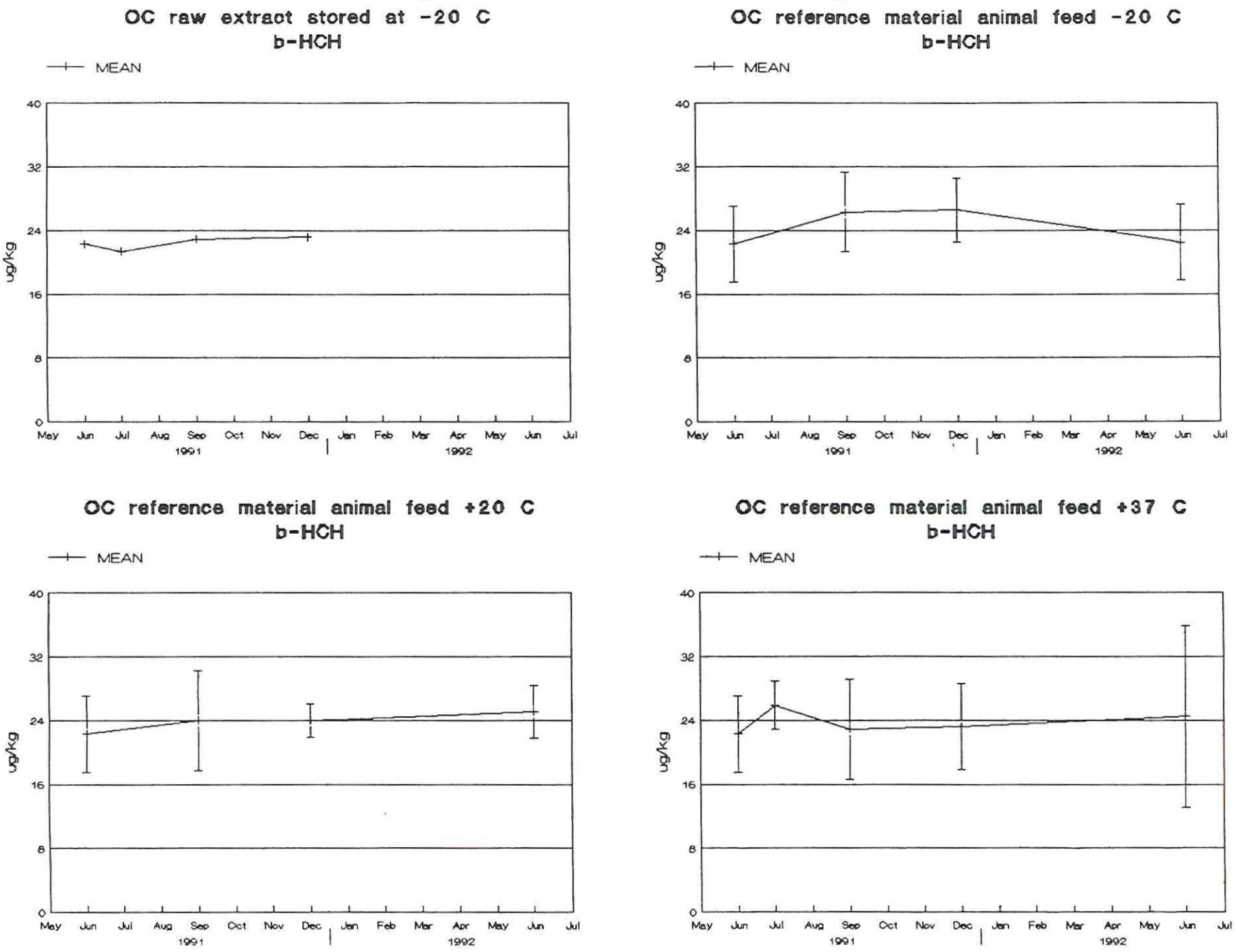
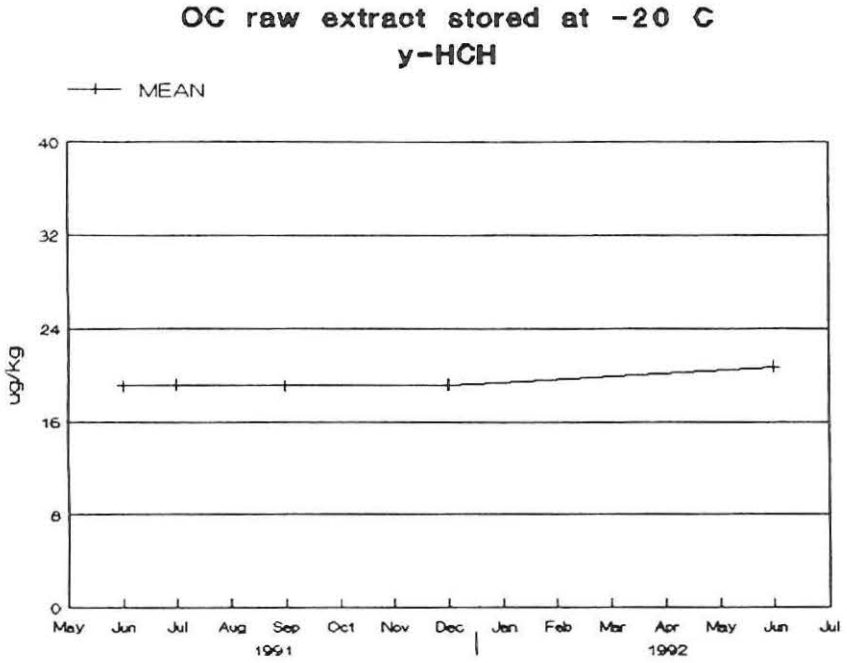
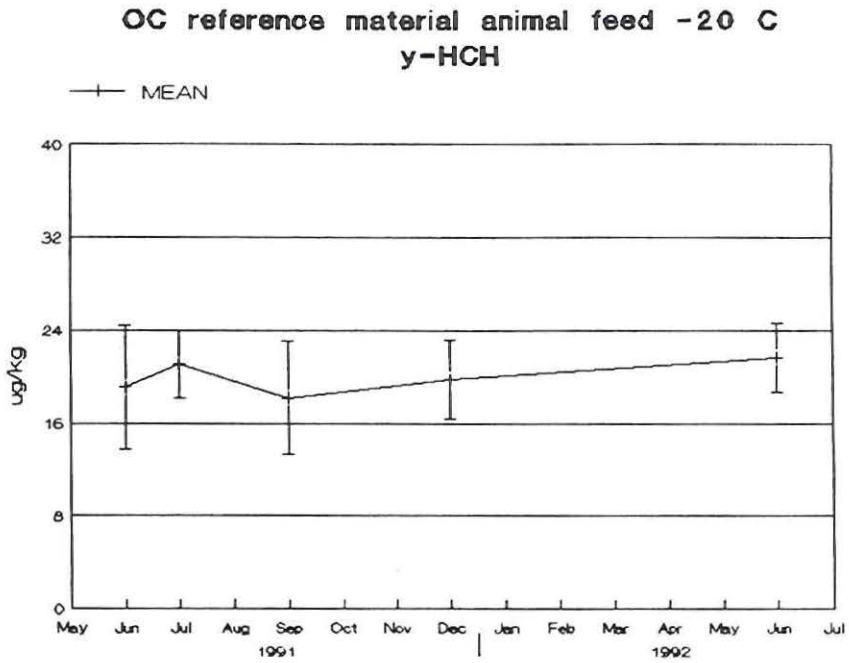
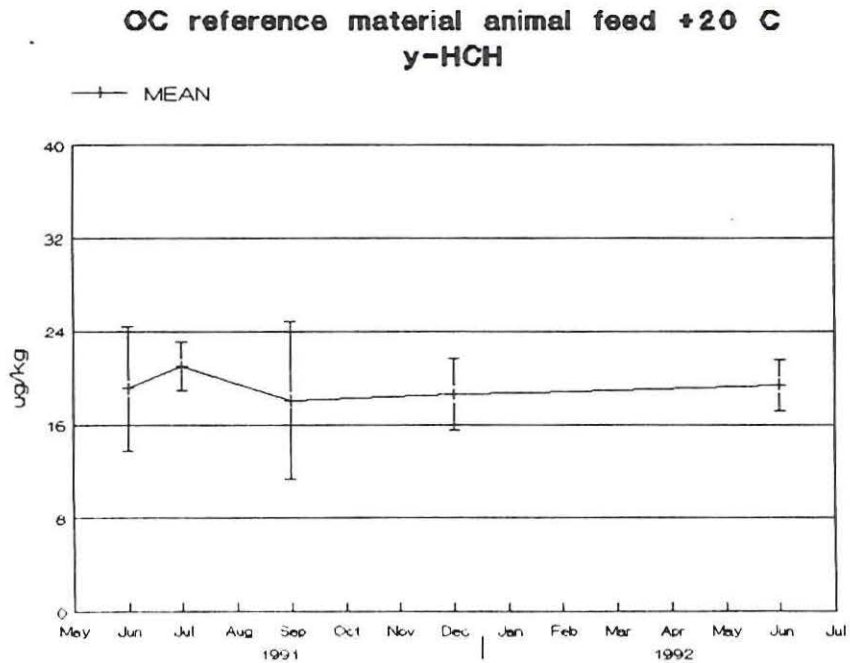


Fig. 3. Results of β-HCH in the raw extract and samples (µg/kg dry mass).







