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UPTAKE AND LOSS OF DIELDRIN BY MARINE ORGANISMS by K W Wilson, J E Thain and Jean Yardley, Ministry of Agriculture, Fisheries & Food, Fisheries Laboratory, Burnham-on-Crouch, Essex.

INTRODUCTION

Assessing the biological problems of releasing a new substance into the marine environment is a complex but necessary procedure. Clearly there is no single test that can demonstrate the hazards associated with all substances and the experimental programme of investigation for each compound should include the measurement of short and long term toxicity, its ability to accumulate in organisms directly or through food chains, and the rates of physical, chemical and biological change. The latter includes microbial degradation and metabolism by higher organisms. As part of this assessment it is necessary to understand the rate and extent to which a compound can be taken up from water and eliminated, and how physical, chemical and biological parameters affect these kinetics. This paper describes experiments to determine the rate of uptake from water and the loss of dieldrin by a variety of marine organisms.

MATERIALS AND METHODS

Test animals

The following test species were used:

Species	Average wet weight (g)	Number per tank
Mytilus edulis	2.78	20
Ostrea edulis	2.11	20
Crangon crangon	0.60	30
Agonus cataphractus	1.82	15

The animals were collected from the estuary of the River Crouch, Essex and maintained in running aerated sea water in the laboratory. They were not fed during the acclimation period or during the experiments.

Test compounds

The test compound used was analytical grade (>99.5% purity) dieldrin, 1, 2, 3, 4, 10, 10- hexachloro - 6, 7 - epoxy - 1, 4, 4a, 5, 6, 7, 8, 8a - octahydro - exo - 1, 4 - endo - 5, 8 - dimethanonaphthalene. Stock solutions of dieldrin were made up in 3:2 distilled water and Analar grade acetone.

Bioaccumulation and elimination

The continuous flow apparatus of Connor and Wilson (1972) was modified by

replacing most of the PVC delivery tubing with glass capilliary tubing. The test animals were held in 10 litre Perspex tanis receiving flows of sea water of 10 1/hour and of dieldrin solutions of 2 ml/hour. Nominal concentrations of dieldrin in water of 0.1 to 20 µg/1 were tested; actual concentrations of dieldrin in the water were determined at least once every day.

Routinely the accumulation of dieldrin was monitored for 20 days. The dieldrin flow was then stopped and clean sea water passed through the tanks for up to 30 days. Samples of animals were taken daily. They were weighed after surface drying (wet weight) and stored at -20°C to await analysis. Only the soft parts of mussels and oysters were weighed and analysed; shrimps and fish were analysed whole. Animals were analysed individually.

Throughout the experiments the sea water was maintained at 13°C and salinity at 30-32°/oo.

Chemical analysis

Sea water was extracted with a suitable volume of n-hexane and an aliquot of the hexane was then injected directly onto the GLC. For the determination of dieldrin in the animals, the tissue was first cut into small pieces and transferred to a 250 ml round bottomed flask fitted with a splash head and downward delivery condenser. With shrimps and fish samples only 10 ml of 2N NaOH was added and the tissue allowed to hydrolyse for 30 minutes. For all tissues 100 ml distilled water, 4 ml of antifoam compound and 10 ml of n-hexane were added to the flask and approximately 50 ml water distilled off together with the hexane. This hexane layer was removed by pippette and a sample injected directly onto the GLC.

When fat content was also determined, the tissue was first mixed with anhydrous sodium sulphate, placed in a thimble and soxhlet extracted with hexane for 2 hours. After cooling, the extract was diluted to 100 ml with n-hexane; from this solution 1 ml was cleaned up on an alumina column (Holden and Marsden 1969) before analysis for dieldrin on GLC. The remainder of the solution was used to calculate fat content.

