NOTES

Influences of Dieldrin on the Growth and Body Composition of Fingerling Rainbow Trout (*Salmo gairdneri*) Fed Oregon Moist Pellets or Tubificid Worms (*Tubifex* sp.)[1]

GLENN R. PHILLIPS[2] AND DONALD R. BUHLER

Department of Fisheries and Wildlife, Department of Agricultural Chemistry and Environmental Health Sciences Center, Oregon State University, Corvallis, Oreg. 97331, USA

PHILLIPS, G. R., AND D. R. BUHLER. 1979. Influences of dieldrin on the growth and body composition of fingerling rainbow trout (*Salmo gairdneri*) fed Oregon Moist Pellets or tubificid worms (*Tubifex* sp.) J. Fish. Res. Board Can. 36: 77–80.

Fingerling rainbow trout (*Salmo gairdneri*) fed tubificid worms (*Tubifex* sp.) grew faster than those fed Oregon Moist Pellets (OMP) over a similar range of rations. Faster growth resulted because trout incorporated protein and fat from tubificids more efficiently than from OMP. Exposure to a sublethal concentration of dieldrin in water (0.18 μg/L) did not affect the protein elaboration of trout fed either diet or fat metabolism in trout fed OMP. However, dieldrin reduced fat elaboration in trout fed tubificids. The dieldrin concentrations in trout exposed to dieldrin in water increased as feeding level (and therefore body fat) increased. The different responses to dieldrin by fish fed these two diets provides evidence that the diet offered to fish can significantly influence the outcome of chronic toxicity tests.

Key words: Rainbow trout, tubificid worms, Oregon Moist Pellets, dieldrin, growth, fat metabolism

PHILLIPS, G. R., AND D. R. BUHLER. 1979. Influences of dieldrin on the growth and body composition of fingerling rainbow trout (*Salmo gairdneri*) fed Oregon Moist Pellets or tubificid worms (*Tubifex* sp.) J. Fish. Res. Board Can. 36: 77–80.

Les fingerlings de truite arc-en-ciel (*Salmo gairdneri*) nourris aux vers tubificides (*Tubifex* sp.) croissent plus rapidement que ceux nourris aux boulettes mouillées d'Orégon (OMP) pour une même gamme de rations. Cette croissance plus rapide est due au fait que les truites incorporent les protéines et les lipides des tubificides plus efficacement que celles des OMP. L'exposition à une concentration sublétale de dieldrine dans l'eau (0,18 μg/L) n'affecte pas la formation de protéines chez les truites nourries à l'un ou l'autre régime, ou le métabolisme des lipides chez les truites nourries aux OMP. Cependant, la dieldrine réduit la formation de lipides chez les truites nourries aux tubificides. Les concentrations de dieldrine dans les truites exposées à cette substance dans l'eau augmentent à mesure que le niveau d'alimentation (et, partant, de lipides corporels) augmente. Ces réactions différentes à la dieldrine de poissons nourris à ces deux régimes sont une preuve que le régime alimentaire offert aux poissons peut influencer de façon significative les résultats d'essais de toxicité chronique.

Received August 16, 1978
Accepted October 27, 1978

Reçu le 16 août 1978
Accepté le 27 octobre 1978

ALTHOUGH the effects of diet on responses of mammals to stress have been well documented (Lee et al. 1964; Tinsley 1966; Boyd 1969; Stoewsand et al. 1970; Shackman 1974), few studies have been conducted that investigate how diet composition influences the response of fish to stress. Mehrle et al. (1974) showed that the acute toxicity of chlordane to rainbow trout was influenced by the type of diet fed before exposure. During chronic exposure, Mehrle et al. (1977) found that toxaphene influenced the growth of channel catfish (*Ictalurus punctatus*) differently depending on the Vitamin C content of the diet.

Our studies using rainbow trout (*Salmo gairdneri*) fed tubificid worms (*Tubifex* sp.) obtained locally from hatchery rearing ponds or a production diet (OMP)[3]

[1]Technical Paper No. 3456 from Oregon Agricultural Experiment Station.

[2]Present address: Cooperative Fishery Research Unit, Montana State University, Bozeman, Mont. 59717, USA.

[3]Bio-vita Oregon Pellets, Bioproducts, Inc., Warrentown, Oreg. 96146, USA.

Printed in Canada (J5349)
Imprimé au Canada (J5349)

were undertaken to observe how these diets, either alone or in combination with a dieldrin stress, might influence growth, protein elaboration, and fat accumulation. Three feeding rates were used because growth response may vary with the plane of nutrition.

