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ACCUMULATION AND DISPOSAL OF SOME BYPRODUCTS FROM VINYL-CHLORIDE PRODUCTION IN COD LIVER

by

B. Braaten and E. Egidius Møllerud x)

Introduction

Last year a new type of pollution was discovered along the Norwegian coast, the North Sea and parts of the North Atlantic (Jensen et.al. 1970). This pollution was byproducts of vinylchloride production, and the major part consists of chlorinated aliphatic hydrocarbons (Cl-C). Relatively little is known of the harmful effects of this heterogenous mixture, but studies of Jensen et.al. (1970) showed a tendency for accumulation in living organisms. Further, "the data indicated that the highest concentration of Cl-C found in the open sea was about one tenth of the lowest value found to cause an unequivocal acute biological effect" (cit.).

The present study is a preliminary study included in a series of experiments designed to show eventual harmful effects of Cl-C on several species of the Gadidae. The liver was supposed to be the main organ for storage of Cl-C. Accumulation in this organ and some other tissue was followed, also after the fish were transferred to fresh seawater. It must be emphasized that the experiments deals with direct uptake of Cl-C from the water.

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x)
Institute of Marine Research,
Directorate of Fisheries,
Bergen, Norway.

This work is part of a project worked by the Pollution Group consisting of: G. Berge, B. Braaten, L. Føyn, E. Egidius Møllerud and K.H. Palmork.

Material and methods.

The experimental fish pollack, Pollachius pollachius L. and cod Gadus morhua L., caught in the vicinity of Bergen, were used as test animals. In a preliminary experiment (nr. 1) with pollack, the animals were kept a few days for acclimation before use. In the two other tests, the cod were maintained in running sea-water in outdoor concrete tanks of approximately 36 000 l until the experiment started.

The cod in experiment 2 was caught in December 1970 and fed herring twice a week until the experiment started (April -71). The fish was overfed resulting in a big and mostly light coloured liver.

The cod in experiment 3 was collected in May 1971, fed with saithe and acclimated for two weeks. These animals were slender with a small and often dark coloured liver.

Table 1 presents the biological data for the animals tested.

Experiment 1 was performed in a static test solution of 100 l (0.7 ppm). The aquaria, 150 l PVC containers, were coated with a plastic film and aerated. The toxic solutions were changed after 24 hours, and only 10 fish were used in each test.

PVC aquaria of a 1 000 l size were used in experiment 2 and 3, containing 720 l of a continuous flowing toxic solution. The experimental set up with use of a precision dosage pump, similar to the type described by Swedmark et.al. (1971) was used. The total waterflow was adjusted to 1 000 ml/min with additional aerating.

The waterflow and dosage of toxic solution were checked daily and adjusted if necessary. The temperature was recorded daily, the salinity was not controlled since the water used is a more or less constant in respect to salinity throughout the whole year (34.6 $\pm$ 2 °/oo).

Table 2 gives information about the experimental conditions.

Test material

The present investigation covers studies of some byproducts from Norwegian vinylchloride production. A preliminary study was carried out on the vinylchloride crude waste (EDC-waste) (batch 1). A destillate from the EDC tar was used in the other experiments (batch 2), with approximate

composition of the following observed components: 1.2 dichloroethane 49 % (1), 1.2 dichloropropane 28 % (2), 1.2.3 trichloropropane 7 % (4), 1.1.2 trichloroethane 1 % (7) and pentachloroethane <0.5 % (5). The number in brackets refers to peak number. The chemical composition of the EDC tar used will be reported later (Jensen et.al. 1972 (in prep.).

Stock solutions in experiment 1 and 2 were calculated by weight pr. volume, but later analysis showed that the actual concentrations were lower. On the basis of these results, stock solution in experiment 3 was increased 2.5 times. Table 3 gives the theoretical and actual concentrations used, the latter based on average values of the stock solution.

Table 3. A summary of the actual concentrations used ("average" values) compared to the theoretical (expected) values, and the concentrations of the stock solution.

Exp.	Conc. (ppm)		Actual conc. (ppm)			"Average"
nr.	Stock solut.	Theoret.	Comp. 1.	Comp. 2.	Comp. 4.	All comp.
1	-	1	-	-	-	0.7
2	50	1	0.34	0.40	0.58	0.4
2	50	0,1	0.03	0.04	0.06	0.04
3	125	2.5	0.92	1.07	1.43	1
3	125	0.25	0.09	0.1	0.14	0.1

Stock solutions were prepared every second or third day, and samples for analysis were taken the day after. Water samples from the tanks containing 0.1 and 1 ppm (experiment 3) were taken daily.

