Technical Report 373
November 2002

Effects of Selenium Accumulation on Larval Rainbow Trout (Oncorhynchus mykiss)

Steven M. Bay

Doris Vidal

Daniel Schlenk

Southern California Coastal Water Research Project

Effects of Selenium Accumulation on Larval Rainbow Trout (*Oncorhynchus mykiss)*

Steven Bay [1]
Doris Vidal [1]
Daniel Schlenk[2]

[1] *Southern California Coastal Water Research Project*
7171 Fenwick Lane Westminster, California 92683
www.sccwrp.org

[2] *Department of Environmental Sciences, University of California, Riverside, Riverside, CA 92521, USA*

December 09, 2002

Technical Report #373

ABSTRACT

Elevated selenium (Se) concentrations in water (>10 µg/L) have been measured in San Diego Creek, Orange County. The objective of this study was to determine if Se accumulation has the potential to cause significant impairments to fish populations in San Diego Creek. A 90-day test was conducted to determine the effects of selenomethionine (SeMe) on the growth, survival and whole body Se accumulation in larval rainbow trout. The reduced and oxidized glutathione and thiobarbituric acid reactive substance levels were also measured in the livers of the trout to assess oxidative damage caused by Se. Se was administered orally using food spiked with SeMe to contain 4.6, 12 and 18 µg/g (dry weight) of Se. Fish exposed to SeMe through their food for 90-days exhibited a reduction in body weight and fork length at all levels of exposure when compared to control samples. Whole body total Se concentrations significantly increased in fish fed 18 µg/g SeMe after 60-days when compared to controls. A significant increase in the whole body Se concentration was also observed after 90 days at all levels of exposure. Lipid peroxidation and GSH:GSSG ratios were unchanged by the Se treatments. Based on reduced growth after 90 days, a dietary Se LOEC value of 4.6 µg/g was obtained, as well as a body burden LOEC of 0.51 µg/g (wet weight).

ACKNOWLEDGMENTS

The authors would like to acknowledge the invaluable assistance of Mr. David Thomason and Rickey Kim (University of California at Riverside) for their help with the experiment and chemical analyses. The authors also thank Gary Williams of the Mojave Hatchery (California Department of Fish and Game, which provided the fish and fish food used in this experiment. Financial support for this project was provided by the State Water Resources Control Board (contract 00-191-180-0).

TABLE OF CONTENTS

Abstract ..... *i*

Acknowledgments ..... *ii*

Introduction 1

Methods 4
Fish Exposure 4
Selenium Analysis 4
Hepatic Lipid Peroxidation and Hepatic GSH: GSSG 5
Data Analysis 6

Results 7
Fish Survival and Growth 7
Se Accumulation 8
Hepatic Lipid Peroxidation and Hepatic GSH: GSSG 9

Discussion 10

Literature Cited 12

Appendix: Fish Data 26

INTRODUCTION

Selenium (Se) is an element that occurs naturally in many soils and sediments, and is an essential micronutrient for fish, birds, humans, and many microorganisms. In experiments preformed with rats and chickens, Se has been shown to have a role in preventing dietary hepatic necrosis and exudative diathesis (Schwarz *et al.*, 1957) and its deficiency is characterized by cardiomyopathy and muscular weakness (Chen *et al.*, 1980). Despite the fact of being a required micronutrient, Se is toxic at concentrations only slightly greater than the nutritional requirements (Hilton *et al.*, 1980). Se can cause adverse effects on aquatic organisms including fish and birds that eat contaminated prey since it bioaccumulates through the food chain (Sappington, 2002).

Elevated Se water concentrations (>10 µg/L) have been found in San Diego Creek (Orange County). These concentrations exceed the 5 µg/L criteria for fresh water systems established by the U.S. EPA (CRWQCB 2001). Se levels in San Diego Creek sediment, which range between 0.2 and 3 µg/g, are similar to the values established for sediment quality that range between 2 and 4 µg/g (CRWQCB 2001). Se levels in fish from San Diego Creek vary between 2 and 7 µg/g and do not exceed the values established for the protection of human health (20 µg/g wet). However, these levels are similar to the level of concern (>4 µg/g) for toxicological effects on wildlife (CRWQCB 2001).

Se undergoes a biogeochemical cycle analogous to the sulfur cycle (Shrift, 1973). SeO_4^{2-} and SeO_3^{2-} are the most common Se oxyanions found in soil and natural waterways (Dungan and Frankenberger, 1999). Se can also undergo four types of transformations: reduction (assimilatory and dissimilatory), oxidation, methylation, and demethylation (Dungan and Frankenberger, 1999). Se exists in many natural soil and water environments around the world, but anthropogenic activities such as irrigated agriculture on Se-laden soils has created environmental problems with respect to this element.

Inorganic Se, along with other salts, can leach out of the soil and enter aquatic habitats via agricultural drainages and ground water discharges. For example, irrigation drainage waters of the San Joaquin Valley often have Se values of 140-1400 µg/L, with some local values reaching as high as 4,200 µg/L (Presser and Barnes, 1985). Once in the waterways, microbial organisms can reduce SeO_4^{2-} as a terminal electron acceptor and incorporate it into organic compounds, such as the amino acid conjugates selenomethionine and selenocysteine (Weiss *et al.*, 1965). Se passes through the food chain primarily in organic forms, such as selenomethionine and selenocysteine (Fan *et al.*, 2002). Exposure of fish to free-amino acid SeMe has been shown to closely mimic the toxicity of Se produced by the consumption of Se-contaminated prey species, thus SeMe is a good model for naturally incorporated Se in the aquatic food chain (Hamilton *et al.*, 1990). The contamination of food chain organisms with Se can have adverse effects on fish populations (Maier and Knight 1994; Lemly 1993; Hamilton *et al.*, 1990). Reduced fish growth, whole body concentrations of Se and survival were strongly correlated with Se in diets of Chinook salmon (Hamilton *et al.*, 1990).

Despite previous research the mechanism of selenium toxicity in fish is poorly understood. It is known that flavin-containing monooxygenases (FMO) play a role in the activation of organoselenides to selenoxides in mammals initiating the oxidation and depletion of glutathione (GSH) (Chen *et al.,* 1994). GSH is a low molecular weight thiol-containing tripeptide, which has an important role in the maintenance of cell membrane integrity, drug and chemical metabolism, and protection from oxidative stress (Rogers and Hunter, 2001).

Depletion of hepatic GSH and the induction of lipid peroxidation have been observed in numerous mammalian tissues following treatment with organoselenides (Hoffman, 2002). Measurement of the ratio of reduced GSH to oxidized GSH (GSSG) and the relative amount of lipid peroxidation are sublethal endpoints that can be used to provide an indication of oxidative stress within organisms (Baker *et al.,* 1990 Jentzsch *et al.,* 1996).

