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Influence of Diet and Body Lipids on the Bioconcentration of Endrin from Water in the Fathead Minnow ('Pimephales promelas')

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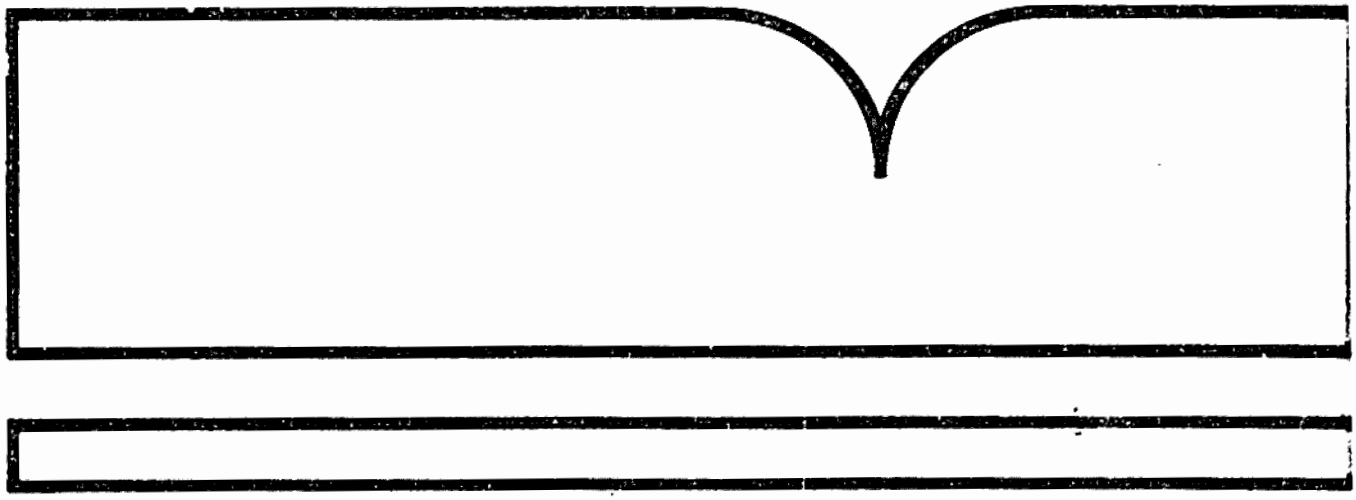

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INFLUENCE OF DIET AND BODY LIPIDS ON THE BIOCONCENTRATION OF ENDRIN FROM WATER IN THE FATHEAD MINNOW (Pimephales promelas)

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16. ABSTRACT

The purpose of this study was to quantify the importance of the fathead minnow's (Pimephales promelas) body lipid content and its composition in the bioconcentration of a lipophilic chemical (endrin) from water.

For three months prior to exposure, six groups of fish were fed reference research diets containing 0, 10, 15 or 20% (dry weight diet basis) lipids added as corn oil and/or salmon oil. Two other groups were fed frozen brine shrimp (Artemia salina) at two ration levels.

Bioconcentration tests at two concentrations of endrin in water (0.11 and 0.19 $\mu g\ L^{-1}$) produced mean bioconcentration factors (BCFs) of 15,000x after 14 days and 23,000x after 29 days when expressed on a wet weight, whole body basis. Corresponding mean BCFs expressed on a lipid, whole body basis were 190,000x and 340,000x.

Whole body BCFs expressed on a wet weight basis ranged 8,000x - 21,000x after 14 days exposure and 5,000x - 30,000x after 29 days exposure. Independent of diet composition, whole body BCFs expressed on a wet weight basis were positively correlated to the concentration of total fish body lipids. When BCFs were expressed on a lipid basis, they were instead negatively correlated to the concentration of total fish body lipids. From the limited number of samples examined for each diet group, no influence of diet lipid source (corn oil, salmon oil and brine shrimp lipids) could be found.

Results from this study were interpreted as follows: the greater the fishes' body lipid content the more endrin it bioconcentrates rapidly and directly from the water. Thus, fatter fish take a longer time to reach equilibrium.

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Results from this study were interpreted as follows: the greater the fishes' body lipid content to more endrin it bioconcentrates rapidly and directly from the water. Thus, such fish take a longer time to reach equilibrium.

