TO: Linda Logan
From: David DeForest
Sept. 01

#24

RESPONSE TO COMMENT #16

Comment #16 states that lead and zinc concentrations in cutthroat trout exceed their respective tissue screening concentrations (herein termed Shephard TSCs) derived by Shephard (1999). Before commenting on the applicability of these TSCs to cutthroat trout, it is important to emphasize that, as the name suggests, these are just screening concentrations. This is a particularly important consideration for mobile fish species (e.g., trout) that may be exposed to metals that are ubiquitous in urban environments. In these cases, it is not possible to unequivocally link the measured metal concentration to an individual source. The remainder of this response focuses on the applicability of the TSCs derived by Shephard to trout and developments of alternative fish-specific TSCs that we believe are more applicable. Note that the TSC terminology is also used for these alternative values provided below to emphasize that any tissue-based toxicity value for fish should be considered a screening concentration and does not provide conclusive evidence of potential risk or link potential risk to an individual chemical source.

The Shephard TSCs reported in comment #16 (as the basis of Dr. Strand's contention that lead and zinc are chemicals of concern) are 0.32 and 100 mg/kg dry weight (dw) for lead and zinc, respectively. While neither of these values appear in the citation in Dr. Strand's declaration (Shephard 1999), they do appear to be based on a wet weight to dry weight conversion (assuming aquatic organisms are 80 percent moisture) of the TSCs published in Dyer et al. (2000). While the general approach used by Shephard is scientifically valid, we propose that the direct application of the Shephard TSCs to fish tissue concentrations from Miller Creek is not appropriate. We instead use data relevant to cutthroat trout with Shephard's methodology to calculate fish-specific TSCs for use in this evaluation. In order to present our approach and results, it is necessary to first summarize the TSC methodology used by Shephard.

Overview of Shephard Methodology for Deriving TSCs

Shephard derived TSCs using the chronic U.S. EPA water quality criterion (WQC) and bioconcentration factor (BCF) for a given chemical. A BCF is the ratio of a chemical's concentration in the tissue of an organism (e.g., whole body fish) to the aqueous chemical concentration to which the organism was exposed, and is an indication of the accumulation of a substance in a target organism. Accordingly, multiplication of a chemical's WQC by an estimate of its BCF can be used to estimate the TSC [put in an equation]. As discussed below, however, there are several obstacles in using this approach to derive TSCs applicable to cutthroat trout that make the direct application of Shephard's TSCs inappropriate.

There are three primary reasons we feel the Shephard TSCs are not applicable to tissue residue data for cutthroat trout. First, the Shephard TSCs are based on a WQC which is designed to be protective of 95 percent of the species in an aquatic community. For metals, the WQC is often driven by sensitive invertebrates (e.g., U.S. EPA 1985a,b). Accordingly, application of these values to cutthroat trout assumes that cutthroat are among the most sensitive species to a given metal. Second, metal BCFs are highly

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species-specific because of the wide range of mechanisms aquatic biota have to regulate and/or store metals (e.g., Phillips and Rainbow 1989). Thus, without more information on the basis of the BCF used to derive the TSC, it is uncertain whether the BCF is relevant to cutthroat trout. The third issue is also related to the BCF and how, for metals, it tends to be highly dependent on the exposure concentration. For most metals and species, the BCF and exposure concentration are inversely related (i.e., the BCF increases as the exposure concentration decreases) (Brix and DeForest 2000). Therefore, it is always suspect when applying an individual BCF for metals. Based on all three of these issues, we independently derived what we feel are more appropriate estimates of metal TSCs for cutthroat trout.

Alternative Methodology for Deriving Fish-Specific TSCs

The method we used for deriving lead, zinc, and copper[1] fish-specific TSCs for cutthroat trout used (1) chronic toxicity data for trout or salmon species; (2) fish-specific BCFs; and (3) these fish-specific BCFs expressed as a function of the exposure concentration of interest (in this case the chronic toxicity value identified for trout or salmon species). For comparative purposes, we also identified chronic toxicity studies for some of these metals in which whole fish body concentrations were directly measured at conclusion of the test. Whole fish body metal concentrations could thus be directly related to the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) measured in the chronic toxicity test.