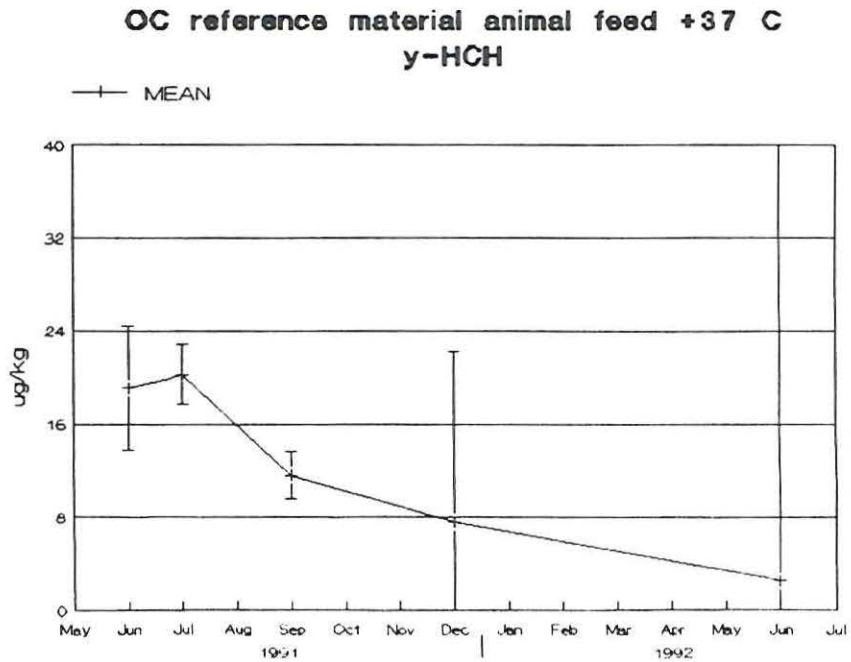
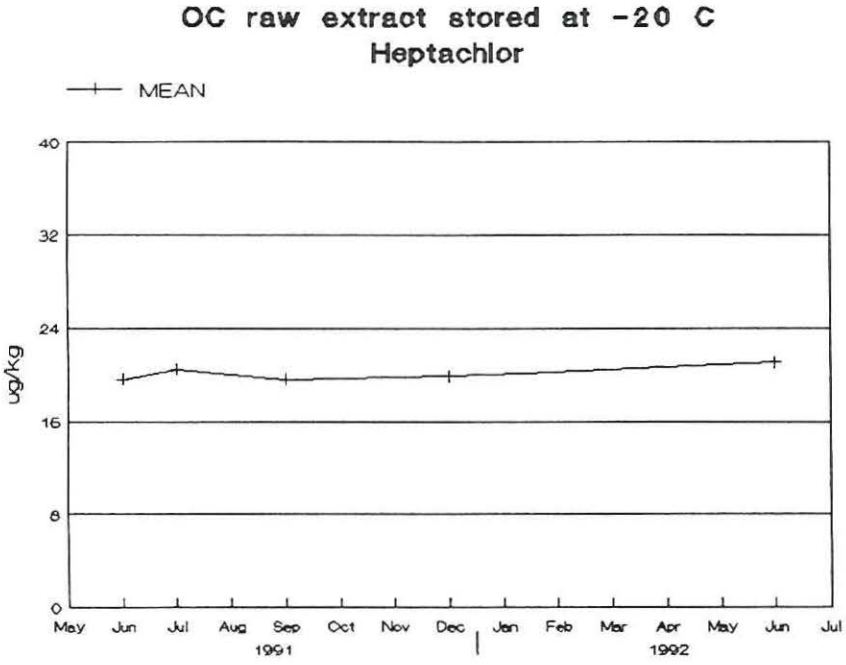
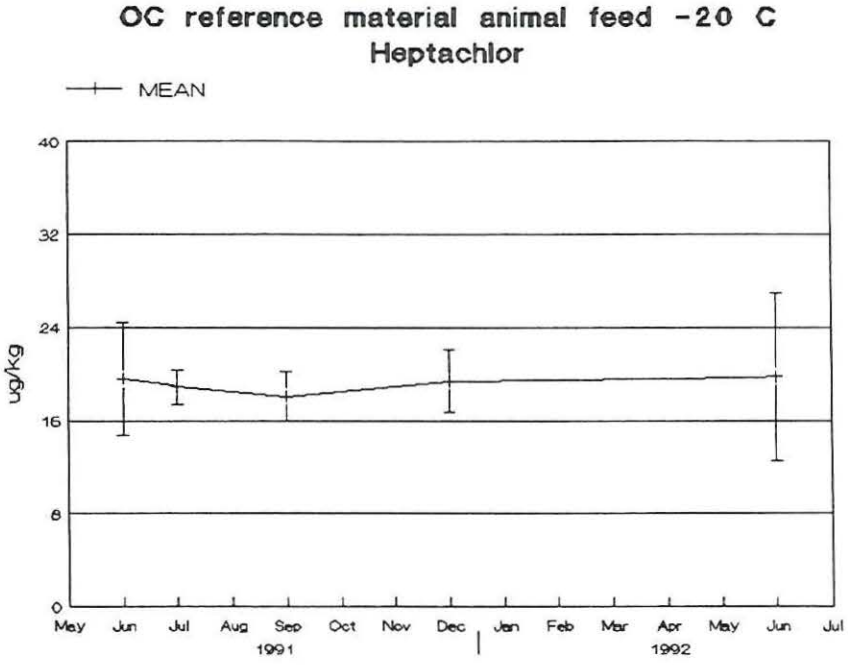
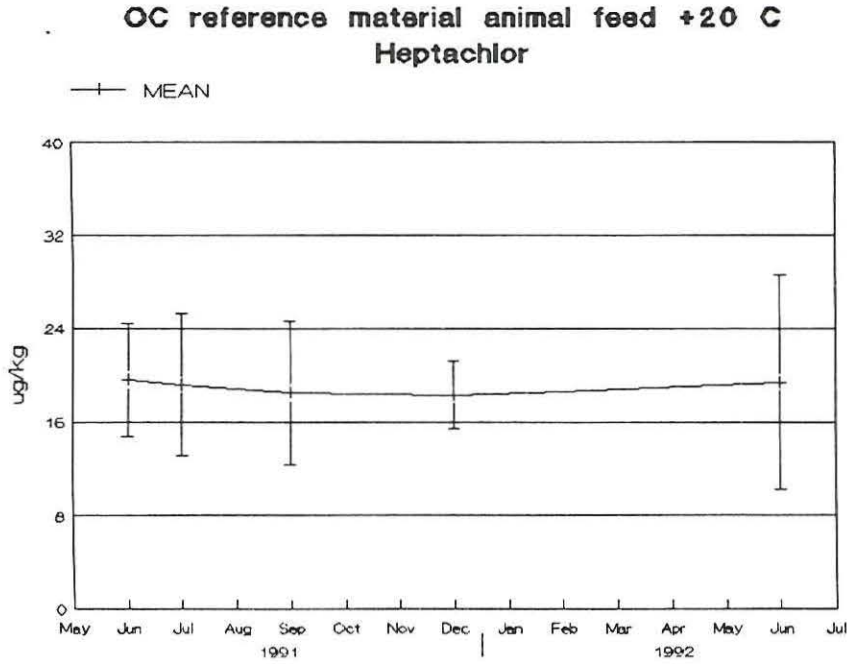


Fig. 4. Results of γ-HCH in the raw extract and samples (μg/kg dry mass).