Materials and methods — Fertilized rainbow trout eggs were obtained from the Oregon Game Commission's Roaring River Fish Hatchery located near Scio, Oregon. The trout were reared on OMP (Hublou 1963) after hatching, and kept at Oregon State University's Oak Creek Laboratory until reaching a size of approximately 3–4 g. Before the experiment, fish of approximately the same size were individually fed one of the diets for 7 d. The experimental group was chosen from these on the basis of diet acceptance and uniform weight.

Trout were kept in 20-L aquaria divided into four separate 5-L compartments, each compartment housing one trout. Filtered creek water and dieldrin were metered to the experimental aquaria via a series of distribution boxes and manifolds equipped with adjustable siphons. Technical dieldrin (Shell Chemical Corporation 1959) was introduced by passing water through a column of inert sand coated with the pesticide (Chadwick and Kiigemagi 1968). Flow rates through all the aquaria were maintained near 100 mL/min (exchange time 3.3 h). Dissolved oxygen was at least 82% of saturation, temperature averaged 15.1°C, and daily photoperiod was 16 h light:8 h dark. Weekly gas-chromatographic analyses confirmed that the concentration of dieldrin eluted from the column remained relatively constant (80–92 μg/L) throughout the experiment. Biweekly analyses of water from the aquaria showed that the mean dieldrin concentration in the aquaria was 0.18 μg/L (SE = 0.05) and ranged from 0.12 to 0.21 μg/L; dieldrin was not detectable in control water.

At the beginning of the 61-d experiment, four fish were killed, weighed, and placed in a vacuum drying oven for 5 d at 80°C. The initial dry weights of the experimental fish were estimated on the basis of the wet to dry weight ratios of this group. Forty-eight fish were used in the experiment; 24 were held in dieldrin-free water and 24 were exposed continuously to dieldrin. Twelve of the fish in each sub-group were fed tubificid worms and 12 were fed OMP. Feeding rates were calculated as percentages of body weight (dry to dry basis); nominal rates included 2½, 4, and 5½% of body weight per day. Rations were readjusted to fixed percentages of body weight after each weighing period.

Fish were fed each day during the experiment except for the day before weighing. The amounts of OMP and tubificid worms to be fed were weighed to the nearest 0.01 g. Excess moisture on tubificid worms was removed before weighing by blotting with paper towels. Dry weight of subsamples of OMP and tubificid worms were determined each week; the dry weights of food consumed by each fish could then be determined because the fish always consumed the entire ration.

Fish were individually weighed at the onset of the experiment and every 7–10 d thereafter. Before each weighing period, fish were starved for 24 h to allow time for evacuation of previously ingested food. Each fish was blotted dry with a soft, moist cloth, placed in a 1000-mL graduated cylinder containing 50 mL of water and weighed to the nearest 0.01 g; no anesthetic was used.

At the end of the experiment, dried samples of the initial fish, the final experimental fish, the OMP, and the tubificid worms were pulverized in a Wiley Mill and preserved in a desiccator for subsequent proximate analyses.

The proximate composition of each fish was determined in duplicate. The biweekly samples of each diet were individually pooled and analyzed in triplicate. Lipids were extracted by the chloroform–methanol method of Bligh and Dyer (1959); protein was determined using a modification (Ballentine 1957) of the microkjeldahl method; and ash content was determined by burning a 0.1-g sample in a muffle furnace at 600°C for 15 h. Carbohydrate contents of tubificids and OMP were calculated by difference. The energy contents of dry samples were calculated from conversion factors (Baker 1974) for fat (38.5 kJ/g), protein (20.5 kJ/g), and carbohydrate (17.2 kJ/g). Rates of growth, food consumption, protein elaboration, protein consumption, and fat elaboration were computed for each sample and comparisons made between treatments. Fat elaboration rate was compared to food consumption rate rather than fat intake since lipid deposition can be derived from dietary constituents other than lipid. Statistical significance was judged using Student's *t*-test (Steel and Torrie 1960); comparisons were based on treatments having similar consumption rates of a particular dietary constituent.

Results and discussion — Both diets contained nearly the same percentages (on a dry basis) of protein and carbohydrates, but tubificids had a higher percentage of fat whereas OMP contained more ash (Table 1). Others (Embody and Gordon 1924; Phillips et al. 1954; Wood et al. 1957) have found a similar relationship between natural and artificial (production) diets.