Biological samples were taken at certain intervals, packed in staniol and deep-frozen for later gas chromatography analysis. The present material consisted of regular samples of liver and random samples of brain, heart, muscle, gonad and gallbladder. All analysis were made using a Perkin Elmer 900 gas chromatograph equipped with an electron capture detector (3 H tritium) and coupled to an Infotronics digital integrator (model CRS 100).

Results

Experiment 1. Accumulation of EDC crude waste.

Fig. 1 illustrates the accumulation of an unknown compound (x) from the EDC crude waste of batch 1 measured pr. gram pollack liver. The curve is based on average values of two samples, except after $\frac{1}{2}$ hour (4 samples) and 12 hours (3 samples).

The calculation of concentrations has been done by comparing peak height of the unknown compound to a standard of 262 ng/l. Concentrations are therefore relative and inaccurate. It seems that accumulation takes place within the first 24 hours, after which a level is reached.

Only few studies were carried out concerning accumulation in other organs part from liver. Average values obtained from 5 individuals, tested in 10 ppm in 24 and 96 hours showed the following order of accumulation: liver > brain > lateral muscle > heart > gonad > gall-bladder. The concentration ratio calculated pr. gram tissue was for liver/brain 9, liver/lateral muscle 30, brain/lateral muscle 5 and lateral muscle/heart 1.5.

The results are only indicative, and further studies are needed.

The concentration in the water was roughly calculated to be 0.7 ppm (peak x). By using liver concentration attained after 48 hours, an accumulation factor of 540 was found.

Results from this test are not directly comparable with the following experiments since the measured compound was unknown.

Experiment 2. Accumulation of EDC tar distillate in fatty cod livers.

Great variations in the liver weight were found in the fish used in this experiment. The concentration of the stock solution also varied to a great extent, although accurate amounts of EDC tar distillate were used. Considering these circumstances, it was not possible to get more than a rough estimate of accumulation.

The experiment showed a clear relationship between liver weight and the amount of Cl-C accumulated, for the components studied (2 - 4 - 5 and 7). The method used gave quantitative results of component 4 - 5 and 7 only. The remaining components were either not measurable in the liver, or too difficult to separate from other peaks with the temperature used on the gas chromatograph. A maximum level of accumulation was attained rather fast fig. (2). After reaching a level, further variations in the concentrations seemed to vary according to differences in the test solution. This was indicated in fish from 0.4 ppm, but not from 0.04 ppm (fig. 2). A comparison of concentrations in the liver from fish exposed to 0.4 and 0.04 ppm respectively are given in table 4. The value for each component represents the average value for all the fish tested in the given concentration.

Table 4. Residues of three components from the EDC tar distillate measured in µg pr. gram liver. C_1/C_2 represent the ratio between the highest (C_1) and the lowest (C_2) concentration (experiment 2).

Test conc. (ppm)	Average concentrations in µg pr. gram liver		
	comp. 4	comp. 5	comp. 7
0.4 (C_1)	12.720	0.0403	2.430
0.04 (C_2)	1.290	0.0026	0.174
Ratio C_1/C_2	9.8	15.5	14
Expected ratio C_1/C_2	10	10	10

The actual ratio found between the two concentrations tested was in good agreement with the theoretical value. This indicates that the amount of Cl-C taken up is in proportion to the concentration in the water. For component 4 and 7, following accumulation factors were found.

0.4 ppm	7 - 192	0.04 ppm	7 - 181
	4 - 326		4 - 368

Experiment 3. Accumulation of EDC tar distillate in lean cod livers.

Since the test material in experiment 1 and 2 were small and variable, a third experiment was designed in order to verify the results of the two previous tests. The selection of test fish were better, giving a more homogenous liver material. A better control of the test concentrations were also encountered, although uncontrolled variations appeared (fig. 3).

Fig. 4 represent a comparison of the amount accumulated of component 4 in the total liver, pr. gram liver and the corresponding variations in medium concentration and stock solution. In accordance with the other tests an immediate uptake in the liver was found, and a maximum level was reached within 12 hours. On continuing the exposure no further accumulation seemed to take place.

Component 4, 5 and 7 diminished rapidly after transferring the fish to fresh seawater, and was untraceable within 48 hours with the method used (table 5).