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INTRODUCTION

In aquatic animals bioconcentration of chemicals can take place directly from water and from food. When Jarvinen and Tyo (1978) exposed fathead minnows (Pimephales promelas) to endrin, both through water and through food (consisting of previously exposed clams) for 300 days, they found maximum bioconcentration factors (BCFs) of 0.8x from food and 13,000x from water. The conclusion from their study is that the uptake from water was the predominant route for endrin in the fathead minnow.

Prediction of the bioconcentration factor, BCF (= concentration in fish divided by concentration in water at steady state), from the partition coefficient between octanol and water (P) has been thoroughly investigated (Neely et al., 1974; Veith et al., 1979; Konemann, 1979; Renberg and Sundstrom, 1979). In these studies the general equation: Log BCF = a_0 + a_1 x Log P has given good correlations for a wide variety of chemicals. However, in estimates made on the same product, factors like source of fish and/or experimental error, species and test temperature have produced variations in BCF (for Aroclor 1016) of 2, 4 and 12 times, respectively (Veith et al., 1979).

The purpose of this study was to estimate the relative importance of some nutritional factors on the fathead minnow's ability to bioconcentrate a lipophilic chemical, endrin, from water. The nutritional factors studied were dietary lipid content and composition and ration level. The influence of these factors and of starvation on the toxicity of endrin has been reported in a simultaneous study (Dave, 1981).

CONCLUSIONS

The results from this study have shown that the body lipid content had a significant effect on the bioconcentration of a lipophilic chemical, endrin, in the fathead minnow. The fatter the fish was, the more endrin it bioconcentrated directly from water. Bioconcentration factors (BCFs) after 29 days exposure ranged 5,000x - 30,000x (6-fold difference). when expressed on a whole body wet weight basis. When instead expressed on a lipid basis, BCFs after 29 days exposure ranged 250,000x - 450,000x (2-fold difference).

BCFs calculated on a wet weight basis were positively correlated to the lipid content of the fish, but BCFs calculated on a lipid basis were instead negatively correlated to the lipid content, indicating that the fatter the fish was, the longer was the time needed to reach a steady state.

RECOMMENDATIONS

Bioconcentration factors (BCFs) for lipophilic chemicals should be expressed on a lipid as well as a wet weight basis. Since BCFs for lipophilic chemicals like endrin estimated in 30-day bioconcentration tests can be expected to be significantly affected by the lipid content of the fish. A fatter fish is expected to show a higher residue than a lean fish. Also, twenty-nine-day BCFs expressed on a lipid basis showed less variation (as C.V.) than BCFs expressed on a wet weight basis. Since BCFs expressed on a lipid basis are expected to be more uniform, perhaps lipid-based BCFs could reduce variations observed within the same species and also between different species.

MATERIALS AND METHODS

A detailed description of the materials and methods used have been given previously (Dave, 1981). Therefore, only a brief presentation of the experimental design and the method for residue analysis is provided here.

One-month-old fathead minnows were separated into eight groups (I-VIII), which were kept in separate tanks for three months prior to exposure to endrin. Diets fed to group I-VI were reference research diets (Brauhn and Schoettger, 1975; National Research Council, 1973; 1977) containing different percentages or sources of lipids. Group VII and VIII were fed a high (ad lib) and a low (1/6 of ad lib) ration of frozen brine shrimp, respectively. Diet compositions are given in Table 1.

After this feeding period, exposure to endrin through water was performed simultaneously for all groups by enclosing them in screened compartments in a flow-through exposure system. Water temperature was 25.1±0.7°C (mean±S.D.). Alkalinity ranged 39.5-41.0 mg L^{-1} as $CaCO_3$, hardness 43.5-45.5 mg L^{-1} as $CaCO_3$, and pH ranged 7.3-7.5 as determined weekly on the laboratory source (Lake Superior water). Calculated mean endrin concentrations in water based on chemical analyses are given in Table 2.

For the residue analysis composite, whole fish samples were homogenized with 70 g of granular anhydrous Na_2SO_4 (Mallinckrodt, Inc.). The powdered homogenate was transferred to a 300-ml chromatographic column and eluted with 250 ml of redistilled hexane. The eluate was collected in a 250-ml volumetric flask. No cleanup procedure was performed. A 10-ml aliquot of the sample was transferred to a culture tube and stored in the freezer to await analysis by gas chromatography. The remaining 240 ml were used for lipid content measurements.