In general, as ration increased, fish increased (%) in fat and ash and decreased in protein and water; fish samples did not contain carbohydrate. Fish fed the low rations of both diets gained little or no weight over the 2 mo while the average weight increase (wet weight basis) of fish in the high ration treatments ranged from 78.1 to 98.9%. At the end of the experiment the mean proximate composition for individual treatments were as follows:

	% fat	% protein	% ash
low ration	7.2–9.0	78.2–82.0	12.2–13.3
medium ration	7.7–15.1	73.6–80.5	12.6–15.2
high ration	11.0–14.7	73.2–80.9	14.9–16.5

A comparison of control fish — Control fish fed the highest ration of tubificid worms exhibited significantly higher ($P < 0.10$) energy growth rates (joules of growth

TABLE 1. Proximate composition (dry basis) of the two diets used during the test.

	Percentage composition (dry basis)			
Diet	Fat	Ash	Protein	Carbohydrate[a]
Tubificid	15.0	8.2	59.7	17.1
OMP	10.0	12.2	59.7	18.1

[a]Determined by subtracting total percentage of other three constituents from 100.

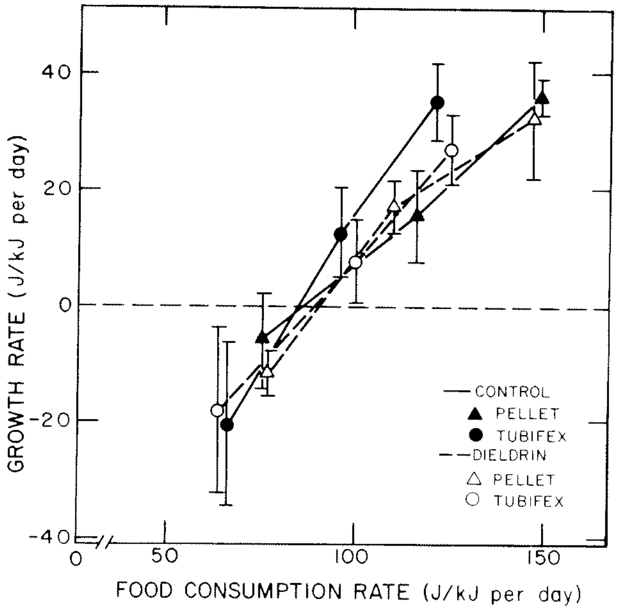

FIG. 1. Influence of dieldrin on the relationship between growth rate and food consumption rate in rainbow trout (*S. gairdneri*) fed Oregon Moist Pellets or tubificid worms; means and 90% confidence intervals shown for each treatment ($n = 4$).

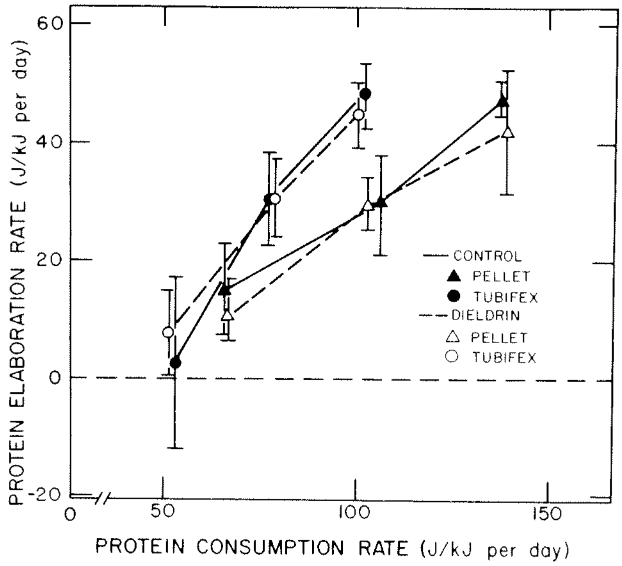

FIG. 2. Influence of dieldrin on the relationship between protein elaboration rate and protein consumption rate in rainbow trout fed Oregon Moist Pellets or tubificid worms; means and 90% confidence intervals shown for each treatment ($n = 4$).

per kilojoule mean trout biomass per day) for a given food consumption rate (joules of food consumed per kilojoule mean trout biomass per day) than fish fed OMP at a comparable rate (Fig. 1). Fish fed the medium ration of OMP averaged a 52.0% weight increase while fish consuming tubificids at a similar rate averaged a 92.6% increase. This occurred because protein and fat from tubificids were more efficiently elaborated than protein and fat from OMP ($P < 0.05$) at the higher feeding rates (Fig. 2 and 3).