As discussed above, metal BCFs and exposure concentrations tend to be inversely related. Furthermore, in log space[2], the relationship between the BCF and exposure concentration for most metals is typically linear. These available BCF-exposure concentration relationships for copper, lead, and zinc in fish were then used to relate chronic NOECs and LOECs to whole body fish tissue residues. The following summarizes the development of fish-specific TSCs for copper, lead, and zinc to use in the evaluation of cutthroat trout data reported in Dr. Strand's declaration.

Results

<u>Lead</u>

A chronic lead toxicity study with brook trout (*S. fontinalis*) was identified in which whole body lead concentrations were measured (Table 2a). The whole body lead concentrations associated with the chronic NOEC and LOEC of 58 and 119 µg/L were 12.7 and 20.1 mg/kg dw, respectively. For comparison, the Shephard lead TSC reported in comment #16 was 0.32 mg/kg dw.

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[1] Although copper concentrations in cutthroat trout were not identified as exceeding the copper TSC in comment #16, it was included in this evaluation because the copper concentration in trout was nevertheless reported.

[2] Measuring when each variable is transformed by taking the logarithm of water and tissue concentrations.

Rainbow trout (*Oncorhynchus mykiss*) appear to be more sensitive than brook trout based on aqueous NOECs and LOECs, but no whole body concentration data were available for rainbow trout. Consequently, the fish-specific BCF-exposure concentration relationship for brook trout was used to estimate whole body lead concentrations in rainbow trout at the chronic value (Figure 2). The aqueous chronic value for rainbow trout at a hardness of 18 mg/L is 18.9 μg/L. The estimated whole body lead concentration at this aqueous concentration is 4.9 mg/kg dw (Table 2b). The lead chronic value for rainbow trout normalized to a hardness of 50 mg/L is 69.3 μg/L and the whole body lead concentration associated with this chronic value is 14.8 mg/kg dw (Table 2b). Accordingly, the fish-specific TSCs for rainbow trout (4.9 mg/kg dw at 18 mg/L hardness and 14.8 mg/kg dw at 50 mg/L hardness) are also much greater than the reported lead TSC of 0.32 mg/kg dw. The lead concentrations measured in Miller Creek cutthroat trout (0.31, 0.74, 0.34 mg/kg dw) are over an order of magnitude below the measured and estimated fish-specific TSCs presented here.

<u>Zinc</u>

No chronic zinc toxicity studies were identified in which whole body zinc concentrations could be related to a chronic toxicity threshold. Thus, the BCF-exposure concentration relationship for zinc and Atlantic salmon (*Salmo salar*) was used to estimate whole body zinc concentrations (Figure 3). Several chronic zinc toxicity studies were identified for salmon and trout species. The aqueous chronic values from these studies were used to estimate fish-specific TSCs for zinc. The most sensitive chronic value identified was 277 μg/L at a hardness of 26 mg/L, reported by Sinley et al. (1974) for rainbow trout (*O. mykiss*). Application of the BCF-exposure concentration relationship for zinc to this chronic value results in an estimated whole body zinc concentration of 182 mg/kg dw (Table 3). The chronic value normalizes to 482 μg/L at a hardness of 50 mg/L. The estimated whole body zinc concentration based on the normalized chronic value (188 mg/kg dw) is very similar to the estimated whole body concentration when the aqueous chronic value is not hardness-normalized (Table 3). For comparison, the Shephard TSC reported in comment #16 was 100 mg/kg dw. The zinc concentrations measured in Miller Creek cutthroat trout (137, 145, 129 mg/kg dw) do not exceed the estimated fish-specific TSCs estimated here.

<u>Copper</u>

No chronic copper toxicity studies for salmon or trout species were identified that measured whole body copper concentrations in exposed fish. However, a study by Lind et al. (Unpublished) was identified that measured whole body copper concentrations in the fathead minnow (*Pimephales promelas*) following a chronic toxicity test. The NOEC and LOEC from this toxicity test were 9 and 13.1 μg/L, respectively. The whole body copper concentrations associated with the NOEC and LOEC were 19.8 and 25.2 mg/kg dw (Table 1a).