Table 5. Average concentrations in µg pr. gram cod liver. Duration of experiment 13 days in 1 ppm, and 5 days in clean sea-water. Mean of three samples.

Time (days)	Liver weight (in g)	Average conc. µg/g liver of the components		
		4	5	7
½	1.62	10.5	0.032	1.9
1	1.47	4.7	0.036	1.5
2	1.47	9.0	0.030	2.0
3	1.32	4.3	0.018	1.2
4	1.65	12.3	0.058	2.7
7	1.35	8.06	0.033	2.7
13	1.03	2.5	0.012	1.2
½	0.7	1.2	trace	trace
1	1.12	trace	trace	trace
1½	0.83	trace	trace	trace
2	0.95	0	0	0
4	0.62	0	0	0
5	1.01	0	0	0

By comparing the curves for test concentration and concentration in the liver obtained for component 7 in 0.1 ppm, a real time lag was observed (fig. 5). Discrepancies are probably caused to uncertainty in the analysis, since the measured level of concentration was in the lower detectable range of the gas chromatograph.

An accumulation factor was determined for component 4 and 7 based on average values obtained during the exposure to 1 ppm. The values being 72 and 59 respectively.

The stock solution in experiment 3 was 2.5 times stronger than the stock solution in experiment 2, and a similar relationship was expected to be found in the concentration in the liver. Table 6 shows that values obtained in experiment 3 were far below the theoretical ones as seen by the C_1/C_2 ratio.

Table 6. Residues of three components from the EDC tar-distillate measured in ug pr. gram liver. The results are based on average values from the fish tested in each concentration. C_1/C_2 represent the concentration ratio between experiment 1 (C_1) and experiment 2 (C_2).

Test conc. (ppm)	Average conc. in ug. pr. gram liver		
	comp. 4	comp. 5	comp. 7
1 (C_1)	7.34	0.0313	1.90
0.4 (C_2)	12.72	0.0403	2.43
Ratio C_1/C_2	0.58	0.78	0.78
Expected ratio C_1/C_2	2.5	2.5	2.5

When comparing the C_1/C_2 ratio for 1 and .1 ppm with increasing exposure time, the ratio changed from 11 to 15 ($\frac{1}{2}$ day), 2.1 to 5.8 (1 day) to 0.28 to 0.82 (13 days), for component 4 and 7 respectively. The following accumulation factors based on single observations were found in 0.1 ppm.

Component 7 : $\frac{1}{2}$ day - 49, 1 day 467 and 13 days - 600
Component 4 : $\frac{1}{2}$ day - 128, 1 day 68 and 13 days - 106

Although an increase in the test concentration with time was observed, the amount of Cl-C found in the liver was exceedingly higher than expected (fig. 5). The present data do not reveal the reason such findings.

Discussion and conclusion

The present study showed that relatively small amounts of Cl-C were taken up by the organism, compared to the accumulation of for example Dieldrin in the tissues examined (Lane and Livingstone, 1970). Both the chlorinated aliphatic hydrocarbons and the organochlorine insecticides are of liphophilic character, and will easily be stored in fatty tissue. The main organ for biotransformation of foreign compounds is usually the liver. However very little information is available concerning detoxication mechanisms and metabolism of foreign compounds in fishes (Warren, 1971, Scheline, 1962).

In the Gadidae the liver is the main organ for storage of fat. It was therefore expected that the greatest amount of Cl-C were found in this organ. The different accumulation factors found in experiment 2 and 3 is possibly due to the liver conditions, the more fat, the more Cl-C stored. The fact that the C_1/C_2 ratio found for lean (C_1) and fatty (C_2) livers was far below the theoretical values, also indicate the importance of the fat content.

The comparison of "fingerprints" (chromatograms) in the water and the tissue in the present material and other studies (Jensen et. al. 1970) seems to indicate that no, or only minor changes took place within the organism. The disappearance of Cl-C from the liver and the muscle tissue (which was seen in experiment 2) after transfer to clean seawater, is possibly due to elimination through lipoidal membranes of the gills. Whether the components are metabolized or not, in the liver is difficult to determine at this stage with the present method.

A state of equilibrium was probably reached within 12 hours in a lean liver, and within 48 hours in a fatty liver, for the components studied.

The complexity of the waste product, and the lack of information on the mechanism of toxicity makes it difficult to evaluate the biological consequenses for the fish, exposed to small concentrations of Cl-C in seawater.

