AN ABSTRACT OF THE THESIS OF

Pamela J. Shubat for the degree of Master of Science in the Department of Fisheries and Wildlife presented on July 18, 1984

Title: Ration and Toxicant Preexposure Influence Dieldrin Accumulation by Rainbow Trout

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Whole body accumulation and tissue distribution of dieldrin (HEOD[1]) in rainbow trout was studied through oral dose disposition tests and subchronic exposures via water (0.04 and 0.08 µg dieldrin/l) and/or diet (0.087 µg dieldrin/g fish/day). Growth and maintenance rations of 4 and 2% body weight/day were employed. Subchronic testing produced apparent steady state residue concentrations dependent on ration and exposure concentration after eight weeks exposure. At 16 weeks, mean whole fish residue concentrations ranged from 120 to 1400 ng dieldrin/g fish. Exposure through food and water under conditions of growth produced the highest values (1300-1400 ng dieldrin/g fish). Maintenance rations apparently limited accumulation to a maximum of 360 ng dieldrin/g fish. Whole body dieldrin concentration calculated on the basis of total lipids greatly reduced the differences in residue levels due to ration.

Disposition tests were conducted on fish administered dieldrin via the diet for 2, 4 and 6 weeks and naive fish (no pretreatment). The

[1] HEOD: endo exo isomer of 1,2,3,4,10,10-hexachloro-6,7-expoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene

disposition of a single oral dose of radiolabelled dieldrin was determined 48 hours following administration.

Retention of the dose was relatively constant at 0, 2, and 6 weeks with a downward trend for most tissues and a 2 and 5 fold increase in retention of label in the bile of fish receiving maintenance and growth treatments, respectively. After 4 weeks pretreatment, label concentrations in all tissues except the bile, gut, and gut contents increased 3 to 30 fold compared to label retained by naive fish.

Ration and Toxicant Preexposure Influence Dieldrin Accumulation by Rainbow Trout

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RATION AND TOXICANT PREEXPOSURE INFLUENCE DIELDRIN ACCUMULATION BY RAINBOW TROUT

INTRODUCTION

Accumulation of organochlorine compounds in terrestrial and aquatic biota has been of interest because of their toxicity to nontarget organisms and persistence following agricultural or industrial use. Research on accumulation of organochlorine insecticides in components of aquatic systems has focused on transfer of chemicals through food chains and the relative contributions of contaminated food and water to total accumulation. Experiments with reticulate sculpins (Chadwick and Brocksen, 1969) and guppies (Reinert, 1972) indicated dieldrin in water was more available for accumulation than dieldrin in the diet. Eberhardt et al. (1971) studied DDT applied to a freshwater marsh and concluded maximum residues were associated with food chain transfer, a conclusion shared by Macek and Korn (1970) based on laboratory experiments with DDT and trout. Jarvinen and Tyo (1978) concluded endrin accumulated by fathead minnows exposed via diet was additive to that accumulated from water. Food chain transfer of DDT was rejected by Hamelink et al. (1971) in favor of accumulation based on partition coefficients between water, blood and lipid. The authors concluded that it was not possible to state which source, food or water, provided the greatest quantity of residues in fish, but that the critical factor in reducing accumulation would be reduction of the concentration of toxicant in water.

Dieldrin residues in freshwater fish have been monitored nation-wide since the mid-1960s and levels greater than 0.1 µg/g have been regularly measured in areas where aldrin, dieldrin's parent compound, has been applied as a soil insecticide (Schmitt et al., 1981). Although use of dieldrin and aldrin has been restricted since 1975 (Schnoor, 1981), higher order consumers may provide a reservoir of the chemical which will reenter the environment. For example, dieldrin is thought to be responsible for bat mortality in Missouri maternity colonies as recently as 1981 and is implicated in concurrent macroinvertebrate mortality (Clark et al., 1983).

This study attempted, not a resolution of conflicting information on organochlorine accumulation, but a unified examination of some of the many possible conditions influencing dieldrin accumulation in trout, Salmo gairdneri. Exposure conditions of dieldrin contaminated food and/or water were combined with two ration levels to examine the influence of growth on dieldrin accumulation. Accumulation under these various conditions was evaluated through acute and subchronic exposures. Disposition tests were conducted to determine how a single dose of dieldrin was distributed throughout the body of fish with or without prior exposure to dieldrin.

MATERIALS AND METHODS

Juvenile rainbow trout (Salmo gairdneri) from Oregon State University or from the Alsea Fish Hatchery (Oregon Department of Fish and Wildlife) were utilized in exposures. Six-month-old Shasta strain (nonmigratory) rainbow trout (2.8g) were exposed to acute concentrations of dieldrin in water and six-month-old Alsea steelhead (anadromous) trout (1.8g) were exposed to acute dietary concentrations. Seven-month-old Alsea trout of two size classes (3.0g and 5.1g) were used for subchronic exposures and fed growth or maintenance rations during the 16 week test. Fourteen month old Alsea trout (34-86g) were used for dispositon studies. Fish were fed Oregon Test Diet (Sinnhuber et al., 1977) before and during testing.

