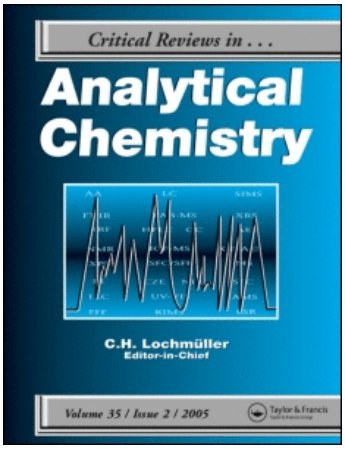

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BIOACCUMULATION OF WATERBORNE 1,2,45-TETRACHOROBENZINE IN TISSUES OF RAINBOW TROUT

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INTRODUCTION

During the past 3 years we have been studying the rates of uptake by fishes of selected synthetic organic chemicals, foreign to natural systems, known as xenobiotics. We have conducted several tests using an automated respirometer in which we have exposed fish to 1,2,4,5-tetrachlorobenzene (TCB). Our principal test animal has been the rainbow trout (*Oncorhynchus mykiss*), although we have also tested other fish species to compare results. One of our long-range objectives is to determine to what extent uptake and depuration rates of xenobiotics by fishes may be related to their respiration rates, in the hope of developing a simple predictive model describing the rates of uptake and depuration of xenobiotics as a function of the oxygen uptake rate and chemical properties of the chemical in question. In this presentation we will describe our approach to this research and discuss some of our results.

METHODS

Test Conditions

Many of our preliminary tests were conducted at Fisheries Bioassay Laboratory, Montana State University (MSU), and most of our respirometry experiments were conducted in a modified Brett-type respirometer at the Zoology Department, University of British Columbia (UBC). All fish tissue and water analyses were conducted at MSU. The UBC respirometer was designed and built specifically for our studies, and has been described in some detail by Gehrke et al. (1990). The respirometer can test fishes up to 2 kg in size and at swimming speeds as great as 2.5 m/second. Experiments can be conducted over several days with water velocity, temperature, pH, dissolved oxygen, and carbon dioxide all computer-controlled at predetermined levels, and data monitored continuously by the

computer as well. For the tests at UBC described here, fish were sacrificed immediately after removal from the respirometer, and whole fish or fish tissues, and test water samples, were frozen and stored at -20°C until shipment to MSU for chemical analysis; shipment was by overnight air carrier with samples packed in dry ice. Fish tested at MSU were prepared for analysis the same as those tested at UBC. All samples were stored at -20°C at MSU prior to analysis.

TCB Analysis

After receipt at MSU, small whole fish (< 10 g), subsamples of homogenates of larger fish, and individual organ tissues, were blended to a fine powder with the aid of dry ice and anhydrous Na_2SO_4 while still frozen. At least two separate samples were prepared from homogenates of larger fish. Only single samples were prepared from smaller fish and fish tissues because of limitations in sample quantity. TCB was soxhlet extracted (≥ 8 hours) from each sample using hexane. Surrogate (1,2,3,4-TCB) in amount comparable to the amount anticipated for TCB results (usually 100 μg) was added at the initiation of the extraction step. Lipids were removed from portions of this extract using florisil column chromatography; TCB and surrogate were eluted with 5% methyl-*t*-butyl ether/hexane. Pentachlorobenzene (PCB) internal standard (0.25 μg) was added to the purified extract and component concentrations were determined by electron capture gas chromatography (ECD-GC). Packed GC columns (1.8 m x 2 mm i.d.) containing 3% SE-30 or 3% Carbowax on 100/120 Supelcoport™ were used. A multipoint calibration curve (0.5 - 10 μg/l) was employed during quantitation. Both standards and sample extracts were injected in duplicate. When plasma samples were required, the caudal peduncle was severed immediately after the fish was sacrificed and blood was collected from the caudal artery into heparinized capillary tubes. Plasma was separated by centrifugation and 5.0 μl of plasma was removed by means of a 10 μl glass syringe and transferred to a 10 ml volumetric flask containing 0.05 μg internal standard (PCB). This solution was made to volume with hexane, and the contents mixed for approximately 1 minute using a vortex mixer. TCB was extracted from test water samples using hexane, and internal standard was added to the diluted extracts. Both plasma and water samples were analyzed for TCB as described above.

