UPTAKE, METABOLISM, AND ELIMINATION OF DDT AND DIELDRIN BY FRESHWATER MUSSELS

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ABSTRACT

UPTAKE, METABOLISM, AND ELIMINATION OF DDT AND DIELDRIN BY FRESHWATER MUSSELS

By

James Bedford

Freshwater mussels were exposed to several concentrations of DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane) and dieldrin (hexachloroepoxyoctahydro-endo, exo-dimethanonaphthalene) in reconstituted distilled water, dechlorinated tapwater, and natural lake water under continuous flow and constant temperature conditions.

The mussels concentrated DDT approximately 1000 fold in distilled water and 2400 fold in lake water. They concentrated dieldrin about 1200 fold in lake water. The concentration of insecticides in the mussels reached equilibrium with the level in the water faster in lake water than in distilled water and the insecticides also had a shorter half-life in lake water. Dieldrin's half-life was 4.7 days in lake water, about one-third that of DDT's half-life.

The insecticide concentrations were highest in the digestive and reproductive tissue and low in the muscle, mantle, and gill tissues. The concentrations were very low in the

marsupia in tests run in distilled water but were almost as great as the digestive and reproductive tissue in lake water.

Increasing the temperature of the water increased the rate of uptake and elimination of the pesticides by the mussels and increased the equilibrium concentration in dechlorinated tapwater from 5-20^{o}C.

UPTAKE, METABOLISM, AND ELIMINATION OF DDT AND DIELDRIN BY FRESHWATER MUSSELS

By

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INTRODUCTION

Currently there is much concern over the contamination of our waterways by pesticides and other chemicals (Stickel, 1968). Very small concentrations of these contaminants have been found to have deleterious effects. For example a water concentration of 1-3 parts per trillion (ppt) DDT in Lake Michigan has resulted in several species of fish surpassing the Food and Drug Administration's temporary tolerance level for human consumption of 5 parts per million (ppm) (Reinert, 1970). Butler (1966) found that small concentrations of insecticides (e.g., 10 parts per billion (ppb) DDT) in the water resulted in a large decrease in the shell growth of oysters.

The Federal Water Quality Administration Surveillance System maintains 131 sampling stations at which water quality data, including the identification and measurement of organic pollutants are collected (Breidenbach et al., 1966). Since it would be impossible to set up elaborate monitoring stations to keep a check on all possible sources of insecticide pollution, the use of biological monitors has been suggested and tried.

The most widely used group has been the bivalves (Class Lamellibranchia). The eastern oyster (Crassostrea virginica) has been used extensively as an insecticide monitor in the marine environment (Casper, 1967; Bugg et al., 1967; and U. S. Department of Interior, 1965). Several species of freshwater mussels (Unionidae) have been used in various freshwater situations (Miller et al., 1967; Goodsil and Johnson, 1968; Bedford et al., 1968; and Fetterolf and Willson, 1970). The freshwater mussel is currently (fall, 1970) being used by state agencies in Michigan, Wisconsin, Minnesota, and Indiana to monitor Great Lakes' tributaries.

In spite of this recent extended use of freshwater mussels as biological monitors little is known about the uptake, metabolism, and elimination of insecticides by these organisms, or even what significance certain concentrations in the mussels have in terms of the concentration in the environment from which they were removed.

Thus the purpose of this study is to increase our understanding of this potentially important biological tool in the monitoring of insecticide pollution by accomplishing the following objectives:

1. Correlation of the amount of insecticide concentrated by the mussel with the concentration in the water.
2. Determination of the primary locations in the mussel

where the insecticides and any metabolites formed are stored.

3. Determination of the time required for the insecticide concentration in the mussel to reach equilibrium with the concentration in the water.
4. Determination of the half-life of the insecticide in the mussel after the insecticide introduction is terminated.
5. Determination of the effect of temperature on the uptake and elimination of insecticides by the mussel.

METHODS

Mussels used for this study were collected by hand from local streams and lakes. Anodonta grandis and Elliptio dilatatus were collected from the Red Cedar River, Ingham County, and the Looking Glass River, Clinton County, Michigan. Lampsilis siliquoidea was collected from Gun Lake, Barry County, Michigan. The mussels were held in a recirculating tank (Frigid Units, Inc.) at 9°C until tested.

Two methods were utilized to provide a continuous flow of a known concentration of insecticide. The first involved the daily premixing of the insecticide solution in a 190 l stainless steel tank. The solution was then introduced into the test aquaria via siphoning action. The second method utilized a Beckman solution metering pump (Model 746). A filter flask was used as a mixing flask into which the insecticide was metered and the water was siphoned from a constant head source. The constant head was provided by continuously overflowing a 190 l stainless steel tank.

In both methods the insecticide was dissolved in absolute ethanol before addition to the water. The insecticide stock solution was made sufficiently concentrated so that the ethanol concentration in the water never exceeded 10 ppm.

All experiments were run in aquaria partially submerged in constant temperature water baths. The water containing the insecticide was preconditioned to the temperature of the bath before addition to the aquaria by flowing through stainless steel coils submerged in the bath (Figure 1). Either glass or Teflon tubing was used throughout the system. The water was then siphoned out of the aquaria into a second filter flask, thus maintaining a constant level in the aquaria (Figure 1).

Pre-sterilized silica sand was used as a substrate for the mussels in all experiments.

Mussels from the test aquaria were prepared for insecticide analysis as follows: The living mussels were removed from their shells, drained, and weighed to the nearest mg. The mussels were then blended in a Sorvall Omni-Mixer for three min. at 10,000 rpm with 50 ml of hexane-acetone (2:1). The solvent mixture was decanted and the sample blended twice more with 50 ml aliquots of additional solvent. The combined extract was then washed with a 10% NaCl solution to remove the acetone. The extract was dried over anhydrous Na_2SO_4 and a 10 ml aliquot was removed for determination of per cent fat, by evaporation of the solvent in a vacuum oven at 60°C. The remaining extract was concentrated to approximately 5 ml in a water bath at 80°C for introduction onto a clean-up column.

Figure 1. Schematic diagram of experimental apparatus for exposing mussels to insecticides.

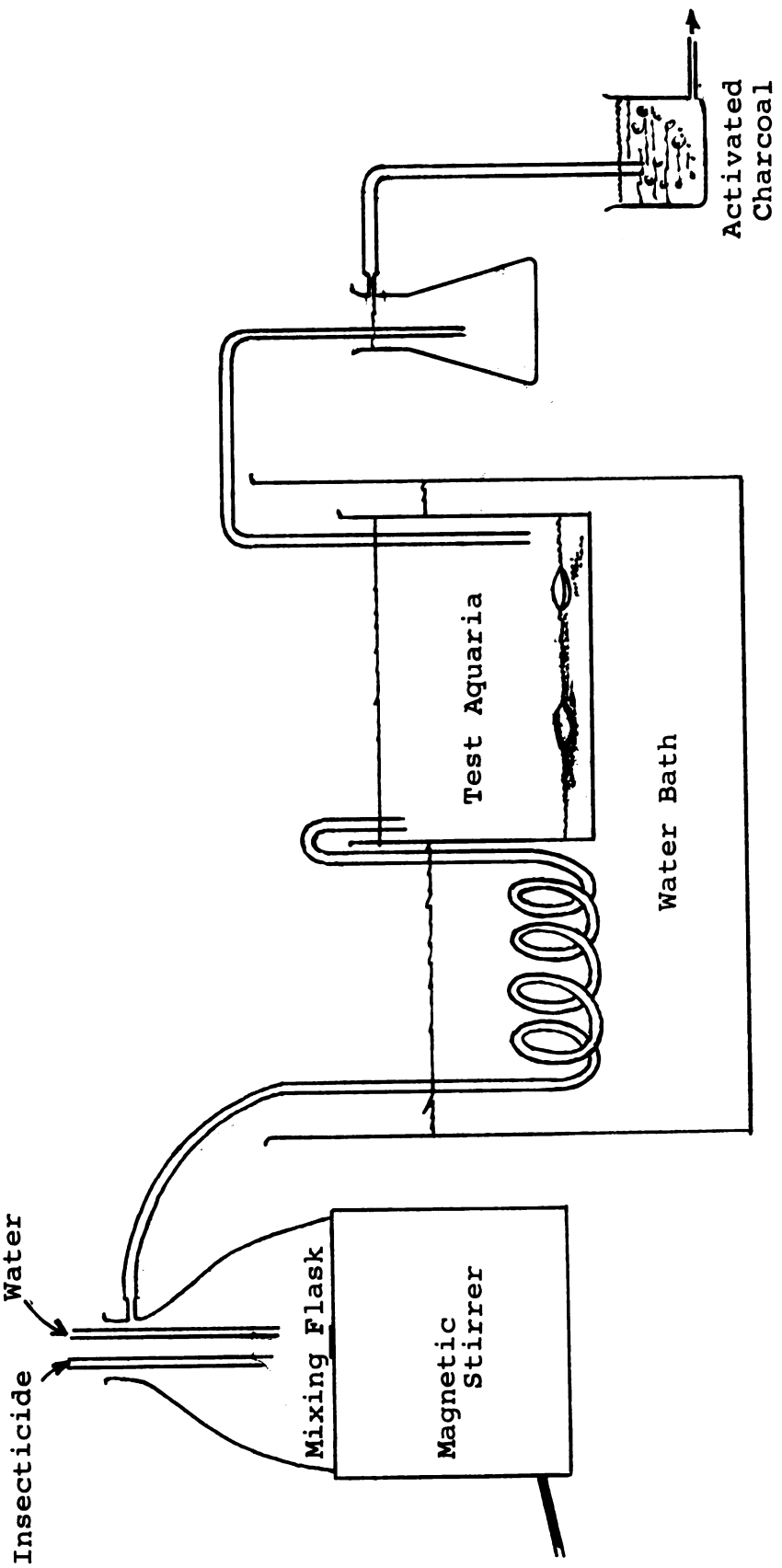

Insecticide
Water
Mixing Flask
Magnetic Stirrer
Test Aquaria
Water Bath
Activated Charcoal

Figure 1

Pyrex columns, 2.4 x 50 cm, fitted with a fritted-glass disk, were packed with 10 g of Florisil-Celite (5:1) with a layer of anhydrous Na_2SO_4 above and below the packing. The Florisil, activated at 649°C by Floridin, Inc., was deactivated with approximately 10% distilled water and the mixture was calibrated before use to ensure conformation to the elution procedure used. Each sample was eluted with 200 ml of n-hexane and then reconcentrated to a volume of 5 ml. These extraction and clean-up procedures generally follow those recommended by Shell Development Company (1964) with several modifications.

One liter water samples, taken periodically from the outlets of the test aquaria, were extracted successively in 2 l separatory funnels with 100, 50, 50, 50, and 50 ml of hexane. The combined extract was dried over anhydrous Na_2SO_4 and concentrated to 5 ml for introduction into the gas chromatograph. All solvents were re-distilled before use.

A Beckman GC 4 chromatograph, equipped with a discharge electron capture detector was used for the analyses. It was fitted with a 6 ft (1.83 m) x 1/16 in (1.59 mm) Pyrex column packed with 11% QF-1 and 3% DC 200 on Gas Chrome Q (60/80 mesh) and was operated at a column temperature of 220°C and a 30 ml/min helium (99.995% pure) flow. The injection temperature was 250°C and the detector temperature, 275°C. Standards were injected at the beginning of each run, after

every six samples, and at the end of the run. Quantitations were based on peak height and the concentrations were based on the wet weight of the mussel.