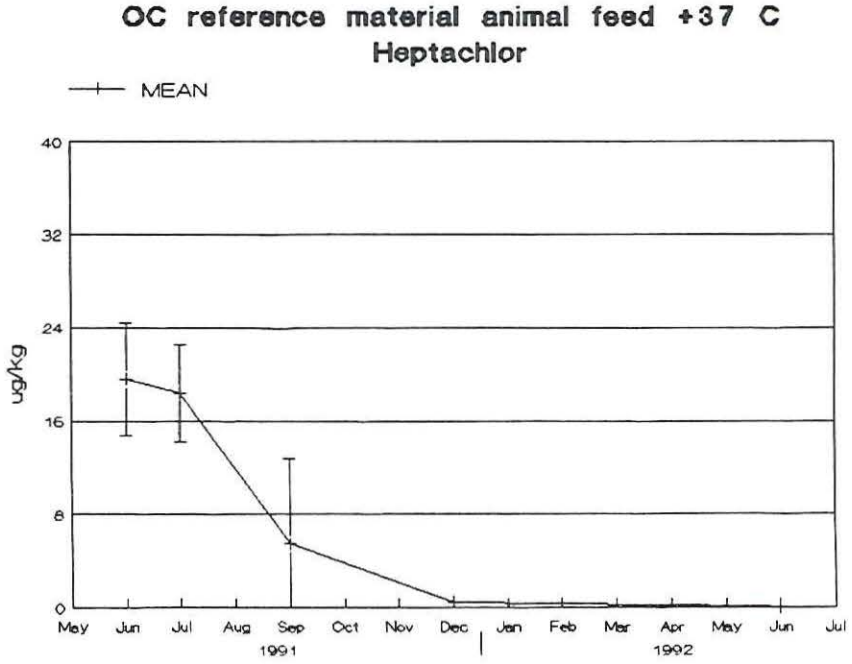
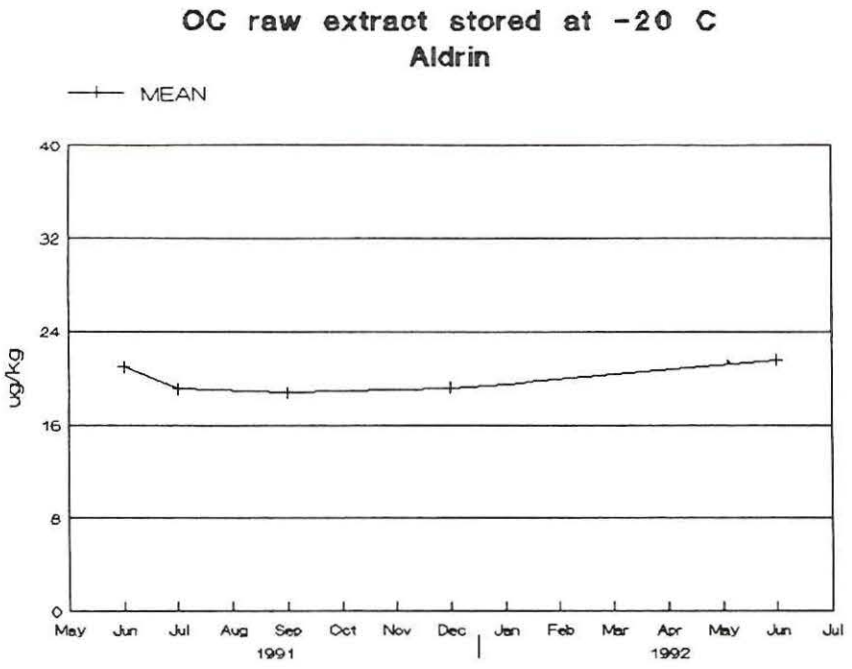
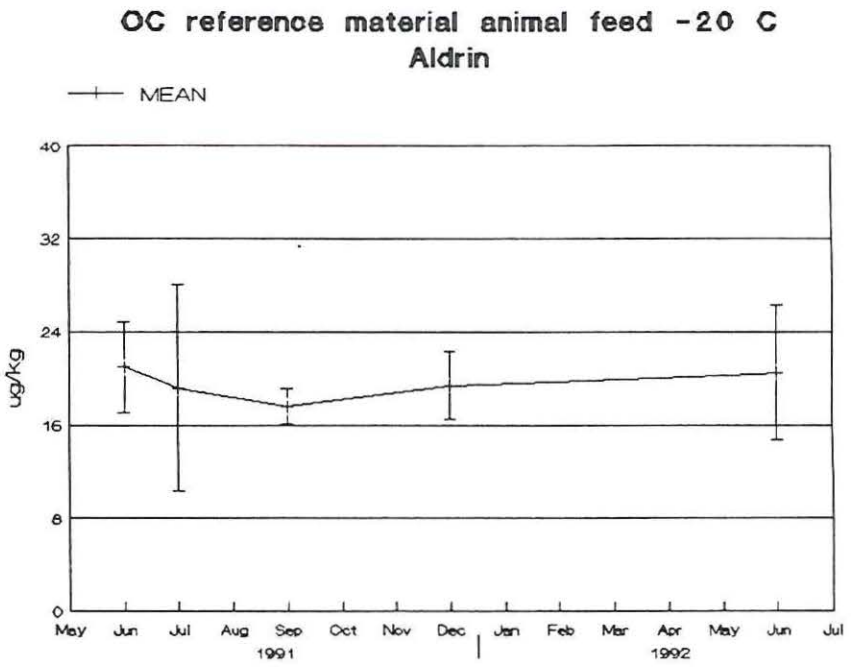
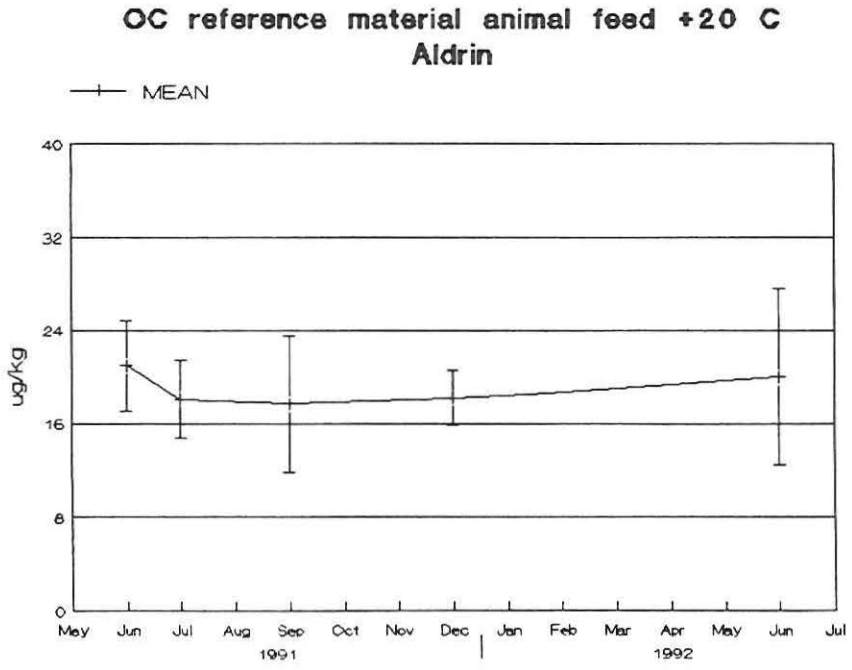


Fig. 5. Results of Heptachlor in the raw extract and samples (µg/kg dry mass).







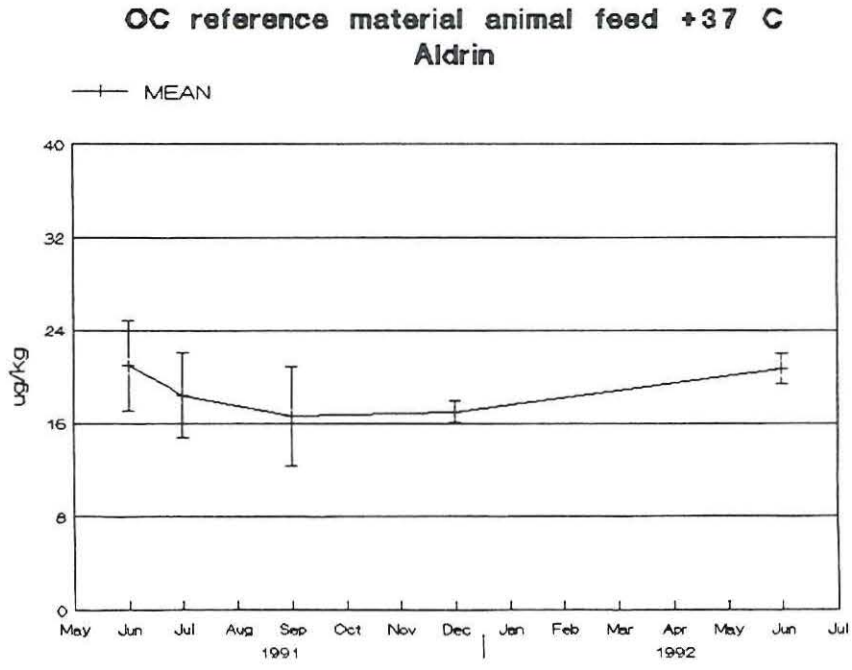
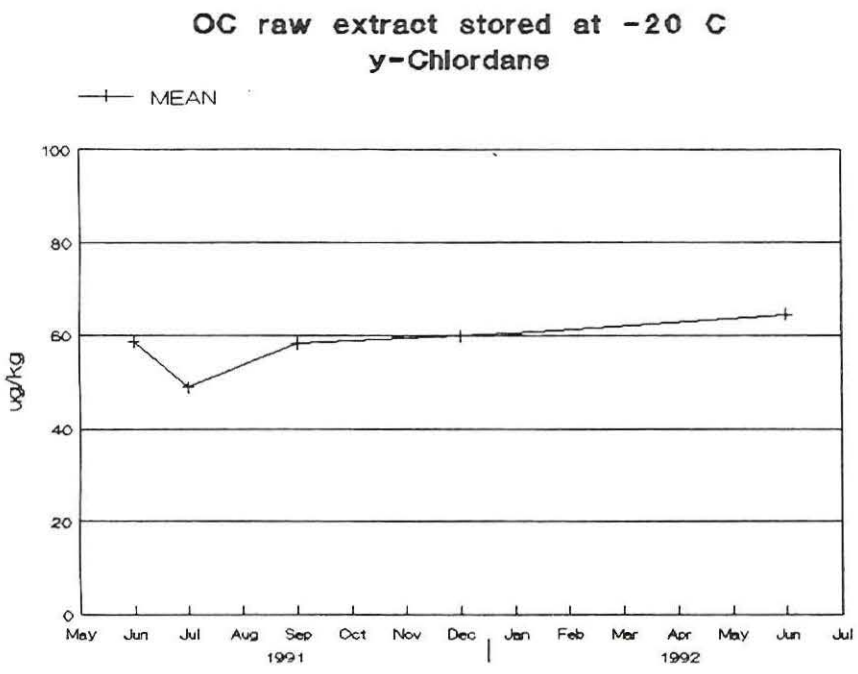
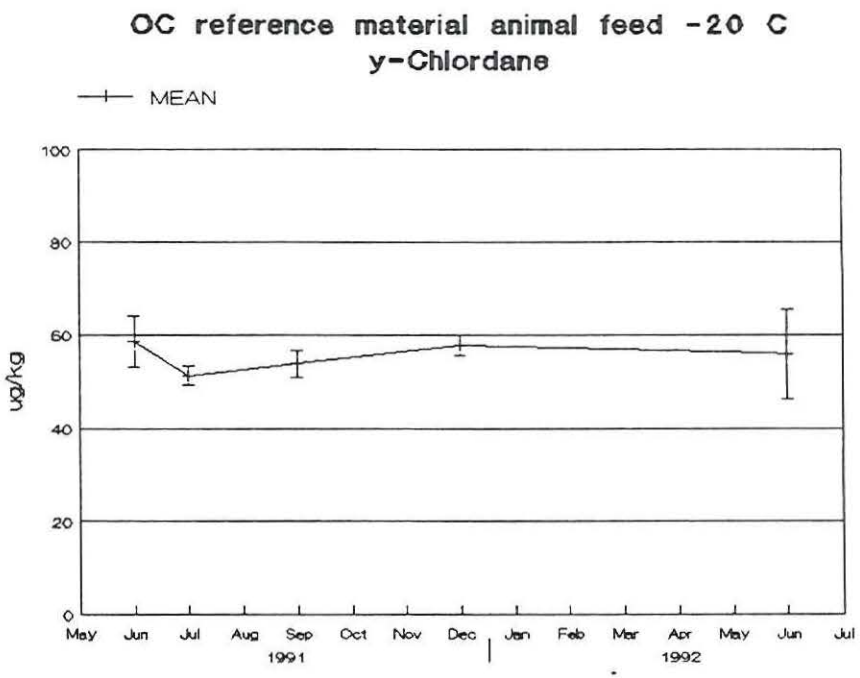
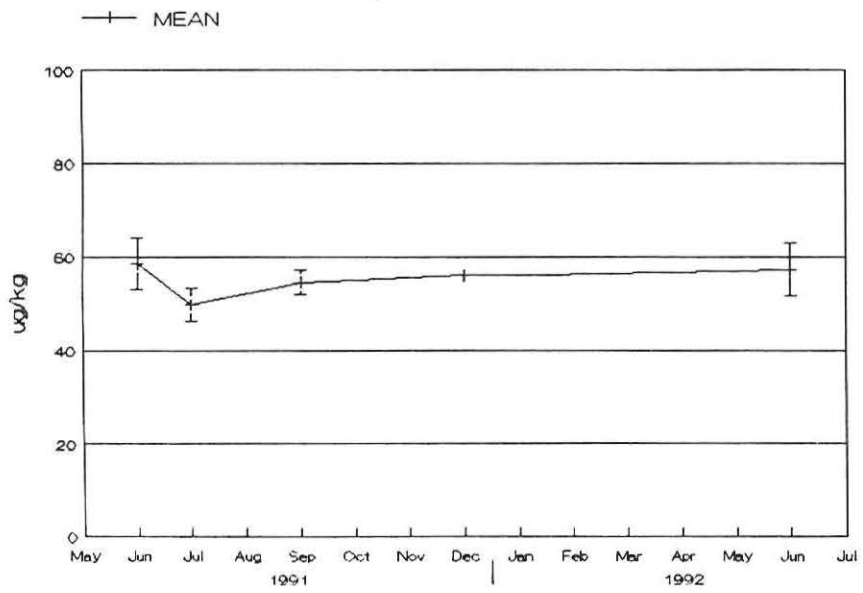