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Lipid Analysis

Total lipid content in whole small fish (< 10 g) or samples from homogenized larger fish (> 30 g) was determined by a modification of a method described by Folch et al. (1957). While still frozen, small whole fish or subsamples of tissue homogenates from larger fish were ground to a powder using dry ice and anhydrous Na_2SO_4. Lipids were extracted by blending this mixture twice for 3 minutes with 2:1 chloroform:methanol. Both extracts and rinsings were vacuum filtered and combined into a 500 ml separatory funnel. Methanol was partitioned from the chloroform into aqueous KCl solution. The chloroform layer was vacuum filtered using a 0.2 μ Nylon-66 filter and the volume adjusted to 250 ml. A measured aliquot was transferred to a tared beaker and chloroform was removed using low heat and a gentle stream of dry N_2. The residue was dried ($\geq$ 12 hours) in a vacuum desiccator and weighed.

TESTS CONDUCTED, RESULTS, AND DISCUSSION

In several of our initial experiments rainbow trout were exposed to TCB for different time periods up to 6 hours under either static test conditions at MSU or forced swimming conditions in the respirometer at UBC. After test, fish were sacrificed and different tissues analyzed to determine any apparent sequence among them in increase of toxicant concentration. Tissues analyzed were blood plasma, gills, liver, kidney, brain, spleen, heart, upper gut, lower gut, white muscle, pink muscle, and adipose. We also reviewed the data to determine if the rate of toxicant concentration increase in any one tissue might be representative of the fish as a whole. Because TCB is lipophilic, this presupposes one tissue might have a fat content representative of the fish as a whole, or at least with a fairly constant ratio to that of the fish as a whole. Standard deviations from the mean concentrations of TCB among the tissues analyzed were so great that we were unable to detect any sequential build-up among the tissues. Indeed, concentrations were not appreciably different between 1 and 6 hours except in the case of adipose tissue and possibly muscle (Table 1). We also looked at the tissue/blood plasma ratio of the test fish, and once again did not see any consistent ratio (Table 2).

It was apparent we would need to measure TCB concentrations in whole fish, and our next tests were designed to compare blood plasma TCB concentration against that of the whole body over time. In our first experiment the concentrations of TCB in blood plasma and whole body were very similar after 2 hours exposure, but blood plasma concentration at 6 hours had not increased, whereas whole body concentration had more than doubled (Figure 1). The obvious conclusion was that TCB equilibrium between exposure water and blood plasma was achieved within 2 hours or less, and after reaching saturation the blood was acting as a conduit to pass TCB from gills to body tissues. Thus, blood plasma could not be used to indicate either total body content or uptake rate of the toxicant.

In our next experiment 5-g rainbow trout were exposed to nominal TCB concentrations of 10, 50, and 100 μg/liter in holding tanks for several days, during which they were transferred to a fresh solution every 24 hours. Four fish were removed from each tank at 1, 2, 4, and 8 days, sacrificed, and blood plasma and whole body TCB concentrations measured. A tight pattern of TCB increase over time emerged for both blood plasma and whole body, and these patterns were also correlated with exposure concentrations (Figure 2), but once again equilibrium between TCB in exposure water and whole body lagged behind TCB equilibrium reached in blood plasma.

Earlier studies at MSU had shown the rate of uptake of some of the chemicals with which we might be working can be extremely rapid, with equilibrium between whole body and water sometimes reached in just a few hours. Tischmak (1984) demonstrated that equilibrium for 2,4,6-trichloroaniline between fathead minnows (*Pimephales promelas*) and their water environment was achieved in less than a day (Figure 3), and although equilibrium for TCB required 6-8 days, it was also apparent that the rate of uptake of TCB decreased considerably between initial exposure and 24 hours into test (Figure 4).