The identities of the insecticides and their metabolites found were confirmed gas chromatographically using columns packed with 5% DC 11 on Gas Chrome Q and 11% QF-1-OV-17 (1.3:1) on Gas Chrome Q. Selected samples were also spotted on Brinkman pre-made silica gel thin layer plates, developed with hexane-diethyl ether (4:1) and detected with Rhodamine B.

The insecticides used were DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane), obtained from City Chemical Corp., N. Y., N. Y., and dieldrin(hexachloroepoxyoctahydro-endo,exo-dimethanonaphthalene), obtained from Shell Chemical Co., N. Y., N. Y. Both were recrystallized and 99+% pure.

Recovery for DDT from lake water was 89.2±4.4% and from tapwater was 81.0±3.8%. For dieldrin recovery from lake water was 69.1±2.4% and from tapwater was 69.0±2.3%. Each per cent recovery was based on four spiked samples.

RESULTS AND DISCUSSION

Mussels are held as biological monitors of pesticides during all seasons in streams with a broad range of water quality. To determine some of the effects these conditions had on uptake and elimination of pesticides three different series of experiments were run in which freshwater mussels were exposed to known concentrations of DDT or dieldrin. In the first series of tests two species of mussels were exposed to various concentrations of DDT in distilled water with 50 ppm Ca^{++} added as $CaCl_2$ for shell maintenance. The purpose of this series of tests was to determine the uptake and elimination of the insecticide under conditions where there were no stimuli to feed. This condition could occur in small cold streams which have very little plankton or suspended material and do not naturally support mussel populations.

In the second series of tests, mussels were exposed to various concentrations of DDT or dieldrin in lake water to ascertain the uptake and loss of the insecticides under simulated natural conditions.

The third series involved the exposure of the mussels to DDT or dieldrin at different temperatures to determine

the effect of temperature on the up-take and loss of the insecticides.

Reconstituted Distilled Water

For the first experiment (1) thirty mussels (Elliptio dilatatus) were collected from the Looking Glass River. These were held for several months without food at 9°C before being placed in the test aquaria. Three control mussels were analyzed for pesticides immediately prior to the test and found to contain low levels of DDT and its metabolites TDE and DDE (Table 1). The remaining mussels were placed into the test aquaria and exposed to a mean concentration of 0.38±0.11 ppb DDT for three weeks at 20±1°C. After the introduction of DDT was stopped the mussels were exposed to reconstituted distilled water for one additional week. DDT at a concentration of 0.14 ppb was still present in the water leaving the aquaria at the end of the fourth week. Considerable mortality (50%) occurred during the experiment, and, for this reason, the experiment was terminated after the fourth week.

Three mussels were removed from the aquaria after each week and analyzed for insecticide content. The total DDT and its metabolites, TDE and DDE increased nearly fourfold after one week's exposure, continued to increase during the second week and then remained about the same for the following week

Table 1. Concentrations of DDT and its metabolites (ppm, wet weight) in Elliptio dilatatus exposed to 0.38±0.11 ppb DDT at 20°C for three weeks.

Weeks	Weight (g)	Fat (per cent)	DDE	TDE	DDT	Total
0	17.890	0.70	0.022	0.010	0.009	0.041
	16.848	1.10	0.029	0.024	0.048	0.101
	10.481	1.14	0.018	0.011	0.017	0.045
Mean		0.98	0.023	0.015	0.022	0.062
1	17.763	0.76	0.014	0.113	0.105	0.231
	11.579	1.08	0.018	0.105	0.102	0.226
	11.906	1.23	0.010	0.100	0.146	0.255
Mean		1.02	0.014	0.106	0.118	0.237
2	19.922	1.04	0.009	0.187	0.226	0.421
	17.508	1.07	0.006	0.105	0.121	0.232
	12.160	1.17	0.009	0.216	0.346	0.571
Mean		1.10	0.008	0.169	0.231	0.408
3	14.917	0.87	0.008	0.163	0.237	0.408
	12.201	0.75	0.005	0.173	0.128	0.306
	6.955	0.30	0.018	0.298	0.147	0.463
Mean		0.97	0.010	0.211	0.171	0.392
Stop DDT introduction						
4	12.586	1.00	0.008	0.189	0.154	0.351
	14.654	1.15	0.007	0.244	0.079	0.329
	10.962	0.70	0.009	0.226	0.153	0.388
Mean		0.95	0.008	0.220	0.128	0.356

(Table 1). The concentration in the mussels decreased about 10% after the introduction of DDT was halted (Table 1). The increase in total DDT and metabolites was due to an increase in DDT and TDE as the concentration of DDE decreased from that of the controls. There was no reduction in the per cent fat of the mussels even though they were being starved during the experiment.

In the second experiment (2) two changes were made in order to solve the mortality problem. The temperature was decreased from 20° to 15°C and the flow rate was increased from 30 ml/min to 120 ml/min.

For this experiment (2) 40 mussels (Anodonta grandis) were collected from the Looking Glass River just prior to experimentation. Three mussels were analyzed for pesticide content and were found to have 5 to 10 times the concentration of DDT found in any of the other mussels used in these experiments (Table 2).

The remaining mussels were exposed to a mean concentration of 0.27±0.08 ppb DDT for a period of five weeks. The experiment was continued for four weeks after the introduction of DDT was terminated. The DDT concentration in the water decreased to 0.09 ppb at the end of the sixth week and traces (< 0.05 ppb) were still present after the ninth week.

Three mussels were removed and analyzed weekly for insecticide content. As in the first experiment the DDT

Table 2. Concentrations of DDT and its metabolites (ppm, wet weight) in Anodonta grandis exposed to 0.27±0.08 ppb DDT at 15°C for five weeks.

Weeks	Weight (g)	Fat (per cent)	DDE	TDE	DDT	Total
0	49.222	0.70	0.006	0.062	0.165	0.233
	44.955	1.17	0.008	0.034	0.119	0.161
	24.661	0.84	0.005	0.050	0.213	0.268
Mean		0.90	0.006	0.049	0.166	0.220
1	31.024	1.07	0.009	0.107	0.267	0.382
	45.678	0.58	0.012	0.082	0.244	0.338
	21.704	0.76	0.012	0.099	0.327	0.439
Mean		0.80	0.011	0.096	0.280	0.386
2	25.877	0.49	0.014	0.246	0.696	0.956
	18.898	0.31	0.008	0.106	0.397	0.471
	9.605	0.74	0.026	0.558	1.228	1.813
Mean		0.51	0.016	0.304	0.760	1.080
3	40.947	0.78	0.015	0.158	0.410	0.582
	15.644	1.27	0.024	0.359	0.757	1.140
	35.749	0.41	0.012	0.213	0.507	0.732
Mean		0.82	0.017	0.243	0.558	0.818
4	38.633	0.46	0.005	0.136	0.259	0.400
	32.438	0.35	0.005	0.186	0.325	0.516
	10.259	0.63	0.008	0.283	0.246	0.536
Mean		0.48	0.006	0.202	0.276	0.484
5	40.451	0.64	0.014	0.306	0.637	0.957
	14.775	0.65	0.009	0.257	0.552	0.818
	20.268	0.55	0.008	0.232	0.405	0.644
Mean		0.61	0.010	0.265	0.531	0.806
Stop DDT introduction						
6	24.253	0.48	0.014	0.393	0.818	0.225
	24.993	0.69	0.009	0.178	0.357	0.545
	10.866	1.06	0.028	0.814	1.166	2.007
Mean		0.74	0.017	0.462	0.780	1.259

continued

Table 2--continued

Weeks	Weight (g)	Fat (per cent)	DDE	TDE	DDT	Total
7	40.455	0.69	0.019	0.218	0.654	0.891
	15.701	0.99	0.022	0.260	0.847	1.130
	6.774	1.38	0.035	0.377	0.998	1.410
Mean		1.02	0.026	0.285	0.833	1.144
8	39.920	0.48	0.012	0.217	0.343	0.571
	33.106	0.57	0.024	0.333	0.893	1.250
	36.392	0.80	0.018	0.515	0.665	1.198
Mean		0.62	0.018	0.355	0.634	1.006
9	34.110	0.48	0.011	0.278	0.326	0.615
	24.911	0.40	0.014	0.298	0.378	0.689
	16.991	0.51	0.026	0.612	0.617	1.256
Mean		0.46	0.017	0.396	0.440	0.853

concentration increased during the first two weeks and then leveled off except for a drop after four weeks (Table 2). Again the increase was due mainly to an increase in DDT and TDE. However, the concentrations reached in this experiment were considerably higher than the first even though the mussels were exposed to a slightly lower mean concentration of DDT at a lower temperature. When the introduction of DDT was terminated after five weeks the mussels showed a sudden increase in insecticide content and then slowly decreased (Table 2).

Although the fat levels were lower and more variable than in the first experiment (0.70±0.27% vs 1.00±0.19%), there was again no indication of reduction due to starvation. Mortality was reduced to about 10%.

Approximately the same DDT concentration (0.34±0.17 ppb) was employed for the third experiment (3) as in the first two in order to try to ascertain why there was such a large difference in the equilibrium concentrations of the first two experiments. In this experiment the mussels were dissected into the combined intestinal tract, associated digestive gland and the gonad (viscera); the muscle, mantle, and gill tissues (muscle); and tissue fluids. These three combinations were analyzed separately. The marsupia from gravid mussels were also analyzed separately for insecticide content.

Forty specimens of Anodonta grandis were collected from the Red Cedar River and held at 9°C for several weeks prior

to placement in the test aquaria. Three controls analyzed just before the experiment started were found to have low concentrations of DDT and its metabolites (Table 3).

DDT was introduced into the aquaria for a period of four weeks and the experiment continued for three weeks after the DDT introduction was stopped. The DDT level dropped rapidly to 0.012 ppb in the water following termination of its introduction but did not fall below 0.10 ppb for the remainder of the experiment.

Three mussels were removed for analysis each week. The concentration increased rapidly during the first two weeks and then leveled off (Table 3). The plateau concentration in the mussels was very close to that of the first experiment, about 0.4 ppm total DDT and its metabolites.

The highest concentrations of insecticides were found in the combined digestive and reproductive systems (viscera) in most mussels analyzed (Table 3). The concentrations in the combined muscle-mantle-gill portion were generally 1/2 to 2/3 that of the viscera while the marsupia usually contained considerably less than 1/2 the concentration in the muscle. A similar distribution was found in oysters by Butler (1966).

When the concentrations were based on the fat content of the tissues, there was a much smaller variation between tissues (Table 3). This indicated that there was little selective storage of the insecticides in the mussel. However, there was a gradual decrease in the proportion of

Table 3. Mean concentrations of DDT and its metabolites (ppm) in Anodonta grandis exposed to 0.34±0.07 ppb DDT at 15°C for four weeks.