Quantification of malondialdehyde (MDA) has been used in the past decades as a marker of lipid peroxidation. One of the most widely used methods involves the use of 2-thiobarbituric acid (TBA or 4,6-dihydroxy-2-mercapto-pyrimidine). The adduct formed during the reaction can be measured by spectrophotometry and by spectrofluorometry (Jentzsch *et al.*, 1996). Biological specimens contain a mixture of thiobarbituric acid reactive substances (TBARS), including lipid hydroperoxides and aldehydes, which increase as a result of oxidative stress. The concentration of TBARS returns to normal levels over time, depending upon the presence of anti-oxidants. In practice, TBARS concentration is expressed in terms of malonaldehyde (MDA) equivalents (Kwon and Watts, 1964).

Organoselenides are good substrates for FMO oxidation (Goeger *et al*., 1993). Moreover, the primary uptake of selenium in fish is through the food (Sandholm *et al*., 1973). In addition, exposure of fish to free-amino acid SeMe has been shown to closely mimic the toxicity of Se produced by the consumption of Se-contaminated prey species, thus SeMe is a good model for naturally incorporated Se in the aquatic food chain (Hamilton *et al.*, 1990). SeMe is commonly found in aquatic animal foods (Spallholz *et al.,* 2002).

The objective of this study was to determine whether the accumulation of Se in a model fish species could potentially cause a significant impairment of fish populations in San Diego Creek (Orange County). The present study addressed this objective by exposing larval rainbow trout to dietary SeMe in a 90-day toxicity test, with food as the primary route of exposure to:

- Determine the effects of SeMe on the survival of the fish
- Determine the effects of s SeMe on larval growth
- Determine SeMe accumulation in the fish
- Assess whether SeMe exposure caused oxidative liver damage by measuring reduced and oxidized glutathione (GSH; GSSG) and

thiobarbituric acid reactive substance (TBARS) levels as an endpoint of lipid peroxidation

By determining the effects of SeMe on larval trout, this project will help to demonstrate the effects that elevated concentrations of selenium could potentially have on resident fish populations in San Diego Creek.

METHODS

FISH EXPOSURE

Larval rainbow trout (24-day old) were obtained from the Mojave Hatchery (California Department of Fish and Game). Selenium (Se) spiked food was prepared by thoroughly mixing dry fish food (provided by the hatchery) with appropriate amounts of selenomethionine (SeMe) in a methanol solution to produce Se concentrations of 4.6, 12, and 18 µg/g (dry weight). The control treatment contained (fish food with no additional SeMe) contained 0.23 µg/g of Se. Each treatment had five replicates and each replicate contained 12-16 larval trout. The total number of fish used in the experiment was 300. Each fish was fed an average of 10 mg of fish food daily for 30 days, between 30 and 60 days of exposure they were fed 25 mg per fish, and fed 40 mg per fish thereafter. The analysis of the fish diet showed that other elements in the food were present at typical concentrations (Table 1).

The study took place in September to November (2001) and was conducted in aerated, replicate ten-gallon aquaria, which were partially submerged in a refrigerated water bath (at 10±2°C). Each replicate received >1 liter of 12±2°C replacement water every minute. To reduce water borne accumulation of SeMe from fish excretory products and dissolution of diet, one third of the water in each bath was replaced with filtered water on a daily basis. Se concentration in the water column was below levels of detection (0.05-0.1 µg/L) throughout the study. Water quality (pH, DO, conductivity, ammonia, and temperature) was measured weekly. Water parameters remained within normal ranges during the exposure (Table 2). Ammonia values ranged between 0 to 1.0 mg/L, pH values ranged from 7.0-8.4, and conductivity values ranged from 0.01 to 1.11 µS/cm. Temperature was constant (10±1ºC).

The glass aquaria had glass lids and were exposed to partially shaded, natural light (12/12 light regime). Fish were acclimated for three days before exposure began. Wet weight and fork length of a sample of three to five fish from each aquarium were measured as controls before the exposure began.

Ten fish from each treatment (two fish per aquarium) were sampled after 30, 60 and 90 days of exposure. Their fork length, body weight, tissue Se concentration and hepatic GSH and TBARS were measured. All of the fish were euthanized with MS-222 (3-amino-benzoic acid ethyl ether, Sigma). Daily observations of mortality were made.

SELENIUM ANALYSIS

The control diet was analyzed by Calscience Environmental Laboratories for 18 elements including Se following EPA 6010B and EPA 7471A methods. Fish tissue analyses were conducted at the University of California, Riverside. Samples of trout tissue (0.5-3.5 g) were digested in 4 ml nitric acid, 4 ml hydrogen peroxide, and 2 mL DI water at 170°C

and ~200 psi in a microwave digester for 30 minutes. In order to change the oxidative state of Se to Se (VI), the samples were heated at 90°C for 20 minutes in 12 ml HCl and 0.2 ml of 0.2 M $K_2S_2O_8$ to a final HCl concentration of 6 N. The samples were analyzed in a Perkin-Elmer atomic absorption spectrophotometer. The calibration standard concentrations used were 0, 5, 10, and 15 µg/L.

The tissues from the 30 and 60-day exposure times were analyzed without the liver (preliminary studies demonstrated that they were no differences in whole body Se concentration between the samples with and without the liver). The 90-day exposure samples were analyzed as whole-body.

For quality assurance and quality control, a dried tomato leaf reference material (National Institute of Standards and Technology) and matrix spikes were analyzed with the samples. The percent recovery for the tomato leaves ranged from 68% to 85%. The percent recovery for the spiked samples was >79%. The detection limit for the Se analysis ranged from 0.003 to 0.017 µg/g. All metal concentrations from fish were reported on a wet weight basis, whereas food was measured as dry weight.

HEPATIC LIPID PEROXIDATION AND HEPATIC GSH: GSSG

Total GSH and GSSG were measured using the method of Baker *et al.*, (1990). Trout liver tissue samples were homogenized in 1:9 v/v 5 % 5-sulfosalicyclic acid (SSA) on ice. The homogenate was centrifuged and triplicate 10 µL samples of supernatant (~1.0 mg of tissue) or standard was added to a 96-well microplate. Two hundred µL of reaction buffer were then added to the wells for measurement of GSH. Reaction buffer consisted of 5.40 mL NaH_2PO_4/EDTA buffer, 2.80 mL 1 mM DTNB (0.15mM final), 3.75 mL of 1 mM NADPH (0.2mM final) and 0.050 mL of 12U GSSG reductase (1 U per mL final). The microplate was read at 10-second intervals over two minutes at 405 nm at room temperature (25°C). The GSSG assay was similar to the above, except that the assay system included 20 µL triethanolamine (10%) and 2 µL of 2-vinylpyridine (97%).