Each sample was analysed on a Varian Aerograph model 1700 fitted with twin 3H electron capture detectors. Details of the columns etc are given below:

glass columns 6 ft x ¼ in O.D.
channel A 4% DC 200 + 6% QFI)
channel B 8% DC 200) both on 80-100 chromasorb W AW DMCS
injector temperatures 240°C
column oven 180-185°C
detector oven 220°C
nitrogen flow 40-50 ml/minute

RESULTS

Water levels

In all cases the levels of dieldrin in solution in the tanks were less than the levels calculated from the flows of sea water and toxin solution. It must be assumed therefore that there was a steady loss of dieldrin from the water to silicone tubing, Perspex, glassware, etc before it reached the test tanks.

Once established, the dieldrin levels in each tank remained very constant (Table 1) and no attempt was made to adjust them to the original nominal concentrations.

TABLE 1 Concentration of dieldrin in water during the 20 day accumulation period

Test animal	Mean concentration (µg/l)	Standard error	Daily observations
Mytilus	0.93	0.024	21
	3.78	0.30	21
	5.41	0.16	19
	15.5	0.58	21
Crangon	0.047	0.002	21
	0.72	0.006	21
	1.27	0.03	21
	6.68	0.33	10
Agonus	1.53	0.88	11

Levels in control animals

For each experimental series a control tank received sea water and stock solution without added dieldrin. Control animals from these tanks were sampled daily and analysed. Despite the fact that the animals were not fed during the experiments there was no apparent increase or decrease in tissue levels of dieldrin with time. Mean values for dieldrin concentrations in control animals are given in Table 2.

TABLE 2 Concentration of dieldrin in control animals from loss experiments

Test animal	Mean tissue level (µg/g)	Standard error	Number of samples
Mytilus	0.036	0.004	29
Crangon	0.036	0.005	29
Ostrea	0.0081	0.0012	22
Agonus	0.029	0.010	11

Accumulation

The pattern of uptake of dieldrin by Mytilus from different concentrations in sea water is shown in Figure 1. There was clearly an increase in tissue concentrations of dieldrin with time and the rate of increase was related to the water level.

Attempts were made to fit the data to an exponential equation of the form $Ca_t = C_{ss}$ (1-exp(-kt) but no significant relationships could be established. However linear regressions were significant (Table 3).

Similar results were obtained with shrimps (Figure 2). At each water level there was a linear increase in concentration with time. Again no equilibrium or steady state was reached during the 20 day exposure period. From the uptake data it is possible to derive some of the accumulation parameters. These are given in Table 3.

TABLE 3 Accumulation coefficients of dieldrin

Species	Concentration in water	Concn. at day 20 (µg/kg)	Rate of accumn. (µg/kg/day)	AF	Accumulation equation
Mytilus	0.93	4,103	207	223	Ca=0.21t-1.037
	3.78	14,998	810	214	Ca=0.81t-1.202
	5.41	24,210	1,311	242	Ca=1.311t-2.01
	15.5	44,041	2,174	140	Ca=2.17t-0.561
Crangon	0.047	145	5	106	Ca=0.005t-0.045
	0.072	197	12	167	Ca=0.012t-0.043
	1.271	3,030	150	118	Ca=0.150t+0.03
	6.68	10,357*	1,102	165	Ca=1.102t-0.663
Agonus	1.53	12,464*	1,042	682	Ca=1.042t+2.044

Loss experiments

In these experiments the animals were exposed to dieldrin concentrations for 20 days and then transferred to clean sea water. The results for the four different test species are shown in Fiture 3. In all cases there was a decline in tissue concentration with time and the results gave a significant fit to an exponential equation of the form, $C_a = C_o$ (exp-kt). From these data it has been possible to calculate the biological half-life (t50) and the time for the elimination of 90% of the accumulated level (t90) in all species. These data are given in Table 4.

TABLE 4 Elimination coefficients of dieldrin

Species	Water level on uptake (µg/l)	-K	t50 (days)	t90 (days)
Mytilus	0.8	0.086t	8.1	27
		0.057t	12.2	40
Crangon	0.65	0.133t	5.2	17
Agonus	0.1	0.068t	10.2	34
Ostrea	1.8	0.112t	6.2	21

Effect of length of uptake period

Mussels exposed to the same concentration of dieldrin (2 µg/l) were transferred to clean sea water after different exposure periods. The rate of loss of dieldrin from these mussels is shown in Figure 4. There was an exponential decrease in dieldrin concentrations with time. Estimates of biological half-life ranged from 9 to 20 days but bore no relationship to the length of the exposure period.