Weeks	Tissue[2]	Per cent Fat	Wet Weight DDE	TDE	DDT	Total	Fat Weight Total
0	Viscera[3]	1.49	0.007	0.007	0.022	0.036	2.42
	Muscle[4]	0.49	0.005	0.003	0.016	0.024	4.90
	Marsupium(1)	0.41	0.003	0.002	0.009	0.013	3.17
	Fluids[5]				0.003	0.003	
	Whole mussel	0.80	0.005	0.004	0.017	0.027	3.37
1	Viscera	1.03	0.006	0.019	0.058	0.083	8.06
	Muscle	0.67	0.004	0.002	0.066	0.092	13.73
	Marsupium(1)	0.51	0.002	0.004	0.010	0.016	3.14
	Fluids				0.003	0.003	
	Whole mussel	0.76	0.004	0.020	0.060	0.084	11.05
2	Viscera	1.07	0.041	0.174	0.478	0.692	64.67
	Muscle	0.67	0.020	0.109	0.358	0.487	72.69
	Marsupium(1)	0.39	0.005	0.023	0.074	0.102	26.15
	Fluids				0.005	0.005	
	Whole mussel	0.72	0.026	0.124	0.391	0.541	75.14
3	Viscera	1.21	0.021	0.147	0.273	0.440	36.36
	Muscle	0.70	0.012	0.109	0.270	0.391	55.86
	Marsupium(1)	0.46	0.006	0.041	0.070	0.117	25.43
	Fluids				0.005	0.005	
	Whole mussel	0.84	0.014	0.107	0.266	0.415	48.82

continued

Table 3--continued

Weeks	Tissue[2]	Per cent Fat	Wet Weight DDE	TDE	DDT	Total	Fat Weight Total
4	Viscera	1.19	0.023	0.189	0.372	0.584	49.07
	Muscle	0.90	0.012	0.142	0.316	0.470	52.22
	Marsupium(2)	0.49	0.007	0.054	0.144	0.205	41.84
	Fluids				0.007	0.007	
	Whole mussel	0.85	0.014	0.136	0.266	0.415	48.82
Stop DDT introduction							
5	Viscera	1.20	0.020	0.241	0.480	0.742	61.83
	Muscle	0.72	0.008	0.124	0.244	0.354	49.17
	Marsupium(2)	0.46	0.004	0.062	0.051	0.117	25.43
	Fluids				0.003	0.003	
	Whole mussel	0.73	0.012	0.144	0.291	0.447	61.23
6	Viscera	1.23	0.020	0.293	0.420	0.732	59.51
	Muscle	0.74	0.013	0.201	0.280	0.494	66.76
	Marsupium	0.55	0.006	0.101	0.120	0.226	41.09
	Fluids				0.008	0.008	
	Whole mussel	0.77	0.011	0.179	0.244	0.434	56.36
7	Viscera	1.41	0.020	0.333	0.438	0.791	56.10
	Muscle	0.74	0.005	0.123	0.126	0.254	34.32
	Marsupium	0.51	0.005	0.102	0.089	0.197	38.63
	Fluids				0.003	0.003	
	Whole mussel	0.76	0.008	0.154	0.167	0.329	43.29

[2]Mean of three mussels unless otherwise noted.

[3]Combined digestive and reproductive tissue.

[4]Includes gills and mantle.

[5]Not included in whole mussel concentrations.

insecticide in the muscle portion to the viscera as the experiment progressed. This indicates some movement of the insecticide from the gills and mantle where it was initially absorbed to the viscera.

The tissue fluids generally contained about ten times the concentration of DDT present in the water, considerably less than any of the tissues and neither TDE or DDE were detected.

As in the two previous experiments the concentrations in the mussels dropped very slowly after the introduction of DDT was stopped. However, the ratio of DDT to TDE decreased indicating that, although the insecticide was not being eliminated, it was being converted to TDE (Table 3). The same trend, but not as pronounced, was also observed in the second experiment (Table 2). Again the fat levels remained about the same throughout the experiment (Table 3).

In the final experiment (4) with distilled water the insecticide concentration was reduced to 0.08±0.02 ppb. The mussels (Anodonta grandis) which were collected from the Red Cedar River and held in the laboratory for several months at 9°C, were exposed to this concentration for four weeks at 15°C.

Three control mussels were analyzed initially and three mussels were analyzed after each week. The results show very little increase in insecticide concentrations from those of the controls (Table 4) suggesting that DDT levels in the

Table 4. Concentrations of DDT and its metabolites (ppm, wet weight) in Anodonta grandis exposed to 0.08±0.02 ppb DDT at 15°C for four weeks.

Weeks	Weight (g)	Fat (per cent)	DDE	TDE	DDT	Total
0	42.801	0.51	0.006	0.009	0.016	0.031
	32.285	0.51	0.007	0.009	0.017	0.033
	9.159	0.60	0.017	0.025	0.038	0.080
Mean		0.54	0.010	0.014	0.024	0.048
1	39.070	0.53	0.008	0.014	0.027	0.049
	49.056	0.39	0.005	0.014	0.015	0.034
	19.699	0.51	0.011	0.027	0.015	0.054
Mean		0.47	0.008	0.018	0.019	0.046
2	69.307	0.36	0.004	0.010	0.009	0.024
	34.789	0.68	0.007	0.021	0.037	0.065
	18.247	0.48	0.008	0.022	0.019	0.050
Mean		0.51	0.007	0.018	0.022	0.046
3	50.537	0.53	0.008	0.017	0.037	0.062
	41.390	0.61	0.007	0.018	0.015	0.040
	18.949	0.79	0.010	0.025	0.032	0.068
Mean		0.64	0.008	0.020	0.028	0.056
4	28.656	0.73	0.007	0.019	0.024	0.049
	28.860	0.64	0.009	0.034	0.025	0.069
	21.376	0.53	0.011	0.040	0.029	0.080
Mean		0.63	0.009	0.031	0.026	0.066

Red Cedar were probably around 0.08 ppb in the area where the mussels were collected. The concentration factor (mussel: water) here was slightly less (ca. 700 vs 1000) than the factor for the previous experiments.

The same general pattern, an initial, rapid uptake, subsequent leveling-off, and a very slight decrease when DDT introduction was terminated was seen in each of the first three experiments (Figure 2). The large difference between the equilibrium concentration of the second experiment (2) and the other two (1 and 3), despite the very similar mean water concentrations of DDT in the three experiments, is difficult to explain. The main differences were in the initial "physiological state" of the mussels and the species of mussel. The mussels in the second experiment had much higher concentrations of DDT when collected (0.220 vs 0.062 and 0.027 ppm) and were placed in the test aquaria a few days after collection while the others were held for considerable lengths of time at 9°C before experimentation. The initial concentration should in theory have no effect on the equilibrium concentration unless it is higher than the equilibrium concentration. In distilled water there seems to be a very slow elimination of DDT so a high initial concentration in the mussel could affect the equilibrium concentration. The fact that the mussels in the second experiment were fresh from natural conditions might result in greater filtering activity initially than those mussels conditioned to no food and cold

Figure 2. Mean concentrations of DDT and its metabolites in mussels exposed to 0.27 to 0.38 ppb DDT in reconstituted distilled water.

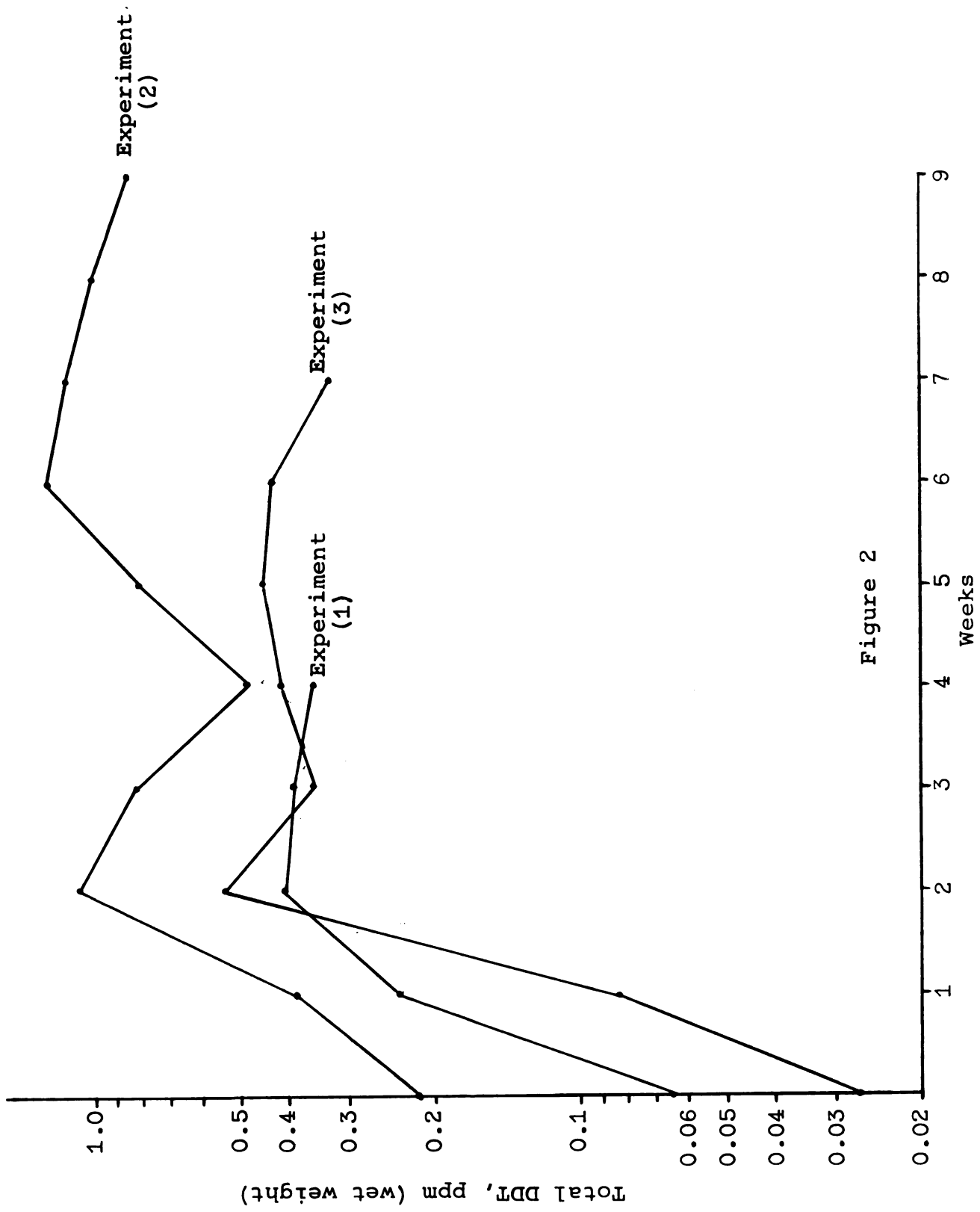

Experiment (2)
Experiment (3)
Experiment (1)
Total DDT, ppm (wet weight)
1.0
0.5
0.4
0.3
0.2
0.1
0.06
0.05
0.04
0.03
0.02
1
2
3
4
5
6
7
8
9
Weeks

Figure 2

temperatures prior to experimentation. Thus they may have concentrated the insecticide to a greater extent because of greater contact.

The difference in species did not correspond with the difference in equilibrium concentrations as Elliptio in experiment (1) and Anodonta in experiment (3) reached similar concentrations while Anodonta in (2) was much higher. The absence of differences between species of mussels in concentrating pesticides was also found in previous studies (Bedford et al., 1968).

In all of the experiments run in distilled water profuse filamentous fungal colonies developed on the aquaria walls, substrate, and mussel shells. Presumably the fungi were living on the waste products of the mussels. In the last two experiments (3 and 4) the growths were especially abundant and samples were collected on Whatman No. 1 filter paper, dried, weighed and extracted with hexane. The fungi samples were found to contain ten times the concentrations of DDT and its metabolites found in the mussels (Tables 5 and 6). When the introduction of DDT was terminated in the third experiment (3) the DDT concentration in the fungi remained about the same for one week and then dropped sharply the following week (Table 5). In the last experiment (4) the levels were approximately the same for all three samples of fungi and were slightly lower than the final concentration in the third experiment (3). This follows, as the DDT

Table 5. Concentrations of DDT and its metabolites (ppm, dry weight) in fungal growth removed from test aquaria during Experiment 3.