Malondialdehyde (MDA), a byproduct of lipid peroxidation was measured utilizing the thiobarbituric acid reactive substance (TBARS) assay (Jentzsch *et al.*, 1996). Trout liver tissue was homogenized in buffer containing 0.1 M Tris, 0.15 M KCl and 1 M EDTA. A 40 µL sample of homogenate (6.67 mg/ml of tissue), 60 µL cold DI water, 600 µL of 1 % phosphoric acid and 200 µL of 0.9 % thiobarbituric acid were added. The samples were then flushed with nitrogen to remove oxygen from the reaction (to prevent formation of lipid peroxides). Samples were cooled for five minutes and then extracted with 900 µL of n-butanol. A spectrophotometer was used to measure the absorption of the n-butanol fraction at 535 nm and 520 nm (to correct for the baseline absorption). MDA concentration was calculated by subtracting the absorption of each sample at 520 nm, from the absorbance of the samples at 535 nm, and dividing the result by the slope of the standard curve.

DATA ANALYSIS

To determine whether or not the health of larval rainbow trout was adversely affected by SeMe exposure, the condition index (CI) was calculated. The index was calculated from both body weight and fork length measurements as follows:

$$CI = \text{body weight}/\text{fork length}^3$$

Trout body weight, fork length, condition index, tissue Se concentration, GSH:GSSG ratio, and TBARS data were analyzed with analysis of variance (ANOVA) to determine the statistical differences between the treatments ($p<0.05$). Dunnet's test was performed if significance among different means was observed, to determine which different groups were significantly different from the control.

The Lowest Observable Effects Concentration (LOEC), was determined as the lowest food or tissue concentration producing a statistically significant adverse effect.

RESULTS

FISH SURVIVAL AND GROWTH

Most mortalities (except two) occurred in the first 17-days of exposure (Figure 1). Thirty percent of the fish died in the control treatment by the end of the experiment. The lowest selenium (Se) treatment of 4.6 µg/g presented the same 30% cumulative mortality as the control treatment. Cumulative fish mortality in the two highest treatments of 12 and 18 µg/g was lower, 6 and 13% respectively. The fish mortality did not show a significant relationship with the Se concentrations used in this experiment.

Fish growth was clearly impacted after 90 days at all Se treatments when compared to control fish. A two-way ANOVA analysis showed that both body weight and fork length were affected by the Se concentration and also by the length of exposure (concentration significance $p= 0.062$; time of exposure significance $p <0.0001$; exposure vs. Se interaction $p= 0.025$). Due to the significant interaction between exposure time and concentration, a one-way ANOVA analysis was done for each different exposure time to determine if the treatments had significant differences in body weight and fork length.

Control fish at the end of the experiment had an average fork length of 7.70 cm while the length for SeMe exposed fish ranged between 6.84 and 7.37 cm. A variable relationship between fork length and time was observed. Fish fork length increased throughout the experiment, but the rate of increase slowed markedly after 60 days in all SeMe exposed treatments (Figure 2). Fork length was significantly reduced ($p <0.05$) after 90 days in all SeMe exposed fish (Table 3).

Trout body weight at the end of the experiment averaged 5.17 g for control fish and ranged between 3.45 and 3.82 g for exposed fish (Table 3). One-way ANOVA analysis demonstrated that there were significant differences in weight after 90 days at the concentrations of 4.6, 12 and 18 µg/g of SeMe exposure ($p <0.001$). Fish in all treatments exhibited a similar rate of weight gain during the experiment (Figure 2).

The weight of the larval fish in the two highest treatments was significantly greater than the control weight at the beginning of the experiment. To reduce the influence of these initial size differences and to improve the detection from SeMe induced growth effects, net body weight gain was calculated by subtracting the initial (day 0) average body weight of a sample of fish in each exposure from the body weight of the sampled fish at 30, 60 and 90 days of exposure. The net weight gain results showed a similar pattern of effect as for the total weight data; a significant reduction in net weight was present at 90 days for all groups of SeMe exposed fish (Figure 3).

SeMe effects on fish growth were also assessed using the calculation of the condition index (calculated from the body weight and fork length). Condition index values ranged between 0.00741 and 0.01329 g/cm^3 during the study. Significant effects were found at time 0 for the two highest concentrations of 12 and 18 µg/g, which had a higher condition

index than the control ($p < 0.05$) due to size variability among the fish populations (Table 3). The condition index decreased in SeMe exposed fish during the first 60 days and increased thereafter (Figure 4). The control fish also showed a marked increase in condition between 60 and 90 days of exposure.

Significant effects (reduced condition index relative to the control) were observed for fish in the 12 and 18 µg/g treatments after 30 days ($p < 0.0001$). However no significant differences were observed for any of the treatments at 60 days when compared to control fish ($p = 0.19$). All of the treatments were significantly different from the controls after 90 days of exposure ($p < 0.05$) (Table 3). When the condition index data within each treatment were analyzed over time, the results showed that index values at 60 days were significantly reduced compared to all the other exposure times (ANOVA: $p < 0.0001$; Dunnet's $t = 2.178$, $p = 0.05$).

Se ACCUMULATION

A positive relationship between Se dose and accumulation was observed at each sampling time (Table 4, Figure 5). Total tissue Se accumulation was not significantly different from the control fish after 30 days, although mean Se concentrations were two to four times higher than the control ($p > 0.05$). After 60 days fish exposed to the highest SeMe treatment contained a significantly higher concentration of Se in their tissue (ANOVA: $p = 0.027$; Dunnet's $t = 2.449$, $p < 0.05$). Significantly higher concentrations of Se were also measured in fish from all SeMe exposed treatments after 90 days (ANOVA: $p < 0.0001$; Dunnet's $t = 2.449$, $p < 0.05$).

The average concentration of Se in the fish tissue appeared to decrease between 60 and 90 days in all treatment groups including controls (Figure 5). Statistical analysis of the data for the control fish showed that the Se concentration in tissue at 60 days was significantly greater than the controls at 30 and 90 days. There was no significant difference between the 30 and 90 days controls. A reanalysis of the 90-day tissue samples confirmed that the values for SeMe exposed fish had decreased when compared to previous sampling periods. The 90-day reanalysis results also showed a significant dose response relative to the control; tissue concentrations in all three Se exposure groups were significantly different from the control.

HEPATIC LIPID PEROXIDATION AND HEPATIC GSH:GSSG

Lipid peroxidation and GSH:GSSG ratios were unchanged by SeMe treatment (Table 5). Lipid peroxidation after 90-days exposure ranged from 0.03 to 0.08 nmol/ml MDA Eq among the treatments. GSH: GSSG ratio ranged from 1.71 to 2.04 after 90 days of exposure. These differences were not statistically significant.