The 240-ml samples were transferred to 250-ml beakers and concentrated to 10 ml. Clean, solvent-rinsed 25-ml beakers were heated to 110°C for 30 minutes, cooled in a desiccator for 30 minutes and weighed to four decimal places. The samples were then quantitatively transferred with methylene chloride to the tared beakers and allowed to evaporate to near dryness. The beakers were then heated to 110°C for 30 minutes, cooled in a desiccator for 30 minutes and reweighed to four decimal places. The lipid content was based on the following calculation:

$$\text{Lipid (percent)} = \frac{(\text{gross} - \text{tare}) \times 100}{\text{tissue weight} \times 0.96}$$

The stored 10-ml samples were re-adjusted to volume and screened on the gas chromatograph to determine the proper dilution ratio. After necessary dilutions were made, 1.5-ml aliquots of the samples were transferred to Hewlett-Packard injection vials for quantitation by gas chromatography.

The gas chromatographic analysis was performed on a 5730A Hewlett-Packard gas chromatograph with an auto sampler and a Hewlett-Packard 3354B lab automation data system. The gas chromatograph was equipped with a ^{63}Ni electron capture detector held at 300°C, and the injection port temperature was 250°C. The 6 ft. 2 x 3 mm (OD) column was packed with 1.5% SP-2250/1.95% SP-2410 on 100/120 mesh Supelcoport. The carrier gas was 5% methane in argon and a flow rate of 40 ml/min. The tissue samples were analyzed at a column temperature of 210°C. The percent recovery of spiked samples was 99.9$\pm$2.3 percent with n = 3. Results from the analyzes are given in the Appendix.

EXPERIMENTAL PROCEDURES

The exposure to endrin was started with 10 fish from each diet group (I-VIII). The three highest concentrations (called 1, 2 and 3) delivered from the diluter (Mount and Brungs, 1967) were used to measure toxicity, concentrations 4 and 5 were used to measure bioconcentration. Concentration 6 was the control (conc. = nil). Fishes for residue analyses were sampled after 14 and 29 days. They were killed by immersion in 0°C water for 30 seconds, weighed to the nearest 0.01 g and deep-frozen in glass liquid scintillation vessels to await residue analysis.

During the exposure period, mortalities of fish were 4/80 in conc. 4; 5/80 in conc. 5 and 1/80 in the control. Only live fishes were used for residue analysis.

No food was given for the first 10 days of exposure or for 24 hr prior to sampling. The other days, the previously fed diets were given at an average ration of 2.8 g dry food/100 g live fish body weight per day to groups I-VII and 1.5 g dry food/100 g live fish body weight per day to group VIII. For the entire exposure periods (14 and 29 days) the calculated average daily ration for the first 14 days was 0.6 g dry food/100 g fish in group I-VII and 0.3 g dry food/100 g fish in group VIII, and for the 29 days exposure corresponding figures are 1.6 and 0.9. Daily rations were corrected for the reduced number of fish due to sampling after 14 days (4-5 fish in each group) but not for mortalities. Calculated rations above are based on measured amounts of food and calculated averages for body weights of sampled fish (given in the Appendix).

Because of the errors in feeding a group as opposed to individual fishes and the variations in fish size within and between the different groups, no

detailed analysis of body weight gain or loss during exposure seemed valid. The diet type provided during the three months prior to and during exposure was the same and the over-all ration given during exposure was approximately a maintenance ration.

RESULTS

Mean concentrations of endrin in water during exposure for 14 and 29 days are given in Table 2. These concentrations and corresponding whole body residues of endrin and whole body lipid concentrations given in the Appendix were used for calculations of BCFs (bioconcentration factors). BCFs calculated both on a wet weight and on a lipid basis are given in Table 3 for 14 days exposure and in Table 4 for 29 days exposure.

Mean values for whole body lipid contents (from Appendix) and whole body water contents (from Dave, 1981) for groups I-VIII are given in Table 5. Mean lipid contents (Table 5) and analytical data in the Appendix suggest that individual variations in whole body lipids among these fish are too great to show a direct relationship to dietary lipid content. However, independent of dietary lipid there is a correlation between the mean whole body lipid contents for the different groups of fish (I-VIII) and their whole body water contents (shown in Figure 1). This relationship between lipids and water has been repo.ted for several other fish species (review by Love, 1970). The present study shows that such a relationship, the fat-water line, also exists in the fathead minnow.