Weeks	Weight (g)	DDE	TDE	DDT	Total
3	0.1704	1.995	8.509	124.119	134.623
4	0.4065	1.353	4.354	105.781	111.488
5	0.1663	2.706	8.720	120.260	131.686
6	0.1493	0.636	2.009	22.438	25.084
7	0.0590	0.932	4.746	16.949	22.627

Table 6. Concentrations of DDT and its metabolites (ppm, dry weight) in fungal growth removed from test aquaria during Experiment 4.

Weeks	Weight (g)	DDE	TDE	DDT	Total
2	0.1497	1.169	2.438	14.696	18.303
3	0.1649	0.940	0.910	13.645	15.494
4	0.1749	0.715	0.457	15.037	16.209

concentration in the water was still slightly higher (0.10 ppb) at the end of the third experiment (3) than the mean concentration (0.08 ppb) of the last experiment (4).

The presence of the fungal growth offers a possible explanation of the relatively high levels of DDT remaining in the water for three weeks following the termination of DDT introduction. The fungi apparently released considerable DDT when the introduction was stopped unless they metabolized it to a non-detectable metabolite. The release of DDT by the fungi could also have occurred in the second experiment (2) and may explain the increase in DDT concentrations in the mussels the week following termination of the DDT introduction.

Lake Water

Lake Lansing, a shallow, warm water, eutrophic lake located in Ingham County, Michigan, provided the water for this series of experiments. Analysis showed that the water contained small amount of DDT (< 0.05 ppb) but no detectable dieldrin. Water was pumped periodically to a stainless steel tank which fed a constant-head tank. This provided a constant flow of lake water to the mixing flask where the insecticide solution was metered.

Mussels (*Lampsilis siliquoidea*) from Gun Lake were used in the first two experiments and were collected just prior

to the tests. In the first experiment (5) the mussels were exposed to a mean concentration of 0.57±0.12 ppb dieldrin for three weeks and then the concentration was approximately doubled to 1.16±0.13 ppb for one week. Introduction of dieldrin was then terminated and the mussels exposed to Lake water for three additional weeks. One week following the termination of the dieldrin introduction the insecticide level in the water had decreased to 0.11 ppb in the tank overflow and at the end of the experiment it was 0.05 ppb.

Three control mussels were analyzed at the beginning of the test and found to have small concentrations of dieldrin (Table 7). Three mussels were removed from the test aquaria each week and analyzed for insecticide content.

The mussels appeared to reach equilibrium concentrations in one week or less as the highest concentration was attained after one week and then decreased slightly the following two weeks (Table 7). When the water concentration of dieldrin was doubled the concentration in the mussels also doubled (Table 7).

When the introduction of dieldrin was stopped the dieldrin concentrations in the mussels dropped very rapidly for the first two weeks and then remained nearly constant (Table 7). This was in contrast to the experiments with DDT in distilled water when after introduction of DDT was terminated the concentrations in the mussels remained approximately the same.

Table 7. Concentrations of dieldrin (ppm, wet weight) in *Lampsilis siliquoidea* exposed to 0.57±0.12 ppb dieldrin in lake water at 20°C for three weeks.

Weeks		Weight (g)	Fat (per cent)	Dieldrin
0		16.501	0.71	0.018
		7.566	1.08	0.011
		3.162	1.23	0.000
	Mean		1.01	0.010
1		9.399	1.18	0.677
		6.782	1.01	0.644
		3.111	1.06	0.775
	Mean		1.08	0.699
2		12.701	0.62	0.436
		4.657	1.06	0.754
		3.429	1.00	0.828
	Mean		0.89	0.672
3		8.714	0.99	0.545
		5.418	1.15	0.705
		4.304	1.10	0.587
	Mean		1.08	0.612
Water concentration increased to 1.16±0.13 ppb dieldrin.				
4		12.715	0.73	1.113
		6.667	0.91	1.485
		3.173	1.08	1.467
	Mean		0.91	1.355
Stop dieldrin introduction				
5		12.217	0.63	0.304
		7.643	0.90	0.360
		3.630	0.96	0.238
	Mean		0.83	0.301

continued

Table 7--continued

Weeks	Weight (g)	Fat (per cent)	Dieldrin
6	14.087	1.01	0.052
	5.582	0.80	0.082
	1.952	1.37	0.128
Mean		1.06	0.088
7	2.678	2.29	0.080
	4.366	1.30	0.084
	2.325	1.02	0.056
Mean		1.54	0.073

A semi-log plot of the dieldrin residue values following stoppage of the dieldrin introduction, were linear with a negative slope (Figure 3). The equation for this relationship is as follows:

$$(1) \quad Y = (A)\ (-B^{X})$$

where:

Y = concentration of dieldrin in the mussel (ppm, wet weight)

A = regression intercept (ppm)

X = weeks following cessation of dieldrin introduction

B = relative rate of residue loss

or for the rectified data:

$$(2) \quad \text{Log } Y = \log A - (\log B)\ X$$

Log B, the regression coefficient, estimates the logarithmic rate of residue loss. In this case the log of the dieldrin concentration in the mussel decreased at an estimated uniform rate of 0.437 and the residue half-life calculated from equation (2) was 4.7 days. If one eliminates from the regression analysis the third week values, where the mussels appear to be in equilibrium with the dieldrin that still remained in the water, the log of the concentration in the mussels decreased at an estimated rate of 0.607. The half-life value is then decreased to 3.5 days.

In the second experiment (6) the mussels (Lampsilis siliquoidea) were exposed to a mean dieldrin concentration

Figure 3. Loss of dieldrin from Lampsilis siliquoidea in lake water.

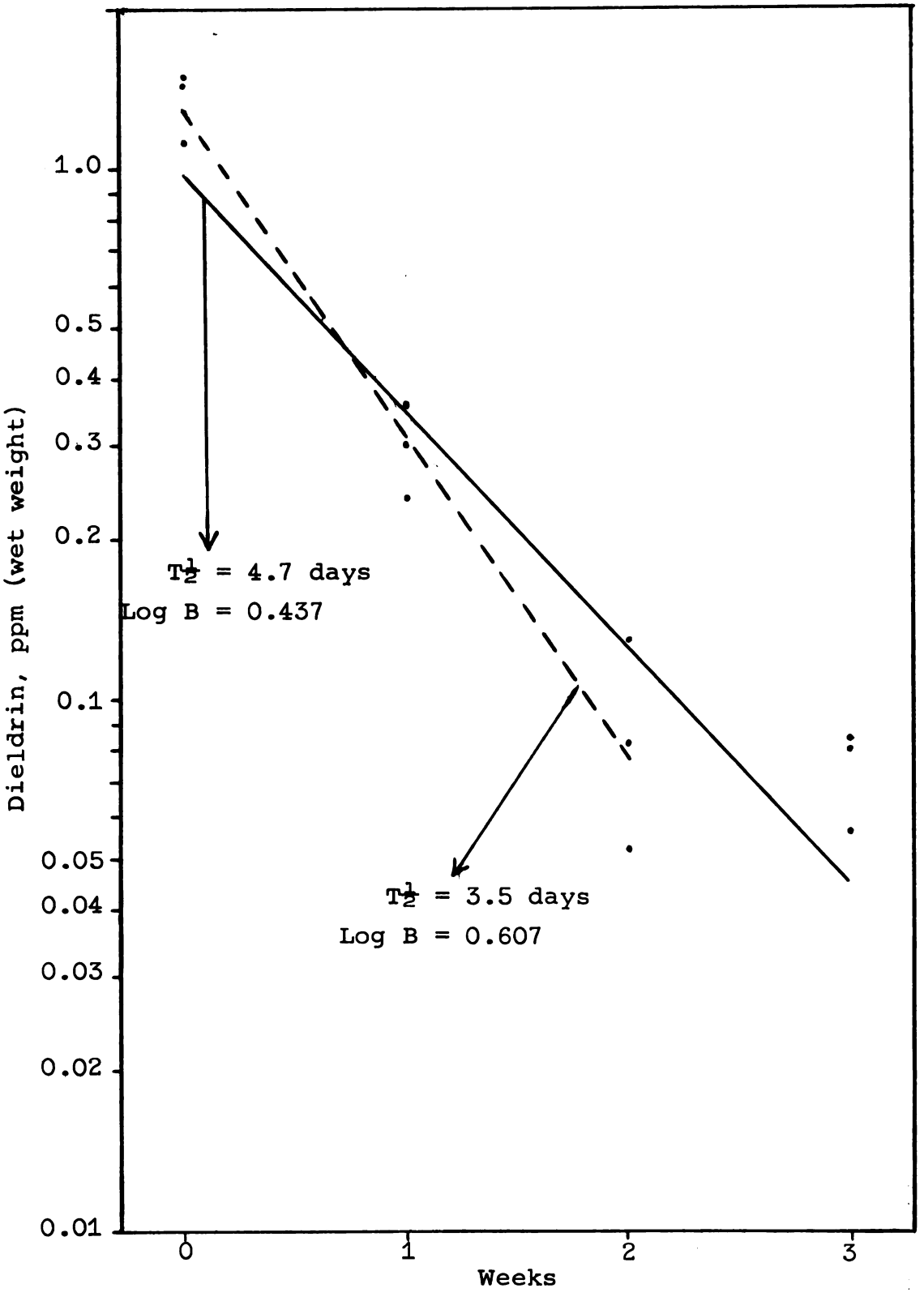

Dieldrin, ppm (wet weight)
1.0
0.5
0.4
0.3
0.2
0.1
0.05
0.04
0.03
0.02
0.01
$T\frac{1}{2}$ = 4.7 days
Log B = 0.437
$T\frac{1}{2}$ = 3.5 days
Log B = 0.607
0
1
2
3
Weeks

Figure 3

of 0.06±0.01 ppb for three weeks. The control mussels contained no detectable dieldrin. As with the first experiment (5) the mussels attained their highest dieldrin concentration after the first week's exposure (Table 8). The concentration in the mussel then dropped about 40% the following week and rose to a level midway between the first and second after the third week. In both experiments the fat levels remained relatively constant.

Equilibrium concentrations in the mussels were plotted against the corresponding water concentrations and fitted with a least squares line (Figure 4). The slope of the line or regression coefficient was 1.19 with the concentration in the mussel in ppm and the water concentration in ppb. Thus the mussels were concentrating the dieldrin about 1200 times over the water concentration. The correlation coefficient (r) of 0.976 indicated a very close fit and a highly significant correlation between the concentrations ($F_{exp.} = 376.8$, $F_{.995} = 10.1$). However the assumptions of variance homogeneity and independence of means and variances were not met due primarily to the small mean and variance of those mussels exposed to 0.07 ppb dieldrin.

The same experimental apparatus used for the dieldrin experiments was employed for DDT in lake water. Mussels (Anodonta grandis) were collected from the Red Cedar River and exposed to three different concentrations of DDT.

Table 8. Concentrations of dieldrin (ppm, wet weight) in Lampsilis siliquoidea exposed to 0.06±0.01 ppb dieldrin in lake water at 20°C for three weeks.