DISCUSSION

The fish survived well in the different selenium (SeMe) exposure concentrations. Most of the mortalities were registered during the first days of the study and were not proportional to the Se exposure level, suggesting that these mortalities were due to preexisting conditions or perhaps initial stress from the laboratory environment.

The GSH:GSSG ratio and the quantification of lipid peroxidation yielded no significant differences, indicating that the Se concentrations used did not cause oxidative stress. The lack of response of these sublethal endpoints indicates that the observed growth impacts were due to mechanisms other than changes in the GSH:GSSG ratio or lipid peroxidation. Although prone to selenoxide formation and GSH oxidation via flavin monoxygenase activation in vitro, GSH did not appear to be depleted in the livers of any of the exposed animals indicating either redox cycling did not occur or other cellular defenses against oxidative stress were available to prevent injury. These results contrast studies in avian species where GSH:GSSG ratios have been shown to be reduced by Se (Hoffman et al. 1989; Spallholz and Hoffman 2002; Hoffman 2002). It is well recognized that Se induces GSH peroxidase, which catabolizes lipid hydroperoxides to the corresponding lipid hydroxide (for review see Burk 1997). Therefore, one may speculate protection against lipid peroxidation may occur in Se-treated animals. However, the lack of GSH consumption in the current study is unclear and suggests the induction of other feedback loops enhancing either reducing equivalent (i.e. NADPH) or GSH synthesis. Clearly, more work is needed to understand the molecular mechanisms of selenium toxicity in non-mammalian organisms.

This study demonstrated that both the length and concentration of dietary SeMe exposure negatively impacted larval trout growth. After 90 days the fish showed reduced growth at the 4.6, 12 and 18 g concentrations, resulting in a dietary threshold for effects (LOEC) of 4.6 g. These results coincide with other studies, which have shown that rainbow trout fed 13 µg/g of selenite for 20 weeks exhibited reduced growth and reduced survival (Hilton *et al.*, 1980). Another study with rainbow trout showed that fish fed 11 µg/g (dry diet) for 16 weeks had reduced body weight when compared to those that were fed 7 µg/g of selenite (Hilton and Hodson, 1983). Hamilton *et al.* (1990) showed that dietary Se was toxic to fish in the range of 3 to 5 µg/g. A value of 4 µg/g for invertebrates has been recommended as a threshold for a hazing program for birds by the California Department of Fish and game (see Hamilton 2002 for review).

The results of the present study showed a direct relationship between Se tissue accumulation and exposure concentration. The relationship between Se accumulation and time of exposure was not as clear. The fish contained lower concentrations of total Se after 90 days when compared to tissue samples from previous exposure times. A similar trend was also seen in the controls, although there was no change in tissue Se concentration between 30 and 90 days. The data trends indicate that a physiological change may have occurred in the fish during the latter half of the experiment. Evidence

of such a change is shown by the condition index and length data, which showed abrupt changes in the control and treatment groups between 60 and 90 days of exposure.

Larval rainbow trout mature into juveniles between the sizes of 2.5 to 13 cm (Morrow, 1980), the same size range of the fish used in this experiment. Furthermore, young trout experience body elongation at 60-90 days (Westers, 2001), which becomes evident in the results from the growth condition index. The divergences in condition index and Se body burden observed between 60 and 90 days may have been the result of a change in Se uptake or excretion produced by the fish becoming juveniles during that period of time. Other researchers have observed that essential metals accumulated in fish decreased after a certain time of constant chronic exposure. Perkins *et al*. (1997) observed that hepatic concentrations of copper in catfish exposed to aqueous copper sulfate for 10 weeks reached a maximum concentration at 6 weeks and then declined. Hamilton *et al*. (1990) observed a similar phenomenon with Chinook salmon demonstrating a 20% reduction of total Se in control animals after 90 days. Since Se is an essential nutrient, its rate of uptake, metabolism, or excretion may have been influenced by physiological changes in the fish.

The growth results for the 90-day experiment indicate a body burden threshold (LOEC) of 0.51 µg/g (wet weight basis). This value is substantially below tissue residue thresholds reported by others (for review see Hamilton 2002). De Forest *et al*. (1999) reported a whole body Se tissue threshold of 3.3-4 µg/g (dry weight) for effects on early life stage rainbow trout. The threshold value obtained in the present experiment is also less than the value of 4 µg/g recently proposed as a tissue-based criteria for adverse effects in fish (Maier and Knight 1994; Hamilton 2002). The lower body burden effects concentration obtained in the present experiment may have been due to reduction of Se uptake or enhanced Se elimination at the 60-90 day period of development. Other possibilities for the lower threshold include the use of younger fish, an ambient light:dark cycle, a lack of Se in the gut contents of fish during whole body measurement (although feeding behavior was never impaired throughout the study), or the use of a wet weight determination of total Se.

Recent studies of fish tissue Se in San Diego Creek and other channels indicate that resident fish species may be impacted by selenium. Tissue Se concentrations for red shiner, measured by the Toxic Substances Monitoring Program (TSMP), ranged from 0.4 to 1.6 µg/g (wet weight), which is above the tissue effects threshold obtained for larval trout (Figure 6). The TSMP data represent adult fish of a different species, which may be more tolerant than the larval trout used in this experiment. The use of the data from this experiment to estimate the risk from Se in San Diego Creek would be aided by studies that include the measurement of Se in the larval stages and diet of resident fish.

In summary, rainbow trout experience sublethal effects on growth, weight and condition following 90 days of dietary exposure to SeMe. The dietary LOEC was 4.6 µg/g Se. The body-burden threshold value for adverse effects was 0.51 µg/g Se. Adverse effects were not related to oxidative stress as measured by hepatic lipid peroxidation and GSH:GSSG ratios indicating other mechanisms may be involved in the toxicity of Se in fish. More

studies are needed to examine the uptake and disposition of Se in its various organic forms in fish at different stages of development to better understand its toxicity in wildlife.

Literature Cited

Baker, M.A., G. J. Cerniglia and A. Zaman. 1990. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. *Anal Biochem 190*: 360-365.

Burk R.F. 1997. Selenium-dependent glutathione peroxidases In: F.P Guengrich volume editor; Biotransformation Volume 3 Comprehensive Toxicology (ed. I.G. Sipes; C.A. McQuenn, A.J. Gandolfi) New York, NY; Elsevier pp 229-242.

California Regional Water Quality Control Board (CRWQCB), Santa Ana Region. 2001. Toxics total maximum daily loads (TMDLs) for the San Diego Creek/Newport Bay watershed. Selenium TMDL Draft. California Regional Water Quality Control Board, Santa Ana, California. 40 pp.

Chen, G.P. and D.M. Ziegler. 1994. Liver microsome and flavin-containing monooxygenase catalyzed oxidation of organic selenium compounds. *Archives of Biochem Biophys* 312: 566-572.