Because of the great individual variations in lipid contents as well as in endrin residues, the data were tested for possible correlations between lipid contents and BCFs (independent of dietary treatment). In Figure 2 we have presented the linear regression of BCFs expressed on wet weight basis and whole body lipid concentrations for the samples from all diet groups. The values for a_0 (lipid content = 0) after 14 and 29 days are almost identical (7, 516 and 7,423), but the calculated value for a_1 for 29 days exposure is more than twice that for 14 days exposure (2,269 and 908,

respectively). These significant correlations must mean, that the fattier the fish is, the more endrin it bioconcentrates directly from water. Furthermore, the higher value for a_1 after 29 compared to 14 days exposure must mean, that the fattier the fish is, the longer is the time required to reach equilibrium.

In Figure 3 we have presented the linear regression of BCFs expressed on a lipid basis and whole body lipid concentrations for the samples from all diet groups. The inverse relationships suggest, that the fattier the fish is, the less saturated is its lipid pool with endrin after identical exposures. Or in other words, the fattier the fish is, the longer the time it takes to reach equilibrium.

The source of lipid in the diet is of nutritional importance because it determined the dietary fatty acid composition and the proportions of triglycerides, cholesterol, phospholipids, etc. The lipid sources used in the present study were corn oil, which is high in $\omega 6$ fatty acids, salmon oil, which is high in $\omega 3$ fatty acids, and the brine shrimp lipids (Artemia salina, San Fransisco Bay variety, analyzed by Gallagher and Brown, 1975). The present study did not indicate that the source of dietary lipid had any influence on the results obtained. However, it might have affected the lipid composition of the experimental fish, but this was not investigated.

DISCUSSION

The results from this study have shown that the body lipid content had a significant effect on the bioconcentration of a lipophilic chemical, endrin, in the fathead minnow. The fattier the fish was, the more endrin it bioconcentrated directly from water. Bioconcentration factors (BCFs) after 29 days exposure ranged 5.000x -30.000x (6-fold difference), when expressed on a whole body, wet weight basis. When instead expressed on a lipid basis, BCFs after 29 days exposure ranged 250.000x - 450.000x (2-fold difference).

Among different species of fish, there are considerable variations in body fat deposit distributions, total amount and composition. Furthermore, there are variations within a species caused by age, availability of food, season, sex, size and strain (review by Love, 1970). Considering these variations, estimates of BCFs for lipophilic chemicals solely on a wet weight basis can be expected to produce considerable variations. Estimates of BCFs both on a wet weight and a lipid basis would contribute to a better understanding of causes for variations in bioconcentration within the same species and perhaps to some extent also between different species of fish.

The results from this study reveal a positive correlation between BCFs expressed on a wet weight basis and total body lipid contents, indicating a higher uptake from water in fat compared to lean fishes. When BCFs were expressed on a lipid basis, they were instead negatively correlated to the total fish body lipid content, indicating that fattier fishes needed a longer time than leaner fishes to reach equilibrium. This implies that estimates of steady state BCFs would require different periods of exposure depending on the total lipid pool. However, other species-related factors, like gill surface area and detoxification capacity, are probably also of great

importance for the time to reach equilibrium. Estimates of bioconcentration in different species of fish under identical exposures, would probably reveal the relative importance of such species-related factors. Such comparative estimates would probably also extend the possibilities to predict bioconcentration of lipophilic chemicals in different species of fish and under different physiological conditions related to age, nutritional status, season and sex. Because of the importance of the lipid content in the bioconcentration process, BCFs should be expressed both on a wet weight and a lipid basis in such studies.

The present study dealt only with uptake directly from water. Under natural conditions uptake of chemicals can take place also from food. From an energetic point of view, a fat compared to a lean fish of the same age and body weight and within the same population (environment and type of food being the same) should have consumed more food. Because of this greater food consumption, a fattier fish under natural conditions would be expected to have experienced a greater uptake both through food (due to bioenergetics) and through water (due to partitioning).