Our next step was to conduct a series of short term tests in which we exposed rainbow trout to TCB in the UBC respirometer to measure TCB uptake vs. oxygen consumed. We chose a 2-hour test period for several reasons, but principally because this was long enough to measure oxygen differences in the exposure water for as little as 100 g of test fish, and short enough so that rate of toxicant uptake by the test fish would not measurably vary between start and finish of the test period. The uptake of TCB by rainbow

trout per gram of fish for three different weight classes is shown in Figure 5. Because of the lipophilicity of TCB, we also measured lipid content in whole fish samples from each weight class, and the data were replotted as uptake of TCB against total lipid (Figure 6). Finally, we looked at uptake of TCB in relation to oxygen consumption rate for each of the three different weight classes of rainbow trout, each tested at two different swimming speeds (Figure 7). This latter curve demonstrates that there was a striking correlation between oxygen uptake and TCB uptake by these rainbow trout under our test conditions.

Separate from these chemical uptake studies, we have compiled a file of data from the published literature on respiratory oxygen requirements of fishes (Thurston and Gehrke, 1992). This file, called OXYREF, contains data from over 6800 individual laboratory tests in which oxygen consumption was measured. The data in our file include fish species, fish weight, certain test water conditions, measured respiratory oxygen requirements, and mode at which each fish was tested: "standard" (resting), "routine" (moving about), or "active" (measured swimming rate).

From OXYREF, we plotted oxygen consumption vs. weight for fishes in an active swimming mode, which is that mode under which we tested our fish at UBC in these TCB uptake experiments. If we now superimpose the data from our rainbow trout TCB experiments on the active swimming mode curve from OXYREF, one sees an excellent fit; the bulk of those data points from the higher swimming speed tests are slightly above the OXYREF curve, and the data points from the slower swimming speed tests are slightly below the curve (Figure 8).

There is a clear correlation between the rate of oxygen uptake and the rate of uptake of TCB among the freshwater fish species we tested. If this correlation holds for other representative fish species, and if the oxygen uptake rate and the water concentration of a chemical to which a fish is exposed are known, it may be possible to predict uptake rates of that chemical during exposure. We are continuing to expand OXYREF as more data become available in the literature, but in the present laboratory study if we can establish correlations between oxygen consumption and toxicant uptake and depuration, then OXYREF can provide a powerful tool for the prediction of bioaccumulation by fishes of xenobiotics.

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REFERENCES

Folch, J., M. Lees, and G.H.S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226:497-509.

Gehrke, P.C., L.E. Fidler, D.C. Mense, and D.J. Randall. 1990. A respirometer with controlled water quality and computerized data acquisition for experiments with swimming fish. Fish Physiology and Biochemistry, 8:61-67.

Tischmak, D.J. 1984. Separate and simultaneous bioconcentration in fathead minnows of five organic chemicals. M.S. Thesis, Department of Chemistry, Montana State University, Bozeman, Montana, USA.

Thurston, R.V., and P.C. Gehrke. 1992. Respiratory oxygen requirements of fishes: description of OXYREF, a data file based on test results reported in the published literature. Second International Symposium on Fish Physiology, Fish Toxicology, and Water Pollution. Sacramento, California (USA), September 1990. U.S. Environmental Protection Agency, Environmental Research Laboratory, Athens, Georgia, USA.

Table 1. Fish tissue concentrations after exposure to TCB.