Chen, X., G.Q. Yang, J. Chen, X. Chen, Z. Wen, and K. Ge. 1980. Studies on the relations of selenium and Keshan Disease. *Biol Trace Elements Res* 2: 91-107.

Dungan, R.S. and W.T. Frankenberger. 1999. Microbial transformations of selenium and the bioremediation of seleniferous environments. *Bioremediation J 3*: 171-188.

Fan, T.W.M., D.E. Hinton, and R.M. Higashi. 2002. The Selenium biotransformations into proteinaceous forms by food web organisms of selenium-l aden drainage waters in California. *Aquat Toxicol 57*: 65-84.

Hamilton, S.J., K.J. Buhl, N.L. Faerber, R.H. Wiedmyer and F.A. Bullard. 1990. Toxicity of organic selenium in the diet to Chinook salmon. *Environ Toxicol Chem* 9: 347-358.

Hamilton, S.J. (2002) Rationale for a tissue-based selenium criterion for aquatic life. *Aqua Toxicol* 57:85-100.

Hilton, J.W., P.V. Hodson, and S.J. Slinger. 1980. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). *J Nutr* 110: 2527-2535.

Hilton, J.W. and P.V. Hodson. 1983. Effect of increased dietary carbohydrate on selenium metabolism and toxicity in rainbow trout (*Salmo gairdneri*). *J Nutr* 113: 1241-1248.

Hoffman, D.J. 2002. Role of selenium toxicity and oxidative stress in aquatic birds. *Aquat Toxicol* 57: 11-26.

Hoffman, D.J., Heinz, G.H., Krynitsky, A.J. 1989. Hepatic glutathione metabolism and lipid peroxidation in response to excess dietary selenomethionine and slenite in mallard ducklings. *J. Toxicol. Environ. Health* 27:263-271.

Jentzsch, A.M., H. Bahmann, P. Furst and H.K. Biesalski. 1996. Improved analysis of malondialdehyde in human body fluids. *Free RadicalBiology and Medicine* 20: 251-256.

Kwon, T. and B. Watts. 1964. Malonaldehyde in aqueous solution and its role as a measure of lipid oxidation in foods. *J Food Sci* 29: 294-302.

Lemly, A.D. 1993. Teratogenic effects of selenium in natural populations of freshwater fish. *Ecotoxicol. Environ. Safety* 26:181-204.

Maier, K.J. Knight, A.W. 1994. Ecotoxicology of selenium in freshwater systems. *Rev. Environ. Contam.Toxicol.* 134:31-48.

Morrow, J. 1980. The freshwater fishes of Alaska. Alaska Northwest Publishing Company. Anchorage, AK.

National Research Council, Committee on Medical and Biological Effects of Environmental Pollutants Selenium. 1976. National Academy of Sciences, Washington D.C.

Perkins, E.J., Griffin, B., Hobbs, M., Gollon, J., Wolford, L. and D. Schlenk. 1997. Sexual differences immortality and sublethal stress in channel catfish following a 10 week exposure to copper sulfate. *Aquat Toxicol* 37: 327-339.

Presser, T.S. and I. Barnes. 1985. Dissolved constituents including selenium in waters in the vicinity of Kesterson National Wildlife Refuge and the West Grassland, Fresno and Merced Counties, California. *USGS Wat Resour Invest Rept* 4220: 84-73.

Rogers, J.M. and E.S. Hunter. 2001. Redox redux: A closer look at conceptual low molecular weight thiols. *Toxicol Sci* 62: 1-3.

Schwarz, K. and C.M. Foltz. 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J Am Chem Soc* 79: 3292-3293.

Shrift, A. 1973. Metabolism of selenium by plants and microorganisms. In: D.L. Klayman and W.H.H. Gunther (Eds.), Organic Selenium Compounds: Their Chemistry and Biology, Wiley, New York. pp. 763-814.

Spallholz, J.E., and D.J. Hoffman. 2002. Selenium toxicity: cause and effects in aquatic birds. *Aquat Toxicol* 57: 27-37.

USEPA (U.S. Environmental Protection Agency). 2002. Total Maximum Daily Loads for toxic pollutants San Diego Creek and Newport Bay, California. USEPA Region 9. Public Review Draft. pp 80.

Westers H. 2001 Production In: Wedemeyer G. A. ed Fish hatchery management (2nd edition). Bethesda, MD: American Fisheries Society. p 31-90.

Weiss, K.F., J.C. Ayres and A.A. Kraft. 1965. Inhibitory action of selenite on *Escherichia coli*, *Proteus vulgaris*, and *Salmonella thompson*. *J Bacteriol* 90: 857-862.

Ziegler, D.M. 1988. Flavin-containing monooxygenases: Catalytic mechanism and substrate specificities. *Drug Metab Rev* 19: 1-32.

Table 1. Trace metal analysis of the unspiked fish food used in the experiment.

Metal (µg/g)	Result	Reporting Limit
Antimony	1.90	0.75
Arsenic	ND	0.75
Barium	5.80	0.50
Beryllium	ND	0.25
Cadmium	ND	0.50
Chromium	1.13	0.25
Cobalt	0.42	0.25
Copper	21.1	0.50
Lead	ND	0.50
Mercury	ND	0.08
Molybdenum	1.76	0.25
Nickel	1.57	0.25
Selenium	0.23	0.75
Silver	ND	0.25
Thallium	ND	0.75
Vanadium	1.23	0.25
Zinc	85.0	1.00

ND= Not detected.

Table 2. Water quality parameters in the Se exposure aquaria. Ranges are shown in the table.

Time (Week)	pH	Ammonia (mg/L)	Conductivity (µS/cm)
1	7.0-8.2	0.0-0.50	0.01-0.05
2	7.7-8.2	0.0-1.00	0.04-0.09
3	7.8-8.3	0.0-1.00	0.03-0.09
4	7.9-8.4	0.0-1.00	0.03-0.09
5	7.7-8.2	0.0-0.05	0.02-1.00
6	7.8-8.1	0.0-0.50	0.03-1.00
7	7.7-8.1	0.0-0.50	0.03-1.00
8	7.6-8.0	0.0-0.05	0.01-0.09
9	7.7-8.1	0.0-0.00	0.01-0.12
10	7.4-7.9	0.0-0.00	0.03-0.09
12	7.5-7.8	0.0-0.05	0.03-1.00
13	7.5-8.0	0.0-0.05	0.01-1.11

Table 3. Rainbow trout growth after four different selenomethionine exposures (SeMe as µg/g). Average values for results at 0, 30, 60 and 90-days. Standard deviation in parenthesis.

