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Bioaccumulation and Chronic Toxicity of Dietary L-Selenomethionine in Juvenile White Sturgeon (*Acipenser transmontanus*)

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Abstract:

An 8-week growth trial was conducted to determine the sensitivity of white sturgeon to the toxicological effects of elevated dietary selenium (Se). Juvenile sturgeon were fed diets supplemented with Se in the form of L-selenomethionine (SeMet), resulting in dietary concentrations of 0.4, 9.6, 20.5, 41.7, 89.8, and 191.1 µg Se/g diet. Effects of dietary SeMet on survival, swimming activity, growth, whole body proximate composition, tissue Se concentrations, and histopathology were determined. Sturgeon survival among treatment groups did not differ significantly with a mean survival rate of 99±0.43% across all groups. A significant decrease ($p<0.05$) in swimming activity and growth rate was observed in sturgeon fed above 20.5 µg Se/g diet. Dietary SeMet concentrations were negatively correlated with whole body protein and lipid contents but positively correlated with ash and moisture contents were positively correlated with dietary SeMet concentrations. Selenium accumulated in the kidney, muscle, liver, gill, and plasma tissues in a dose-dependent manner. Histopathological alterations in the liver and kidneys were observed in sturgeon fed above 9.6 µg Se/g diet. The threshold dietary Se toxicity concentration was estimated at 10 µg Se/g diet but additional studies with more sensitive life stages and multiple stressors are needed in order to determine if dietary threshold should be lowered to minimize the potential impacts of Se on white sturgeon in the San Francisco Bay-Delta.

Keywords: Selenomethionine; Selenium; White sturgeon; Toxicity; Histopathology; Swimming activity

1. Introduction

Field surveys of white sturgeon (*Acipenser transmontanus*) in the San Francisco (S.F.) Bay-Delta over the past 25 years have revealed the presence of elevated selenium (Se) concentrations up to 15 and 30 µg/g dry weight (dw) in muscle and liver samples, respectively (Urquhart and Regalado, 1991; Linville et al., 2002). Although water Se levels concentrations never exceeded 5 µg Se/L, the concentration established by the EPA to be safe to aquatic organisms (USEPA, 1992); these tissue Se concentrations are well above the liver (12 µg Se/g dw) and muscle (8 µg Se/g dw) tissue concentrations shown to cause toxic effects in freshwater and anadromous fish (Lemly, 2002a). The high levels of Se found in white sturgeon are thought to be the result of their feeding habits and life history characteristics (Luoma and Presser, 2000).

The white sturgeon is a bottom feeder which has been found to feed on shrimp, amphipods, mollusks, and fish (Billard and Lecointre, 2001). According to field surveys over the last 40 years, the Asian clam (*Potamocorbula amurensis*) has become the main food source of white sturgeon, which has dominated the benthic macroinvertebrate community in the S.F. Bay-Delta since 1986 (McKechnie and Fenner, 1971; SWRCB, 1991). This clam accumulates Se to unusually high levels due to its efficient filtration of the water column, presumably exposing sturgeon to increased Se levels (Brown and Luoma, 1995; Luoma and Presser, 2000). The white sturgeon is known to be one of the largest sturgeon species, having been recorded to reaching 6.1 m in length and 816 kg in weight (Billard and Lecointre, 2001). The time to puberty is particularly long in wild white sturgeon with males reaching puberty at approximately 12 years of age and females reaching puberty between 16-35 years of age (Billard and Lecointre, 2001). The long life

span of white sturgeon combined with the long time to puberty provides a lengthy period of time for Se bioaccumulation and bioconcentration in the somatic and reproductive organs. Selenium concentrations in the developing ovaries of white sturgeon collected from the S.F. Bay Delta region were found to be as high as 72 µg/g dw in the ovarian and 29 µg/g dw in the eggs (Kroll and Doroshov, 1991). These concentrations were much higher than the threshold concentrations known to cause toxicity in developing larvae of other fish species (Deforest et al., 1999; Lemly 2002a).

Although legislation in recent decades has led to reduced Se concentrations in the S.F. Bay-Delta, the U.S. Bureau of Reclamation has promised farmers to remove the Se-laden agricultural drainage off farmlands and is currently debating whether to dispose of the drainage water into the S.F. Bay-Delta (Luoma and Presser, 2000; Hug et al., 2000). If such a disposal plan is adopted, Luoma and Presser (2000) have forecasted Se concentrations in the major food sources of the white sturgeon to increase between 5-40 times above the generally accepted 3-5 µg Se/g dietary threshold for Se toxicity in fish (Lemly, 2002a; Hamilton, 2004). However, the toxicity of ecologically relevant forms of Se at dietary concentrations forecast by Luoma and Presser (2000) have not been previously investigated in the white sturgeon, a fish species morphologically and physiologically different than previously studied species. This study was conducted to determine the kinetics of Se accumulation and the sensitivity of white sturgeon to dietary Se toxicity.