Whether the Cl-C has any permanent effects on liver function or not has yet to be investigated. Further informations are needed on accumulation through the food chain, compared to direct uptake, as presented in this study. Long term effect experiments in low concentrations must also be investigated. The present material are to be considered as preliminary, and further studies are under investigation.

Abstract

The accumulation and disappearance of byproducts from vinylchloride production in cod liver was studied, using a gas chromatographic method. Preliminary studies were also carried out on other organs. A maximum level of Cl-C accumulation was attained within 48 hours, and disappeared at the same rate when transferred to clean seawater. The fat content of the liver seemed to have great importance on the rate the amount of Cl-C accumulated.

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Table 1. Biological data of test fish used in three bio-assay experiments.

Exp. nr.	Conc. (ppm)	Species	Length (cm)			Weight (g)			Liver weight (g)			Fish in exp.	Parall. pr. analyz.
			Average	max.	min.	Average	max.	min.	Average	max.	min.		
1	0,7	G. pollach.	13,5	15	12	20	-	-	4,5	6,2	2,4	17	2
2	0,4	G. morhua	25,4	29	20,5	166,5	275,6	74,5	12,6	26,4	2,7	10	1
	0,04	"	27,6	32,5	22	108	281	92,8	17,5	42,4	4,2	10	1
	C.	"	26,6	30	20	199	310,5	68,9	15,9	32,5	2,7	9	1
3	1	"	21,2	26	13,5	76,8	134	19,8	1,2	2,5	0,2	39	3
	0,1	"	20,7	28	17	77,5	134,6	48,5	1,1	2,0	0,6	39	3
	C.	"	25,3	29	18	139,6	223,5	41,1	2,5	4,3	0,9	7	1

Table 2. Outline of experimental conditions of three bio-assays.

Experiment nr.	Start date	Total exposure time (days)		Conc. (ppm)	Temp. °C		
		Toxic solut.	Fresh seawat.		Average	+	-
1. Static test	23/11-70	2	-	0.7	11.5	14.3	8.7
2. Cont. flow. test	23/3 -71	44	6	0.04	6.8	7.8	5.7
"		"	"	0.4	6.9	8	5.8
3. "	7/6 -71	13	5	0.1	10.7	12.2	9.1
"	"	"	"	1	10.9	12.5	8.9