Well water was used throughout rearing and testing. Water chemistry parameters of hardness, alkalinity and dissolved oxygen were measured at two week intervals during testing according to standard methods (APHA, 1975) and averaged 130 mg/l Ca_2CO_3, 147 mg/l Ca_2CO_3, and 9.6 mg/l O_2, respectively. Measurements of pH ranged from 7.6 to 8.1. Temperatures ranged from 12 to 13°C during acute tests and 9 to 11°C during subchronic and disposition tests. A 12-hour photoperiod of 120 lux was used throughout testing. Exposures were conducted in screen covered glass aquaria (41 x 20 x 23 cm) filled with 15 l of water. Tanks received a flow rate of 60-80 ml/min (over 6 volume additions per day). Tanks were aerated during static disposition exposures.

For those tests of exposure via water, a 100 µg dieldrin/l solution in water was continuously generated using a column containing dieldrin (Shell Technical grade, 100% purity), a reservoir tank, and a pump circulating water from the reservoir through the column. A diluter similar to that described by Chadwick (1971) delivered the toxicant and control water to exposure tanks.

Dieldrin (Chem. Serv., Inc., 99% purity) was dissolved in salmon oil and incorporated into Oregon Test Diet (OTD) for dietary exposures. Diet concentrations of 6.82, 3.47, and 1.62 µg dieldrin/g food (wet weight) were used for subchronic testing and a diet concentration of 364 µg dieldrin/g food for acute doses. Diet dosages for disposition tests were prepared by spotting a 21.4 µg ^{14}C-dieldrin/l solution (2.36 mCi/mM, Shell, 99+% purity) in ethanol onto cubes of OTD and evaporating the ethanol.

Acute Exposures

1. Exposure via water

Trout were exposed to dieldrin concentrations of 0, 0.15, 0.30, 0.99 and 3.1 µg/l. Fifteen fish were divided between duplicate tanks for each exposure concentration and observed for loss of equilibrium and mortality for 96 hours. At that time, surviving fish from one set of tanks were removed for residue analysis. The remaining fish were fed and observations continued for an additional eight days. Water in exposure tanks was sampled for dieldrin concentration.

2. Exposure via diet

Six doses of contaminated diet 0, 0.32, 0.75, 1.4, 3.0, and 5.7 µg dieldrin/g fish/day were prepared for 17 days of feeding at 25 mg food/g fish/day (1.4% body weight ration). Five fish were individually fed each dose concentration. Fish were held in 15 l tanks partitioned into five sections, one tank per dose. Fish were fed each morning and a record kept of their feeding success and behavior. Uneaten food was removed, offered again later that day, and discarded if still not taken. Fish dying during the test or sacrificed at day 18 were weighed and frozen for dieldrin analysis. Water was sampled on days 3, 6, and 13 for dieldrin concentration.

Subchronic Exposures

A 3 x 2 x 2 test matrix was used to produce combinations of toxic diet, water, and control exposures at two different rations. Concentrations of 0, 0.04, and 0.08 µg dieldrin/l in water and a dose of 0 or 0.087 µg dieldrin/g fish/day in diet were used in six combinations of toxicant or control conditions. A total of twelve test conditions were produced by feeding fish in one set of six tanks a growth ration at 2.5% their body weight per day (equivalent to 4% dry food:dry fish). The second set of six tanks received a maintenance ration of 1.2% (equivalent to 2% dry food:dry fish). Fish were branded before testing to allow calculation of individual growth rates and facilitate random sampling. The thermal marking procedure of Groves and Novotny (1965) was modified by chilling marking tools in an acetone/dry ice mixture. Two weeks after branding, fifteen fish

from one of the two size classes described earlier were placed in each exposure tank. Seven days ration was calculated for fish in each tank and divided into portions for three weekly feedings. Three fish were removed after two weeks exposure for residue analysis. Very large and very small fish were removed at this sample period to achieve a more uniform group of test animals and subsequent sampling at 4 and 8 weeks was random. Fish in each tank were weighed as a group at 4 week intervals to calculate ration increases, and recalculated at 2 week intervals according to each tank's growth record. At 16 weeks, remaining fish were removed for lipid and dieldin analysis. Water was sampled in tanks at 2 week intervals for dieldrin analysis.

Disposition Exposures

Dieldrin disposition tests were conducted on individually housed fish exposed to dieldrin via diet for 2, 4 and 6 weeks before receiving a nominal dietary dose of 0.087 µg ^{14}C-dieldrin/g fish. Preexposures were at ration levels used in the subchronic study with 0.174 µg dieldrin/g fish given on alternate days. Control (naive) fish were also fed maintenance or growth rations for 2 or 4 weeks prior to receiving a single ^{14}C-dieldrin dose. Fish that readily consumed the labelled diet were left undisturbed for 15 minutes then transferred to aerated tanks for monitoring under static conditions. Water was sampled and feces removed for analysis at 0, 6, 12, 24 and 48 hours during exposure. At 48 hours, fish were removed, killed by a blow to the head, and weighed. Tissue samples (approximately 0.1g) of blood, liver, gallbladder and bile, kidney, fat, brain, gill, and

muscle were removed. The gut was sectioned for analysis and activity of the gut contents determined separately. Muscle mass was determined by weighing a fillet and the remaining carcass was ground with distilled water and a sample analyzed for ^{14}C-dieldrin activity. Tissue samples were minced and weighed, digested with Baker LSC Tissue Solubilizer QT and suspended in Fisher Scintiverse II LSC cocktail. Counting was performed on a Packard Tricarb counter with external channel ratio standardization for correction of quench.