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Table 1. Gross Composition of Experimental Diets

Ingredient	Amount of Ingredient (g) in Respective Diet					
	I	II	III	IV	V	VI *
Casein	280	280	280	280	280	280
Gelatin	120	120	120	120	120	120
Dextrin	280	280	280	280	280	280
Vitamin mix.	10	10	10	10	10	10
Mineral mix.	40	40	40	40	40	40
Sum of above	730	730	730	730	730	730
Corn oil	-	100	-	50	75	100
Salmon oil	-	-	100	50	75	100
Sum of above	730	830	830	830	880	930
α-cellulose	230	170	170	170	120	70
Sum of above	1000	1000	1000	1000	1000	1000
Added water	2000	2000	2000	2000	2000	2000
Moisture (%)	66.7	66.7	66.7	66.7	66.7	66.7

*Values for diets called VII and VIII, which were frozen brine shrimp Artemia salina (San Fransisco Bay Brand, Newark, California 94560; Stock #65006) as determined by Gallagher and Brown (1975) were as follows: protein 58% dry weight (Kjeldahl method), crude fat 5.1% dry weight (ether extract) or 19.3% dry weight (methanol-chloroform extract), fiber 3.5% dry weight, ash 20.6% dry weight, at 90% moisture.

Table 2. Concentrations of Endrin in Water (µg L^{-1}) During Exposure of Fathead Minnows

Period of Exposure	Conc. 4*	Conc. 5
Day 0-14	0.18392** (0.13277 - 0.23506)***	0.10829 (0.09158 - 0.13840)
Day 0-29	0.19474 (0.14370 - 0.24578)	0.11466 (0.08461 - 0.14471)

* Conc. 4 and 5 were the two lowest concentrations delivered by the diluter (Mount and Brungs, 1967).

** Mean values calculated from analytical data of regular samples taken from the dipping bird reservoir and exposure tanks. The analytical procedure does only justify two numbers, e.g., 0.18 µg L^{-1}. Calculated mean values in the table have been used for calculations of BCFs.

*** Numbers in parenthesis are 95% confidence limits.

Table 3. Bioconcentration Factors (BCFs) for Endrin After 14 Days Exposure Through Water in Fathead Minnows Fed Different Diets

Diet Group	Endrin in Water $\mu g\ L^{-1}$	Total Body Lipid g/100 g	$BCF_{14\ days}$ W.W. Basis	$BCF_{14\ days}$ Lipid Basis
I	0.18 (conc. 4)	3.64	8,960	246,140
II	0.18 (conc. 4)	10.67	18,100	171,216
III	0.18 (conc. 4)	5.79	14,659	243,154
IV	0.18 (conc. 4)	9.33	18,856	202,099
V	0.18 (conc. 4)	7.88	14,425	183,069
VI	0.18 (conc. 4)	11.81	21,286	180,241
VII	0.18 (conc. 4)	12.75	19,182	150,446
VIII	0.18 (conc. 4)	4.99	14,659	293,769
I	0.11 (conc. 5)	3.26	8,016	245,914
II	0.11 (conc. 5)	8.40	17,232	205,097
III	0.11 (conc. 5)	8.34	15,191	182,104
IV	0.11 (conc. 5)	10.91	15,311	140,364
V	0.11 (conc. 5)	9.15	15,311	167,328
VI	0.11 (conc. 5)	11.49	15,911	138,517
VII	0.11 (conc. 5)	13.54	17,038	125,866
VIII	0.11 (conc. 5)	6.60	11,885	180,072

Table 4. Bioconcentration Factors (BCFs) for Endrin After 29 Days Exposure Through Water in Fathead Minnows Fed Different Diets

Diet Group	Endrin in Water µg L^{-1}	Total Body Lipid g/100 g	$BCF_{14\ days}$ W.W. Basis	$BCF_{14\ days}$ Lipid Basis
I	0.19 (conc. 4)	1.34	5,068	378,248
II	0.19 (conc. 4)	9.90	24,669	249,204
III	0.19 (conc. 4)	5.39	20,782	385,540
IV	0.19 (conc. 4)	8.64	24,653	285,355
V	0.19 (conc. 4)	10.07	25,644	254,647
VI	0.19 (conc. 4)	7.99	27,760	347,438
VII	0.19 (conc. 4)	6.41	28,654	447,006
VIII	0.19 (conc. 4)	8.28	24,423	299,733
I	0.11 (conc. 5)	2.69*	10,291	382,565*
II	0.11 (conc. 5)	7.04	22,100	313,885
III	0.11 (conc. 5)	7.96	30,167	411,174
IV	0.11 (conc. 5)	7.23	20,487	283,360
V	0.11 (conc. 5)	9.29	30,167	324,699
VI	0.11 (conc. 5)	8.91	25,301	283,970
VII	0.11 (conc. 5)	8.20	30,054	366,475
VIII	0.11 (conc. 5)	3.71	13,666	368,394

*Mean lipid value for diet group I and BCF calculated from this value, because lipid value was missing.