Weeks		Weight (g)	Fat (per cent)	Dieldrin
0		12.183	0.63	0.000
		5.430	0.89	0.000
		4.210	1.07	0.000
	Mean		0.86	0.000
1		13.719	0.65	0.053
		4.776	1.01	0.071
		3.846	0.84	0.081
	Mean		0.83	0.068
2		6.504	1.35	0.035
		4.913	1.15	0.050
		3.779	0.96	0.036
	Mean		1.15	0.040
3		9.868	0.89	0.041
		3.750	1.15	0.059
		3.105	0.84	0.060
	Mean		0.96	0.053

Figure 4. Relation between dieldrin concentrations in mussels (Lampsilis siliquoidea) and the concentrations in the water from which they were removed.

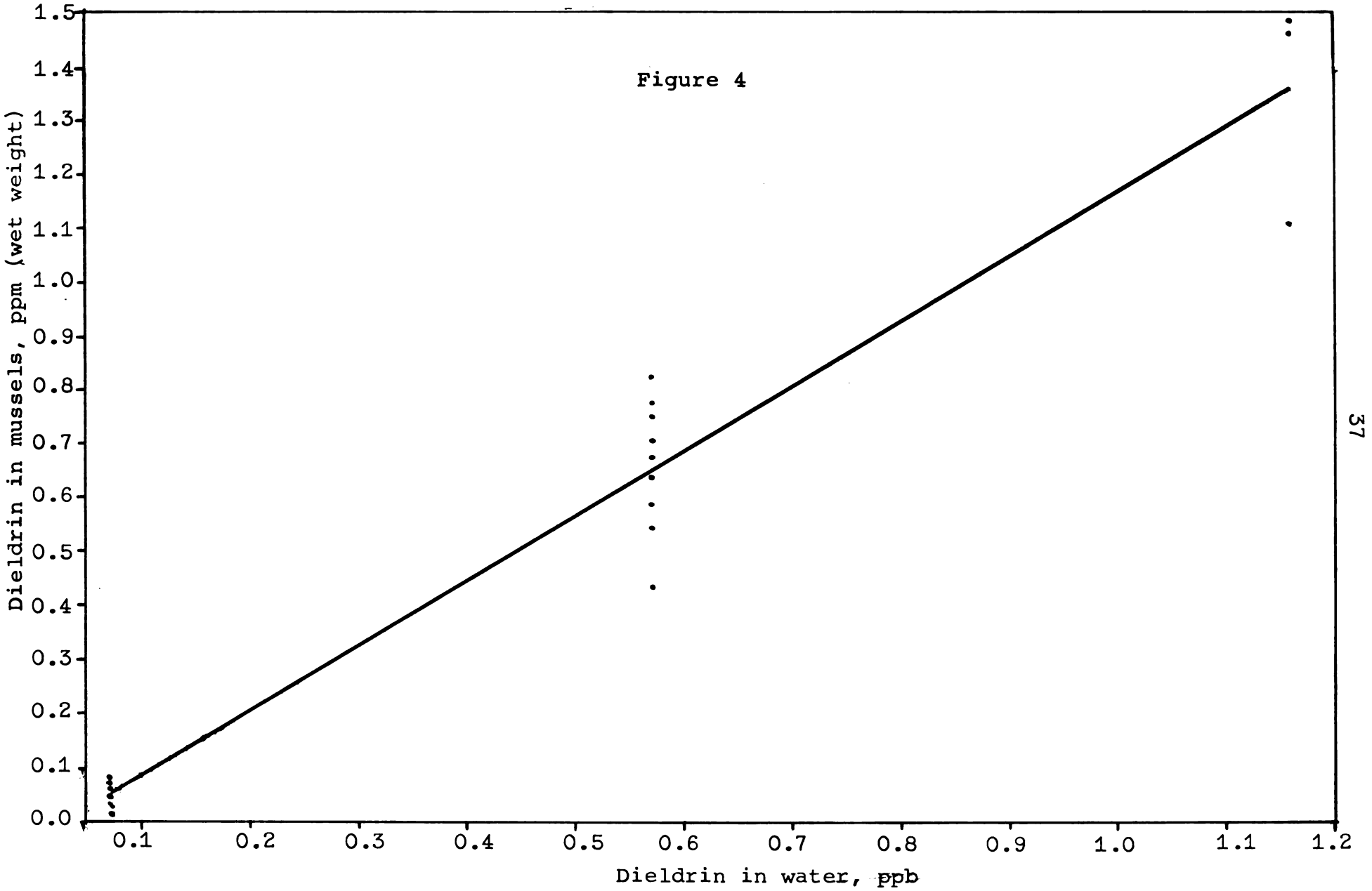


Figure 4
Dieldrin in mussels, ppm (wet weight)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
Dieldrin in water, ppb

In the first experiment (7) the mussels were exposed to a mean concentration of 0.62±0.13 ppb DDT for three weeks followed by five weeks exposure to lake water after the cessation of DDT introduction. The DDT concentration in the water dropped to 0.24 ppb at the end of one week and was down to 0.11 ppb three weeks after termination of the introduction.

The mussels were dissected before insecticide analysis as in the third distilled water experiment (3) except that the body fluids were not analyzed. The control mussels were found to have very low DDT residues (Table 9). After one week's exposure the concentration in the mussels increased sharply to over 1 ppm and stayed at that level the following week before increasing again after three weeks's exposure (Table 9). The sudden increase in DDT residue concentrations after three weeks was due to excessive concentration of DDT in the test tanks during a 2 1/2 day period when the dilution water line became plugged with debris and periphyton. Unfortunately no water samples were obtained when this problem was discovered. Later in the week, following clearing of the line, water samples showed concentrations of DDT to be only slightly higher than the mean for the three weeks.

The concentrations of DDT and metabolites were again much higher in the viscera than in the muscle, mantle, and gills (Table 9). However, in contrast to the distilled water experiment, the marsupia had very high concentrations of insecticides, in most cases nearly as high as the viscera.

Table 9. Mean concentrations of DDT and its metabolites (ppm) in Anodonta grandis exposed to 0.62±0.13 ppb DDT in lake water at 20°C for three weeks.

Weeks	Tissue[2]	Per cent Fat	Wet Weight DDE	Wet Weight TDE	Wet Weight DDT	Wet Weight Total	Fat Weight Total
0	Viscera[3]	0.75	0.013	0.005	0.013	0.031	4.13
	Muscle[4]	0.54	0.008	0.002	0.007	0.017	3.15
	Whole mussel	0.63	0.010	0.004	0.010	0.024	3.81
1	Viscera	1.23	0.055	0.119	1.649	1.823	148.21
	Muscle	0.70	0.030	0.071	0.923	1.024	146.29
	Marsupium(1)	0.44	0.015	0.036	0.563	0.614	139.55
	Whole mussel	0.85	0.039	0.091	1.239	1.368	160.94
2	Viscera	1.04	0.052	0.145	1.251	1.448	139.23
	Muscle	0.76	0.038	0.071	0.592	0.701	92.24
	Marsupium(1)	0.77	0.062	0.198	1.498	1.758	228.31
	Whole mussel	0.90	0.047	0.114	0.941	1.103	122.56
3	Viscera	1.03	0.093	0.218	3.073	3.384	223.79
	Muscle	0.70	0.039	0.102	1.597	1.738	248.28
	Marsupium(1)	0.71	0.058	0.213	2.219	2.490	350.70
	Whole mussel	0.85	0.063	0.156	2.255	2.473	290.94
Stop DDT introduction							
4	Viscera	0.85	0.086	0.296	2.359	2.741	322.47
	Muscle	0.67	0.025	0.109	0.847	0.981	146.42
	Marsupium(2)	0.64	0.063	0.237	1.629	1.929	301.41
	Whole mussel	0.73	0.053	0.198	1.536	1.796	246.03

continued

Table 9--continued

Weeks	Tissue	Per cent Fat	Wet Weight DDE	TDE	DDT	Total	Fat Weight Total
5	Viscera	0.97	0.061	0.225	1.744	2.030	209.28
	Muscle	0.77	0.027	0.115	0.847	0.989	128.44
	Whole mussel	0.85	0.041	0.157	1.194	1.392	163.76
6	Viscera	0.98	0.059	0.191	1.474	1.724	175.92
	Muscle	0.77	0.022	0.074	0.624	0.720	93.51
	Marsupium(2)	0.46	0.034	0.096	1.196	1.326	288.26
	Whole mussel	0.77	0.036	0.115	0.915	1.066	138.44
7	Viscera	1.08	0.055	0.159	0.835	1.049	97.13
	Muscle	0.84	0.024	0.081	0.419	0.534	63.57
	Marsupium(1)	1.37	0.041	0.082	0.773	0.896	65.40
	Whole mussel	0.95	0.036	0.110	0.594	0.740	77.89
8	Viscera	1.20	0.039	0.122	0.490	0.651	54.25
	Muscle	0.86	0.021	0.070	0.242	0.333	38.72
	Whole mussel	1.00	0.028	0.092	0.345	0.465	46.50

[2]Mean of three mussels unless otherwise noted.

[3]Combined reproductive and digestive tissue.

[4]Includes gills and mantle.

When the concentration of insecticide in the mussels was placed on a fat weight basis the difference in concentration in the muscle and viscera portions was much reduced (Table 9). The concentrations in the marsupia, when placed on a fat weight basis, were in some cases considerably higher than the DDT concentration in the viscera owing to the relatively low fat content of the marsupia.

When the introduction of DDT was terminated the mussels showed a steady decline in insecticide concentrations (Table 9). When plotted against time on semi-log paper the levels in the mussels decreased in a linear fashion as occurred with dieldrin (Figure 5). When equations (1) and (2) were applied, the regression coefficient (log B) for total DDT and metabolites was found to be 0.148 and the half-life was 13.6 days. Thus the half-life of DDT was between three and four times longer than the half-life of dieldrin in the mussels. Gakstatter and Weiss (1967) also found a much slower elimination of DDT than dieldrin and lindane in fish. They concluded that uptake and elimination rates were related to the solubility of the insecticide in water. Grzenda et al. (1970) reported a logarithmic rate of loss of 0.0725 and a half-life of 29.5 days for DDT in goldfish. This is about half the elimination rate in mussels. However, the initial body burden was produced in the fish by feeding them contaminated food rather than via the water. This could account for some of the difference, at least initially for most of the fed

Figure 5. Loss of DDT from Anodonta grandis in lake water.