Analytical Procedures

1. Water Samples

Water from exposure tanks was filtered through Bond Elut disposible extraction columns (Analytichem, CA) under vacuum and the dieldrin eluted with ethyl acetate. Samples were analyzed on a Hewlett-Packard model 1500A gas chromatograph with ^{63}Ni electron capture detector. A 2mm id, 1.8 m column packed with 100/20 supelcoport was used. The oven and detector were operated at 210 and 300°C, respectively. Recovery was 90 $\pm$ 8% during acute tests, and 83 $\pm$ 14% during subchronic testing. No correction was made for percent recovery.

2. Tissue Samples

Individual fish were homogenized in acetone using a Brinkman polytron. Homogenate and rinses were filtered through Na_2SO_4 and 120 ml of filtrate collected. Half of the sample was placed in a tared flask for lipid determination. Hexane was added to the remaining sample and evaporated to near dryness under a nitrogen stream. Hexane

was again added and the sample evaporated to 5 ml. This volume was drawn through an activated alumina column and eluted with additional hexane. Elute was evaporated to an appropriate volume for gas chromatography. The lowest detectable quantity of dieldrin was less than 15 ng per fish. Recovery averaged 105% and values were not corrected for recovery. Lipid determination consisted of slow evaporation of the acetone solution, resuspension of lipid in hexane, and transfer to a clean tared beaker. Hexane was evaporated and the weight of lipid determined.

3. Statistical Analysis

Calculations of LD50 and LC50 values were made using the trimmed Spearman Karber method (Hamilton et al., 1977). Analysis of variance was performed on subchronic and disposition data sets, followed by multiple comparisons including treatment contrasts, Dunnetts procedure, and Student-Newman-Kuels. A significance value of $p<0.05$ was set. All calculations were performed on a Hewlett Packard 9816 series 200 computer and its statistical software.

RESULTS

Acute Exposures

Exposures of rainbow trout to dieldrin in water resulted in a 96 hour LC50 value of 0.62 µg dieldrin/l with a 95% confidence interval (CI) of 0.53-0.72 µg/l and a 12 day LC50 value of 0.26 µg/l (95% CI 0.19-0.36 µg/l). There were no deaths at the control or lowest exposure level (0.15 µg/l). Whole body dieldrin residues measured in fish exposed for 96 hours or less were proportional to both exposure concentration and duration of exposure. Residues in fish exposed for 96 hours to 0.15 µg/l averaged 0.548 µg dieldrin/g fish (0.087 SE) and residues following exposure to 0.99 µg/l averaged 5.65 µg/g (0.480 SE).

Exposure via diet yielded an 18 day LD50 value of 2.7 µg dieldrin/g fish/day (95% CI 2.0 - 3.7 µg/g/day). Deaths first occurred after 7 days of feeding and by the 15th all fish fed the highest dose, 5.7 µg/g/day, had died. There were no deaths in exposures of 1.4 µg/g/day or less. Fish refused food one or two days before death and some lost equilibrium just prior to death. Dieldrin consumed over 18 days was estimated for comparison with analytical values for whole body residues. Assimilation efficiencies calculated for fish exposed to the two lowest doses (estimated doses of 5.1 and 12 µg dieldrin/g fish) were 17 and 34%, respectively. Fish administered the three higher doses (estimated doses of 23, 36 and 39 µg/g) assimilated 10, 10, and 15% of the dieldrin. Although gut contents were not removed before analysis, fish administered a lethal

dose usually did not eat 24 hours prior to death and all other fish were killed after fasting 24 hours. Mean concentrations in exposure water were 0.018, 0.073, 0.053, 0.12, and 0.19 µg dieldrin/l for the five dose concentrations and no dieldrin was found in the control tank. Tanks were sampled on days 3, 6, and 13 with the highest measured concentrations on day 6.

Results of these tests were used to estimate possible lethal body burdens for calculation of sublethal testing levels for the 16 week (112 days) exposure.

Subchronic Explosures

Mortalities occurred among fish fed maintenance rations and exposed to the high dieldrin water concentration. There were 5 and 4 deaths amongst fish fed the control diet and the dieldrin diet, respectively.

Final mean weights of fish fed growth or maintenance rations were 6.3g and 5.6g, respectively. Fish fed growth rations doubled in size and maintenance fed fish had a 10% weight gain. Within each ration group, no significant difference in fish weight was found between dieldrin treatments, including control.

Lipid quantification at 16 weeks yielded mean values of 1.4% and 3.3% whole body lipid for maintenance and growth ration groups, respectively. Analysis of variance established significant differences in percent lipid among dieldrin treatments within each ration level. No clear trend was apparent, however, from comparison of mean lipid values for each treatment to lipid values for controls (Table 1).