Fig. 6. Results of Aldrin in the raw extract and samples (µg/kg dry mass).







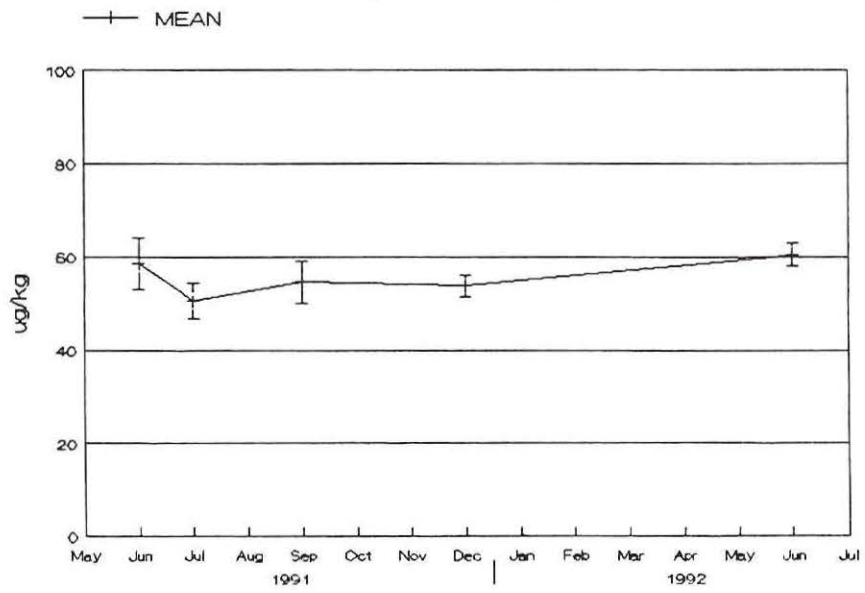
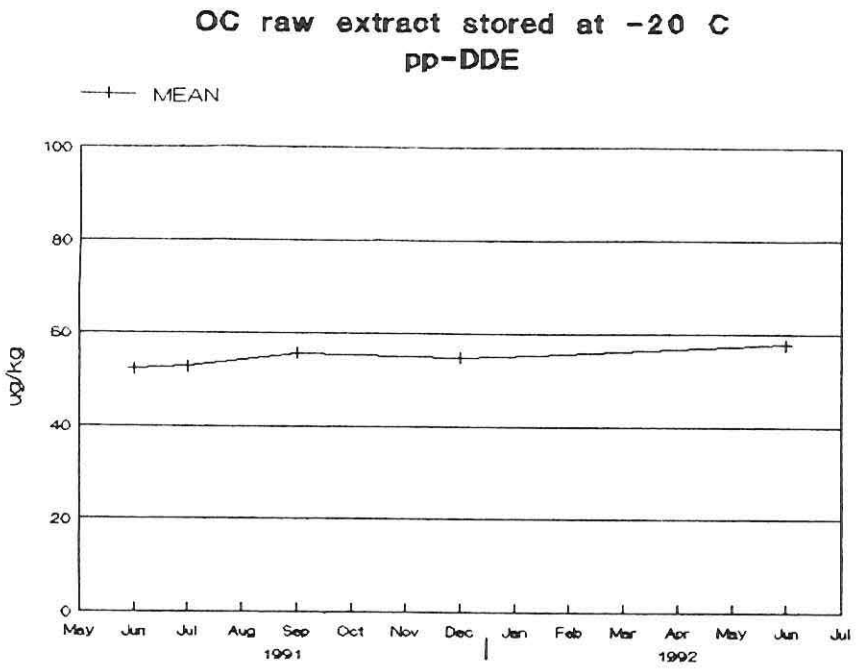
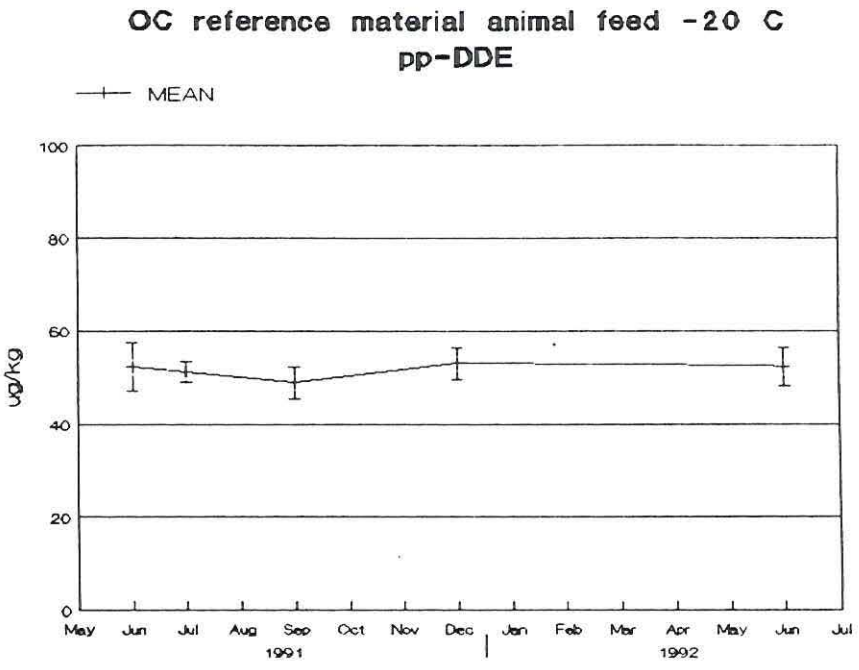
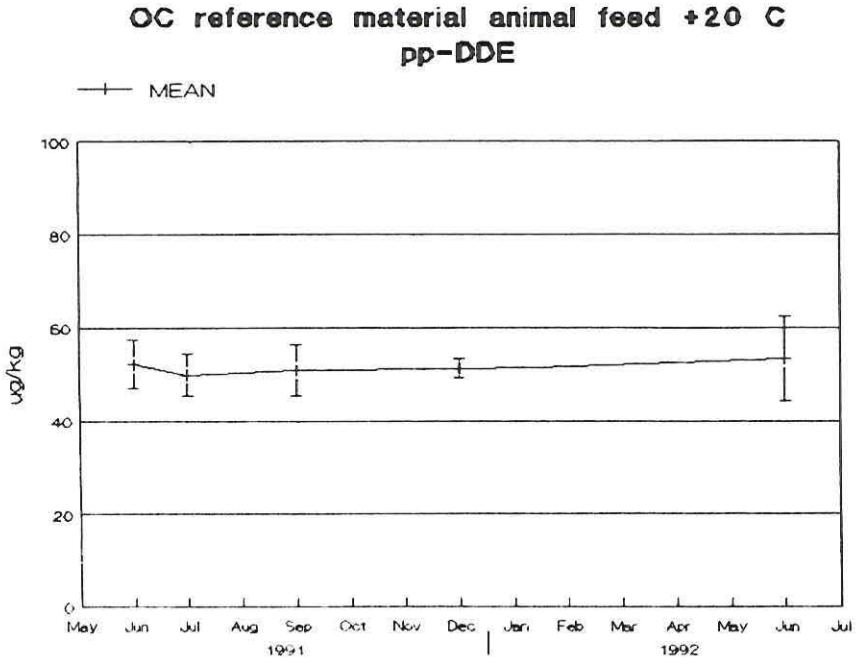