Fat content

It was clear from results of earlier uptake and loss experiments that certain biological parameters were important in determining the residue levels attained in an animal. Figure 5 shows the effect of physiological condition of Mytilus on the rate of uptake of dieldrin over 10 days. Wet weight and condition factor (wet tissue weight per unit internal volume of shell) were both important determinants of tissue level; fat content was not significantly correlated with dieldrin accumulation.

DISCUSSION

Dieldrin has a low solubility in sea water but a high affinity for plastics, PVC etc. However the present experiments have shown that, using a suitable constant flow apparatus, constant water levels can be obtained. To ensure that dieldrin concentrations do not decrease within the test chamber relatively small tanks with high flow rates are recommended.

In the present experiments it was necessary to use a solvent carrier, acetone, to assist solution of the dieldrin in sea water and it is not known if this procedure affects the accumulation characteristics of dieldrin. There was no evidence of any toxic effects of the acetone on control animals but acute toxic effects of dieldrin were observed during the uptake experiments with Agonus and Crangon, as shown in Table 5.

TABLE 5 Toxic effects of dieldrin

Species	Water level (µg/l)	Time to 50% mortality (days)	Animal level at death (mg/kg)
Agonus	0.47	16.2	10.7
	1.06	3.1	3.5
	1.10	3.3	3.8
	1.60	4.2	9.3
Crangon	7.6	2.5	7.0
	6.6	5.2	7.9
	0.9	6.8	-

There was a clear relationship between the concentration in water and time to death, but more interesting was the increase in residue level in the dead animals with increase in the exposure time causing mortality.

Accumulation of dieldrin by all species showed a similar pattern at all concentrations. Accumulation increased linearly with time and even at the lowest concentrations a progression to a steady state situation was not apparent. Under conditions where a steady state is not attained the levels accumulated by the animal are dependent both on water level and exposure time. Discussing this problem, Adema and Compaan (1975) demonstrate the usefulness of the term AF (here defined as accumulation factor) for comparing experimental results. They concluded that for any one species the value of AF was constant, irrespective of water level and time. This has been confirmed in the present study (Table 3). The low value of AF for Mytilus exposed to 15 µg/l dieldrin may be indicative of physiological effects of this high concentration.

The pattern of loss of dieldrin from animals transferred to clean water was similar for all species but, unlike the accumulation experiments, the loss process was shown to be exponential. This result infers that the uptake process also proceeds at an exponential rate but this could not be detected in the short term.

The values for biological half-life of dieldrin, determined from the rate of loss to 50% of the level at the end of the exposure period, were between 5 and 10 days. Except for Crangon it was not possible to confirm if the exponential rate of loss continued and the extrapolated values of t90 were realised. In the case of fish and mussels, determination of t90 would have entailed extending the experiments for several weeks. There are considerable difficulties in prolonging experiments of this nature. For example, the variability in residue levels shown

by Mytilus during the down run after a 30 day exposure period was very much greater than for the shorter exposure times, and this suggests that changes in the physiology of the animals were occurring as a result of prolonged starvation. Certainly in Mytilus, size and condition are both important in determining the level of accumulation, and the lack of correlation between percentage fat content and dieldrin accumulation may have been due to lack of a steady state situation.

For any compound then the value of the biological half-life is dependent on species and upon its physiological state. Attempts to standardise uptake and loss experiments need to take account of these and other factors, such as temperature, feeding rate, etc. These considerations become very important in the longer term studies.