Table 1. Residue concentration and lipid content in fish exposed for 16 weeks to dieldrin in food and/or water and fed maintenance (2% body weight) or growth (4% body weight) rations.[a] Exposure conditions were: no dieldrin in water (OW) or food (OF); dieldrin in water at 0.04 (LW) or 0.08 µg/L (HW); and dieldrin administered in food at 0.087 µg/g fish/day (HF).

Treatment	Whole fish dieldrin concentration (ng/g fish)		Lipid weight dieldrin concentration[b] (ng/mg lipid)		% lipid by body weight	
	Maintenance	Growth	Maintenance	Growth	Maintenance	Growth
OWOF	0	0	0	0	1.2 ± 0.09	3.8 ± 0.18
OWHF	320 ± 62	700 ± 110	20 ± 3.9	22 ± 4.5	1.6 ± 0.03**	3.1 ± 0.14
LWOF	120 ± 19	320 ± 33	7.1 ± 0.74	11 ± 0.69	1.7 ± 0.09**	2.8 ± 0.28**
LWHF	360 ± 38	1400 ± 190	24 ± 2.6	41 ± 3.4	1.5 ± 0.05**	3.3 ± 0.44
HWOF	200 ± 15	710 ± 25	18 ± 1.0	18 ± 0.55	1.1 ± 0.13	4.0 ± 0.25
HWHF	350 ± 43	1300 ± 170	30 ± 3.0	44 ± 2.9	1.2 ± 0.03	3.1 ± 0.44

[a] values are mean ± SE

[b] Total dieldrin recovered from whole fish expressed as ng dieldrin/mg total lipid

** significantly different ($P<0.05$) than control (OWOF)

Ration had its greatest effect following the fourth week of exposure and maintenance rations resulted in decreased accumulation at 16 weeks at all treatment levels. From 8 - 16 weeks, fish fed a 2% ration accumulated less than 360 ng dieldrin/g fish while those fed a 4% ration accumulated dieldrin in a dose dependent manner and residues at 16 weeks ranged from 320 to 1400 ng/g (Table 1). Dieldrin administered in both food and water resulted in residue levels roughly equal to the sum of residues produced by dosing only via food or water. This additive accumulation was most apparent during the first four weeks of exposure (Fig. 1).

Presentation of data as dieldrin per mg total body lipid (lipid-weight dieldrin concentration) reduced the difference in accumulation due to ration (Table 1). As with whole body accumulation, ration produced the greatest difference in lipid-weight dieldrin concentration at 16 weeks. Lipid content was quantified at each time point for two dieldrin treatments, low water (0.04 µg/l) and low water plus food (0.087 µg/g/day). Data was calculated both as ng dieldrin/g fish and as ng dieldrin/mg lipid (Fig. 2). Maintenance rations produced a drop in percent lipid between 4 and 8 weeks in fish exposed to both low water concentration (0.4% drop) and dieldrin in water plus food (0.9% drop).

Dieldrin was present at a level of 0.02 ± 0.008 µg dieldrin/l in exposure tanks receiving dieldrin only via food. Tanks receiving the low water concentration (0.04 ± 0.02 µg/l) plus dieldrin in food averaged 0.07 ± 0.02 µg/l; tanks receiving the high water concentration (0.08 ± 0.03 µg/l) plus in food averaged 0.1 ± 0.01

Figure 1. Mean whole fish dieldrin concentrations during 16 weeks exposure to dieldrin via water and/or diet at two rations. Juvenile rainbow trout were exposed to dieldrin in water (0.08 µg dieldrin/l); diet (0.087 µg dieldrin/g fish/day); or water and diet combined and sampled at the indicated times for residue analysis. Growth and maintenance rations were 4 and 2% of body weight per day. At 16 weeks, maintenance fed fish accumulated 3.3% of the oral dose and growing fish accumulated 7.2%. See Table 1 for lipid corrected data. Three or six fish were sampled at each time point and standard error bars are indicated.