Fig. 7. Results of γ-Chlordane in the raw extract and samples (µg/kg dry mass).







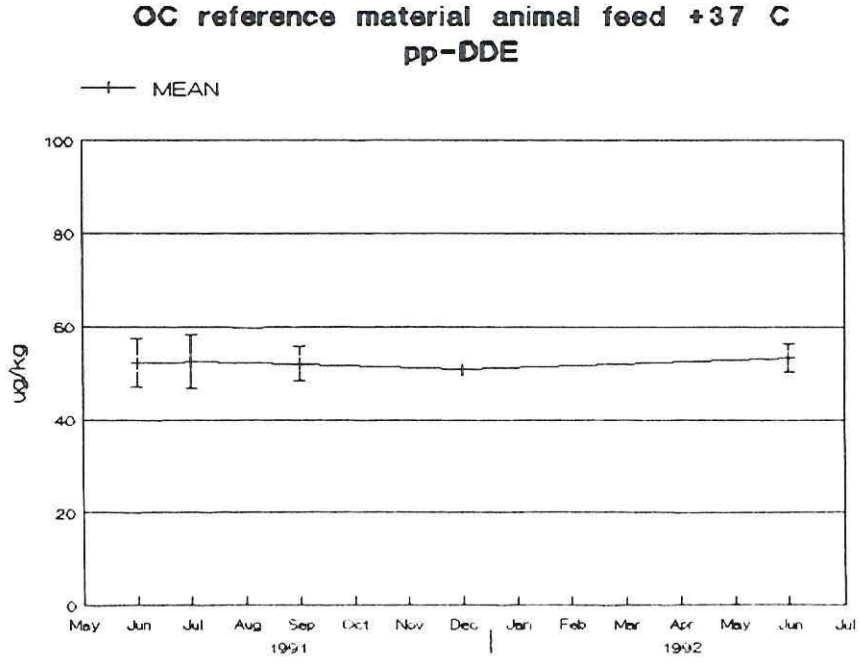
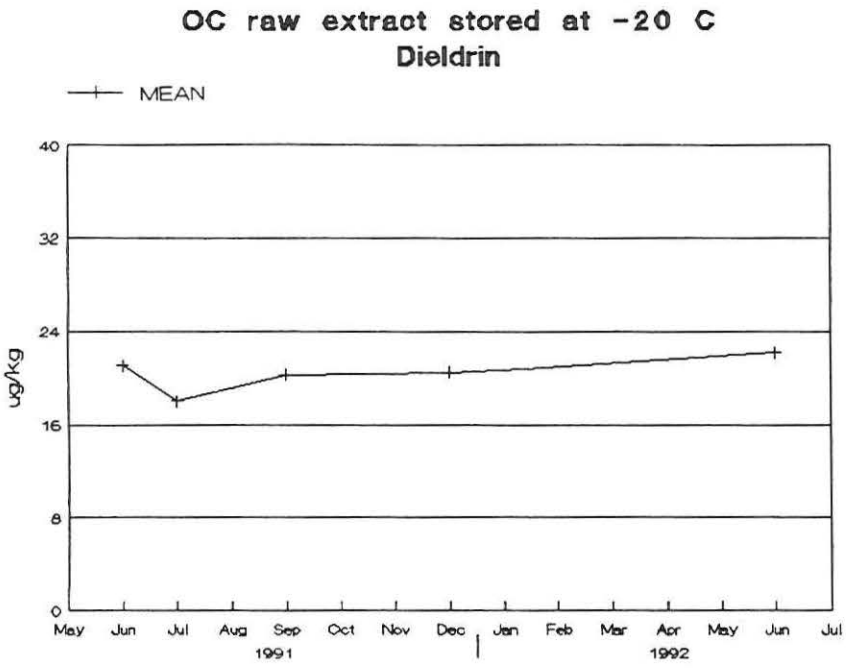
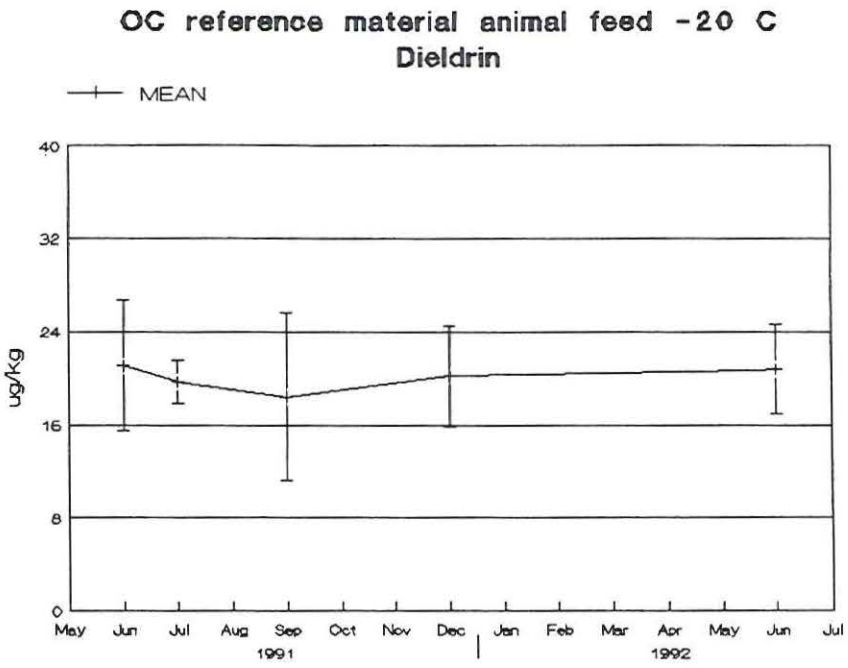
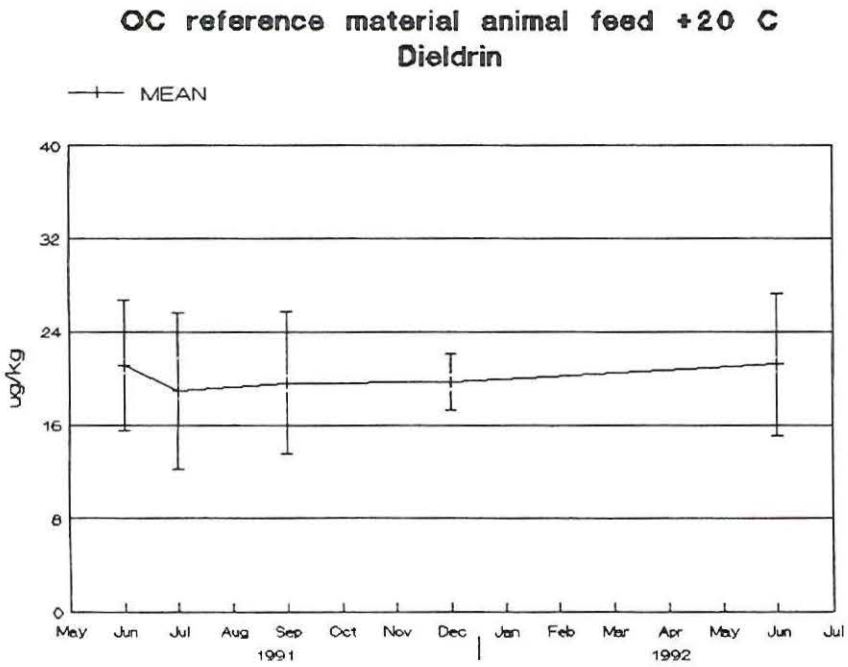


Fig. 8. Results of pp'-DDE in the raw extract and samples (µg/kg dry mass).







