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Growth Rate Reduction of Goldfish (*Carassius auratus*) Exposed to p,p'DDT and Chlorobenzenes in Diets with Differing Lipid Contents

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The inhibitory effect of DDT and other persistent organic compounds on fish growth has been demonstrated in several investigations. Reduced feeding and decreased ability to perceive the presence of food were evident when DDT was incorporated in the diet of young salmon (Buhler and Shanks, 1970). The inhibitory effect of DDT and other organochlorines on coho salmon growth has been demonstrated by Halter and Johnson (1974). Also, there are available a range of papers on the effect of DDT on various physiological parameters which may influence fish growth. For example, DDT-induced biochemical changes on endocrine functioning (Moccia et al, 1977), failure in osmoregulatory system (eg. Hansen et al, 1971.) Janicki and Kinter, 1971), alteration of enzyme activity (eg. Davis and Wedemeyer, 1971., Davis et al, 1972). Moreover, induced behavioural changes (eg. Dill and Sauders, 1974., and Klaverkamp et al, 1976) or morpho-anatomical changes (eg. Weis and Weis, 1975., and Lingaraja et al, 1979) may directly or indirectly influence fish growth.

Limited information is available on the effect of chlorobenzenes on fish growth. Carlson and Kosian (1987) and Smith et al (1991) have shown the inhibition of growth rates in which fish were exposed to sublethal concentrations of chlorobenzenes. With crustaceans, Weis et al (1992) and Mortimer and Connell (1995) demonstrated that exposure to chlorobenzenes can cause various effects including delayed growth, delayed moulting and retardation of limb regeneration. The present investigation involved feeding goldfish with a series of contaminated fish food with differing lipid contents. A mixture of pp'DDT and four chlorobenzenes were used in the food for the purpose of determining the growth inhibition and the influence of lipid content of the food on accumulation of the chemical.

MATERIALS AND METHODS

The chlorobenzenes 1,3,5-trichlorobenzene (135-TCB), 1,2,3,5-tetrachlorobenzene (1235-TCB), pentachlorobenzene (QCB) and hexachlorobenzene (HCB) were obtained from the Aldrich Chemical Company and were of 98% or more purity. The only significant impurities were other chlorobenzene isomers. A commercial mixture of DDT which included pp'DDT, op'DDT and TDE (DDD) was recrystallised in order to obtain a higher proportion of the most abundant component pp'DDT. Chromatographic analysis of

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commercial and recrystallised DDT indicated a significant decrease in the amount of op'DDT and TDE. However the recrystallised DDT still contained a small quantity (about 5%) of op'DDT. The designation of ΣDDT in this study indicates the sum of pp'DDT and op'DDT in the contaminated food and the sum of pp'DDT, op'DDT, TDE (DDD) and DDE in the body.

Three groups of goldfish (*Carassius auratus*) were held in 12 five-litre glass jars (six exposed and six control jars) each closed with a plastic lid containing an aeration tube. The lid was tightly closed except for 1-2 hours during the feeding period each morning after the food was consumed by the fish. The fish were fed six days a week (Monday to Saturday) at a daily rate of 3.5% of their body weight (see Table 1). Samples were collected on Monday each week over ten weeks of the experiment. The fish were fasted on the seventh day before the weekly sampling to enable them to purge their gut. The fish, in both exposed and control jars, were weighed three times during the course of the experiment in order to adjust the daily fish food with the increased body weight.

One fish was sampled at each sampling time and dried with absorbent paper, weighed and kept at -20 C. The fish were homogenised in hexane (10-20 ml) containing sodium sulphate (15 to 30 g) to which the appropriate internal standard had been added. In order to homogenise the specimen an Ultra Turrax (Janke & Kunkel) grinder Model TP 18/10 fitted with a 10N grinding head operated at 20000 rpm was used for 3 minutes. The homogenate was vacuum filtered through a GF/C-Buchner assembly. The filtrate was transferred to a 100 ml measuring cylinder. The filter of residue was rinsed several times with hexane and the rinsings added to the cylinder. The combined filtrates were made up to 60 ml with hexane and mixed. A 40 ml portion of the extract was concentrated to 3 ml using a rotary evaporator. The DDE, DDD (TDE), op'DDT, pp'DDT and chlorobenzenes fraction of the concentrate was isolated by elution of the concentrate with a mixture of 5% diethylether in hexane (50 ml) through a glass column (450mm x 10mm) containing 10 g Florisil topped with 5 g sodium sulphate which was prewashed with 50 ml hexane. Recoveries of chlorobenzenes (1,3,5 TCB, 1,2,3,5 TCB, QCB and HCB) and pp'DDT, op'DDT, DDD and DDE from fish were studied by spiking four fish specimens. The average percentage recoveries for these chemicals were 68, 76, 87, 86, 95, 93, 89 and 79, respectively.

Gas chromatography was carried out on a Varian 3400 equipped with a ^{63}Ni ECD detector fitted with a 20 m x 0.32 mm fused silica capillary column (DB-5 "J&W Scientific"). The carrier gas was ultra high purity helium (Commonwealth Industrial Gases) at a flow rate of 0.5 ml min^{-1}, and the make-up gas was 10% methane in argon (Comm onwealth Industrial Gases). Both gases were dried and deoxygenated with on-line traps.