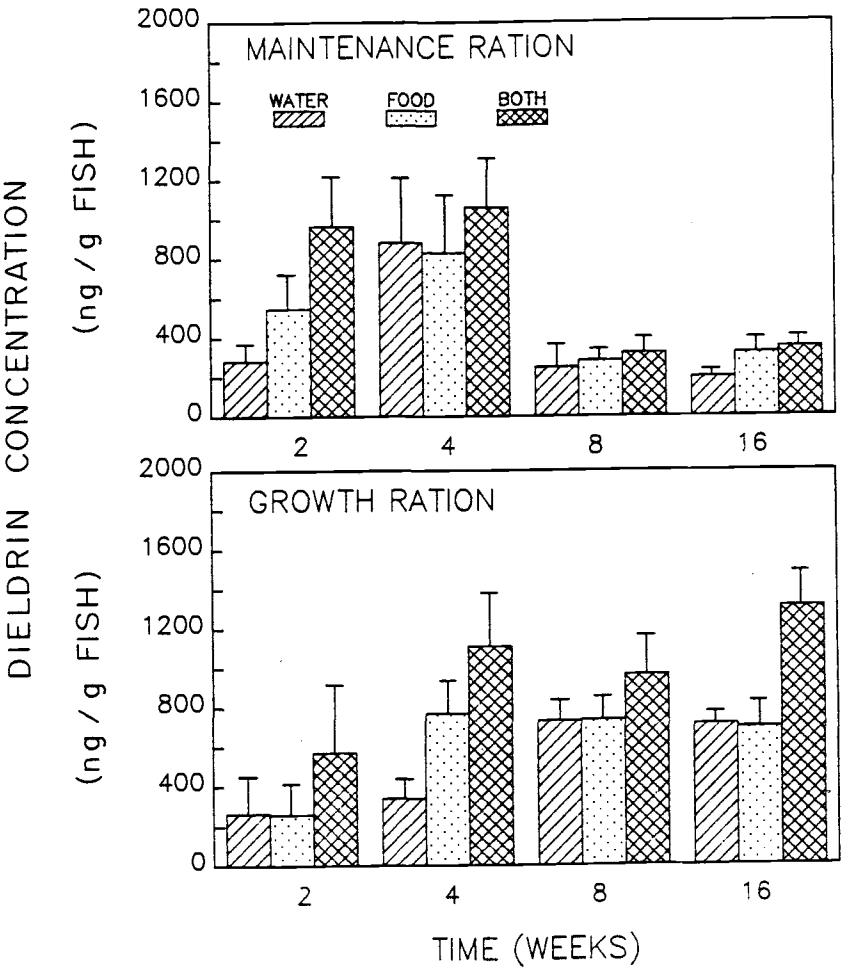
MAINTENANCE RATION
WATER
FOOD
BOTH
GROWTH RATION
DIELDRIN CONCENTRATION
(ng / g FISH)
(ng / g FISH)
2000
1600
1200
800
400
0
2
4
8
16
TIME (WEEKS)

Figure 1.

Figure 2. Mean dieldrin concentrations as whole fish residues are also expressed as lipid-weight concentrations. Juvenile rainbow trout were exposed to dieldrin in water (0.04 µg dieldrin/l) or water plus food (0.087 µg dieldrin/g fish/day) at two rations for 16 weeks and sampled at the indicated times for residue analysis. Growth (G) and maintenance (M) rations were 4 and 2% of body weight per day. Three or six fish were sampled at each time point and standard error bars are indicated.

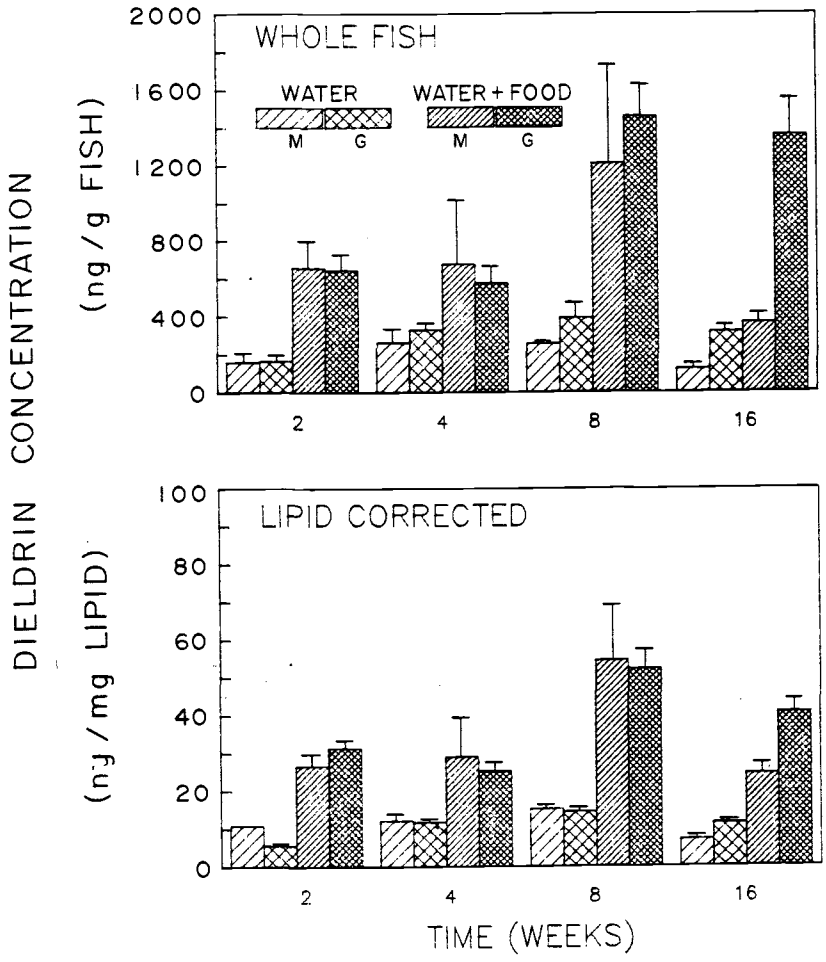
DIELDRIN CONCENTRATION
(ng / g FISH)
2000
1600
1200
800
400
0
WHOLE FISH
WATER
WATER + FOOD
M
G
M
G
2
4
8
16
(ng / mg LIPID)
100
80
60
40
20
0
LIPID CORRECTED
2
4
8
16
TIME (WEEKS)

Figure 2.