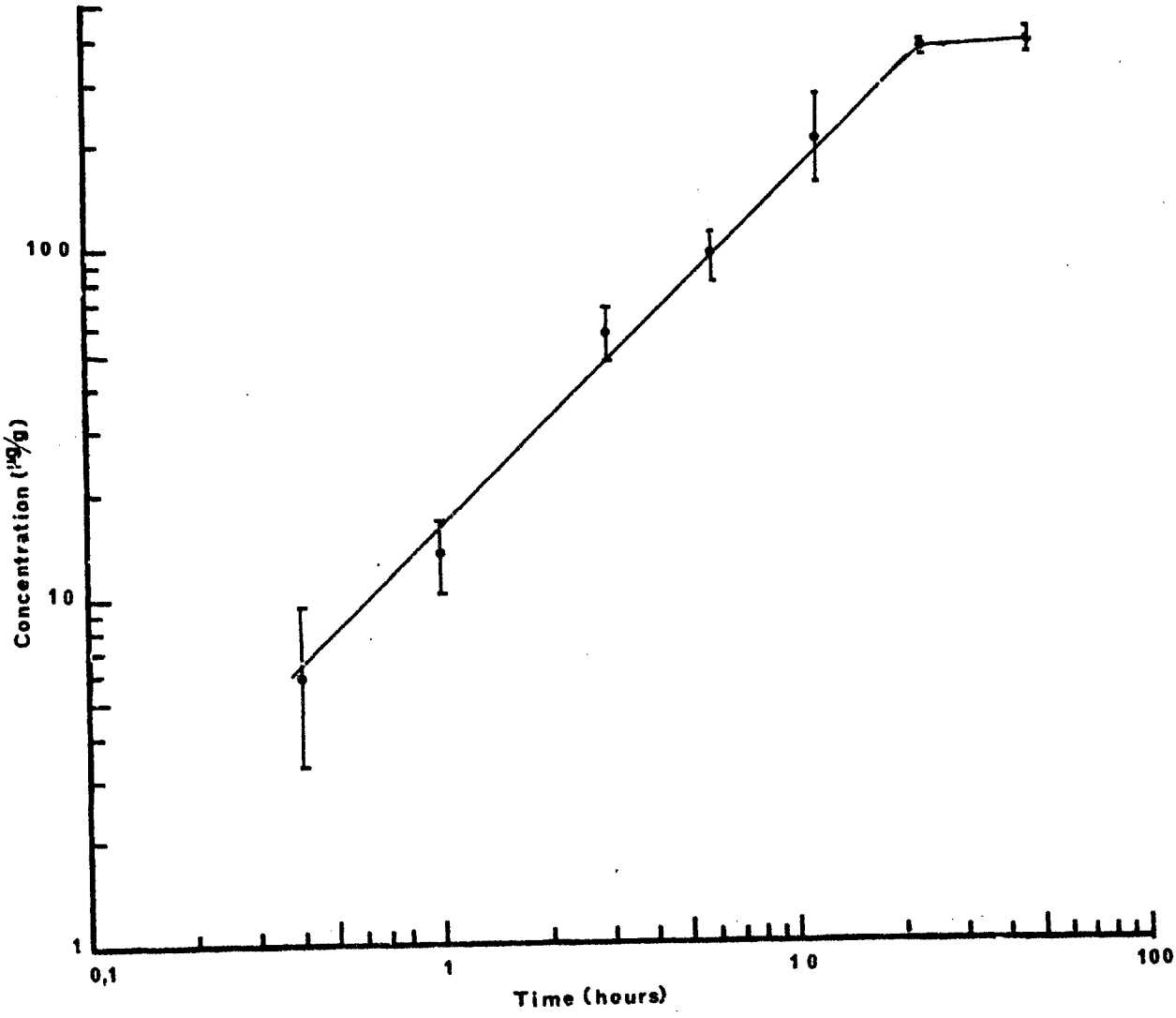
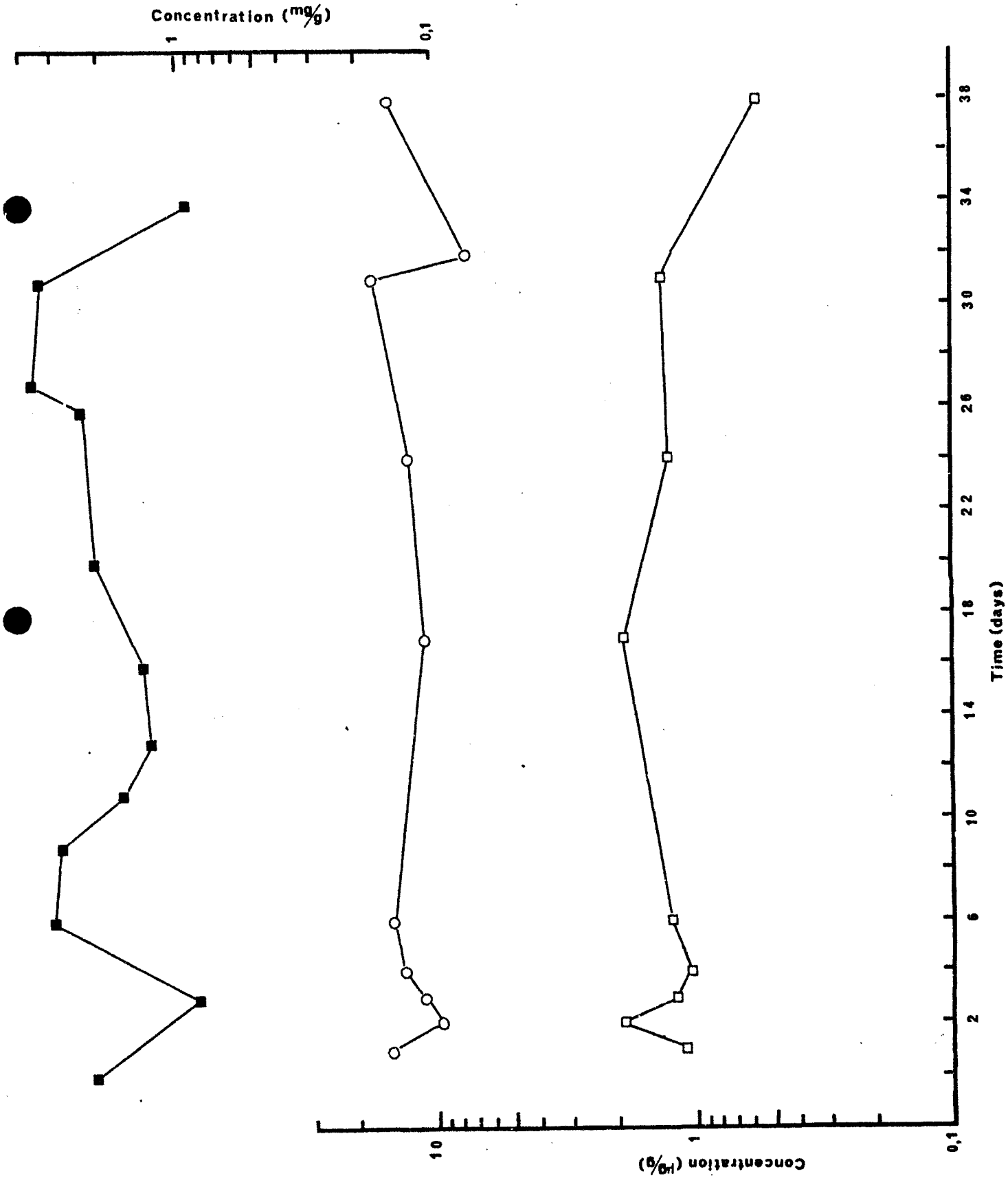