SUMMARY

The kinetics of accumulation and loss of dieldrin in sea water by mussels, shrimps, oysters and fish have been determined. Concentration factors at the end of the 20-day exposure period ranged from 2000 for shrimps to 12000 for fish. Steady state levels were not reached with any species at any water concentration during the accumulation period. The biological half-life of dieldrin was found to be 5 days in shrimp, 6 days in oysters, 10 days in fish and 8-12 days in mussels. Residue levels were related to condition factor and wet weight of mussels but not to their fat content.

REFERENCES

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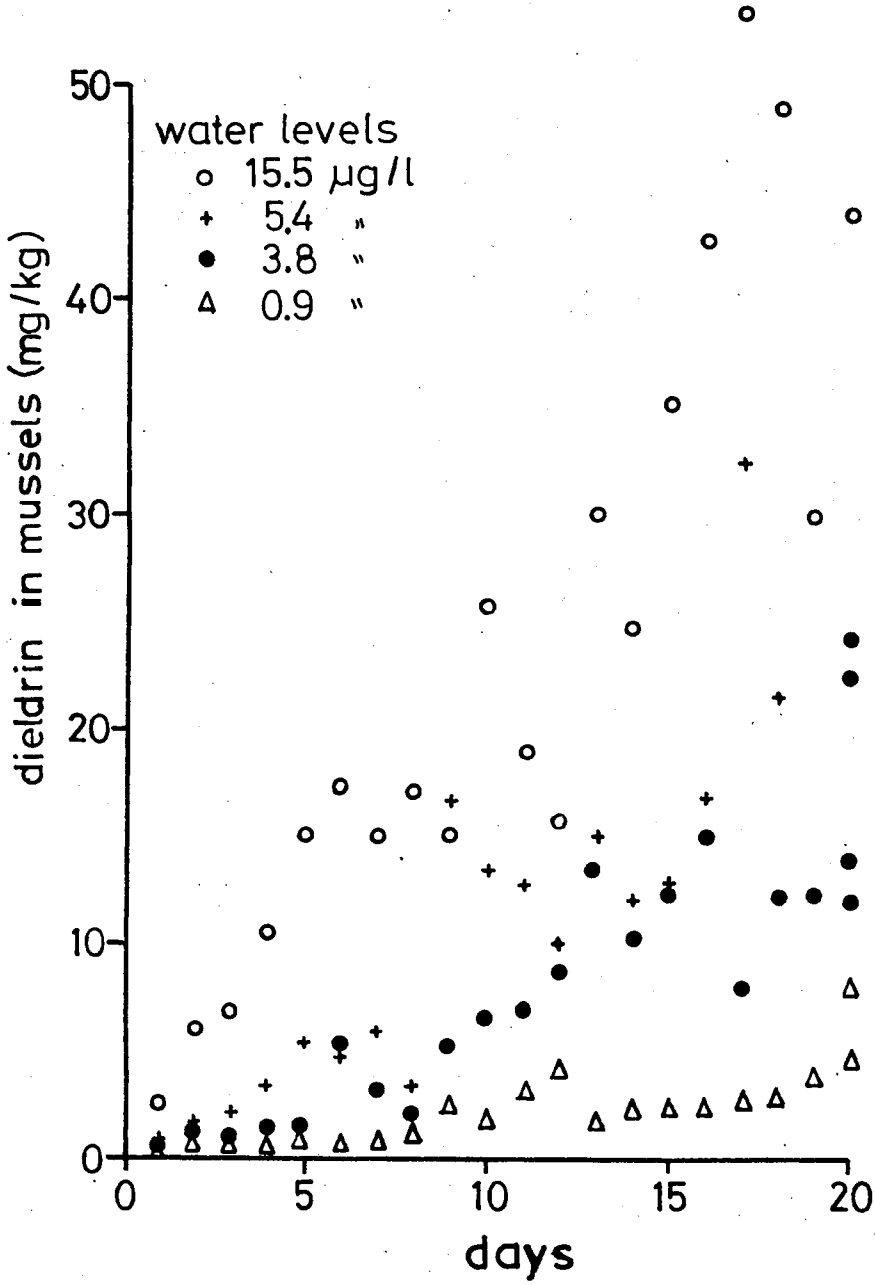

Figure 1. Accumulation of dieldrin from water by mussels, Mytilus edulis.