Appropriate chemicals were added to 900 ml of hexane, and 300 ml of this mixture was added to three separate 100 g portions of fish food. Olive oil was added to two portions of fish foods in order to increase their lipid content from 2.9 to 6.9 and 10.9 per cent. Each mixture was transferred to a modified 1000 ml round bottom flask with several longitudinal projections (pumpkin shaped). The mixture was slowly stirred by a rotary evaporator (without using vacuum) for 6 hours at room temperature to ensure complete mixing. The mixture was transferred to a beaker and the hexane was then removed while being slowly stirred under a gentle stream of nitrogen. A similar procedure was repeated with hexane (without the test compounds) for preparing fish food for the control fish.

RESULTS AND DISCUSSION

The results of a one-way analysis of variance between average fish weight in exposed and control fish did not show any significant difference ($p<0.05$) between fish weights in the twelve jars at the beginning of the experiments. No fish died during the experiments. Table 1 shows average fish body weights at the beginning of the experiment, average lipid contents of the sampled fish and concentration of the test compounds in the contaminated fish food and in the exposed fish. Concentration values in fish are the averages of the last 3-5 measurements for chlorobenzenes. Since uptake for DDT isomers and metabolites was linear, the last value for these chemicals is shown in Table 1. A Student t-test evaluation for fish weights for exposed and control fish indicated that dietary exposure to the test chemicals inhibited fish growth significantly ($p<0.05$) in all three experiments.

Growth rate was calculated by determining the slope of the least squares regression lines fitted to the weight values plotted against time for the test and control fishes (Figure 1). The relatively high values for coefficient of determinations (r^2) in these graphs shows that the growth rates in control fish and the test fish during the 70 days of duration of the experiment were effectively constant. The extent of growth rate inhibition attributed to each treatment was determined as a percentage decrease from the growth rate of the corresponding control treatment (Table 2). The reduced slopes in all three experiments indicate that dietary exposure to the mixture of DDT and chlorobenzenes has influenced goldfish growth rate.

Table 1. A summary of the experimental conditions and results for the growth experiment (ww=wet weight, dw=dry weight).

Conditions	Low Lipid	Medium Lipid	High Lipid
Commencing average fish (g, ww)	3.57(±0.81)	3.33(±1.12)	3.36(±0.93)
Average fish lipid content(%)	4.20(±0.94)	3.95(±0.81)	4.40(±0.72)
Food lipid content(%)	2.90	6.90	10.90
Food concentration (mg/Kg dw)			
135 TCB	58.18	59.81	59.41
1235 TCB	54.92	52.37	54.28
QCB	44.86	43.73	47.39
HCB	61.28	58.79	63.09
∑DDT	3.35	3.23	3.64
Body concentration (mg/Kg ww)			
135 TCB	1.51	1.35	1.42
1235 TCB	8.21	8.44	8.33
QCB	14.51	15.42	14.76
HCB	18.79	19.31	19.67
∑DDT	4.18	4.09	4.91

Comparison between exposed and control fish indicates that overall growth inhibition, as the result of dietary exposure to the mixture of DDT and chlorobenzenes in foods with three different fat contents, is 45.07, 68.18 and 76 percent (Table 2). The magnitude of growth inhibition increases with increase in lipid content of the food. It may be argued that increased lipid content in food could facilitate chemical uptake through coassimilation by lipid which would then cause more growth inhibition. But this is not the case, because the chemical concentration in the fish doesn't demonstrate such a pattern of differences in relation to the lipid content of the food (Table 1).