Exposure (hours)	n	Plasma	Gills	Liver	Kidney	Spleen	U. Gut	L. Gut	W. Mscl	P. Mscl	Heart	Brain	Adipose
					TEST 118	500 - 25 µg/l TCB		0 BL/s					
0.5	2	5.73	4.68	8.70	8.10	<1.5	2.50	3.44	4.03	11.85	10.40	10.60	2.99
1.0	2	10.34	8.65	36.35	13.15	9.84	4.23	10.41	6.82	31.30	15.45	25.90	1.17
3.0	3	10.09	8.58	39.73	17.85	10.55	5.86	16.18	9.49	83.00	19.97	42.00*	20.97
6.0	3	8.39	5.20	14.87	14.20	7.64	7.28	6.19	7.26	63.33	16.00	15.43	23.83
					TEST 105	400 - 50 µg/l TCB		1.25 BL/s					
1.0	2	3.74	4.13	7.85	5.38	2.78	1.93	7.68	3.54	18.50	5.33	11.15	20.59
2.0	2	6.85	4.24	11.25	6.43	2.59	3.32	7.34**	5.20	18.00	6.13	11.29	8.22
6.0	2	3.17	2.96	10.78	9.29	2.21	4.22	no data	7.09	38.55	5.19	8.99	59.75

Plasma µg/mL, Tissue µg/g * = two values, ** = one value.

Table 2. Ratio of fish tissue concentrations to blood plasma after exposure to TCB.

Exposure (hours)	n	Plasma µg/ml	Gills/ Plasma	Liver/ Plasma	Kidney/ Plasma	Spleen/ Plasma	U.Gut/ Plasma	L.Gut/ Plasma	W.Mscl/ Plasma	P.Muscl/ Plasma	Heart/ Plasma	Brain/ Plasma	Adipose/ Plasma
					TEST 118	500 - 25 µg/l TCB		0 BL/s					
0.5	2	5.73	0.816	1.52	1.41	0.0421	0.437	0.600	•0.703	2.07	1.82	1.85	0.523
1.0	2	10.3	0.839	3.53	1.28	0.956	0.411	1.01	0.663	3.04	1.50	2.51	0.114
3.0	3	10.1	0.849	3.93	1.77	1.04	0.580	1.60	0.940	8.22	1.98	3.40	2.08
6.0	3	8.39	0.619	1.77	1.69	0.911	0.860	0.737	0.866	7.55	1.91	1.84	2.84
					TEST 105	400 - 50 µg/l TCB		1.25 BL/s					
1.0	2	3.87	1.07	2.03	1.39	0.720	0.499	1.98	0.915	4.78	1.38	2.88	5.32
2.0	2	6.85	0.619	1.64	0.939	0.378	0.484	1.05	0.759	2.63	0.896	1.65	1.20
6.0	2	3.17	0.934	3.40	2.93	0.699	1.33	no data	2.24	12.2	1.64	2.83	18.8

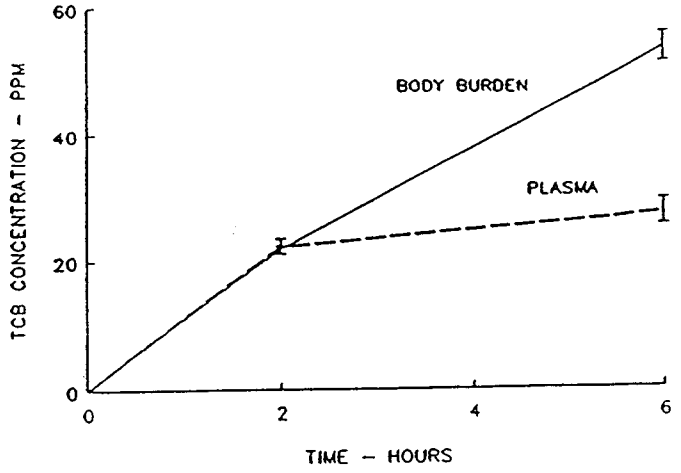

Figure 1. TCB plasma concentration vs whole body burden in rainbow trout.