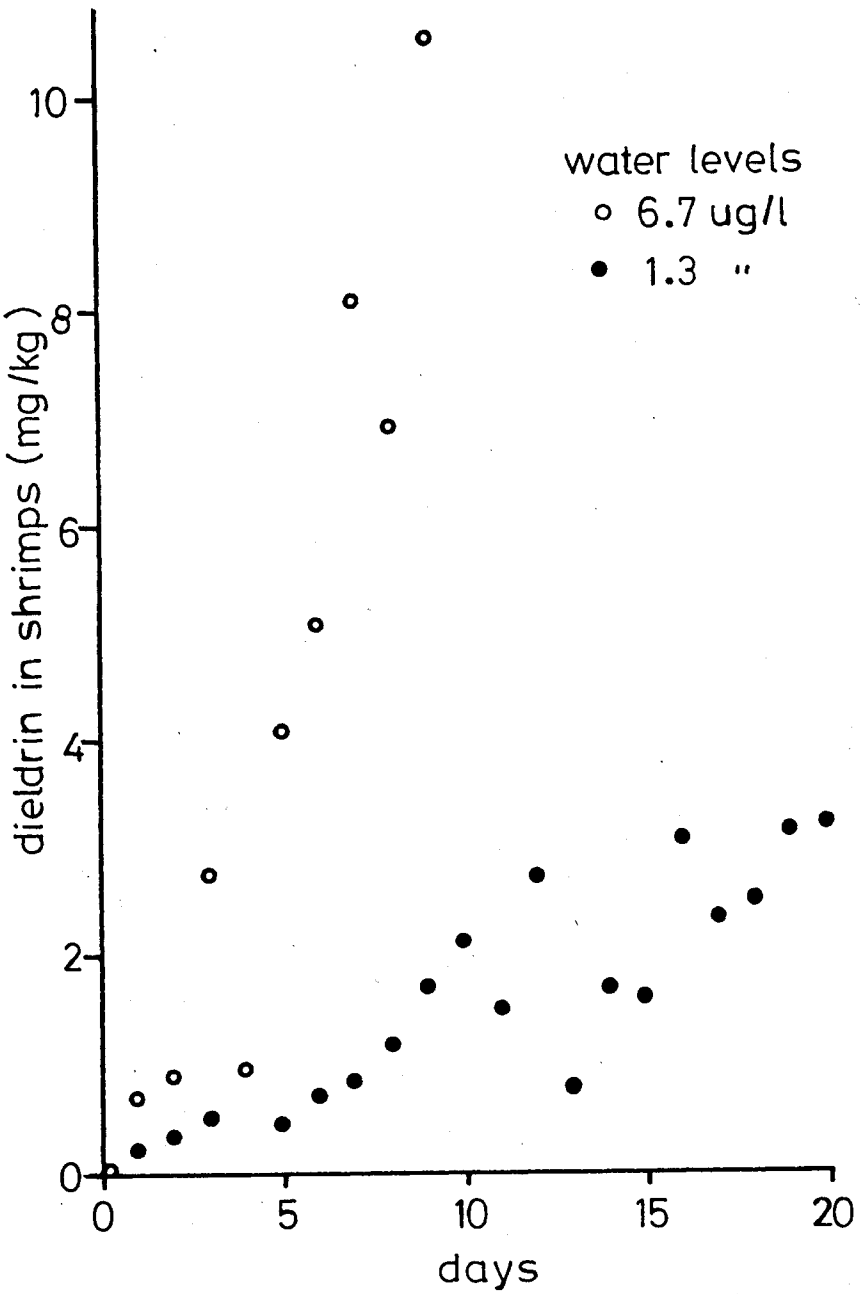

Figure 2. Accumulation of dieldrin from water by shrimps, Crangon crangon.