2. Materials and Methods

2.1. Supply and Maintenance of White Sturgeon

Six-hundred juvenile white sturgeon were obtained from The Fishery (Galt, CA) and acclimated to 18-19°C water for 2 weeks. Fish were fed a commercial diet (Silver Cup, Nelson and Sons, Murray, UT, 2.5 μg Se/g diet) during the acclimation period and were gradually weaned to a purified diet during the week preceding the growth trial. Fish of similar size (29.8 ± 1.0 g) were selected and moved to 24 90-L tanks with 25 fish per tank. Selenium in the water supply was sampled weekly and was undetectable (<1 μg/L) throughout the experiment.

2.2. Dietary Exposure and Sampling Protocol

Six isoenergetic and isonitrogenous experimental diets were formulated and manufactured similar to a sturgeon purified diet (Hung et al., 1987; Hung and Lutes, 1988) which is known to support excellent growth. The experimental purified diet was constituted from the following ingredients (g per 100 g diet): vitamin-free casein (31), wheat gluten (15), egg albumin (4), dextrin (29.5), vitamin mix (0.5), BTM-mineral mix (3), cellulose (4.5), corn oil (6), cod liver oil (6), choline chloride (0.5), and santoquin (0.019). The vitamin mix formulation included the following ingredients(g/kg mix): thiamin mononitrate (3), riboflavin (18), niacin (30), calcium pantothenate (30), pyridoxine HCl (6), vitamin B_{12} crystal (0.012), folic acid (1.08), biotin (0.3), inositol (30), ascorbic acid (300), menadione (12), vitamin A acetate, 20,000 IU/g, (15), vitamin D3, 400,000 IU/g, (0.6); DL-α-tocopheryl acetate, 250 IU/g, (20), cellulose (534). These diets were supplemented with graded levels of Se as L-(+)-selenomethionine (SeMet, Fisher Scientific, Pittsburgh, PA). The resulting dietary Se concentrations were 0.4, 9.6,

20.5, 41.7, 89.8, and 191.1 µg Se/g diet based on the analysis of the diets by perchloric acid digestion/HCl reduction followed by fluorescence and ICP-MS determination (Fan et al., 1998). The concentration range was chosen to span the possible dietary Se concentrations forecast by Luoma and Presser (2000) if the Se-laden drainwater is drained into the S.F. Bay-Delta. L-selenomethionine was chosen as the dietary Se form because the Se found in the natural diet of the white sturgeon is predominantly in an organic rather than inorganic form (Fan et al., 2002). Furthermore, previous studies have shown that the toxic responses in fish fed SeMet were similar to the responses of fish fed diets containing naturally incorporated Se compounds (Hamilton, 2004). The six Se-supplemented diets were randomly assigned to 24 tanks, resulting in four replicate tanks per dietary treatment. The 24 fiberglass tanks (66-cm diameter, 27-cm height, 90-L water volume) were arranged in three rows with eight tanks per row and were supplied with aerated well water at a rate of 1.5 L/min/tank (18-19°C, dissolved oxygen >8.0 mg/L, pH between 7.5-8.0). Fish were weighed once every two wks to monitor growth and to adjust feeding rates. Fish were not fed the day before weighing to minimize weighing discrepancy and stress. The daily ration of feed for each tank was placed in automatic feeders each morning and continuously dispensed into the tanks (Cui et al., 1997). The daily ration was based on the optimal feeding rate equation established by Cui and Hung (1995).

Sturgeon general activity levels were monitored at four and eight wks of exposure to dietary Se. A video camcorder was used to record fish activity during a 5-min interval per tank. Five fish from each tank were randomly chosen and the activity of those fish during the 5-min recording period was quantified. The activity level of each fish was quantified by determining the number of squares traversed on a grid system. Fish were allowed a 5-min acclimation period to the disturbance caused by placing the recording equipment over each tank. The entire

recording procedure was repeated the next day, resulting in two measurements per tank. Feed was withheld from tanks during each 10-min acclimation and recording interval.