Table 5. Lipid and Water Content of Fathead Minnows Fed Six Artificial Diets With Increasing Lipid Contents or Frozen Brine Shrimp at Two Ration Levels

Diet No.	Lipids*	Ration	Fish Body, Mean + S.D. (n)	
			Lipids (%)	Water (%)
I	no lipids	unrestricted	2.69±0.88 (4)	78.8±2.8 (8)
II	10% C.O.	unrestricted	8.99±1.37 (5)	73.3±2.1 (8)
III	10% S.O.	unrestricted	7.06±1.92 (5)	73.5±1.5 (8)
IV	5% C.O. + 5% S.O.	unrestricted	8.80±1.42 (5)	70.1±2.3 (8)
V	7.5% C.O. + 7.5% S.O.	unrestricted	9.24±0.85 (5)	73.1±2.6 (8)
VI	10% C.O. + 10% S.O.	unrestricted	10.33±1.75 (5)	71.6±2.6 (8)
VII	Brine shrimp lipids	unrestricted	10.13±3.01 (5)	68.4±2.9 (8)
VIII	Brine shrimp lipids	restricted ca 1/6 of VII	5.58±1.42 (5)	73.3±2.5 (8)

*C.O. = corn oil; S.O. = salmon oil. Lipid percentages are nominal values on dry weight basis.

Table 6. Mean Bioconcentration Factors (BCFs) of Endrin Through Water in Fathead Minnows Fed Different Diets

Period of Exposure	Endrin Concentration, $\mu g\ L^{-1}$			
	0.18 - 0.19 (conc. 4)		0.11 (conc. 5)	
	BCF W.W. Basis	BCF Lipid Basis	BCF W.W. Basis	BCF Lipid Basis
14 days	16,266* ±3,886 (24%)	210,017 ±49,150 (23%)	14,487 ±3,084 (21%)	173,158 ±39,618 (23%)
29 days	22,707 ±7,511 (33%)	330,896 ±70,220 (21%)	22,779 ±7,683 (34%)	341,815 ±47,241 (14%)

*BCFs given as mean ± S.D. (n = 8) with C.V. (S.D. / mean x 100) in parenthesis. Only two to three significant figures are justified.

Table 7. Linear Regression Analysis*

Y	X	a_0	a_1	r	N	Value of P	S.D.	Data Presentation
BCF after								
14 days (w.w. basis)	Body lipid (%)	7,516	908	0.815	16	0.001	2,105	Table 3; Fig. 2
29 days (w.w. basis)	Body lipid (%)	7,423	2,269	0.795	16	0.001	4,606	Table 4; Fig. 2
14 days (lipid basis)	Body lipid (%)	303,292	-12,909	0.863	16	0.001	24,652	Table 3; Fig. 3
29 days (lipid basis)	Body lipid (%)	430,238	-13,902	0.616	16	0.01	47,372	Table 4; Fig. 3
Mean body water (%)	Mean body lipid (%)	80.51	-0.986	0.846	8	0.01	1,759	Table 5; Fig. 1
Endrin residue (ppm) after								
14 days in conc. 4	Lipid content (%)	1,375	0.1937	0.902	8	0.01	0.334	Appendix
14 days in conc. 5	Lipid content (%)	0.763	0.0899	0.851	8	0.01	0.189	Appendix
29 days in conc. 4	Lipid content (%)	1,207	0.4370	0.833	8	0.02	0.911	Appendix
29 days in conc. 5	Lipid content (%)	0.433	0.3166	0.921	7	0.01	0.346	Appendix