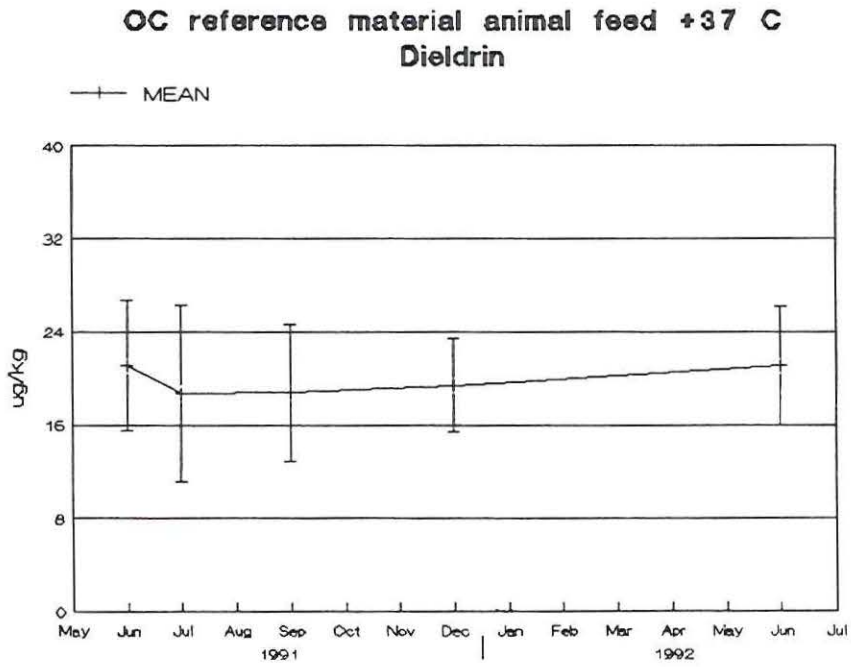
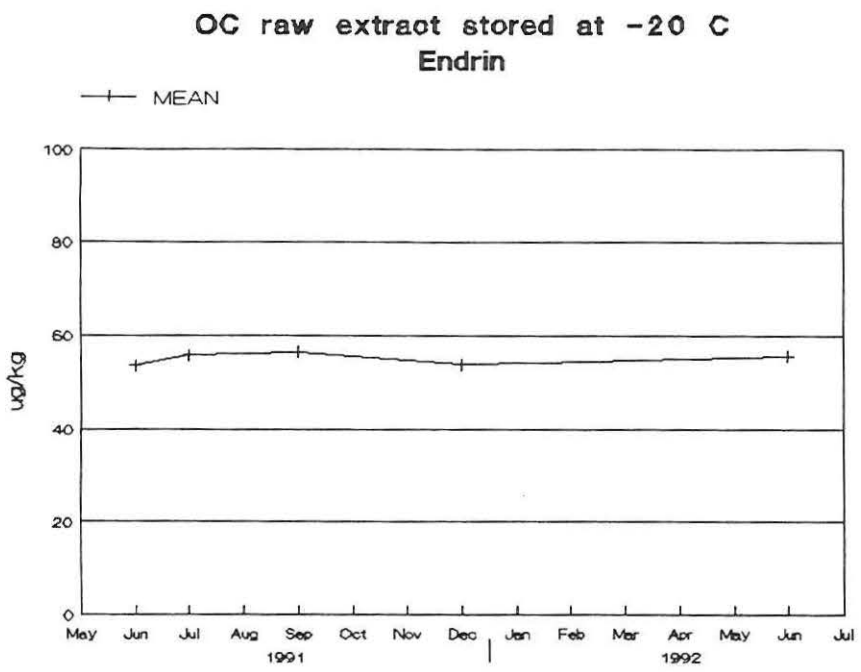
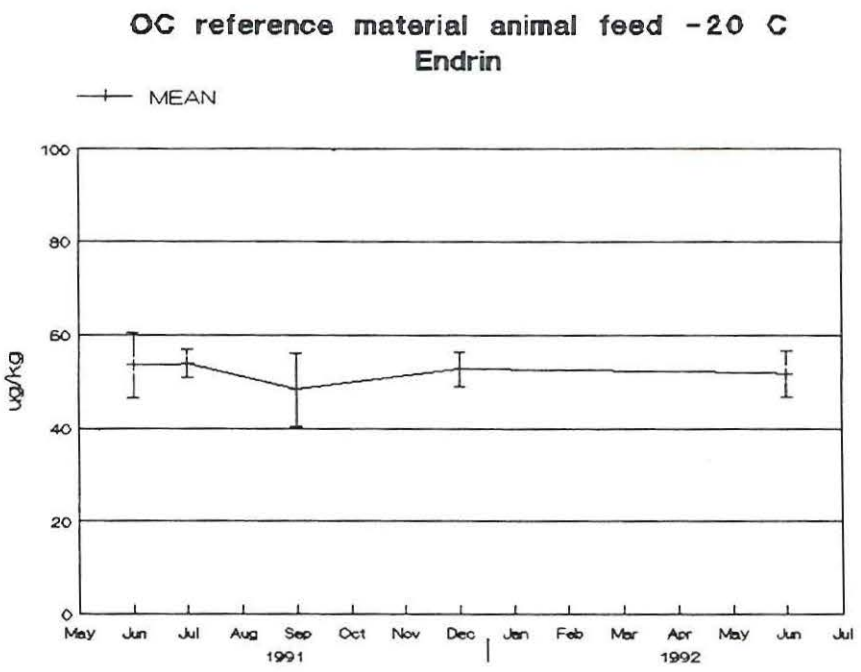
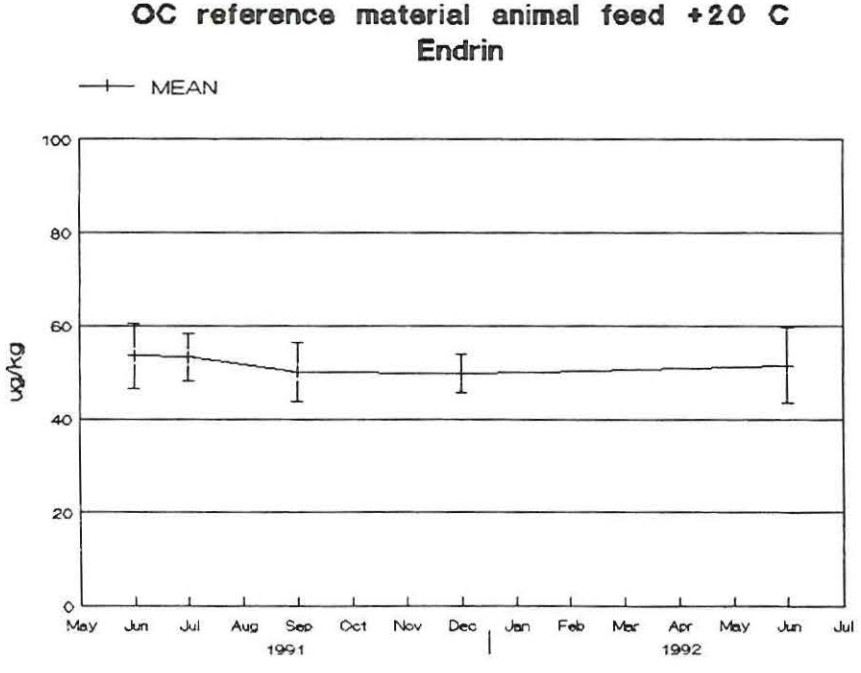


Fig. 9. Results of Dieldrin in the raw extract and samples (µg/kg dry mass).