Time (Day)	Treatment (µg/g)	Weight (g)	Fork Length (cm)	Condition Index (g/cm^3)
0	Control	0.37 (0.30)	3.14 (0.41)	0.01138 (0.0004)
	4.6	0.41 (0.06)	3.17 (0.12)	0.01283 (0.0007)
	12	0.54 (0.01) *	3.44 (0.14) *	0.01329 (0.0013) *
	18	0.55 (0.05) *	3.40 (0.24)	0.01321 (0.0009) *
30	Control	1.33 (0.92)	4.66 (0.41)	0.01283 (0.0008)
	4.6	1.25 (0.21)	4.84 (0.29)	0.01085 (0.0005)
	12	1.33 (0.30)	5.09 (0.46)	0.00992 (0.0008) *
	18	1.31 (0.37)	4.97 (0.50)	0.01045 (0.0011) *
60	Control	2.96 (0.92)	6.91 (0.56)	0.00864 (0.0018)
	4.6	2.33 (0.63)	6.69 (0.67)	0.00747 (0.0002)
	12	2.52 (0.38)	6.88 (0.35)	0.00767 (0.0005)
	18	2.59 (0.24)	6.92 (0.24)	0.00741 (0.0005)
90	Control	5.17 (1.09)	7.70 (0.33)	0.01137 (0.0012)
	4.6	3.45 (0.35) *	6.93 (0.19) *	0.01020 (0.0002) *
	12	3.45 (0.35) *	6.84 (0.68) *	0.01016 (0.0002) *
	18	3.82 (0.62) *	7.37 (0.62) *	0.01019 (0.0004) *

* Statistically significant difference from control ($p \leq 0.05$)

Table 4. Total Se tissue concentration in µg/g wet basis (standard deviation in parenthesis).

Treatment (µg/g)	30-day	60-day	90-day	90-day Rerun
Control	0.46 (0.20)	1.24 (0.54)	0.29 (0.14)	0.32 (0.26)
4.6	1.05 (0.77)	1.70 (0.72)	0.51 (0.19) *	0.66 (0.22) *
12	1.81 (1.04)	1.83 (0.94)	1.08 (0.16) *	1.31 (0.25) *
18	1.60 (0.93)	2.62 (1.22) *	1.06 (0.19) *	1.77 (0.35) *

* Statistically significant difference from control ($p \leq 0.05$).

Table 5. Quantification of hepatic lipid peroxidation (TBARS) nmol/ml MDA Eq and hepatic GSH:GSSG ratio in SeMe exposed larval rainbow trout at 60 and 90 days. Standard deviation in parenthesis.

Treatment	Hepatic Lipid Peroxidation nmol/ml		Hepatic GSH: GSSG	
(μg/g)	60-day	90-day	60-day	90-day
Control	0.18 (0.13)	0.08 (0.07)	1.38 (0.31)	2.04 (0.34)
4.6	0.27 (0.05)	0.07 (0.09)	1.31 (0.37)	1.71 (0.28)
12	0.17 (0.08)	0.03 (0.02)	1.28 (0.31)	1.91 (0.07)
18	0.25 (0.28)	0.06 (0.06)	1.00 (0.04)	1.88 (0.03)

Figure 1. Percent mortality of rainbow trout after 90 days of exposure.

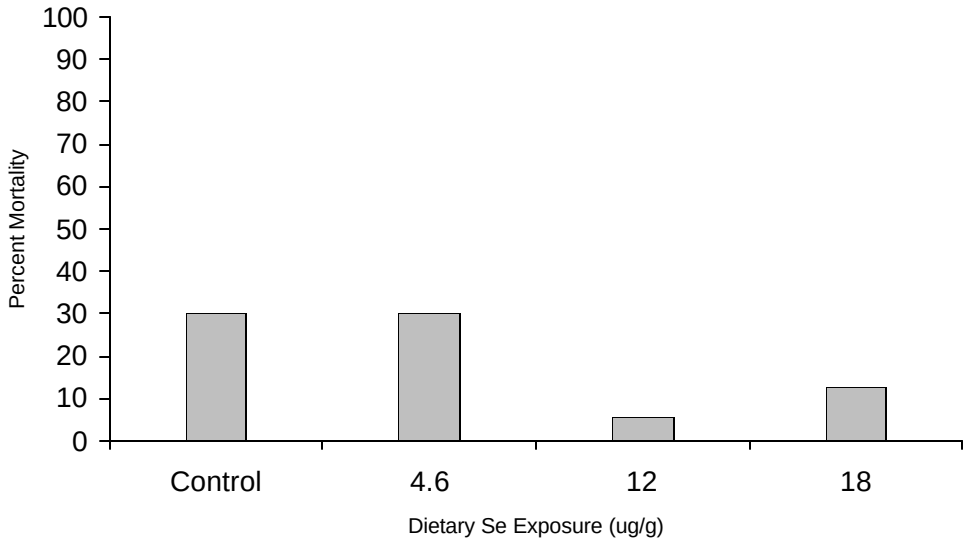


Figure 2. Change in rainbow trout weight (g) and fork length (cm) during 90 days for each treatment.