*$y = a_0 + a_1 \times X$

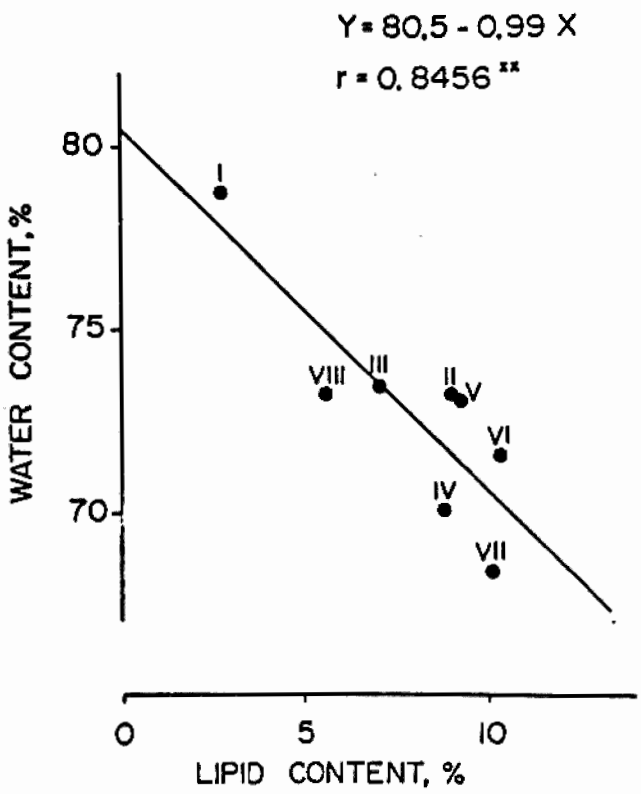

Figure 1. Correlation between mean total body water and mean total body lipid contents for eight groups of fathead minnows fed different diets for at least three months. Roman numerals refer to diet numbers.

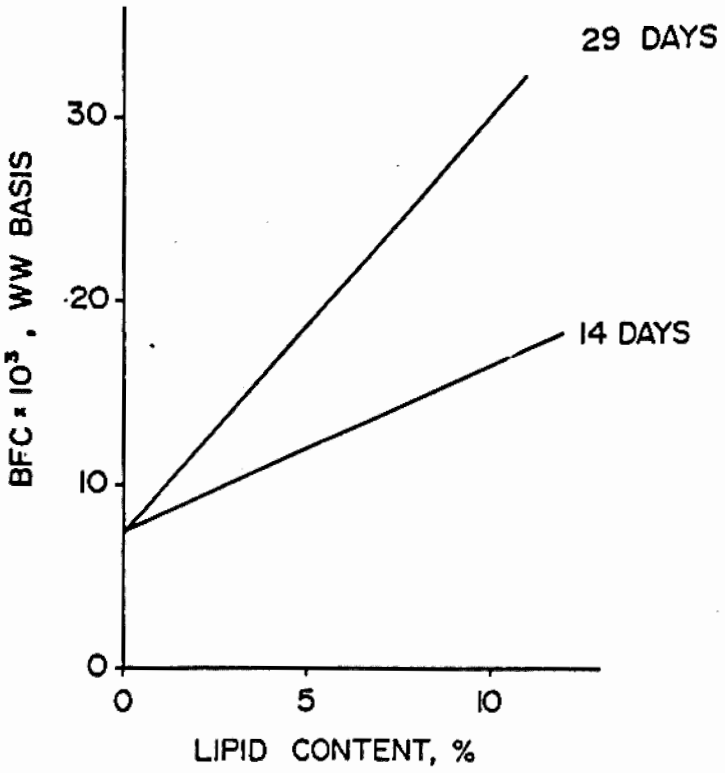

Figure 2. Correlation between bioconcentration factors (BCFs) for endrin from water and total body lipid contents of fathead minnows after 14 and 29 days exposure when expressed on a wet weight basis.