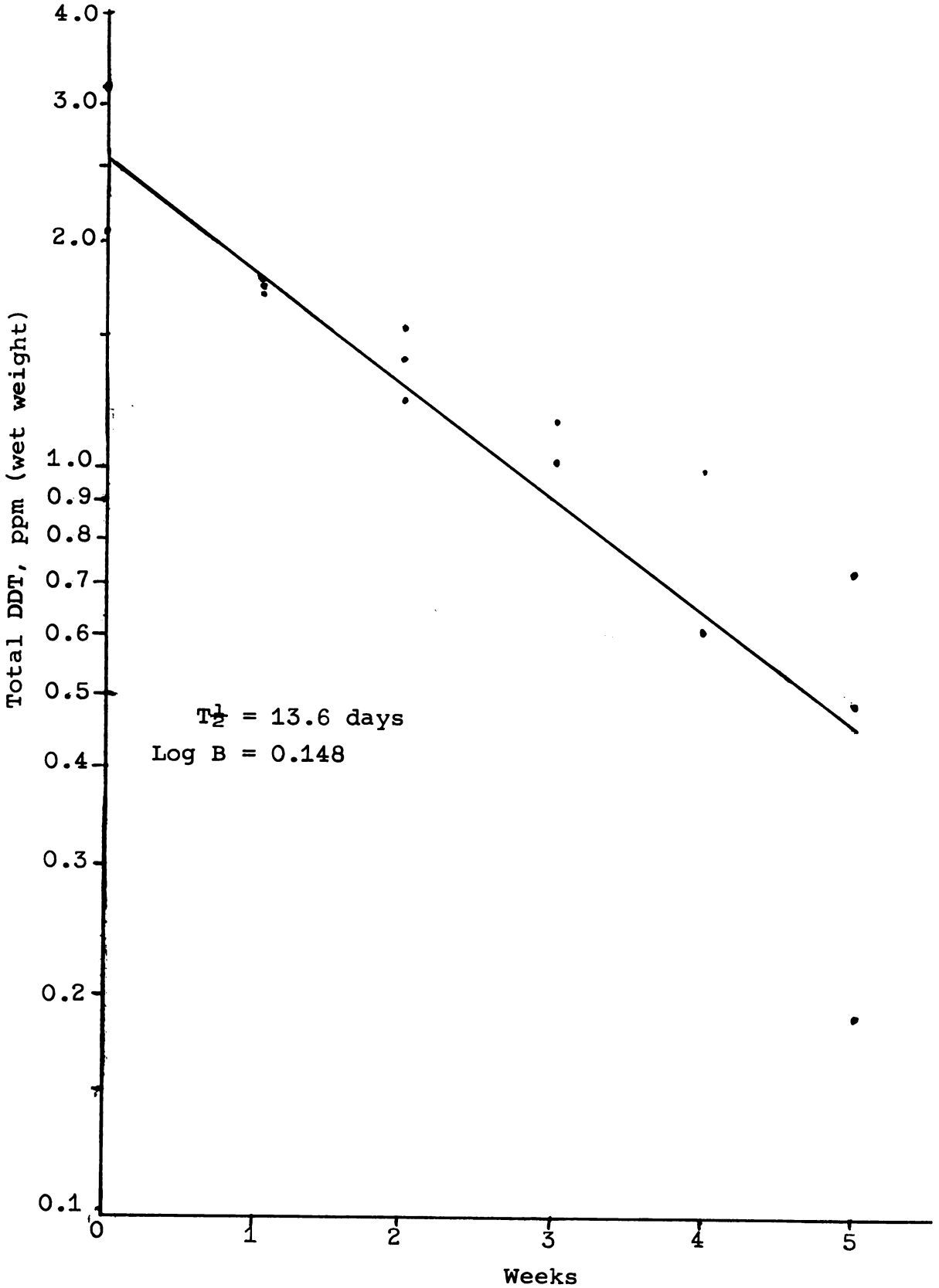

4.0
3.0
2.0
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
Total DDT, ppm (wet weight)
$T_{\frac{1}{2}}$ = 13.6 days
Log B = 0.148
0
1
2
3
4
5
Weeks

Figure 5

insecticide was probably incorporated into the fish tissue while it was possible a significant proportion of the residue level in the mussels was simply adsorbed onto the gills and mantle and was thus more easily lost. The greater fat content (5-29%) and initial body burden (1.2-18.4 ppm) in the fish could also have an effect on the elimination rates.

In the next two experiments (8 and 9) mussels (Anodonta grandis) were exposed to two different concentrations of DDT for two week periods. In the first test (8) the mussels were exposed to a mean concentration of 0.42±0.12 ppb DDT. The residue levels increased greatly after one week and then leveled off in the mussels (Table 10). In the second test (9) a mean concentration of 0.14±0.12 ppb DDT was maintained in the water. The concentration again increased from the controls after one week and then did not change the following week (Table 11).

The equilibrium concentrations for DDT in the mussels were plotted in a manner similar to the dieldrin results and fitted with a least squares line (Figure 6). The regression coefficient or slope of the line was 2.36 with the concentration of DDT in the mussels in ppm and the water concentration in ppb. The mussels were thus concentrating the DDT about 2400 times the concentration in the water or about twice the degree of concentration for dieldrin.

The correlation coefficient (5) of 0.880 was not quite as high as that for dieldrin but still indicated a close fit.

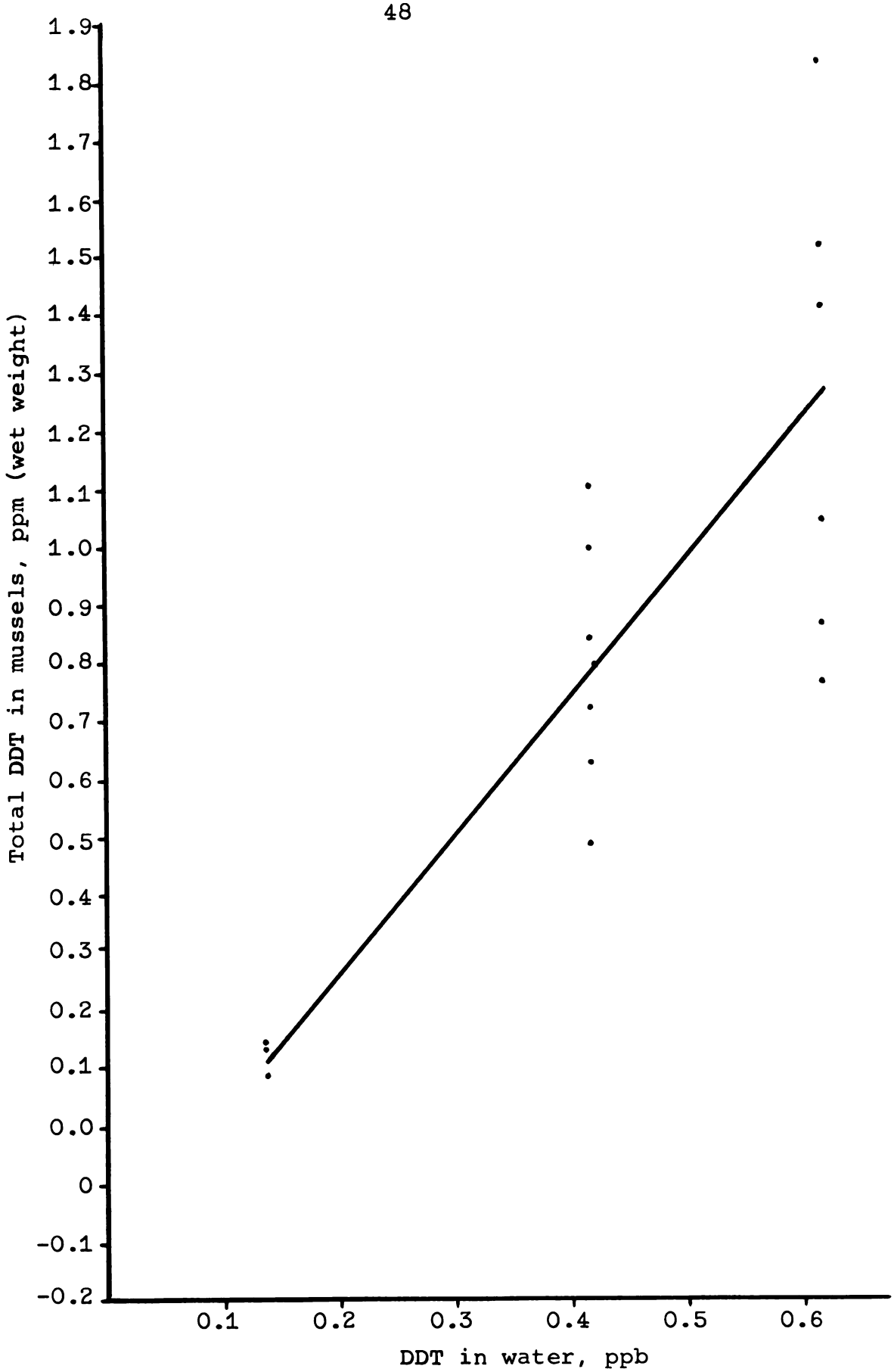
Table 10. Concentrations of DDT and its metabolites (ppm, wet weight) in Anodonta grandis exposed to 0.42±0.12 ppb DDT in lake water at 20°C for two weeks.

Weeks	Weight (g)	Fat (per cent)	DDE	TDE	DDT	Total
0	70.642	0.43	0.003	0.005	0.025	0.033
	63.887	0.66	0.005	0.004	0.020	0.029
	37.712	0.82	0.003	0.007	0.020	0.030
Mean		0.64	0.004	0.005	0.022	0.031
1	89.721	0.34	0.015	0.074	0.535	0.624
	73.109	0.44	0.014	0.051	0.417	0.482
	32.155	0.74	0.022	0.105	0.972	1.099
Mean		0.50	0.017	0.077	0.641	0.735
2	103.355	0.40	0.021	0.093	0.726	0.839
	64.734	0.69	0.031	0.122	0.838	0.990
	75.414	0.48	0.017	0.085	0.617	0.178
Mean		0.52	0.023	0.100	0.727	0.850

Table 11. Concentrations of DDT and its metabolites (ppm, wet weight) in Anodonta grandis exposed to 0.14±0.02 ppb DDT in lake water at 20°C for two weeks.

Weeks	Weight (g)	Fat (per cent)	DDE	TDE	DDT	Total
0	44.813	0.48	0.002	0.006	0.033	0.042
1	67.273	0.64	0.030	0.035	0.078	0.143
	52.750	0.53	0.006	0.016	0.066	0.088
	46.970	0.68	0.006	0.015	0.080	0.102
Mean		0.62	0.014	0.022	0.075	0.111
2	87.602	0.31	0.007	0.014	0.066	0.087
	67.497	0.48	0.011	0.022	0.100	0.133
	55.720	0.58	0.007	0.014	0.063	0.084
Mean		0.46	0.008	0.017	0.076	0.101

Figure 6. Relation between DDT concentrations in mussels (Anodonta grandis) and the concentrations in the water from which they were removed.


Total DDT in mussels, ppm (wet weight)
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
-0.1
-0.2
0.1
0.2
0.3
0.4
0.5
0.6
DDT in water, ppb

The correlation between the concentrations was again found to be highly significant ($F_{exp.} = 54.76$, $F_{.995} = 10.58$) but, as was the case with dieldrin, the assumptions of variance homogeneity and independence of the means and variances were not met owing to the relatively low variance of the mussel concentrations at the 0.14 ppb water concentration.

During both the DDT and dieldrin experiments in lake water several water samples were filtered through Whatman No. 1 filter paper and both the filtrate and the residue were analyzed in order to determine the relative distribution of the insecticide that had been metered into the lake water. The majority of the dieldrin was found in the suspended matter while the DDT was fairly equally distributed between the water and suspended matter (Table 12). This result is somewhat incongruous with the water solubilities of the two compounds. Robeck et al. (1965) reported dieldrin to be several times more soluble than DDT yet a larger proportion of DDT than dieldrin was found in the water filtrate.

Effect of Temperature

Three experiments were run to determine the effects of temperature on the uptake and loss of DDT and dieldrin. Dechlorinated tap water was used as the water source and the insecticide was metered into a common mixing flask. The treated water was divided into the four aquaria maintained

Table 12. Distribution of DDT and dieldrin in lake water (μg/l of water).