Fig. 1

Accumulation of EDC crude waste expressed by the concentration of compound x pr. gram pollack liver. Test concentration 0.7 ppm, exposure time 48 hours.

Fig. 2

Accumulation of EDC tar distillate expressed as component 4 pr. gram cod liver in test solution 0.4 ppm ○——○ and 0.04 ppm □——□ . Variation in stock solution 50 ppm ■——■ . Scale for stock solution on the right side. (Experiment 2).



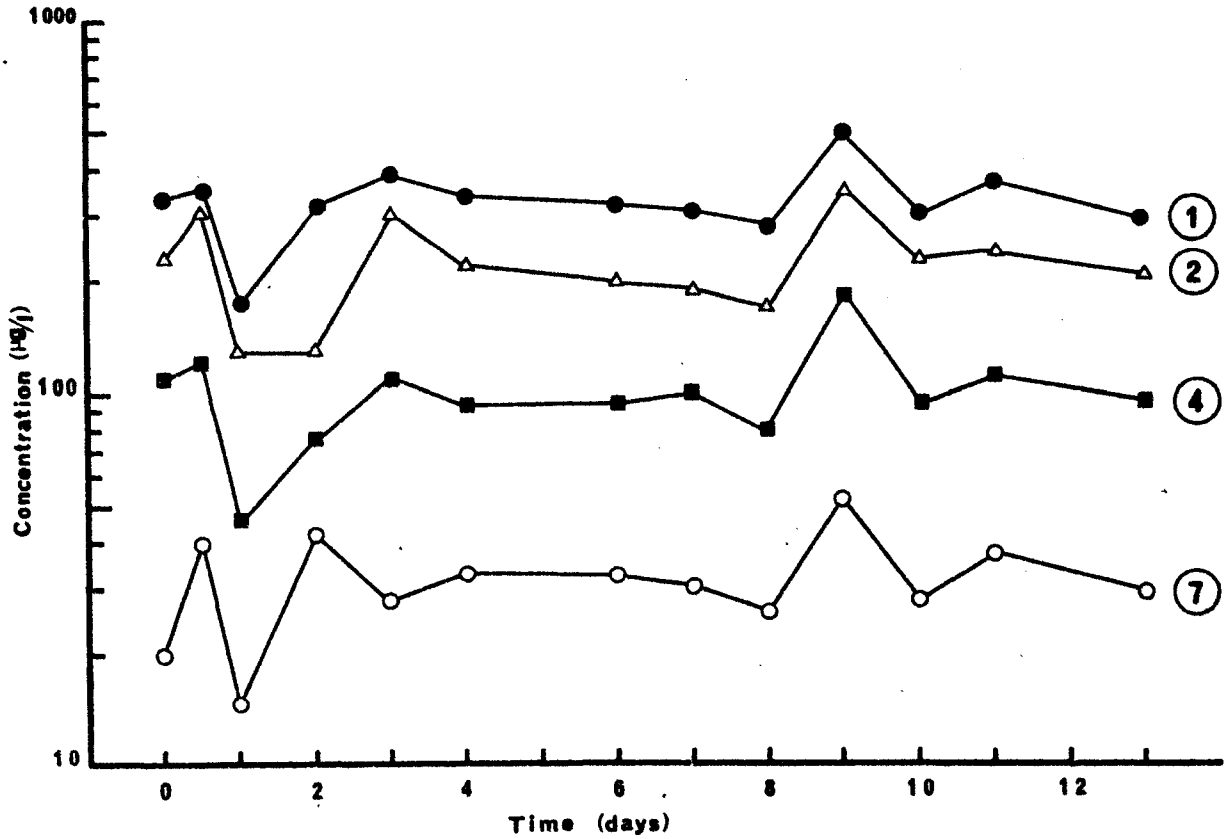


Fig. 3

Variation in the test concentration of four components from EDC tar distillate during a period of 13 days. (Experiment 3). The component number indicated on the figure.