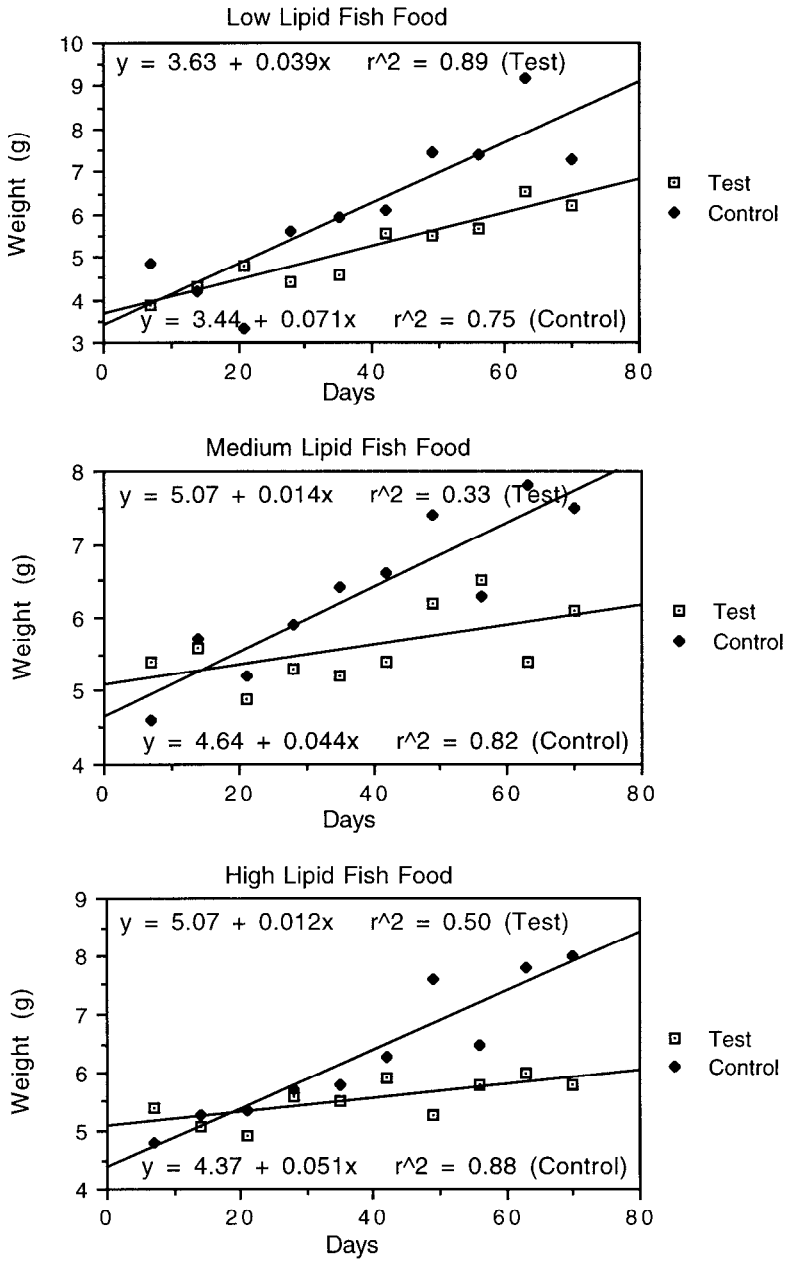

Figure 1. Plots of fish weight against exposure time for goldfish exposed to contaminated food with differing lipid contents.

However, it is possible that increased lipid content of the food may cause a decrease in digestibility of the food through increased faecal egestion (Gobas et al, 1993). The information on growth rates reveals that dietary exposure to the mixture of DDT and chlorobenzenes doesn't completely prevent the growth of fish, but the observed growth in the exposed fish is significantly smaller than for the unexposed fish (Table 2).

Table 2. A comparison of growth rates between exposed and control fish as indicated by the slope of regression lines

Treatment	Slope (g/day)	% Control	% Inhibition
Control	0.071		
Low Fat Food	0.039	54.92	45.07
Control	0.044		
Medium Fat Food	0.014	31.82	68.18
Control	0.050		
High Fat Food	0.012	24	76

Figure 1 demonstrates the weight gain in both exposed and control fish in the three fish groups during the 70 days of the experiments. Generally, the weights of the control fish remained more stable compared to the exposed fish. The coefficients of variation in control fish for high, medium and low lipid experiments were similar and ranged from 0.75 to 0.88. However, similar values for exposed fish demonstrated a wider range of 0.33 to 0.89 indicating more variation than the control fish. This shows that dietary exposure to the chemicals not only reduced the growth but also that the growth was manifested in a more variable manner.

There is little information available in the literature on the influence of chlorohydrocarbons in food on growth rate of fish. When compared with the effects of chlorohydrocarbons in water there is sometimes observed an increase in growth rate. For example in exposing five groups of minnows to PCB, Benglsson (1979) observed increased growth in some of the fish and attributed this growth to a possible disturbance in the hormonal system. Similarly, when fathead minnows (*Aplodinotus grunniens*) were exposed to a low concentration of Mirex a significant increase in growth compared with the control organisms was found. However, at a higher concentration no change in the growth was reported (Buckler et al, 1981). Similar weight gain has been reported for rainbow trout (*Oncorhynchus mykiss*) and goldfish exposed to low concentration of PCB (Seelye and Mac, 1981) and endrin (Grant and Mehrle, 1970., Grant and Mehrle, 1973.). It has been suggested that this apparent increase in growth of exposed aquatic organisms could act through selective elimination of vulnerable individuals to the compound (Murty, 1986). In this current work the growth rate of all individuals in the experiments was taken into account.

From periodic measurements of body weight in treated and control fish in this experiment, we found that dietary exposure to a mixture of pp'DDT and four chlorobenzenes administered in diets with differing lipid contents retarded significantly ($p=0.99$) growth of goldfish during a period of 70 days. The extent of growth inhibition in the three experiments were 45.07, 68.18 and 76 per cent of growth rates in the control fish for low, medium and high lipid content of the food respectively. The increase in the growth inhibition with increase in lipid content of the food can not be attributed to the effect of lipid in uptake of the chemicals because the amount of uptake was independent of lipid content of the foods. Increased lipid content of the food may have affected the fish growth by

decreasing the digestibility of food through increased faecal egestion or from providing a more effective source of energy for growth in control fish.

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