Insecticide	Replicate	Suspended matter	Water filtrate	Total
Dieldrin	1	0.28	0.21	0.49
	2	0.26	0.11	0.37
Mean		0.27	0.16	0.43
Unfiltered				0.42
DDT	1	0.26	0.36	0.62
	2	0.32	0.32	0.64
Mean		0.29	0.34	0.63
Unfiltered				0.63
	1	0.25	0.28	0.53
	2	0.34	0.30	0.64
Mean		0.30	0.29	0.59
Unfiltered				0.64

at different temperatures via glass Y tubes and Teflon tubing. A flow rate of 350-400 ml/min was maintained through each aquaria.

For the first experiment (10) 22 mussels (Anodonta grandis) were collected from the Red Cedar River, divided into groups of five, and acclimated to 5°, 10°, 15°, and 20°C over a period of one week. Two controls analyzed for insecticide content were found to have low levels of DDT and its metabolites (Table 13).

The remaining mussels were exposed to virtually the same concentration of DDT (0.55 to 0.63 ppb) for three weeks. Two mussels were removed from each aquaria after one and three weeks exposure, dissected into visceral and muscle-gill fractions as in previous experiments (3 and 7), and analyzed for insecticide content.

The concentrations in the mussels in the 5°C aquaria remained the same as the controls after one and three weeks exposure (Table 13). The mussels in the 10°C and 15°C aquaria both showed increases from the controls after one week. The mussels at 10°C continued to increase after three weeks but those at 15°C seemed to level off (Table 13). The mussels in the 20°C aquaria increased the greatest amount after one week but mortality of the remaining mussels at about two weeks eliminated the three week sample (Table 13).

A two-way analysis of variance (from Li, 1964) was run to determine if the observed differences in total DDT levels

Table 13. Concentrations of DDT and its metabolites (ppm, wet weight) in Anodonta grandis exposed to DDT (ppb) at different temperatures in dechlorinated tapwater.

Weeks	Temperature	Water Concentration	Tissue[2]	Per cent Fat	DDE	TDE	DDT	Total
0			Viscera[3]	0.71	0.005	0.006	0.019	0.030
			Muscle[4]	0.39	0.003	0.002	0.010	0.014
			Whole mussel	0.52	0.003	0.003	0.013	0.019
1	4.8±0.2°C	0.55±0.08	Viscera	0.71	0.004	0.005	0.021	0.030
			Muscle	0.49	0.001	0.003	0.014	0.018
			Whole mussel	0.59	0.003	0.003	0.016	0.022
	10.0±0.4	0.58±0.06	Viscera	0.71	0.006	0.009	0.030	0.045
			Muscle	0.45	0.002	0.007	0.044	0.053
			Whole mussel	0.55	0.004	0.008	0.038	0.050
	15.1±0.2	0.58±0.07	Viscera	0.68	0.005	0.012	0.039	0.056
			Muscle	0.47	0.002	0.008	0.078	0.088
			Whole mussel	0.52	0.003	0.009	0.064	0.076
	19.5±0.5	0.63±0.09	Viscera	0.75	0.004	0.015	0.029	0.047
			Muscle	0.69	0.001	0.011	0.097	0.109
			Whole mussel	0.71	0.002	0.012	0.071	0.084
3	4.8±0.2	0.55±0.08	Viscera	0.59	0.002	0.004	0.015	0.021
			Muscle	0.36	0.001	0.003	0.015	0.019
			Whole mussel	0.45	0.001	0.003	0.015	0.019

continued

Table 13--continued

Weeks	Temperature	Water Concentration	Tissue	Per cent Fat	DDE	TDE	DDT	Total
3	10.0±0.4	0.58±0.06	Viscera	0.50	0.008	0.016	0.039	0.063
			Muscle	0.45	0.003	0.024	0.078	0.105
			Whole mussel	0.45	0.004	0.019	0.069	0.083
	15.1±0.2	0.58±0.07	Viscera	0.85	0.006	0.028	0.027	0.064
			Muscle	0.50	0.003	0.027	0.042	0.072
			Whole mussel	0.66	0.005	0.028	0.035	0.068

[2]Mean of two mussels.

[3]Combined digestive and reproductive tissue.

[4]Includes gills and mantle.

at different times (1 and 3 weeks) and temperatures (5°, 10°, 15°C) were significant. The results showed no significant difference between either temperature or time (Table 14).

The concentrations attained at 15°C were much lower than one would expect judging from the earlier experiments in distilled water at 15°C. In contrast to the previous experiments, the concentrations in the muscle mantle and gills were as high or higher than in the viscera. Thus it appears that there was much less DDT incorporation into the tissue of the mussel. The mussels were observed to filter very little during the experiment (10). Perhaps this was due to their "physiological state" in the environment from which they were removed and/or insufficient acclimation time. The mussels were collected in midwinter at a water temperature of about 1°C. Another possible reason for this lack of filtering was the discovery of copper (0.05 ppm) in the water after the next experiment (11). Arthur and Leonard (1970) found that lower concentrations than this represented TL_m values for the snail Physa integra (0.039 ppm) and the amphipod Gammarus pseudolimnaeus (0.020 ppm).

Mussels (Lampsilis siliquoidea) for the following two experiments were collected from Gun Lake just prior to each experiment. The mussels were again subdivided and acclimated to 5°, 10°, 15°, and 20°C. In the first experiment the mussels were exposed to mean concentrations of dieldrin from 0.97 to 1.12 ppb (see Table 15) for three weeks and the

Tabld 14. Results of an analysis of variance for the observed differences in mean concentrations of DDT and metabolites in Anodonta grandis with respect to length of exposure and temperature.

Source	SS	DF	MS	$F_{exp.}$	$F_{.95}$
Temperature	0.00313	2	0.00157	6.38	19.0
Time	0.00009	1	0.00009	0.36	18.5
Error	0.00049	2	0.00025		
Total	0.00371	5			

Table 15. Concentration of dieldrin (ppm, wet weight) in Lampsilis siliquoidea exposed to dieldrin[2] (ppb) in dechlorinated tapwater at different temperatures.

Temperature, °C		4.7±0.5		9.7±0.4		14.8±0.4		20.4±0.6	
[2]Water conc.		0.97±0.06		1.01±0.08		1.06±0.12		1.12±0.14	
Weeks		Per cent Fat	Dieldrin	Per cent Fat	Dieldrin	Per cent Fat	Dieldrin	Per cent Fat	Dieldrin
0		0.81	0.024						
		0.89	0.029						
		0.82	0.192						
	Mean	0.84	0.082						
1		0.67	0.054	0.78	0.038	1.10	0.119	0.96	0.255
		0.89	0.086	0.88	0.053	0.94	0.102	1.05	0.260
		0.67	0.078	0.92	0.065	0.92	0.156	0.98	0.244
	Mean	0.74	0.073	0.86	0.052	0.99	0.126	0.99	0.253
2		0.91	0.032	0.70	0.037	0.78	0.203	0.92	0.590
		0.93	0.051	0.91	0.084	0.84	0.069	0.74	0.347
		0.89	0.059	0.96	0.112	1.09	0.240		
	Mean	0.91	0.048	0.86	0.078	0.90	0.170	0.83	0.468
3		0.77	0.047	0.82	0.175	0.85	0.313		
		0.72	0.079	0.78	0.072	0.88	0.382		
		0.87	0.061	0.88	0.190	0.86	0.355		
	Mean	0.79	0.062	0.82	0.146	0.86	0.350		

Stop dieldrin introduction

4		0.71	0.046	0.95	0.104	1.06	0.388
		0.78	0.042	0.77	0.073	0.87	0.300
		0.89	0.046	0.86	0.058	0.80	0.295
	Mean	0.80	0.045	0.86	0.078	0.91	0.327
5		0.76	0.046	0.69	1.044[3]	0.72	0.186
		0.87	0.077	0.92	0.108	0.96	0.202
		0.75	0.057	0.99	0.102	0.93	0.194
	Mean	0.79	0.060	0.87	0.105	0.87	0.194
6		1.03	0.047	0.98	0.056	0.95	0.175
		1.04	0.058	1.23	0.053	0.66	0.158
		0.87	0.074	1.39	0.074	0.92	0.213
	Mean	0.98	0.060	1.20	0.061	0.84	0.182

[3]Not included in the mean.

experiment was continued for another three weeks following termination of the dieldrin introduction. The level of dieldrin in the aquaria dropped to subdetectable levels (0.02 ppb) one week after termination.

Three mussels were analyzed just prior to experimentation for dieldrin content and one was found to have a relatively high dieldrin burden, about seven times the amount in the other two controls, which were quite low as had been the case for mussels previously collected from Gun Lake (Table 15).

As was the case with DDT, the concentrations in the mussels at 5°C remained about the same as the controls throughout the experiment. Both the 10° and 15°C mussels showed increases in insecticide content during the first three weeks followed by a slow decline after termination of dieldrin introduction with the concentrations in the 15°C mussels about twice as high as those in the 10°C mussels (Table 15). Problems of survival were again encountered at 20°C, with the majority of the mussels dying in less than two weeks so that only one and two week samples could be taken. A few mussels were also lost at 15°C (10%) but no mortality occurred in the 5° and 10°C aquaria. The mussels that did survive at 20°C reached concentrations 2-3 times greater than those found in mussels at 15°C for the same length of exposure (Table 15).

A two-way analysis of variance to analyze for the significance of observed differences in insecticide concentrations with respect to time and temperature was again limited to 5°, 10°, and 15°C because of lack of survival of the 20°C mussels for the three weeks. No significant difference was found for either temperature or time of exposure (Table 16).

Following termination of dieldrin introduction at 15°C the residue data when plotted against time on semi-log paper were linear with a negative slope (Figure 7). Upon application of equations (1) and (2) the log of the dieldrin concentration in the mussels was found to decrease at an estimated uniform rate of 0.108 and the half-life was calculated to be 19.3 days. This rate of loss was much slower than found for the mussels in lake water at 20°C (0.108 vs 0.437). Much of this difference is probably due to the lower temperature, however, as will be seen in the next experiment (12), the dieldrin concentration appeared to decrease slowly at 20°C in tap water also.