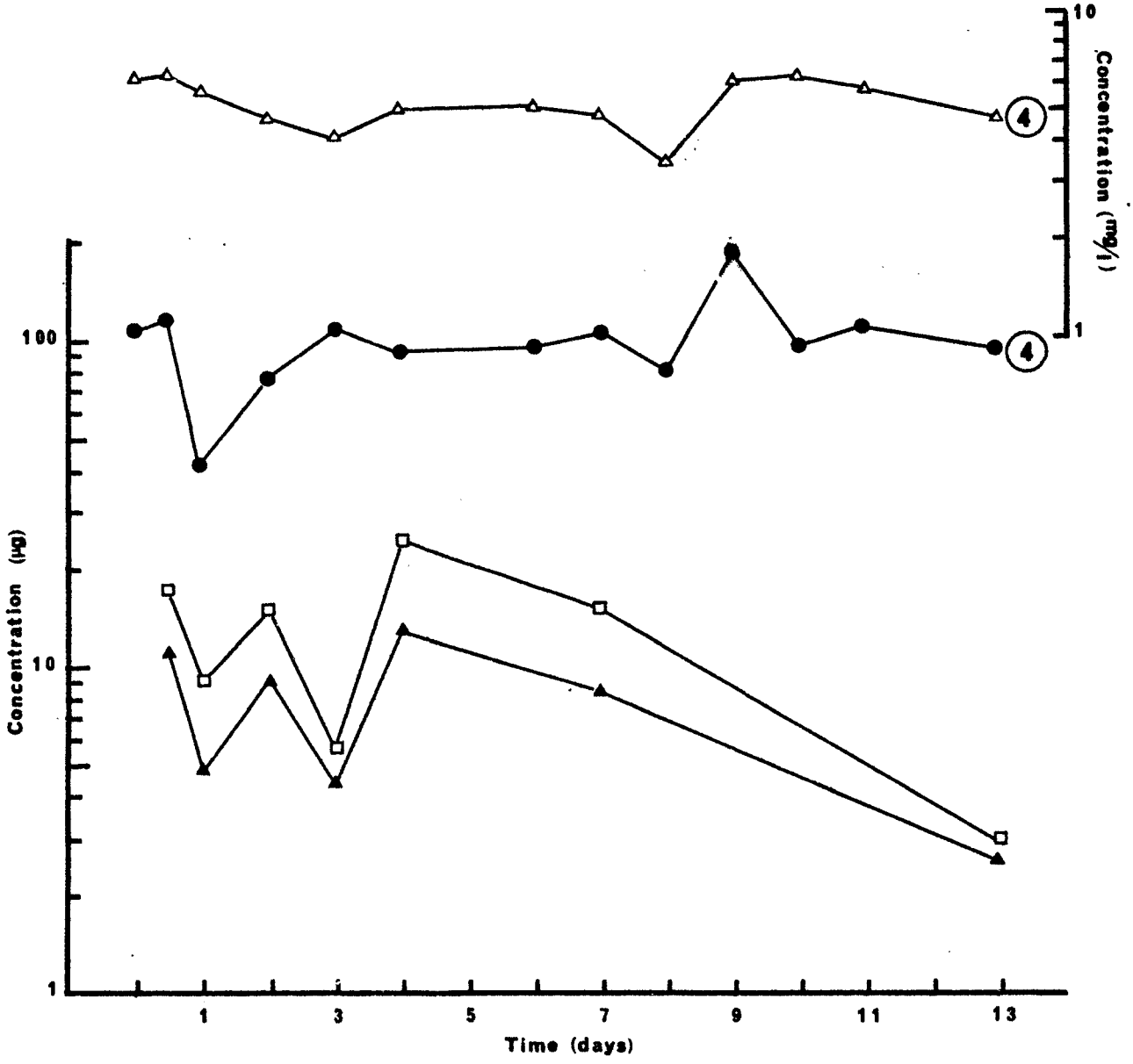


Fig. 4

Accumulation of EDC tar distillate expressed as component 4 in total liver ▲——▲ , and pr. gram cod liver □——□ . Variations in stock solution (125 ppm) △——△ , and test solution (1 ppm) ●——● . Scale for stock solution on the right side. (Experiment 3).