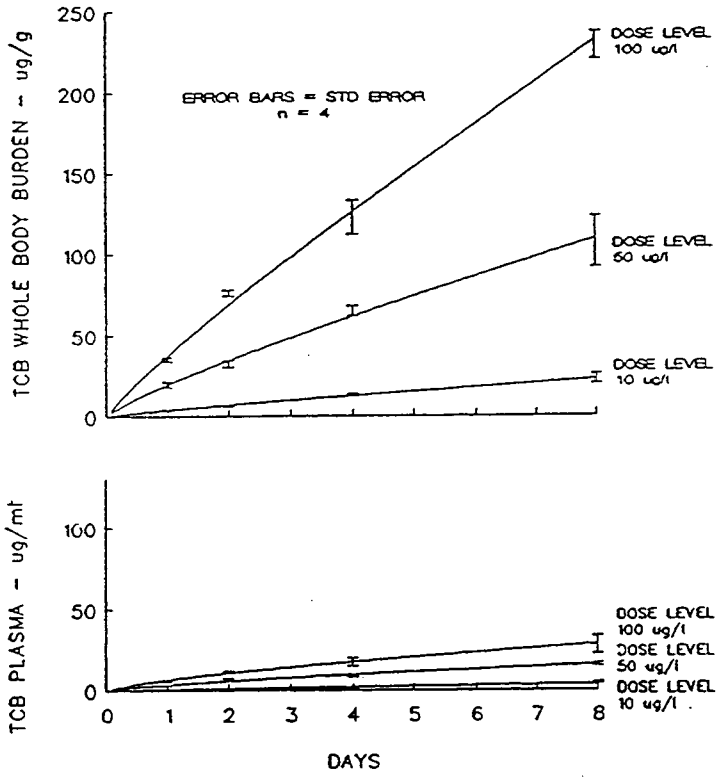

Figure 2. TCB plasma vs whole body burden in rainbow trout.

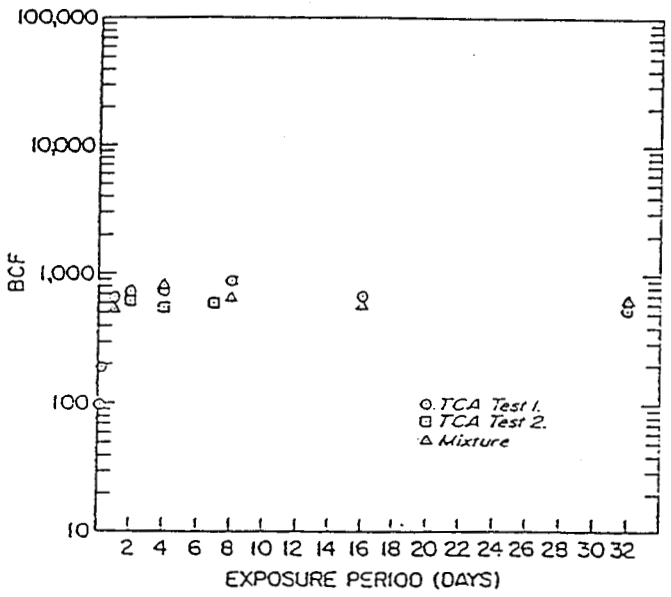

Figure 3. TCA bioconcentration profiles (from Tischmak 1984).

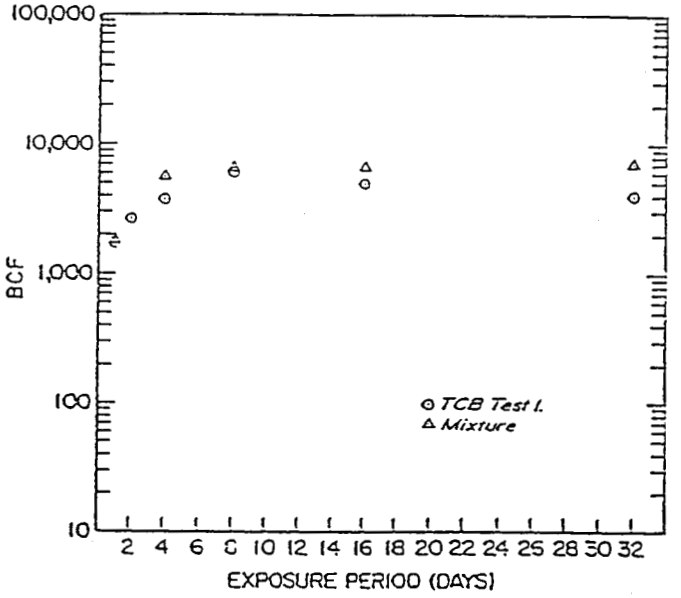

Figure 4. TCB bioconcentration profiles (from Tischmak 1984).