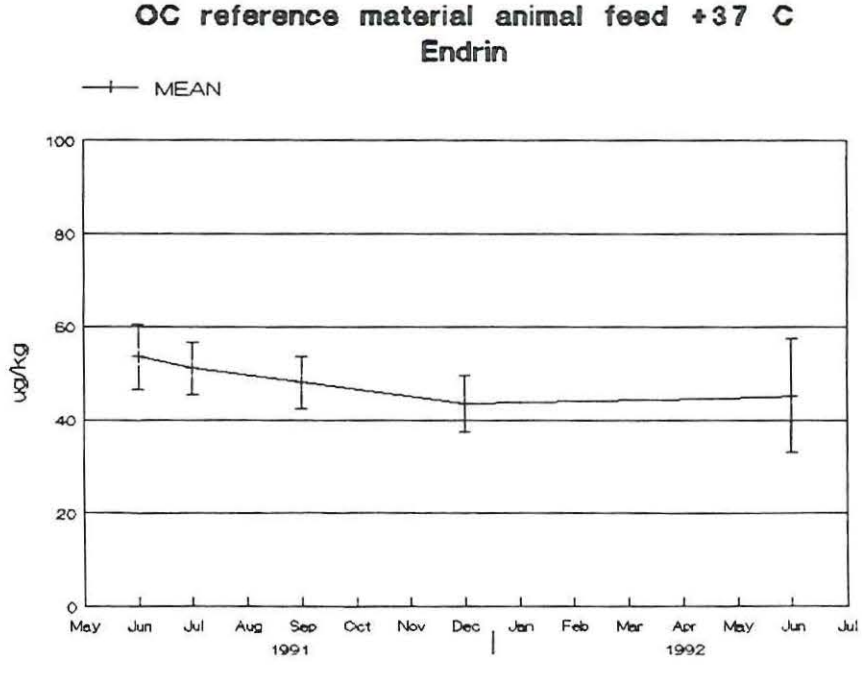
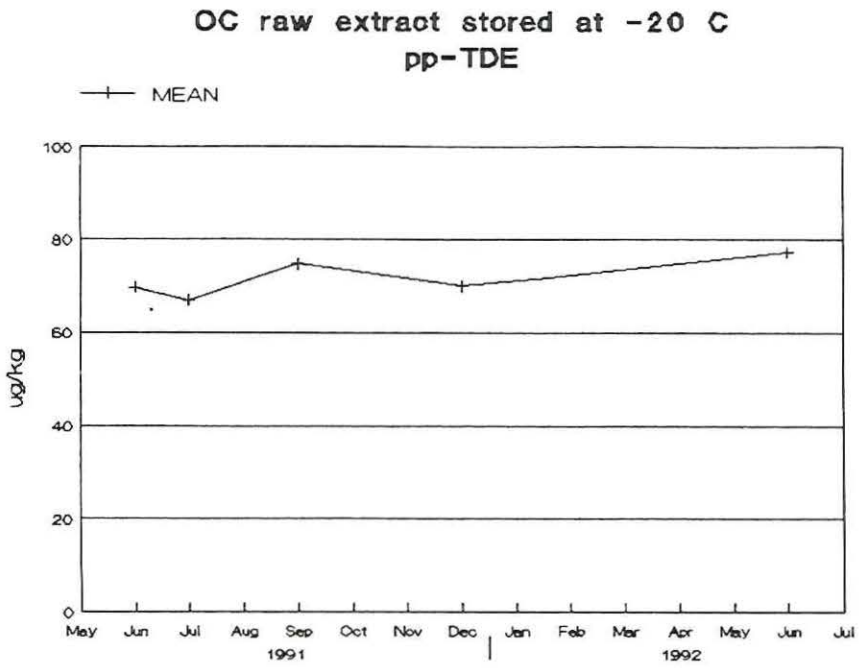
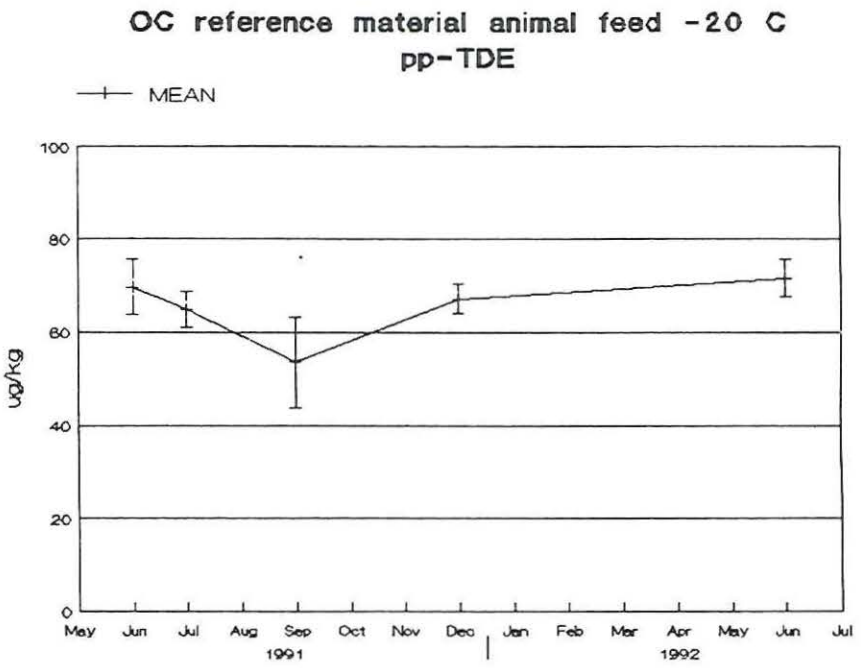
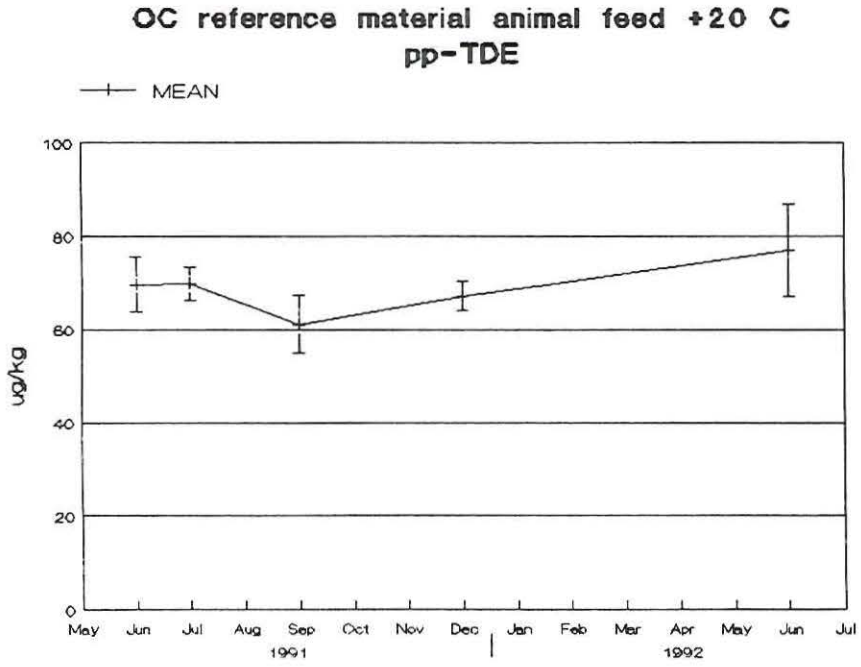


Fig. 10. Results of Eldrin in the raw extract and samples (µg/kg dry mass).







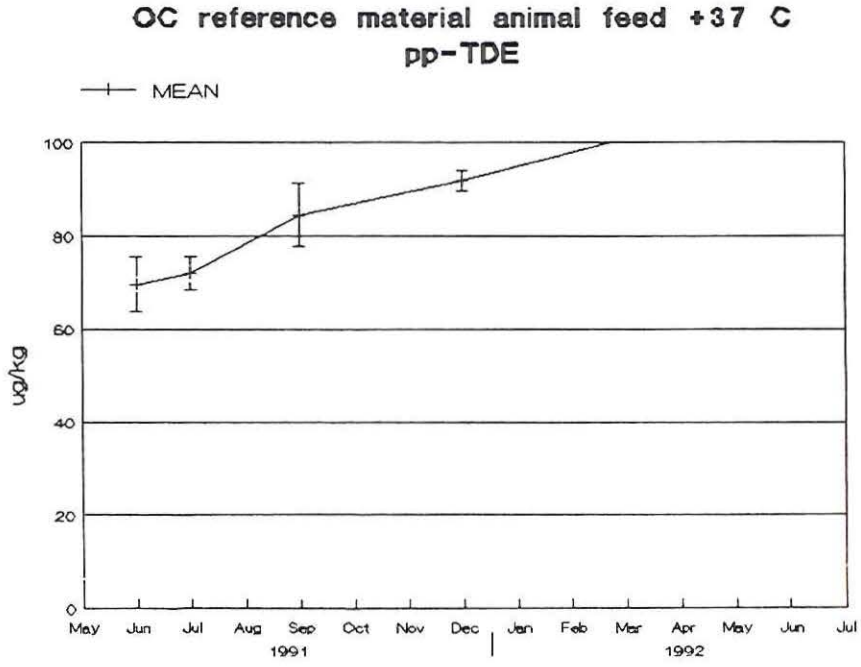
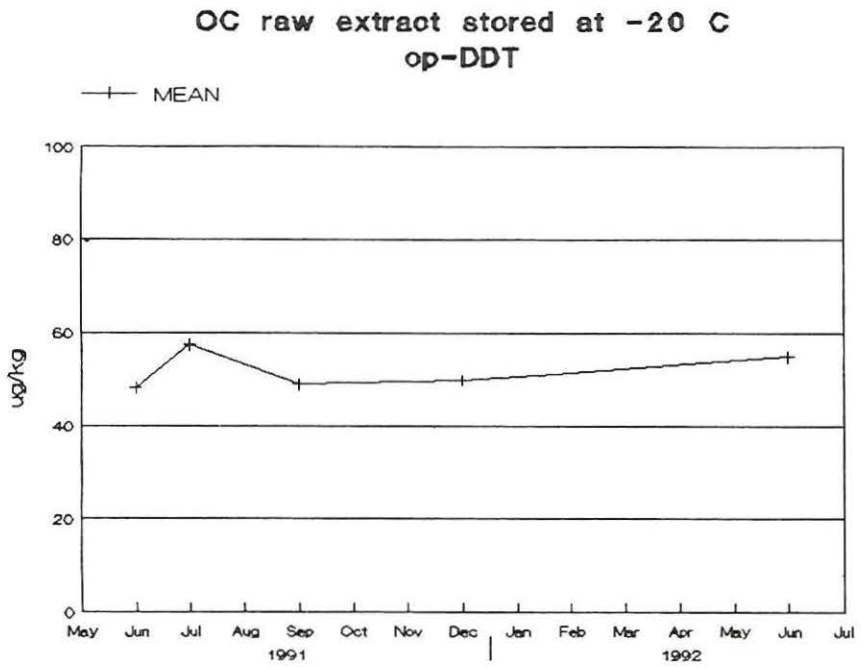
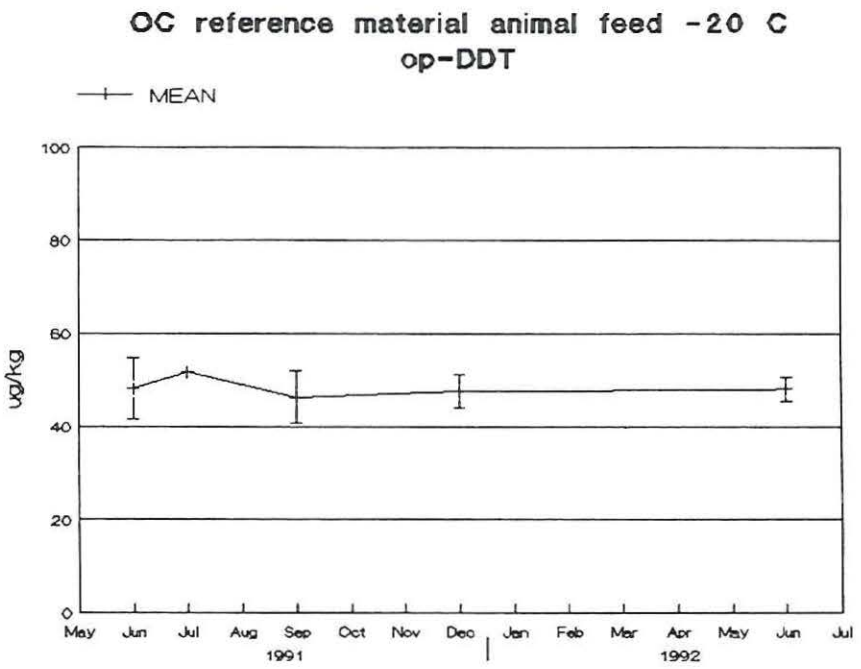
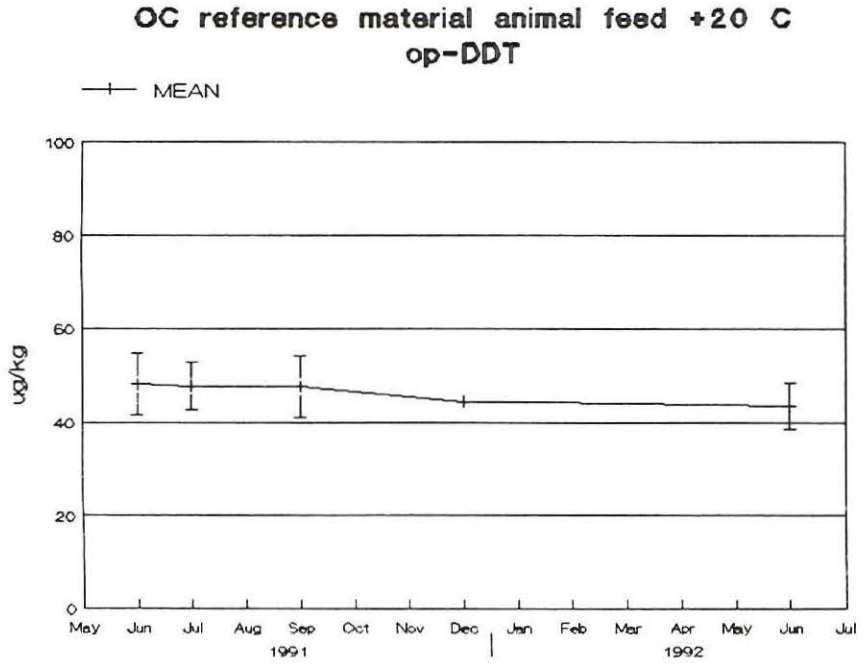