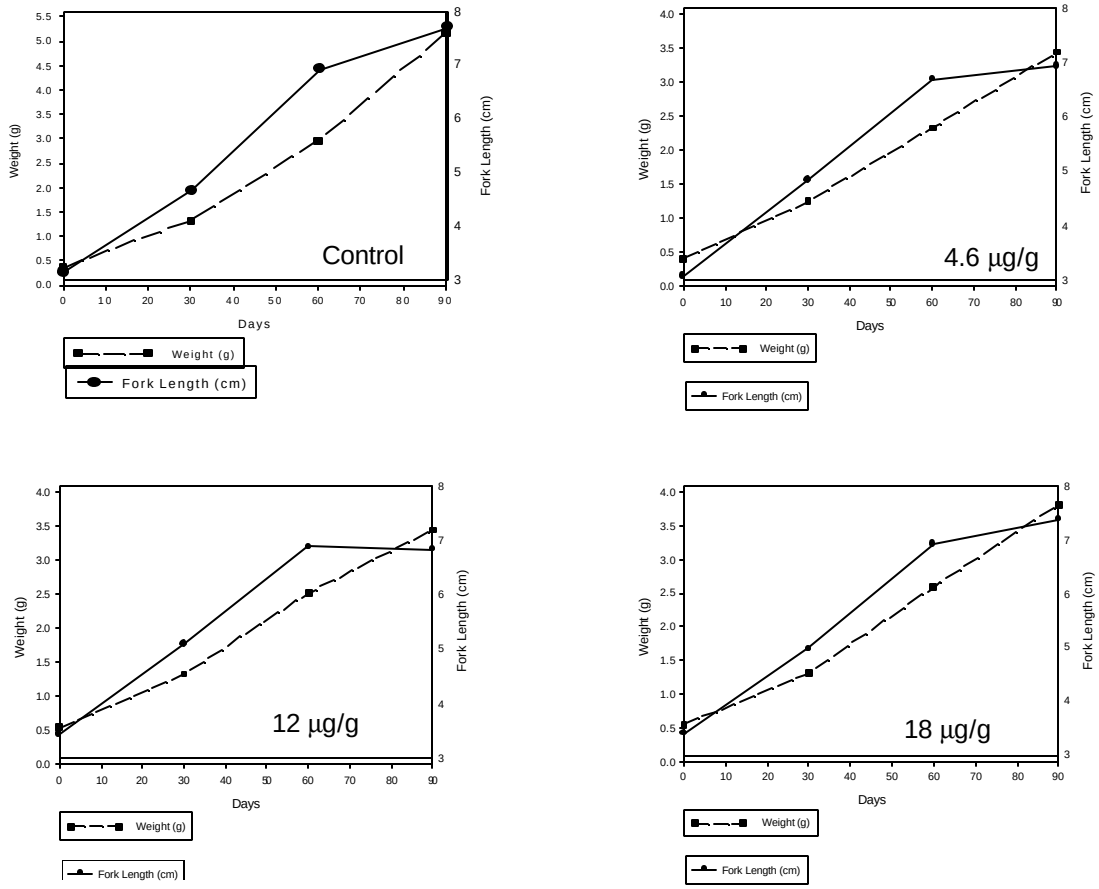
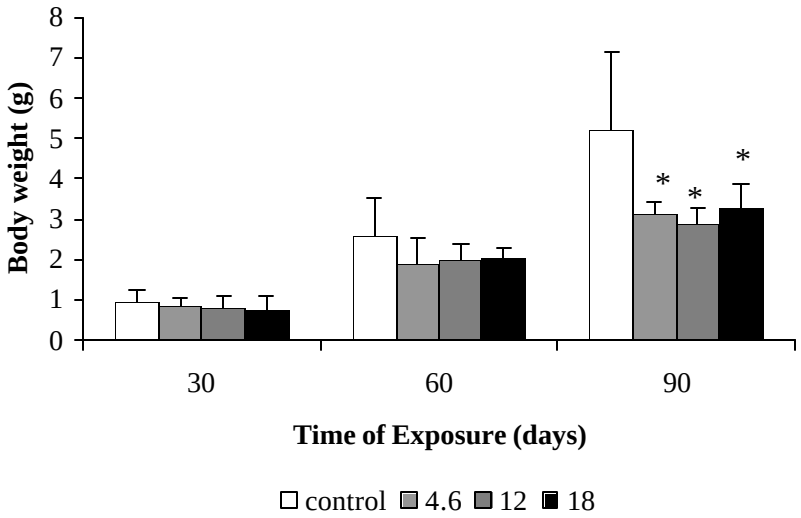


Figure 3. Trout growth as net change in individual body weight at different exposure lengths after exposure to Control, 4.6, 12and 18 µg/g of dietary SeMe (error bars represent the standard deviation).



*= Statistically significant difference from control ($p \leq 0.05$).

Figure 4. Condition index mean values for 0, 30, 60 and 90 days.

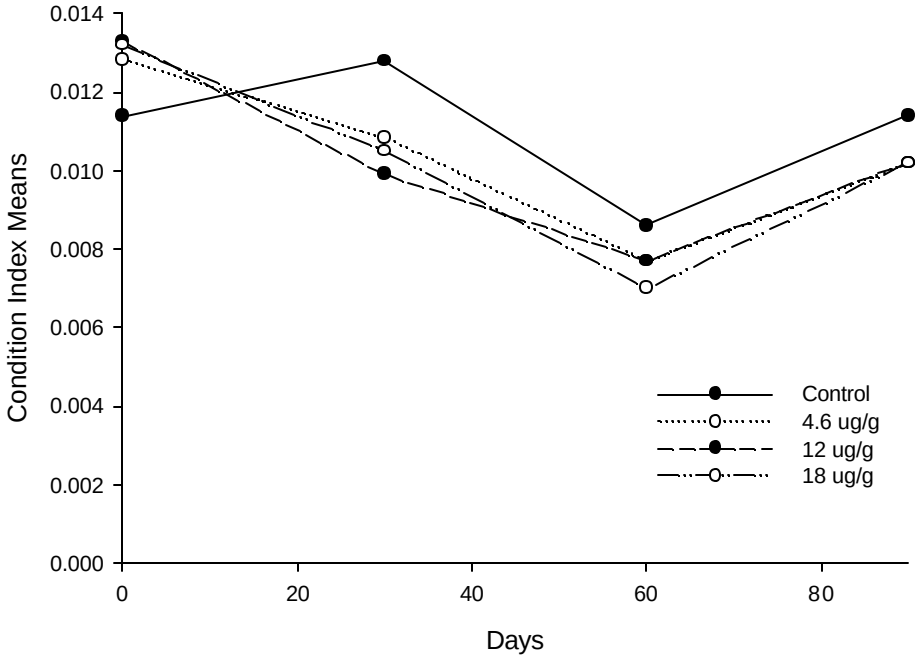
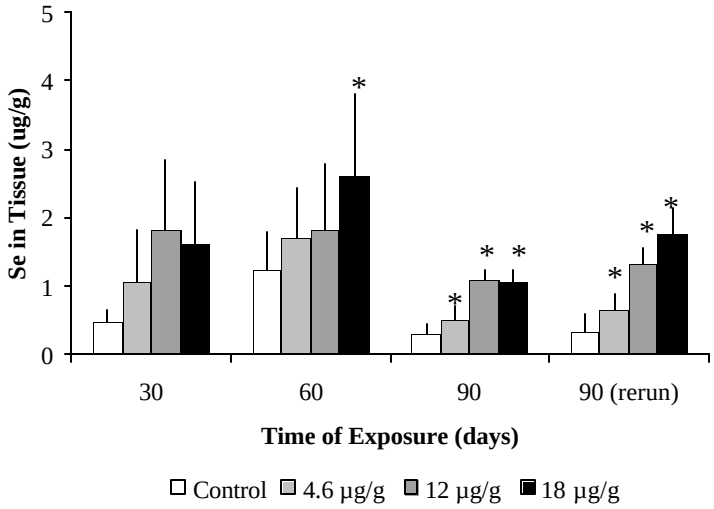
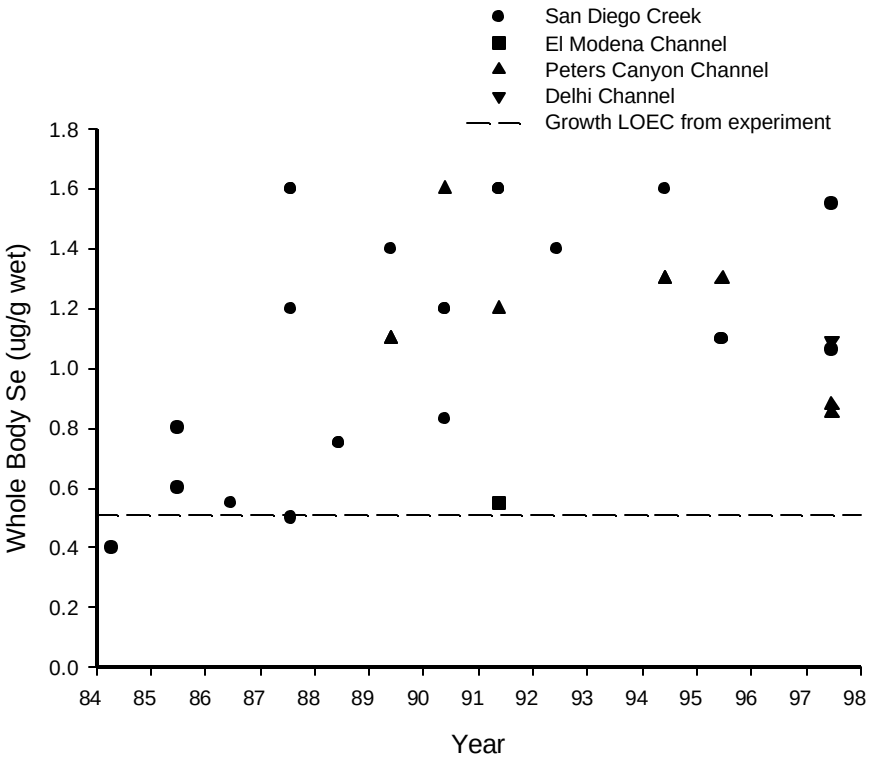