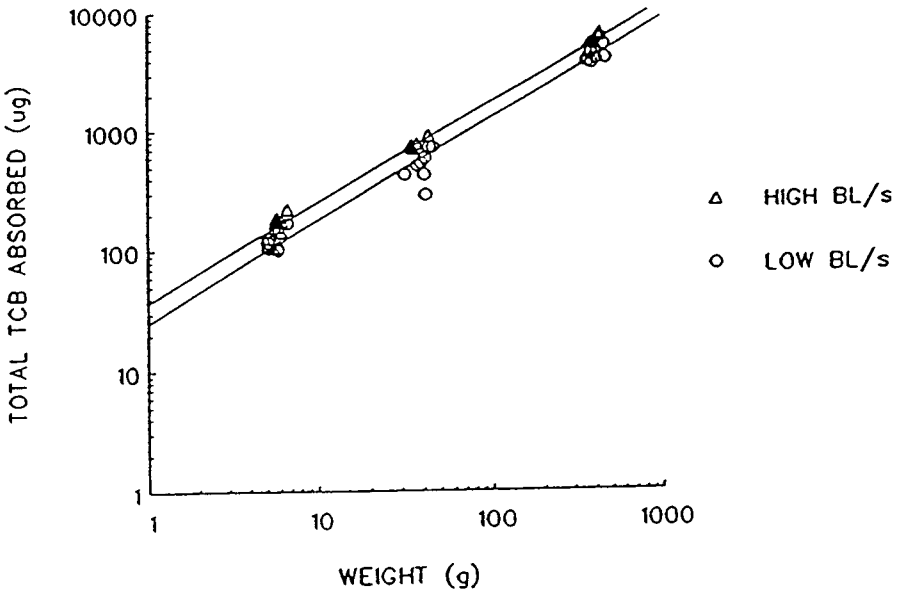

Figure 5. Rainbow trout at two swimming speeds - TCB concentration vs fish weight.

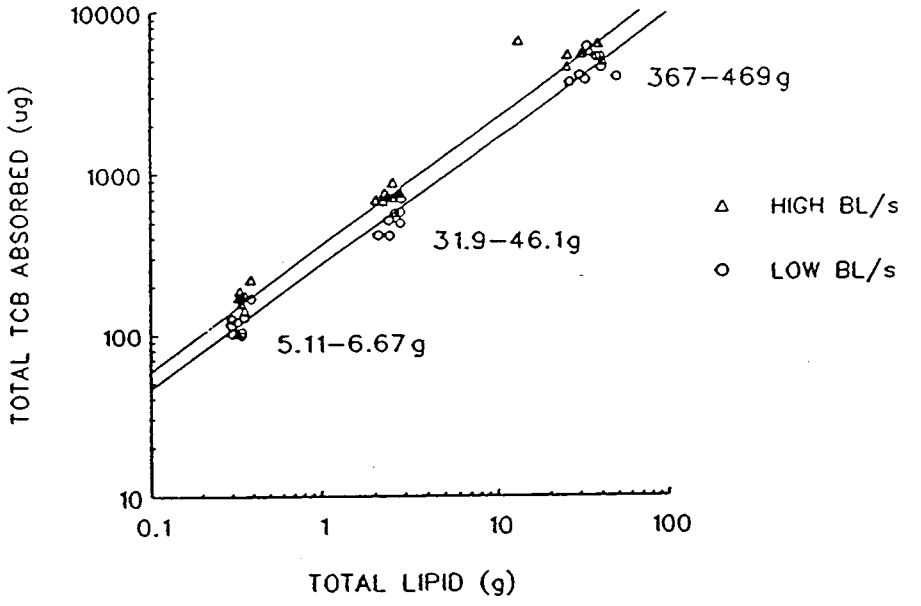

Figure 6. Rainbow trout at three weight classes - TCB concentration vs lipid concentrations.