The BCF-exposure concentration relationship from the fathead minnow study (see Figure 1) was then used to estimate tissue residue-based thresholds from chronic copper toxicity studies with various trout species. The most sensitive trout species identified was brook trout (*Salvelinus fontinalis*), with an NOEC and LOEC of 3 and 5 μg/L, respectively, at a hardness of 37.5 mg/L. Using the fish-specific BCF-exposure concentration relationship for copper, the predicted whole body copper concentration at the chronic value (geomean of NOEC and LOEC) is 9.6 mg/kg dw (Table 1b). Note that if the chronic value is first normalized to a hardness of 50 mg/L, the estimated whole body residue threshold is slightly higher, i.e., 11.7 mg/kg dw (Table 1b). The copper concentrations measured in Miller Creek cutthroat trout (6.5, 6.5, 4.3 mg/kg dw) do not exceed the estimated fish-specific TSCs estimated here.

Conclusions

Overall, we feel the above approach using fish-specific data for deriving fish-specific TSCs is more appropriate for interpreting cutthroat trout tissue residue data than those derived by Shephard for the protection of 95% of the aquatic community. The fish-specific TSCs derived using our method tend to be over an order of magnitude greater than those reported by Shephard. This is primarily driven by using direct measures of trout or salmon sensitivity to metals in deriving the fish-specific TSCs, rather than use of the WQC which are often driven by more sensitive invertebrates. Using this fish-specific approach, the concentrations of lead, zinc, and copper measured in Miller Creek cutthroat trout do not exceed their respective fish-specific TSCs derived here using this approach.

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Table 1a. Copper: Measured tissue thresholds for fathead minnows.

see Lind et al.

Species	Hardness (mg/L)	NOEC (µg/L)	LOEC (µg/L)	ChV (µg/L)	Measured Residue-Based NOEC (mg/kg dw)	Measured Residue-Based LOEC (mg/kg dw)	Measured Residue-Based ChV (mg/kg dw)	Reference
Fathead minnow	45	9	13.1	10.9	19.8	25.2	22.3	Lind et al. Manuscript
Hardness-normalized[3]		*9.8*	*14.3*	*11.9*				

Table 1b. Copper: Estimated tissue thresholds for rainbow, brown, brook, and lake trout.

10^(a+bx) log ChV

Species	Hardness (mg/L)	AWQC NOEC (µg/L)	AWQC LOEC (µg/L)	AWQC ChV (µg/L)	Regression BCF[1]	Estimated Residue-Based ChV (mg/kg ww) ChV * BCF	Estimated Residue-Based ChV (mg/kg dw)[2] assuming 80% moisture	Reference
Rainbow trout	45.4	11.4	31.7	19.0	368	7.0	35.0	McKim et al. 1978
Hardness-normalized[3]		*12.4*	*34.4*	*20.6*	*362*	*7.5*	*37.4*	
Brown trout	45.4	22	43.2	30.8	336	10.4	51.9	McKim et al. 1978
Hardness-normalized[3]		*24*	*46.9*	*33.5*	*331*	*11.1*	*55.5*	
Brook trout	37.5	3	5	3.9	494	1.9	9.6	Sauter et al. 1976
Hardness-normalized[3]		*3.8*	*6.4*	*5.0*	*472*	*2.3*	*11.7*	
Lake trout	45.4	22	42.3	30.5	337	10.3	51.4	McKim et al. 1978
Hardness-normalized[3]		*24*	*45.9*	*33.1*	*332*	*11.0*	*55.0*	

Table 2a. Lead: Measured tissue thresholds for brook trout.

see Holcombe et al.

Species	Hardness (mg/L)	NOEC (µg/L)	LOEC (µg/L)	ChV (µg/L)	Measured Residue-Based NOEC (mg/kg dw)	Measured Residue-Based LOEC (mg/kg dw)	Measured Residue-Based ChV (mg/kg dw)	Reference
Brook trout	44	58	119	83	12.7	20.1	16.0	Holcombe et al. 1976
Hardness-normalized[3]		*68*	*140*	*98*				

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Table 2b. Lead: Estimated tissue thresholds for rainbow trout.

Species	Hardness (mg/L)	NOEC (µg/L)	LOEC (µg/L)	ChV (µg/L)	BCF[1]	Estimated Residue-Based ChV (mg/kg ww)	Estimated Residue-Based ChV (mg/kg dw)[2]	Reference
Rainbow trout	18	13.2	27	18.9	51	1.0	4.9	Sinley et al. 1974
Hardness-normalized[3]		*48.5*	*99*	*69.3*	*43*	*3.0*	*14.8*	

Table 3. Zinc: Estimated tissue thresholds for Chinook salmon, rainbow trout, and brook trout.