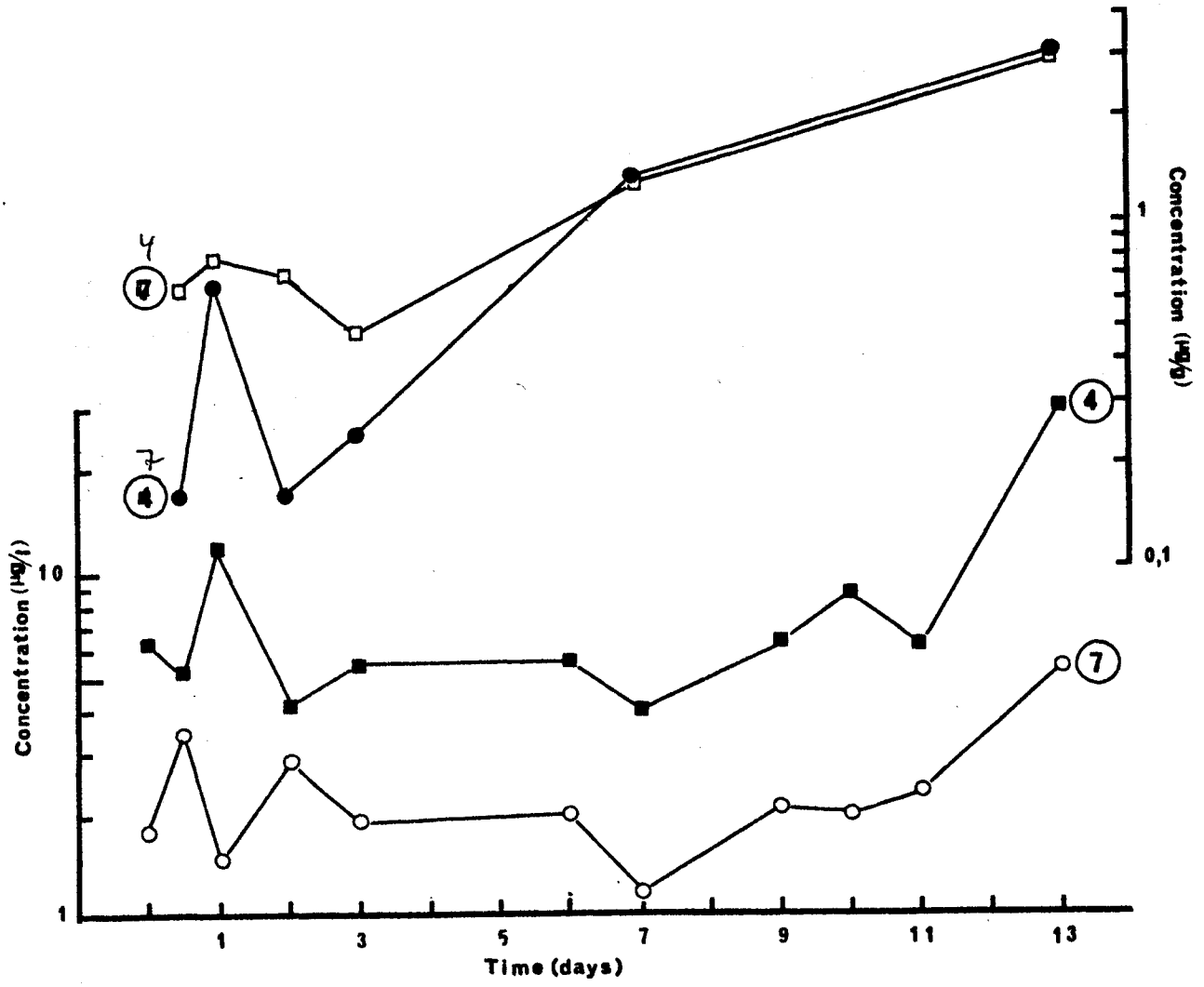


Fig. 5

Accumulation of EDC tar distillate expressed as component 7 ●——● , and 4 □——□ pr. gram cod liver. Variation in test solution 0.1 ppm, component 4 ■——■ , and 7 ○——○ . (Experiment 3).