Gerking (1955) has pointed out that natural diets are more likely to contain the inorganic nutrients and essential factors necessary for good growth than production diets. The poorer growth of fish fed OMP could result from OMP possessing an inferior quality protein or from a deficiency in some important growth factor such as Vitamin C (Mehrle et al. 1977); a more complete assessment of dietary content is essential to interpret these growth responses.

A comparison of dieldrin-exposed fish — The growth rates of fish fed both diets were not significantly affected by the pesticide, but independent examination of the two major components of growth (fat and protein) revealed that dieldrin significantly depressed the lipid elaboration ($P < 0.05$) of fish fed tubificid worms at the two highest rations fed (Fig. 3). However, dieldrin did not influence fat metabolism of fish fed OMP over the entire range of rations. Moreover, the ability of fish to elaborate protein from either diet was unaffected by the pesticide.

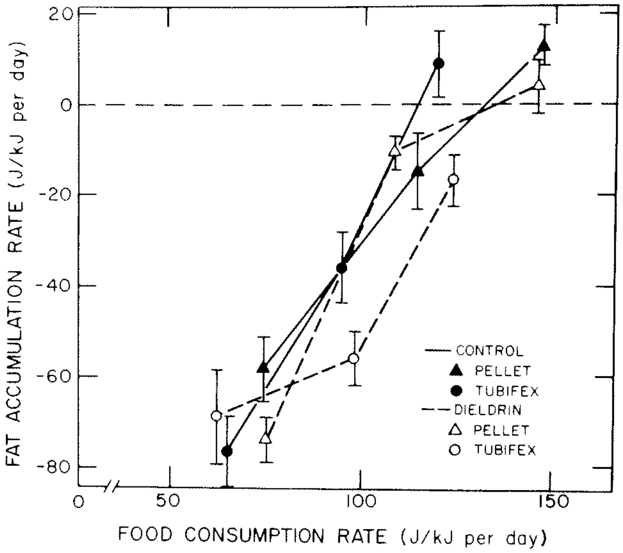

FIG. 3. Influence of dieldrin on the relationship between fat accumulation rate and food consumption rate in rainbow trout fed Oregon Moist Pellets or tubificid worms; means and 90% confidence intervals shown for each treatment ($n = 4$).

Various species of fish show reduced growth rates when exposed to chlorinated hydrocarbon pesticides. Examples include chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) fed DDT (Buhler et al. 1969), rainbow trout fed endrin (Grant and Mehrle

1973), channel catfish (*Ictalurus punctatus*) fed dieldrin (Argyle et al. 1975), and johnny darters (*Etheostoma nigrum*) immersed in 2.3 μg dieldrin/L (Silbergeld 1973).

Reduced growth in mammals resulting from exposure to dieldrin has been attributed to effects on both carbohydrate and lipid metabolism (Bhatia et al. 1972; Kacew et al. 1973). Increased lipolysis and changes in carbohydrate metabolism induced by dieldrin have also been noted in fish. Silbergeld (1973) observed weight loss and a reduced lipid content in johnny darters exposed to 2.3 μg dieldrin/L for 30 d. The elevated level of fat metabolism associated with increased ingestion of dietary lipids may have increased the vulnerability of the fish fed tubificids to dieldrin, resulting in the observed growth depression.

A limited number of dieldrin analyses on fish exposed to dieldrin in water (one fish per treatment) showed that dieldrin accumulation increased as ration (and therefore fat intake) increased. Dieldrin concentrations (μg/g whole wet fish) were 0.53, 1.55, and 1.65 for the low, medium, and high rations of OMP and 0.47, 0.82, and 1.32 for the low, medium, and high tubificid rations. Reinert (1970) noted a similar relationship between pesticide residues and lipid content in great lakes fishes; this trend presumably results from an increase in the size of the storage reservoir available for lipophilic chlorinated hydrocarbon pesticides.

This study shows that various components of fish growth, such as protein and lipid, are not necessarily influenced in the same way by pesticide exposure; moreover, diet composition and ration can have important influences on the growth responses of fish exposed to a pesticide. Dietary stress interaction should be more critically considered by researchers conducting laboratory studies using fish.

Acknowledgments — We wish to thank Dr I. Tinsley and Dr G. Davis for their helpful editing during the preparation of the manuscript, Dr E. Warren for his suggestions regarding experimental design, and Dr R. R. Claeys for performing the dieldrin analyses. This work was supported by training grant No. 572-WP-208 from the U.S. Environmental Protection Agency and by research grants ES-004 and ES-00210 from the U.S. Public Health Service, National Institute of Health.