Species	Hardness (mg/L)	NOEC (µg/L)	LOEC (µg/L)	ChV (µg/L)	BCF[1]	Estimated Residue-Based ChV (mg/kg ww)	Estimated Residue-Based ChV (mg/kg dw)[2]	Reference
Chinook salmon	25	270	510	371.1	100	37	185	Chapman 1975
Hardness-normalized[3]		*486*	*918*	*667.6*	*57*	*38*	*191*	
Rainbow trout	26	140	547	276.7	132	36	182	Sinley et al. 1974
Hardness-normalized[3]		*244*	*952*	*482*	*78*	*38*	*188*	
Rainbow trout	25	444	819	603	63	38	190	Cairns et al. 1982
Hardness-normalized[3]		*799*	*1473*	*1085*	*36*	*39*	*196*	
Brook trout	45.9	534	1368	854.7	45	39	194	Holcombe et al. 1979
Hardness-normalized[3]		*574*	*1471*	*919*	*42*	*39*	*195*	

[1]Based on the metal-specific relationship between BCF and exposure concentration.
[2]Assuming a moisture content of 80%.
[3]Normalized to hardness of 50 mg/L.

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Figure 1. Relationship between copper BCFs and exposure concentrations for the fathead minnow (data from Lind et al. [Unpublished]).