µg/l. Although fish numbers decreased with time, tank concentrations did not. Dieldrin was not detected in feces collected over a five day period.

Disposition Exposures

Approximately 50-60% of a ^{14}C-dieldrin oral dose was recovered from the exposure water, viscera, whole body homogenate and feces of each fish tested. Ration and treatment altered the percentages found in each compartment, but recoveries averaged 15% in water, 17% in whole body homogenates, 20% in viscera and 5% in feces. All values reported here are expressed as ^{14}C-dieldrin concentration or percent administered dose. No attempt was made to distinguish metabolites from parent compound. Other researchers have reported minimal dieldrin metabolism by bluegills (Sudershan and Khan, 1981) and goldfish (Grzenda et al., 1971). Fish tested were ten times larger than those exposed during subchronic testing. Some growth occurred at the lower ration but fish pretreated for 6 weeks on the low ration had less visceral fat, 0.013 g, than high ration, 0.13 g.

The radiolabel was not reliably detected in water until 48 hours following oral dosing. Concentrations in 120 ml samples ranged from 0.034 to 0.11 µg/l and there was no correlation with dose administered. Water concentrations in tanks holding naive fish were the lowest measured, were twice as high for 2 week pretreated fish and held at 0.09 µg/l for 6 week pretreated fish. Water concentrations after 6 weeks pretreatment represented from 9-23% of the administered dose and fish receiving the dose in a maintenance ration (4 µg

^{14}C-dieldrin/g food) released twice as much of the dose into water as growth ration (2 μg ^{14}C-dieldrin/g food). The opposite was found in naive fish and fish dosed after two weeks pretreatment.

Feces collected from exposure tanks contained from 0.7 to 8% of the administered dose. Fish pretreated 4 weeks excreted the smallest quantity of label.

Whole body homogenate concentrations were converted from dry to wet weight and percent of administered dose calculated. There was no correlation between dose and whole body concentration and there was no significant difference due to ration or pretreatment. Concentrations of label in homogenates ranged from 0.010 to 0.026 μg ^{14}C-dieldrin/g fish.

Visceral concentrations were subjected to two way analysis of variance although all but the gut tissue failed Bartlett's test of homogeneity of variance. At 4 weeks, label concentrations in all tissues except bile were significantly different than 0, 2, and 6 week treatments (Table 2). Label concentration in gut tissue of naive fish, however, was not significantly different than that in the pretreated groups, including 4 weeks pretreatment. Ration effect was significant for all tissues except gut, muscle, bile and liver, and label concentration in bile was significantly different at 6 weeks pretreatment. When 4 week values were omitted from the data set, variances of all but kidney, gill, and bile were homogeneous and none showed a significant interaction between length of pretreatment and ration. Significant differences due to pretreatment were established by multiple comparisons for four tissues (blood, kidney, muscle and

Table 2. Disposition of a single ^{14}C-dieldrin dose 48 h after administration in food (0.088 ± 0.002 µg ^{14}C-dieldrin/g fish/day). Fish were pretreated for 2, 4 or 6 weeks with 0.087 µg dieldrin/g fish/day and fed either maintenance or growth rations. Values are mean and standard error of dieldrin concentrations (ng/g) of tissues from 2 or 3 fish. Dieldrin in feces and gut contents are expressed as percent of dose administered.

	Naive 0 weeks		Pretreated 2 weeks		Pretreated 4 weeks		Pretreated 6 weeks	
Tissue	Maintenance	Growth	Maintenance	Growth	Maintenance	Growth	Maintenance	Growth
blood	22 ± 3[a]	29 ± 4	12 ± 0.3	19 ± 2	220 ± 46	420 ± 40	18 ± 3	10 ± 1.1
liver	88 ± 5	62 ± 7	92 ± 17	68 ± 3	500 ± 18	530 ± 45	73 ± 10	79 ± 30
gall bladder and bile	900 ± 200	790 ± 170	2000 ± 560	980 ± 120	2200 ± 420	1400 ± 360	5000 ± 2100	1900[b]
kidney	33 ± 7	26 ± 2	16 ± 1	21 ± 1	570 ± 14	570 ± 53	12 ± 4	17 ± 1
gut	420 ± 68	330 ± 91	270 ± 98	220 ± 14	620 ± 10	440 ± 47	250 ± 110	300 ± 60
gill	55 ± 34	17 ± 5	12 ± 1	12 ± 1	440 ± 60	190 ± 9	10 ± 2	8.0 ± 0.5
muscle	22 ± 4	12 ± 2	10 ± 2	11 ± 1	610 ± 31	430 ± 26	6 ± 2	9.4 ± 0.2
brain	21 ± 4	19 ± 1	18 ± 2	15 ± 1	370 ± 20	480 ± 18	14 ± 3	13 ± 2
fat	540 ± 110	340 ± 71	540 ± 60	370 ± 47	2500[b]	710 ± 32	540 ± 72	340 ± 160
feces	2.3 ± 1.1[c]	1.6 ± 0.4	7.6 ± 3.5	7.0 ± 0.6	0.66 ± 0.06	0.71 ± 0.15	4.5 ± 1.6	6.9 ± 1.4
gut contents	12 ± 4[c]	15 ± 5	13 ± 7	7.1 ± 1.2	21 ± 0.05	11 ± 1	6.0 ± 3.7	9.7 ± 4.7