Argyle, R. L., G. C. Williams, and C. B. Daniel. 1975. Dieldrin in the diet of channel catfish (*Ictalurus punctatus*): Uptake and effect on growth. J. Fish. Res. Board Can. 32: 2197–2204.

Baker, J. C. 1974. Protein and lipid factors in the bioenergetics of swimming in fish (*Oncorhynchus kisutch*). Ph.D. Thesis. Oregon State University, Corvallis, Oreg. 112 p.

Ballentine, R. 1957. Determination of total nitrogen and ammonia, p. 984–995. *In* S. P. Colowick and N. O. Kaplan [ed.] Methods in enzymology. Vol. 3. Academic Press, Inc., New York, N.Y.

Bhatia, S. C., S. C. Sharma, and T. A. Venkitasubramanian. 1972. Acute dieldrin toxicity: Biomedical changes in the blood. Arch. Environ. Health 24: 369–372.

Bligh, E. G., and W. J. Dyer. 1959. Extraction of lipids from animal sources. Can. J. Biochem. Physiol. 37: 911–917.

Boyd, E. M. 1969. Diet and drug toxicity. Clin. Toxicol. 2: 423–433.

Buhler, D. R., M. E. Rasmusson, and W. E. Shanks. 1969. Chronic oral DDT toxicity in juvenile coho and chinook salmon. Toxicol. Appl. Pharmacol. 14: 535–555.

Chadwick, G. C., and U. Kiigemagi. 1968. Toxicity evaluation of a technique for introducing dieldrin into water. J. Water Pollut. Control Fed. 40: 76–82.

Embody, G. C., and M. Gordon. 1924. A comparative study of natural and artificial foods of brook trout. Trans. Am. Fish. Soc. 54: 185–200.

Gerking, S. D. 1955. Influence of rate of feeding on body composition and protein metabolism of bluegill sunfish. Physiol. Zool. 28: 267–282.

Grant, B. F., and P. M. Mehrle. 1973. Endrin toxicosis in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 30: 31–40.

Hublou, W. F. 1963. Oregon pellets. Progr. Fish-Cult. 25: 175–180.

Kacew, S., D. J. B. Sutherland, and R. L. Singhal. 1973. Biochemical changes following chronic administration of heptachlor, heptachlor epoxide, and endrin to male rats. Environ. Physiol. Biochem. 3: 211–229.

Lee, M., K. Harris, and H. Trowbridge. 1964. Effect of the level of dietary protein on the toxicity of dieldrin for the laboratory rat. J. Nutr. 84: 136–144.

Mehrle, P. M., W. W. Johnson, and F. L. Mayer. 1974. Nutritional effects on chlordane toxicity in rainbow trout. Bull. Environ. Contam. Toxicol. 12: 513–517.

Mehrle, P. M., F. L. Mayer, and W. W. Johnson. 1977. Diet quality in fish toxicology: Effects on acute and chronic toxicity, p. 269–280. *In* F. L. Mayer and J. L. Hamelink [ed.] Aquatic toxicology and hazard evaluation. Am. Soc. Testing and Materials, ASTM STP 634.

Phillips, A. M., R. S. Nielson, and D. R. Brockway. 1954. A comparison of hatchery diets and natural food. Progr. Fish-Cult. 16: 153–157.

Reinert, R. E. 1970. Pesticide concentrations in great lakes fish. Pestic. Monit. J. 3(4): 233–240.

Shackman, R. A. 1974. Nutritional influences on the toxicity of environmental pollutants. Arch. Environ. Health 28: 105–113.

Shell Chemical Corporation. 1959. Handbook of aldrin, dieldrin, and endrin formulations. The Woodrow Press, Inc., New York, N.Y. 195 p.

Silbergeld, E. K. 1973. Dieldrin. Effects of chronic sublethal exposure on adaptation to thermal stress in freshwater fish. Environ. Sci. Technol. 7: 846–849.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill, New York, N.Y. 481 p.

Stoewsand, G. S., E. V. Broderick, and V. B. Bourke. 1970. Dietary protein and dieldrin toxicity. Ind. Med. Surg. 39: 45–49.

Tinsley, I. J. 1966. Nutritional interaction of dieldrin toxicity. J. Agric. Food Chem. 14: 563–566.

Wood, E. M., W. T. Yasutake, A. N. Woodall, and J. E. Halver. 1957. The nutrition of salmonoid fishes. II. Studies on production diets. J. Nutr. 61: 479–488.