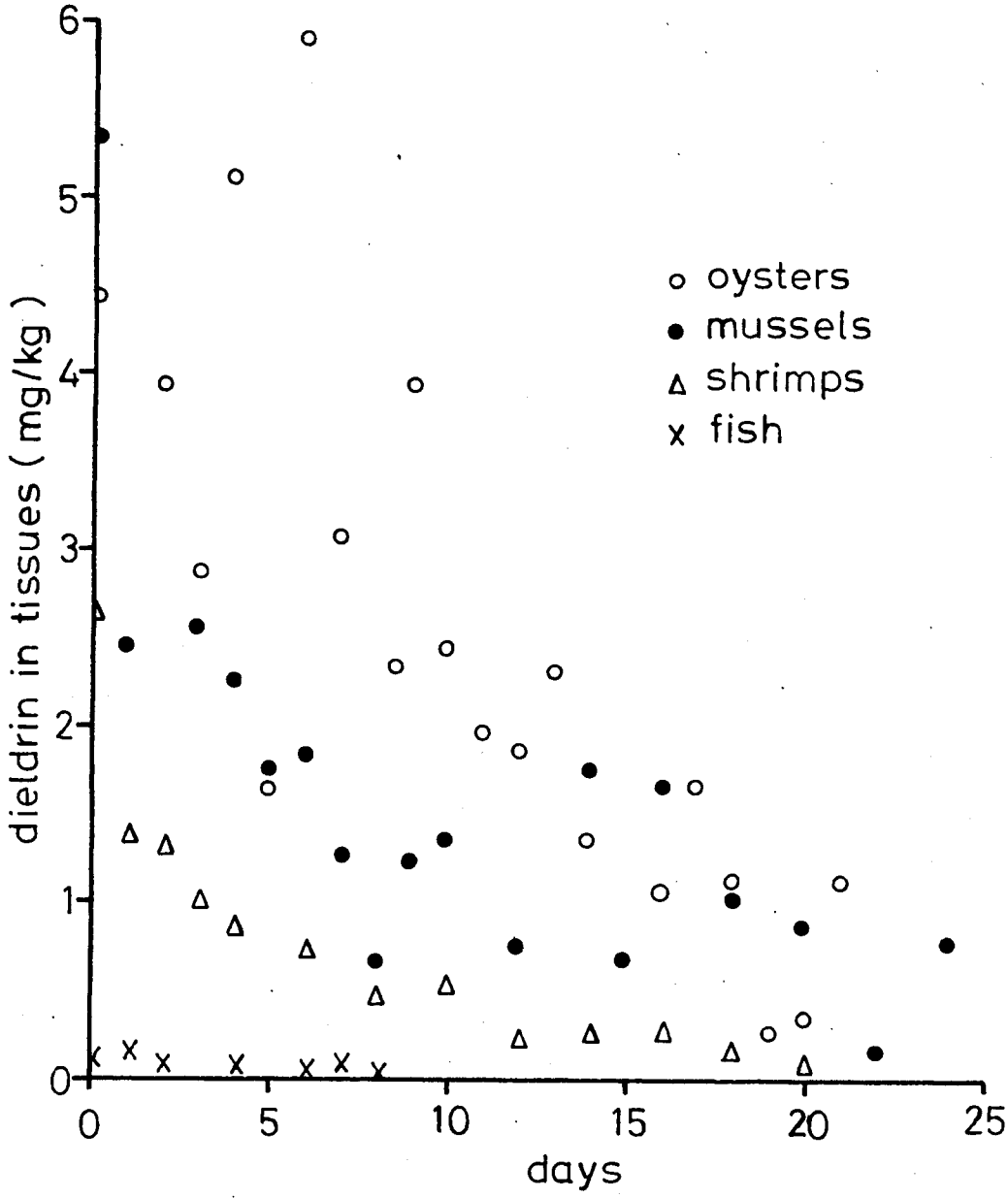

Figure 3. Loss of dieldrin by mussels, oysters, shrimps and fish.