[a]mean and SE, n= 2 or 3

[b]value available for only one animal

[c]% dose administered

bile) with significant main effect F-ratios. Blood, kidney, and muscle tissue concentrations from naive fish were significantly different than concentrations at 2 and 6 weeks. Bile concentration was significantly different at 6 weeks. A trend of decreasing concentration of label was apparent in this revised data, with the exception of bile, fat, gut and liver. Liver concentration remained stable while bile increased with length of dieldrin pretreatment. Fat concentrations were remarkably stable from time point to time point and 0, 2, and 6 week treatments were combined to show a significant difference due to ration.

Gut contents were analyzed at 0, 2, and 6 weeks for comparison with dieldrin food concentration. Except for 6 weeks pretreatment at growth rations, lower gut contents contained a greater amount of radioactivity than upper gut contents. The gut contents of naive and 2 week pretreated fish given maintenance rations contained twice the label concentration as fish fed the growth ration. After 6 weeks pretreatment, label concentrations of gut contents of maintenance and growth fed fish both averaged 0.6 μg ^{14}C-dieldrin/g and represented only 5% of the administered dose. Fish fed 4 μg ^{14}C-dieldrin/g food had 2.3, 1.7 and 0.67 μg ^{14}C-dieldrin/g in gut contents at 0, 2, and 6 weeks respectively, and feces concentrations increased with time.

DISCUSSION

Subchronic testing produced no single value for dieldrin accumulation, but demonstrated exposure level, ration, and route of exposure all influence accumulation. Two different water concentrations produced different residue levels in a dose dependent manner. Administering additional dieldrin via diet increased accumulation. Dose dependent accumulation was most apparent when total residue was divided by lipid quantity rather than whole body weight.

Growth ration produced greater total percent lipid and dieldrin residues than maintenance (no-growth) ration. When accumulation was expressed as whole body burden, growing fish accumulated up to four times as much dieldrin as maintenance fish over a 16 week period. Expression of accumulation as lipid-weight dieldrin concentration reduced the difference in accumulation due to ration to a factor of less than two and dieldrin residues in growth and maintenance fed fish were not significantly different in two treatments. Food and water combined, however, produced significantly different residue levels for growth and maintenance groups.

Exposure route (food or water) did not alter the pattern of accumulation observed over time in either maintenance or control groups. Accumulation was not significantly different between the exposure groups of food only (0.087 µg/g/day) and water only (0.08 µg/l). Choice of any other exposure concentrations probably would have resulted in other accumulation levels. Whether food or water

contribute more to accumulation will depend on the concentrations of dieldrin in food and water along with other factors such as ration and dosing regimen.

Two states of "equilibrium" were produced by 16 weeks, one for growth fish and one for maintenance fish, which could not be explained as simple sequestration of dieldrin in lipids of fish as residues were not equal when expressed as lipid-weight dieldrin concentration (total lipid in these tests was a measure of any tissue constituent soluble in acetone and hexane). If lipid serves as the primary medium for dieldrin storage both the quantity and nature of lipid constituents of tissues must be considered.

A change in lipid quantity after four or eight weeks probably reduced accumulation in maintenance fish (Fig. 1). Lipid content was measured over time in fish exposed to a low water concentration of 0.04 µg dieldrin/l. The largest percent decrease in lipid content occurred between four and eight weeks and continued to drop in the combined food/water dieldrin treatment.

Lipid may undergo some qualitative change as a result of ration and/or dieldrin treatment. Along with reduced lipid tissue available for dieldrin storage, the nature of the lipid membrane and cellular composition may be altered resulting in a lower affinity for dieldrin (increasing dieldrin flux out of the fish or redistribution to tissues with a higher affinity for dieldrin). An example of a qualitative change would be saturation of tissue with dieldrin.

Results of disposition tests indicated as a state of equilibrium was approached, less and less of a single dose was distributed

throughout the tissues and was instead concentrated in the bile. Tissues reaching equilibrium retain a smaller amount of each dose, and displaced unlabelled dieldrin redistributes to tissues with a greater capacity for dieldrin storage. The fate of dieldrin retained by bile was not determined in these tests, but biliary excretion has accounted for 90% of mammalian excretion of dieldrin (Hayes, 1965) and Mayer (1970) found excretion of dieldrin by rainbow trout following an oral dose was primarily via the feces.

Absorption and excretion were not specifically studied, but elimination after oral administration was indicated by dieldrin (0.02 µg/l) measured in tanks of fish given dieldrin via food during subchronic testing. Radiolabelled dieldrin (>0.03 µg/l) was also present in tanks 48 hours following dosing. Feces present in these tanks were one probable source of the ^{14}C-dieldrin. Dieldrin quantities from the lower gut give some indication of absorption of a single dose. The lower gut of two naive fish contained 9-14% of the label while those of 6 week pretreated fish contained less than 5%. If biliary excretion of unlabelled dieldrin is presumed, the lower percentage in pretreated fish may be explained as saturation of feces with unlabelled dieldrin.