Fig. 11. Results of pp'-TDE in the raw extract and samples (µg/kg dry mass).







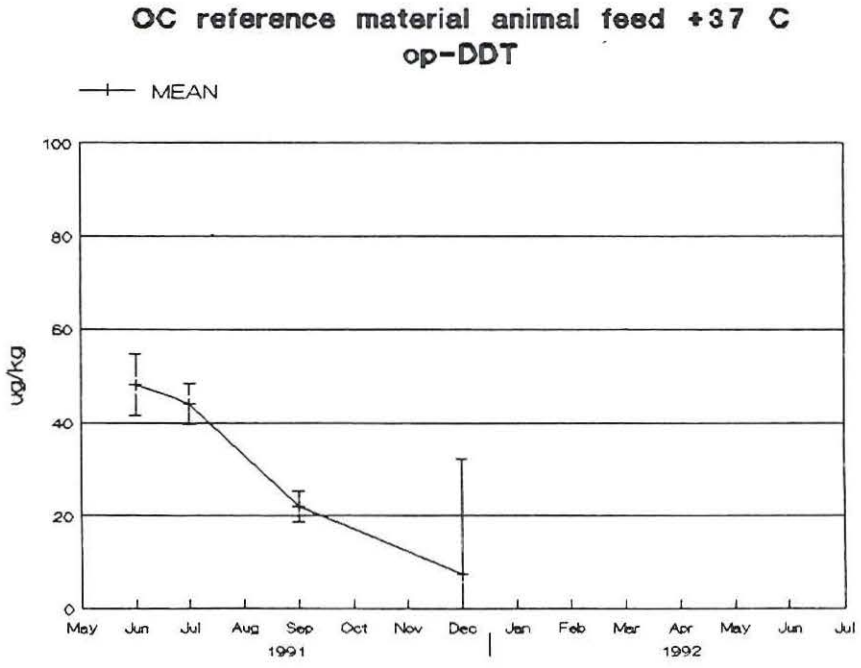


Fig. 12. Results of op'-DDT in the raw extract and samples (µg/kg dry mass).

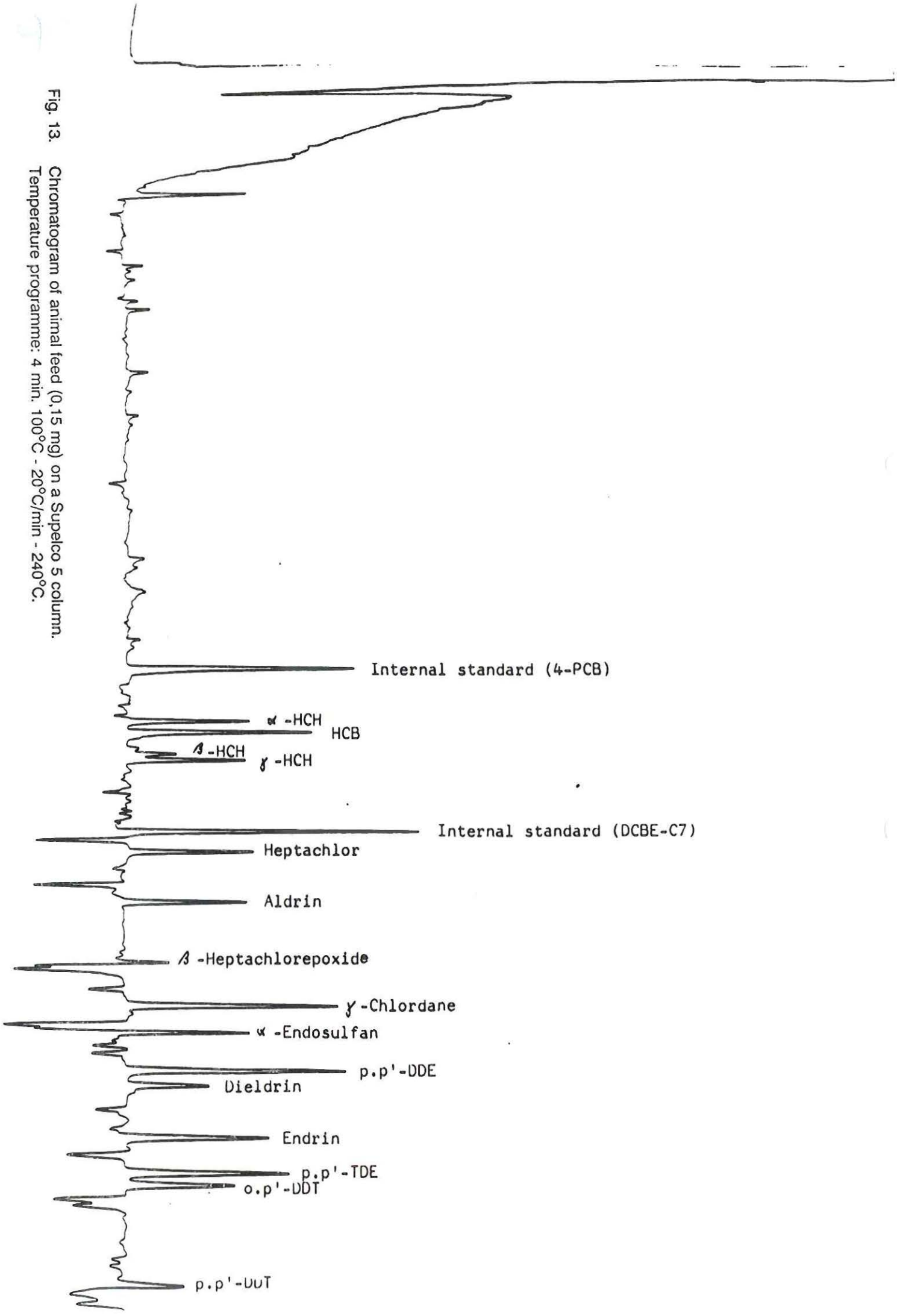


Fig. 13. Chromatogram of animal feed (0,15 mg) on a Supelco 5 column. Temperature programme: 4 min. 100°C - 20°C/min - 240°C.