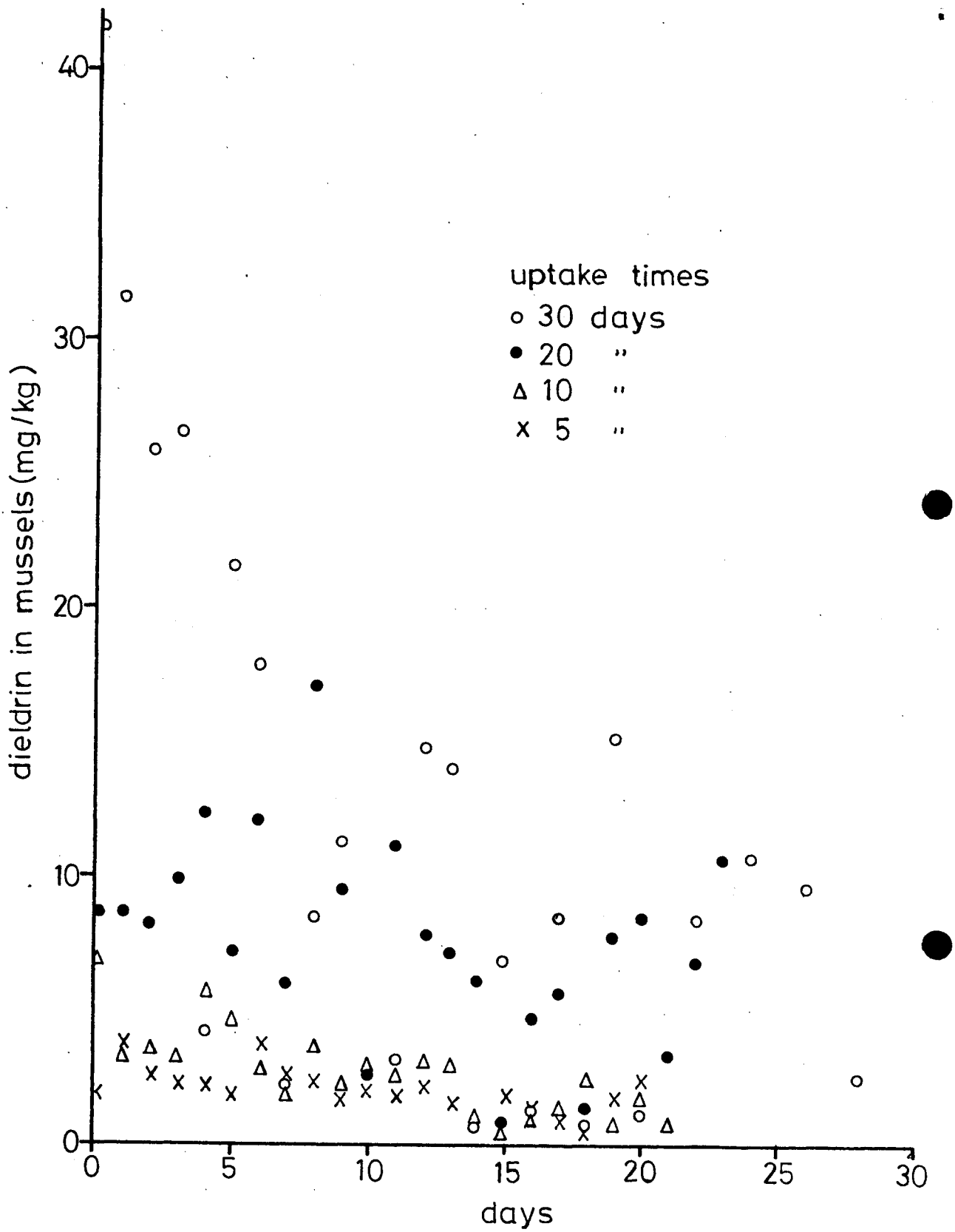

Figure 4. Loss of dieldrin by mussels after different exposure times.