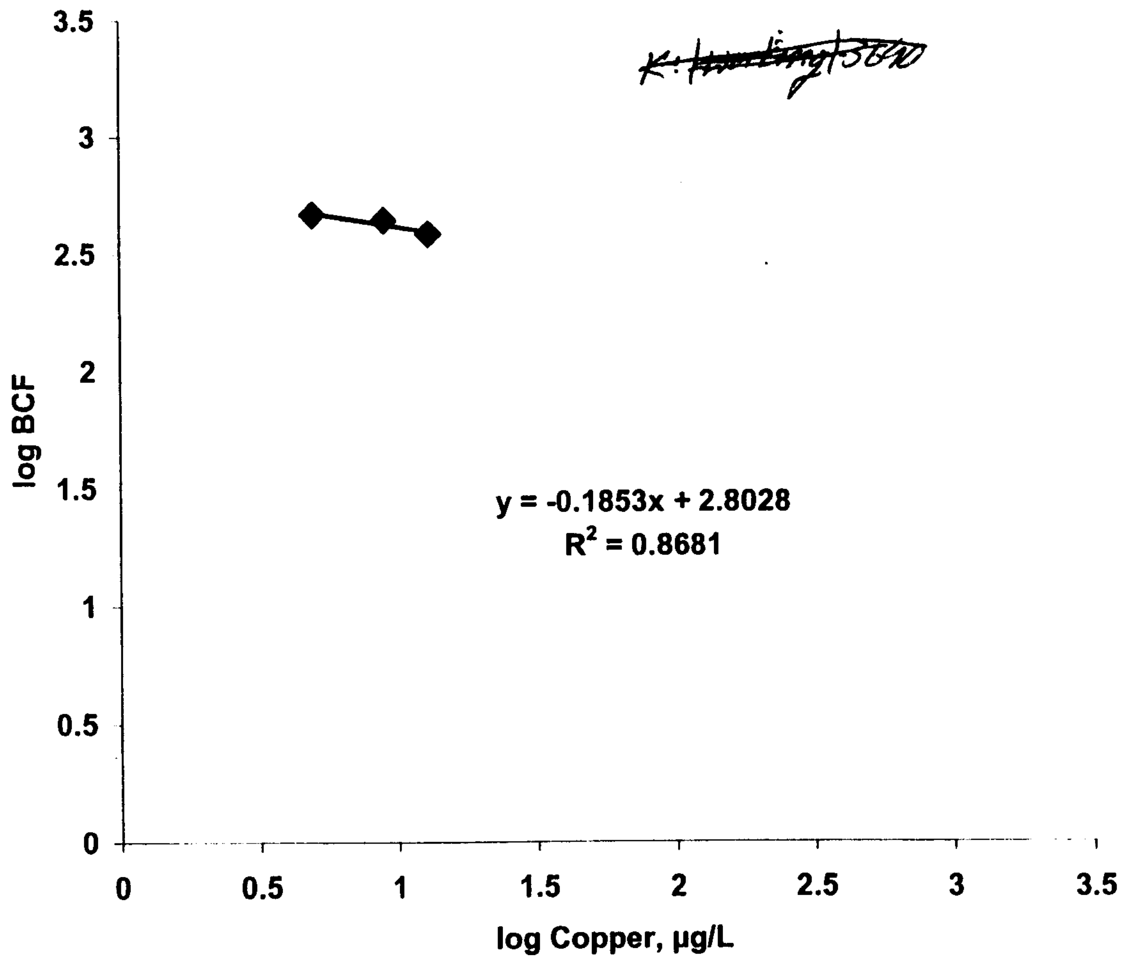

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Figure 2. Relationship between lead BCFs and exposure concentrations for brook trout (data from Holcombe et al. 1976).