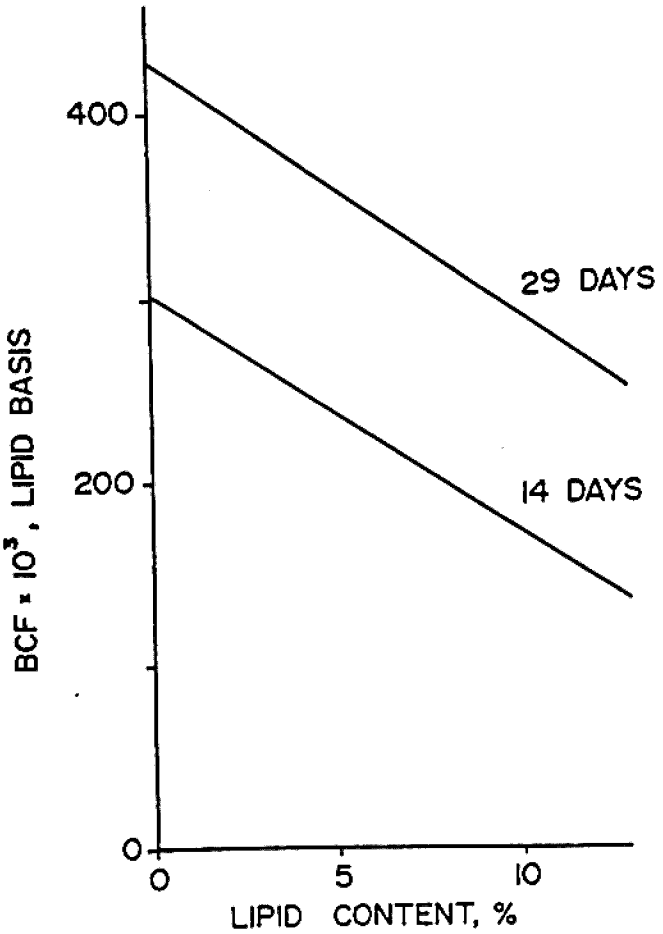

Figure 3. Correlation between bioconcentration factors (BCFs) for endrin from water and total body lipid contents of fathead minnows after 14 and 29 days exposure when expressed on a lipid basis.

APPENDIX

ANALYTICAL DATA FROM ENDRIN RESIDUE AND LIPID CONTENT ANALYSIS

	Sample Name	Wet Tissue Weight (gm)	Endrin (ppm) (µg/gm)	Lipid Content (percent)
5/14	I[4] 1-5	2.49	1.648	3.64
5/14	II[4] 1-5	2.69	3.329	10.57
5/14	III[4] 1-5	2.41	2.696	5.79
5/14	IV[4] 1-5	2.44	3.468	9.33
5/14	V[4] 1-4	1.84	2.653	7.88
5/14	VI[4] 1-4	1.86	3.915	11.81
5/14	VII[4] 1-5	6.81	3.528	12.75
5/14	VIII[4] 1-4	1.47	2.696	4.99
5/14	I[5] 1-5	2.23	0.868	3.26
5/14	II[5] 1-4	2.96	1.866	8.40
5/14	III[5] 1-5	2.83	1.645	8.34
5/14	IV[5] 1-5	3.45	1.658	10.91
5/14	V[5] 1-5	2.41	1.658	9.15
5/14	VI[5] 1-5	2.97	1.723	11.49
5/14	VII[5] 1-5	6.34	1.845	13.54
5/14	VII[5] 1-5	1.49	1.287	6.60
	BlK	-	<0.01	-
5/29	I[4] 6-8	1.84	0.987	1.34
5/29	II[4] 6-9	3.40	4.804	9.90
5/29	III[4] 6-10	3.77	4.047	5.39
5/29	IV[4] 6-10	3.53	4.801	8.64
5/29	V[4] 6-9	2.50	4.994	10.07
5/29	VI[4] 6-8	2.51	5.406	7.99
5/29	VII[4] 6-10	6.47	5.580	6.41
5/29	VIII[4] 6-10	3.64	3.082	5.28

APPENDIX (Continued)

	Sample Name	Wet Tissue Weight (gm)	Endrin (ppm) (µg/gm)	Lipid Content (percent)
	B1K1	-	<0.01	-
5/29	I^5 6	0.27	0.771	-
5/29	I^5 8	0.71	1.589	-
5/29	II^5 6-9	2.54	2.534	7.04
5/29	III^5 6-8	3.56	2.814	5.96
5/29	IV^5 6-10	3.81	2.349	7.23
5/29	V^5 6-10	3.09	3.459	9.29
5/29	VI^5 6-8	1.83	2.901	8.91
5/29	VII^5 6	1.02	4.162	-
5/29	VII^5 7	0.78	7.059	-
5/29	VII^5 8-10	2.51	3.446	8.20
5/29	$VIII^5$ 6-9	2.01	1.567	3.71
	B1K2	-	<0.01	-
5/29	I^6 1-5	2.35	<0.01	2.50
5/29	II^6 1-5	2.84	<0.01	9.03
5/29	III^6 1-5	3.65	<0.01	9.80
5/29	IV^6 1-5	4.62	0.159	7.89
5/29	V^6 1-5	3.54	<0.01	9.82
5/29	VI^6 1-5	3.43	<0.01	11.45
5/29	VII^6 1-55	5.89	<0.01	9.76
5/29	$VIII^6$ 1-5	2.39	<0.01	7.33

Name of samples indicates/days of exposure, diet group (roman), endrin concentration (actual concentrations in Table 2), numbers of fish in the composite sample.