Disposition following 4 weeks pretreatment produced suspect ^{14}C-dieldrin retention values. These values were 3 to 30 times greater than retention values following 0, 2, or 6 weeks pretreatment. However, label recovered from bile, feces, gut and gut contents indicate a possible metabolic change occurred around this time point. The extremely low feces concentration suggest increased efficiency in

absorption or less contamination of feces with ^{14}C-dieldrin (if the radioactivity in feces includes excretion of the labelled dose). Impaired excretion could elevate tissue levels, but this would have to be followed by improved excretion in order to explain decreases in tissues at 6 weeks pretreatment. Increased absorption efficiency might occur after induction of a protein which binds dieldrin, reducing the concentration of free dieldrin in blood and other tissues. Induction of a binding protein with the result of increased tissue levels of a xenobiotic is not without precedent. Metallothionein and ligandin (glutathione-s-transferase) are two proteins found in fish which bind non-substrate xenobiotics, thereby serving a storage and detoxification role. Synthesis of metallothionein is induced by exposure to copper and zinc. Gut contents were not altered substantially at 4 weeks and gut tissue concentrations at 4 weeks were not significantly different than tissue concentrations in naive fish. This suggests doses at each treatment were equivalent and that gut tissues were not affected by 4 weeks pretreatment, perhaps due to saturation at all time points. Bile did not appear to be saturated since label concentration increased at 6 weeks.

Peaks at 4 weeks in the subchronic dietary exposures suggest a change in tissue exchange occurred around 4 weeks which could be thought of as a metabolic change. This was followed by an apparent steady state concentration in whole fish (growth ration) or a decrease to a new steady state concentration (maintenance ration). Peaks of dieldrin accumulation followed by decreases have been reported by Hayes (1965) for mammals.

BIBLIOGRAPHY

American Public Health Association, 1975. Standard methods for the examination of water and waste water. 14th ed. Washington, D.C.

Chadwick, G.G. and R.W. Brocksen, 1969. Accumulation of dieldrin by fish and seclected food fish organisms. J. Wildlife Management 33:693-700.

Chadwick, G.D., J.R. Palensky and D.L. Shumway, 1972. Continuous-flow dilution apparatus for toxicity studies. Proc. of the Pacific N.W. Industrial Waste Management Conference. Portland, OR. 101-105.

Clark, D.R., R.L. Clawson, and S.J. Stafford, 1983. Gray bats killed by dieldrin at two additional Missouri caves: Aquatic macroinvertebrates found dead. Bull. Environm. Contam. Toxicol. 30:214-218.

Eberhardt, L.L., R.L. Meeks, and T.J. Peterle, 1971. Food chain model for DDT kinetics in a freshwater marsh. Nature 230:60-62.

Groves, A.B. and A.J. Novotny, 1965. A thermal-marking technique for juvenile Salmonids. Trans. Am. Fish. Soc. 94:386-389.

Grzenda, A.R., W.J. Taylor and D.F. Paris, 1971. The uptake and distribution of chlorinated residues by goldfish fed a ^{14}C-dieldrin contaminated diet. Trans. Am. Fish. Soc. 100:215-221.

Hamelink, J.L., R.C. Waybrant and R.C. Ball, 1971. A proposal: Exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in lentic environments. Trans. Am. Fish. Soc. 100:207-214.

Hamilton, M.A., R.C. Russo and R.V. Thurston, 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Science and Tech. 11:714-719.

Jarvinen, A.W. and R.M. Tyo. 1978. Toxicity to fathead minnows of endrin in food and water. Arch. Environm. Contam. Toxicol. 7:409-421.

Hayes, W.J., Jr., 1965. Review of the metabolism of chlorinated hydrocarbon insecticides especially in mammals. Ann. Rev. Pharm. 5:27-52.

Macek, K.J. and S. Korn, 1970. Significance of the food chain in DDT accumulation by fish. J. Fish. Res. B. Canada. 27:1496-1498.

Mayer, F.L., Jr., 1970. Dynamics of dieldrin in rainbow trout and effects on oxygen consumption. Ph.D. dissertation.

Reinert, R.E., 1972. Accumulation of dieldrin in alga, Daphnia magna, and the guppy. J. Fish. Res. B. Canada. 29:1413-1418.

Schmitt, C.J., L.J. Ludke and D.F. Walsh., 1981. Organochlorine residues in fish: National Pesticide Monitoring Program. 1970-74. Pesticides Monitoring Journal. 14:136-206.

Schnoor, J.L., 1981. Fate and transport of dieldrin in Coralville Reservoir: Residues in fish and water followng a pesticide ban. Science. 211:840-842.

Sinnhuber, R.O., J.D. Hendricks, J.H. Wales and G.B. Putnam, 1977. Neoplasms in rainbow trout, a sensitive animal model for environmental carcinogens. Annals New York Academy of Sciences. 298:389-408.

Sudershan, P. and M.A.Q. Khan, 1981. Metabolism of ^{14}C dieldrin in bluegill fish. Pest. Biochem. Phys. 15:192-199.