Figure 5. Whole body total Se concentration (µg/g tissue wet weight) in rainbow trout after 30, 60 and 90-d food SeMe exposure (error bars represent the standard deviation).



* Statistically significant difference from control ($p \leq 0.05$)

Figure 6. Whole body concentration of Selenium in red shiner collected from San Diego Creek and other channels in the Newport Bay watershed (data from the Toxic Substances Monitoring Program).



APPENDIX

Fish Data

Table A-1. Individual fish values for weight, fork length and condition index after 0, 30, 60 and 90 days.

Treatment (µg/g)	0 Days			30 Days			60 Days			90 Days		
	Weight (g)	Fork Length (cm)	Condition Index (g/cm^3)	Weight (g)	Fork Length (cm)	Condition Index (g/cm^3)	Weight (g)	Fork Length (cm)	Condition Index (g/cm^3)	Weight (g)	Fork Length (cm)	Condition Index (g/cm^3)
Control	0.350	3.040	0.01186	0.900	4.10	0.01290	2.920	6.90	0.00825	5.215	7.90	0.01050
	0.384	3.280	0.01101	1.575	4.85	0.01384	4.260	7.25	0.01156	7.290	8.52	0.01162
	0.286	2.900	0.01083	1.400	4.80	0.01250	2.490	6.45	0.00885	5.534	8.10	0.01036
	0.343	3.070	0.01140	1.170	4.40	0.01310	1.800	6.30	0.00717	7.225	8.60	0.01300
	0.493	3.400	0.01180	1.620	5.15	0.01179	3.320	7.65	0.00738	---	---	---
4.6	0.473	3.333	0.01230	1.090	4.55	0.01130	2.320	6.65	0.00745	3.400	6.67	0.01022
	0.393	3.170	0.01229	1.030	4.60	0.01039	1.650	5.90	0.00786	3.305	6.88	0.00989
	0.327	3.000	0.01200	1.580	5.25	0.01084	1.775	6.20	0.00742	3.613	6.94	0.01014
	0.380	3.130	0.01265	1.250	4.80	0.01140	3.140	7.50	0.00723	3.968	7.22	0.01054
	0.463	3.233	0.01390	1.295	5.00	0.01030	2.750	7.20	0.00736	3.474	6.92	0.01023
12	0.540	3.333	0.01390	1.070	4.80	0.00970	2.095	6.60	0.00714	2.882	6.10	0.01000
	0.560	3.700	0.01104	1.190	4.70	0.01095	2.730	7.05	0.00778	3.538	6.60	0.01036
	0.560	3.433	0.01389	1.660	5.40	0.01055	2.625	6.80	0.00843	3.490	6.60	0.00991
	0.533	3.370	0.01395	1.660	5.75	0.00873	3.005	7.40	0.00728	3.464	7.90	0.01020
	0.517	3.370	0.01365	1.075	4.80	0.00968	2.165	6.55	0.00770	3.860	7.15	0.01033
18	0.550	3.533	0.01240	1.950	5.75	0.01027	2.580	7.25	0.00675	4.553	8.20	0.00990
	0.600	3.633	0.01267	0.995	4.50	0.01024	2.515	6.75	0.00780	3.660	6.90	0.00985
	0.437	3.570	0.01257	1.245	5.10	0.00938	2.550	6.90	0.00762	4.358	6.70	0.01071
	0.573	3.130	0.01393	1.175	4.90	0.01005	2.340	6.65	0.00791	3.140	7.40	0.01003
	0.620	3.150	0.01447	1.190	4.60	0.01230	2.990	7.05	0.00697	3.362	6.80	0.01036

Table A-2. Individual fish values of total selenium in tissue (wet weight).

Treatment mg/g	Replicate	Day 30	Day 60	Day 90
Control	A	0.642	1.360	0.530
	B	0.402	1.760	0.413
	C	0.503	0.520	0.140
	D	0.299	2.025	0.230
	E	0.286	1.378	0.210
	F	0.309	0.790	0.170
	G	0.797	0.874	0.370
4.6	A	1.790	4.600	0.520
	B	2.330	2.265	0.540
	C	1.200	0.719	0.540
	D	0.780	0.789	0.530
	E	0.208	2.365	0.530
	F	0.523	1.425	0.130
	G	0.534	2.288	0.770
	H		2.060	-------
12	A	0.802	2.045	1.070
	B	2.450	1.980	1.030
	C	3.335	3.712	1.460
	D	1.470	2.195	1.132
	E	2.251	1.697	1.193
	F	2.562	2.445	0.941
	G	0.597	1.440	1.000
	H	0.417	1.375	0.920
	I	2.960	1.456	0.920
	J	1.215	-------	1.120
18	A	4.000	2.678	0.770
	B	1.900	3.311	1.070
	C	1.370	2.909	1.030
	D	1.200	1.855	1.460
	E	0.730	2.835	1.132
	F	0.836	4.595	1.193
	G	1.132	2.735	0.941
	H	1.355	2.640	0.920
	I	1.727	-------	0.920
	J	1.773	-------	1.120