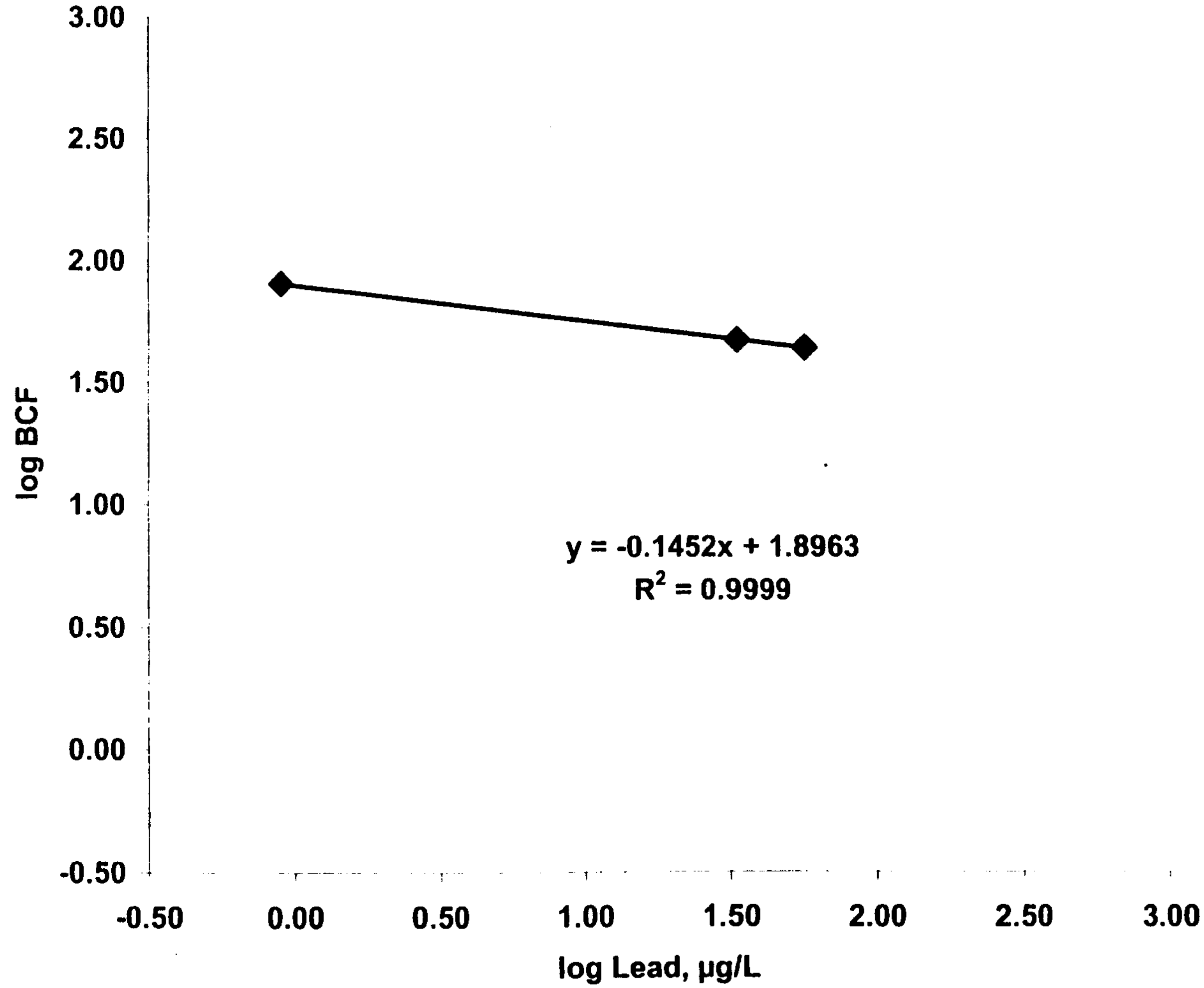

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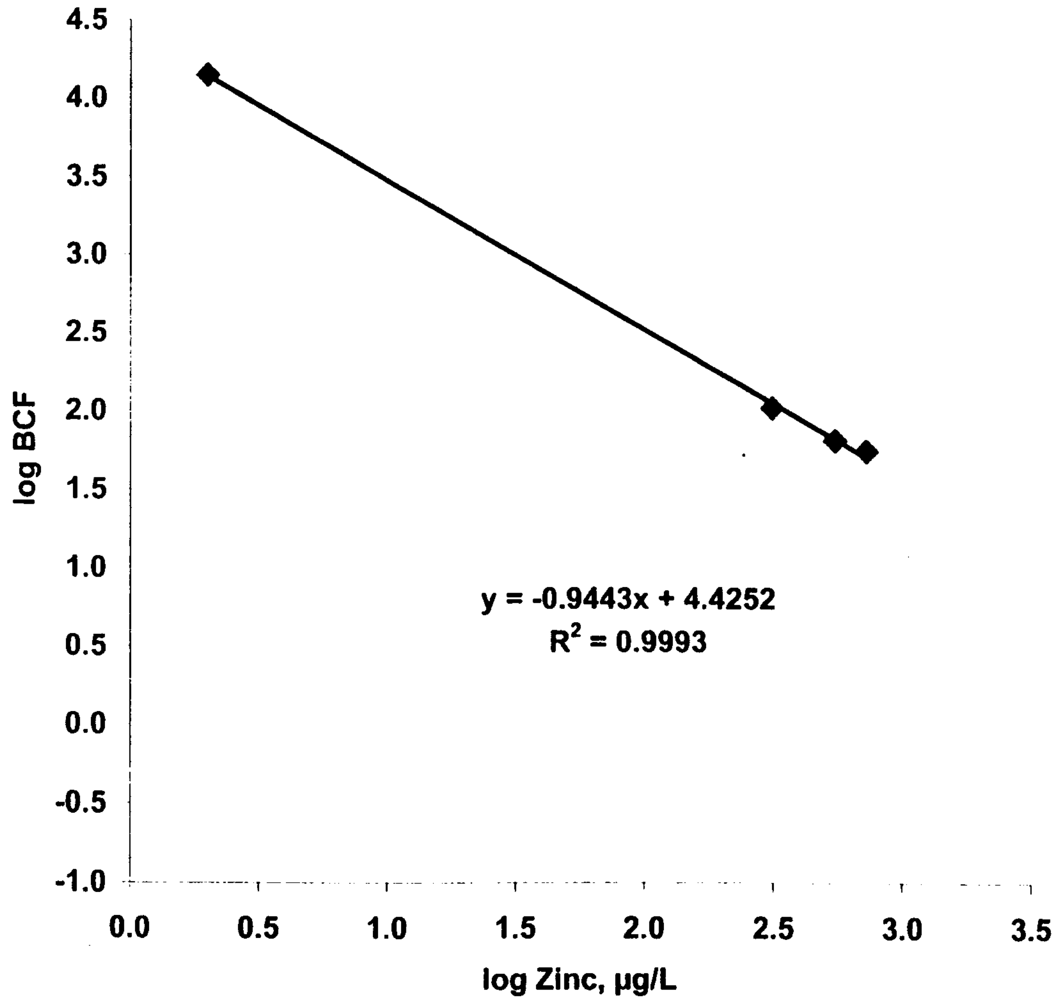
Figure 3. Relationship between zinc BCFs and exposure concentrations for Atlantic salmon (data from Farmer et al. 1979).



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