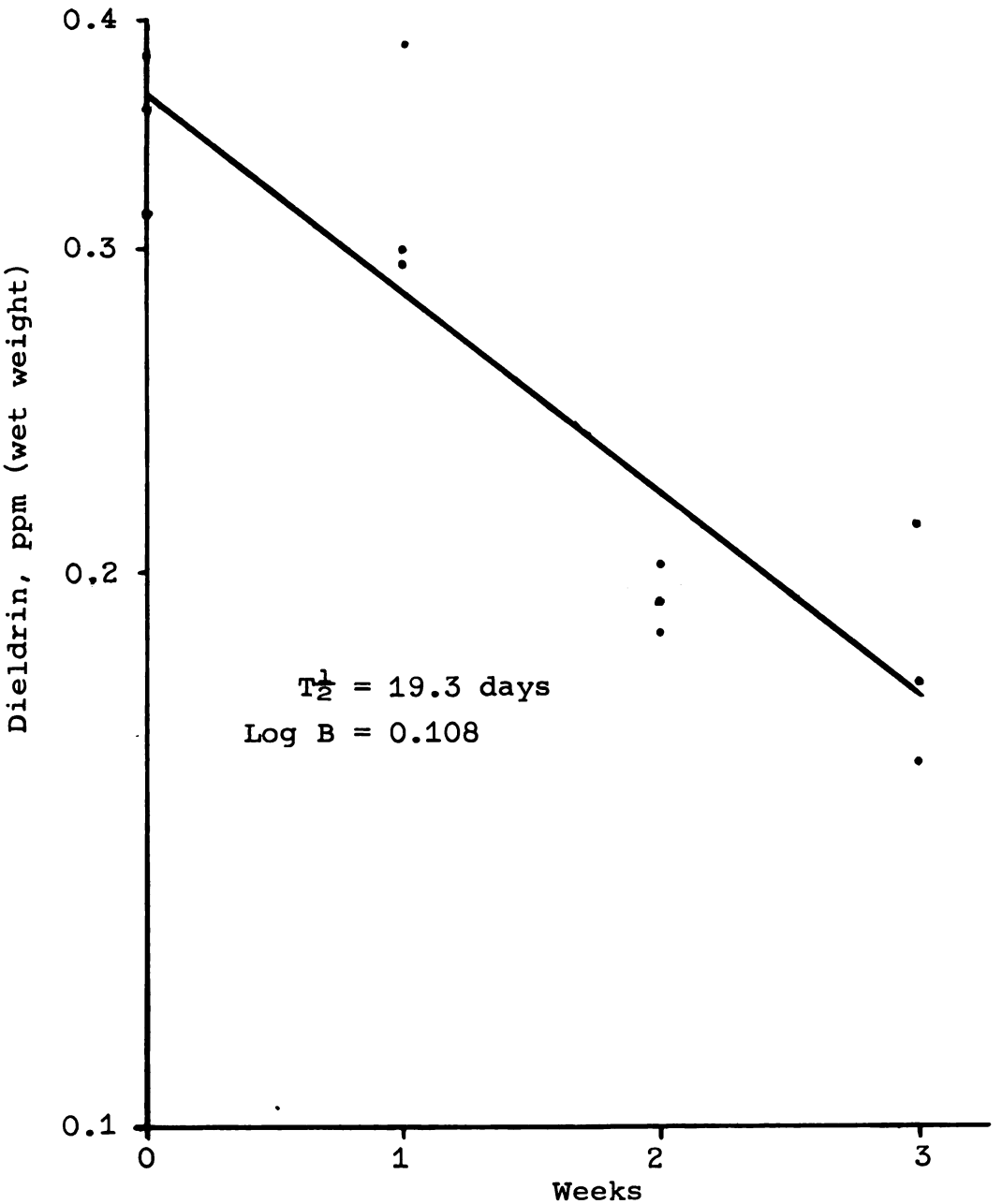
One sample (10°C-5 weeks), which contained ten times the concentration of dieldrin of the other two replicates, was not included in computing the mean for that temperature and time because of suspected contamination.

For the final experiment (12) two changes were made to enhance the survival of the mussels at 20°C. First the copper pipes in the laboratory building were bypassed with flexible plastic pipe. This reduced the copper concentration to 0.004

Table 16. Results of an analysis of variance for the observed differences in mean concentrations of dieldrin in Lampsilis siliquoidea with respect to length of exposure and temperature.

Source	SS	DF	MS	$F_{exp.}$	$F_{.95}$
Temperature	0.03999	2	0.01999	5.38	6.94
Time	0.01832	2	0.00916	2.46	6.94
Error	0.01487	4	0.00372		
Total	0.07319	8			

Figure 7. Loss of dieldrin from Lampsilis siliquoidea in dechlorinated tapwater.


0.4
0.3
0.2
0.1
Dieldrin, ppm (wet weight)
$T_{\frac{1}{2}}$ = 19.3 days
Log B = 0.108
0
1
2
3
Weeks

ppm from 0.05 ppm. Second, the dieldrin concentration was cut in half.

Three control mussels were analyzed and all contained low concentrations of dieldrin (Table 17). The remaining mussels were divided into the four aquaria after acclimation and exposed to mean concentrations of 0.42 to 0.45 ppb dieldrin for three weeks. The experiment was originally intended to study only uptake of the insecticide; however, since there was 100% survival of the mussels at all temperatures, enough mussels remained for another analysis. This sample was taken two weeks after termination of the dieldrin introduction at which time no detectable dieldrin remained in the water.

After one week's exposure the mussels in the 5°, 10°, and 15°C all contained similar low levels of dieldrin only slightly greater than the controls (Table 17). Those at 20°C contained about four times the amount of dieldrin found in the mussels at other temperatures (Table 17). At the end of two weeks there was little change from the one week concentrations except at 5°C where the level almost doubled. The mussels at 5°C again differed from those at the other temperatures after three weeks, only this time there was no change at 5°C and there was substantial increases (40 to 100%) at the other temperatures (Table 17).

Two weeks following termination of the dieldrin introduction the mussels at all temperatures had lower concentrations

Table 17. Concentration of dieldrin (ppm, wet weight) in Lampsilis siliquoidea exposed to dieldrin[2] (ppb) in dechlorinated tapwater at different temperatures.

Temperature, °C	4.6±0.3		10.1±0.2		15.1±0.2		20.8±0.2	
[2]Water conc.	0.42±0.06		0.42±0.08		0.45±0.09		0.44±0.08	
	Per cent Fat	Dieldrin	Per cent Fat	Dieldrin	Per cent Fat	Dieldrin	Per cent Fat	Dieldrin
0	0.78	0.010						
	0.95	0.012						
	0.94	0.018						
Mean	0.89	0.013						
1	0.85	0.053	0.90	0.020	0.75	0.049	0.83	0.230
	0.66	0.036	0.68	0.063	0.70	0.061	0.85	0.202
	0.67	0.078	0.67	0.065	0.78	0.075	0.69	0.283
Mean	0.73	0.056	0.75	0.049	0.74	0.062	0.79	0.238
2	0.92	0.039	1.01	0.037	0.88	0.055	1.04	0.205
	0.95	0.095	1.03	0.043	1.14	0.069	1.08	0.198
	0.88	0.154	1.12	0.072	1.26	0.120	1.00	0.196
Mean	0.92	0.096	1.05	0.051	1.09	0.081	1.04	0.200
3	0.91	0.079	1.07	0.105	1.01	0.115	0.98	0.280
	1.02	0.093	1.03	0.103	0.95	0.107	0.89	0.208
	0.90	0.105	0.89	0.094	0.88	0.257	1.05	0.328
Mean	0.94	0.093	0.99	0.101	0.95	0.160	0.97	0.272
Stop dieldrin introduction								
5	0.80	0.089	0.85	0.091	0.87	0.088	0.92	0.191
	0.86	0.063	0.87	0.079	1.03	0.025	1,12	0.268
	0.88	0.061	0.98	0.111	1.12	0.084	0.92	0.213
Mean	0.85	0.071	0.90	0.094	1.01	0.066	0.99	0.224

of dieldrin with an especially large decrease (ca. 70%) at 15°C (Table 17).

A two-way analysis of variance was run on the means during the uptake period to determine the significance of the observed differences. A highly significant difference between temperatures and a significance difference with time was found (Table 18). Further investigations using Duncan's (1955) new multiple range test showed that most of the difference was due to the much greater concentrations found at 20°C and the large increase between the second and third weeks accounted for most of the significant change with time (Table 18).

In all three experiments (10, 11, and 12) there seemed to be little difference in insecticide uptake at 5° and 10°C. In both dieldrin experiments (11, 12) there was a considerable increase in the concentrations attained at 15°C and, where the mussels survived, an even larger increase at 20°C. Before any final conclusions are drawn however, tests should be run in natural water to see if temperature influences the uptake and elimination of insecticides the same way when food is present.

Conclusions

This study has brought out four general conditions which one must consider when using freshwater mussels as monitors of insecticides in water.

Table 18. Results of an analysis of variance and multiple range tests for the significance of observed differences in mean dieldrin concentrations in Lampsilis siliquoidea with respect to length of exposure and temperature.

Source	SS	DF	MS	$F_{exp.}$	$F_{.995}$	$F_{.95}$
Temperature	0.05472	3	0.01824	33.16	12.92	
Time	0.00739	2	0.00369	6.71		5.14
Error	0.00334	6	0.00055			
Total	0.06545	11				

Temperature	$10^{o}C$	$5^{o}C$	$15^{o}C$	$20^{o}C$
Means[2]	0.067	0.082	0.101	0.237

Weeks	1	2	3
Means[3]	0.135	0.143	0.208

[2]Means not underlined by common line are significantly different (99.5%).

[3]Means not underlined by common line are significantly different (95%).

First the previous conditioning and insecticide level of the mussel may influence the concentration of the insecticide attained by the mussel. This seems to be especially important when they are placed in waters which contain little or no natural food. The mussel's filtering rate is dependent on the quality and quantity of food in the water (Wilbur and Yonge, 1966).

This leads to the second consideration, the type of water into which the mussels are placed. As mentioned above, the level of available suspended matter for food affects the filtering rate. A large difference was found in both uptake and elimination in the experiments between conditions of no food (distilled and tapwater) and essentially unlimited food (Lake Lansing water). Thus mussels placed in cold clear streams would probably yield different results from those in streams which had native mussels populations. It is also possible to have a suspended load that is too great and results in decreased filtering (Loosanoff and Engle, 1947). Poor water quality conditions which cause the mussel to close up and not filter for periods of time, may yield lower than representative concentrations of insecticides in the mussel for the water. This condition apparently occurred in previous field studies (Bedford et al., 1968).

As would be expected, the temperature of the water is another important consideration. At 10°C and lower the mussels appear to concentrate insecticides at the same low

levels but large differences were noted between 10°, 15°, and 20°C, both in rate of uptake and equilibrium concentration.

Finally, the type of insecticide being monitored must be considered. Although both DDT and dieldrin are classed as chlorinated hydrocarbon insecticides, the degree to which they were concentrated and their half-life in the mussels were considerably different.

Most of these limitations would apply to any organism used for monitoring pesticides. Thus the mussel's traits of feeding by filtering large quantities of water, moving very little, and long life span (up to 20 years) makes them especially well adapted as monitors in comparison with other aquatic organisms. Galtsoff (1928) found that adult oysters, of a size similar to freshwater mussels (3-4 in long) siphoned up to 3,000 ml/hr when the water temperature was 25°C and siphoned, on the average, 20 hr a day at a temperature range of 15-22°C. Bovjerg (1957) reported that the mean movement of Lampsilis siliquoidea when well fed was only 2.3 m per week and when not fed the mean movement ranged from 3.4 to 6.7 m per week.

SUMMARY

1. Fresh water mussels were exposed to several concentrations of DDT and dieldrin between 0.05 and 1.0 ppb in reconstituted distilled water, dechlorinated tapwater, and natural lake water under continuous flow and constant temperature conditions.

2. The mussels concentrated DDT approximately 1000 fold in distilled water and 2400 fold in lake water.

3. Dieldrin was concentrated about 1200 fold in lake water by the mussels.

4. The concentration of insecticide in the mussels reached equilibrium with the concentration in the water faster in lake water than distilled water and the insecticide also had a shorter half-life in the mussel in lake water.

5. Dieldrin's half-life was 4.7 days in lake water, about one-third of DDT's half-life in lake water.

6. The insecticide concentrations were highest in the digestive and reproductive tissue and low in the muscle, mantle, and gill tissues. The concentrations were very

low in the marsupia in tests run in distilled water but were almost as great as the digestive and reproductive organs in lake water.

7. Temperature was found to affect the rate of uptake and elimination of the insecticides in dechlorinated tap-water. It also affected the equilibrium concentration in the mussels.

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LITERATURE CITED

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