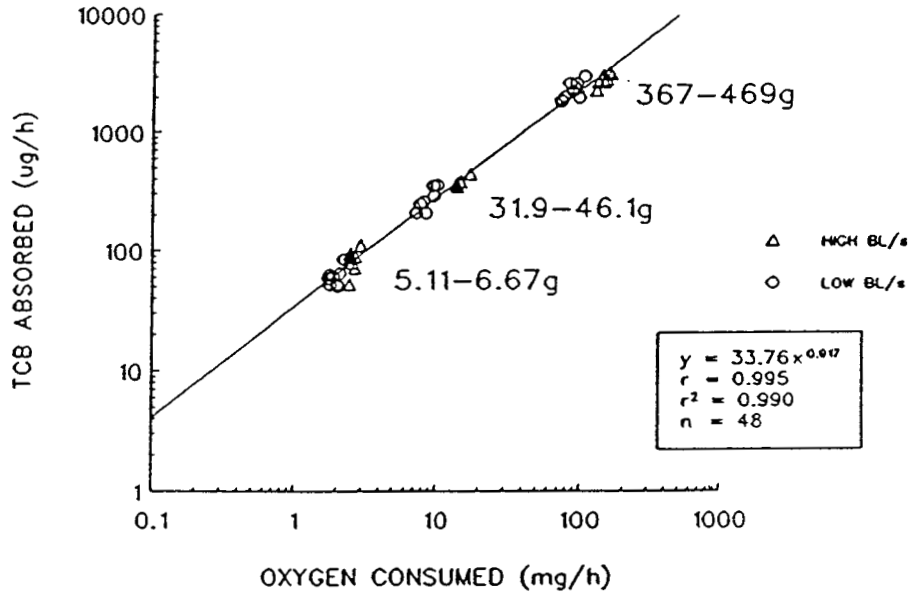

Figure 7. Rainbow trout at three weight classes, TCB concentration vs oxygen consumed.

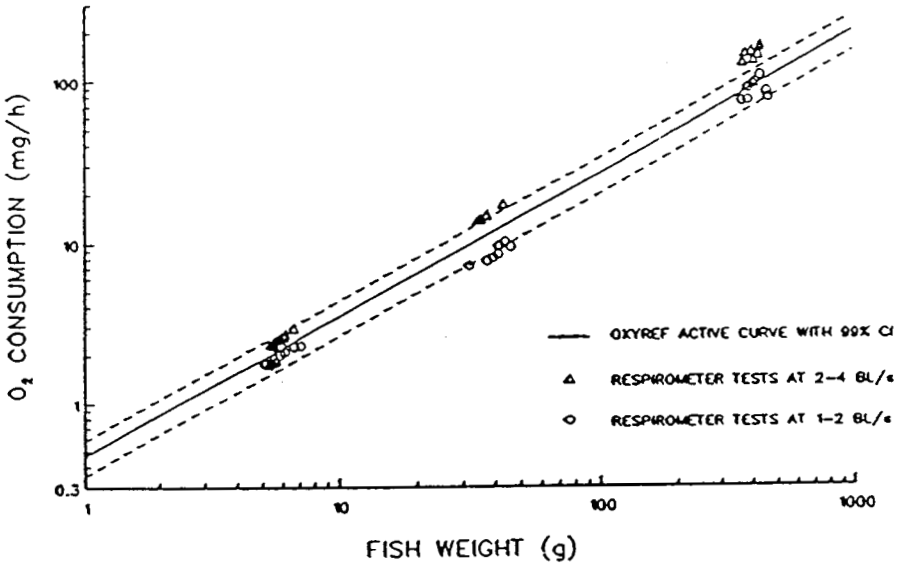

Figure 8. *OXYREF* data file active-swimming regression curve vs rainbow trout oxygen consumption during respirometer tests.