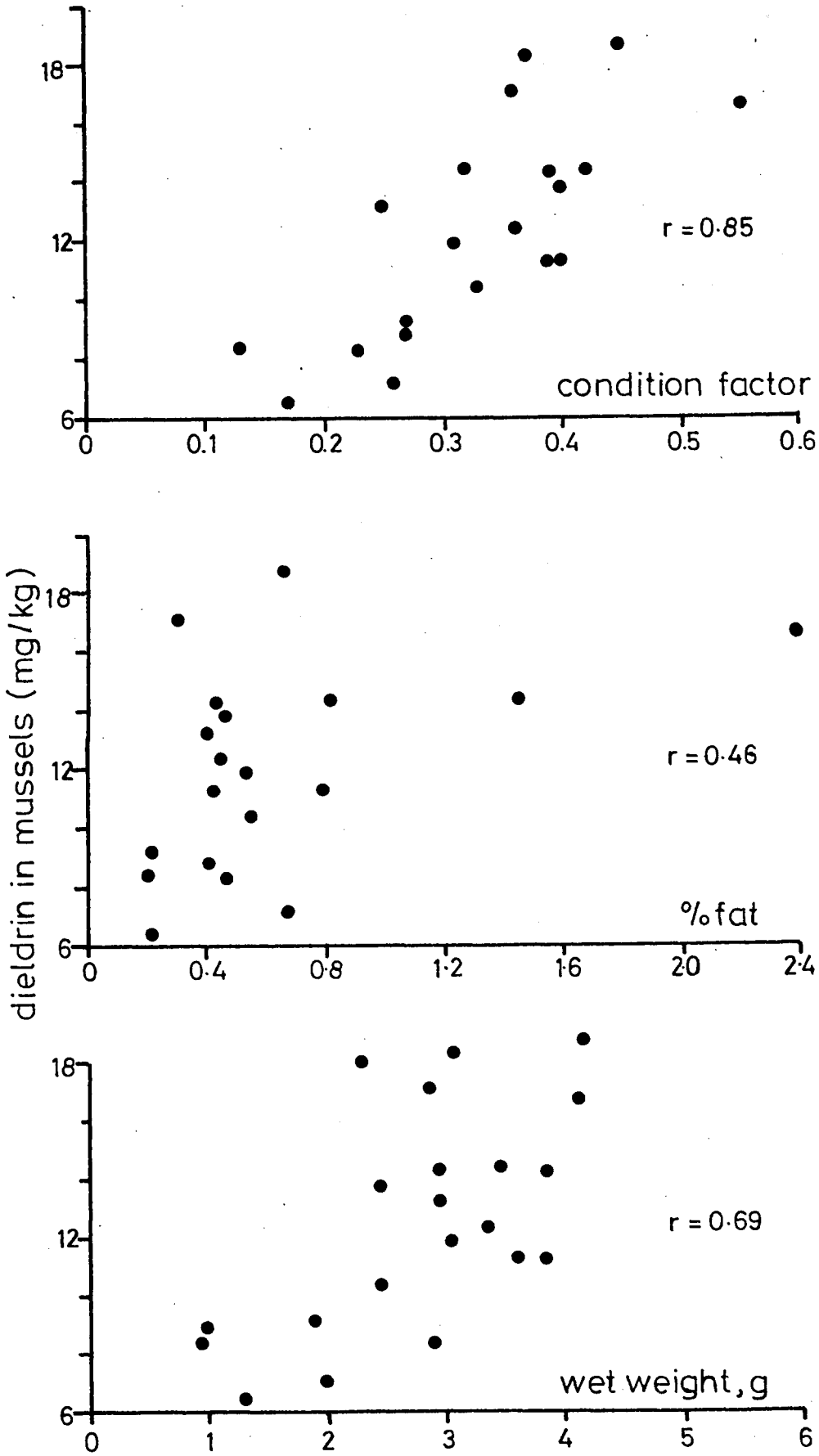

Figure 5. Relationship between condition and dieldrin level in Mytilus after 10 days exposure to 2ug/l