Fish were starved for 24 h prior to samplings. Four fish from each tank were sampled at 0, 4, and 8 wks during the dietary Se exposure to determine fish weight, fork length, liver weight, condition factors (CF), hepatosomatic indices (HSI), and hematocrit (Hct) (Table 1). Fish were euthanized with a tricaine methanesulfonate solution (0.5 g/L, MS222, Argent Chemical Laboratories, Redmount, WA). Following euthanization, blood was sampled by caudal sectioning and centrifuged (4500 x *g*) to obtain plasma. Kidney, liver, gill, and white muscle tissues were removed and divided into a large and a small portion. The larger portion was frozen in liquid nitrogen for selenium analysis and the smaller portion was fixed in 10% neutral buffered formalin for histopathologic examination. Hematocrit was determined by centrifuging microcapillary tubes filled with freshly sampled blood and recording the packed red blood cell content.

2.3. Proximate Composition

Eight fish per treatment at 0 and 8 wks during the dietary Se exposure were sampled to determine whole body Se concentrations and proximate composition. Fish were not fed for 24 h prior to sampling to ensure evacuation of the gastrointestinal tract. Whole body samples were lyophilized and pulverized prior to Se analysis. Proximate composition of the whole body and diet samples were determined according to AOAC, 1984.

2.4. Se Analysis

Plasma samples were directly subjected to Se analyses, while kidney, liver, and muscle tissues were lyophilized and homogenized prior to Se analyses. Tissue Se concentrations were

determined by micro-digestion of samples in nitric acid, followed by the reduction of Se from the +6 to +4 state with HCl and derivatization of Se with 2,3-diaminonaphthalene. Selenium concentrations were then measured by the fluorescence intensity generated by the benzopiazselenol product (Fan et al., 1998). Each sample was analyzed in triplicate with one of the three replicates spiked with an addition of a known standard to verify Se recovery in each sample. A dogfish liver standard (National Research Council Canada) was analyzed simultaneously with experimental samples and the observed concentration (6.89 µg Se/g) was within the certified standard range (7.06 ± 0.48 µg Se/g).

2.5. Histopathology

Liver, kidney, gill, and muscle tissues fixed in 10% neutral buffered formalin were dehydrated in a graded ethanol series and embedded in paraffin. Tissue blocks were sectioned (4 µm thick) and stained with hematoxylin and eosin (H&E). Tissue sections were examined under a BH-2 Olympus microscope for common and/or significant lesions. Lesions were qualitatively scored on a scale of 0 = not present, 1 = mild, 2 = moderate, and 3 = severe.

2.6. Statistical Analysis

Statistical differences among treatment groups were determined using the ANOVA model in the JMP 4.0 statistical package (SAS Institute, Cary, NC). Regression analyses were used to determine relationships between dietary selenium concentration and the various measured variables. The Tukey Honestly Significant Difference test was used for multiple-treatment comparisons to maintain an α=0.05 Type I error rate. All numerical values reported in the text represent mean ± standard error unless noted otherwise.

3. Results

3.1. Survival and Growth

Sturgeon survival did not significantly differ among treatment groups after the 8 wk dietary Se exposure, with a mean survival rate of 99 ± 0.43% across all groups. However, significant differences in growth rate were observed among treatment groups (Table 1). Differences in Hct among treatment groups were insignificant at the 4 wk (mean=21.9 ± 0.7 %) and 8 wk sampling points (mean=22.4 ± 0.6 %). Significant differences in the CF and HSI among treatment groups were found, with the lowest values in sturgeon fed 191.1 µg Se/g diet (Table 1).

3.2. Behavioral Effects and Activity Levels

Sturgeon fed 191.1 µg Se/g diet exhibited abnormal behavior including decreased swimming activity and confinement to tank bottoms after 2 wks of dietary SeMet exposure. A decrease in activity and increased confinement to tank bottoms became more pronounced through time in sturgeon fed 41.7-191.1 µg Se/g diet (Table 1). The presence of food in the tanks caused a burst of feeding activity in sturgeon fed 0.4-20.5 µg Se/g diet, but the intensity of the feeding bursts were lower in sturgeon fed 41.7-191.1 µg Se/g diet.

3.3. Proximate Composition

There were significant differences in the proximate composition of white sturgeon among treatment groups (Table 2). Whole body protein ($r = -0.568$, $p<0.004$) and lipid ($r = -0.780$, $p<0.001$) contents were negatively correlated with dietary SeMet concentrations while ash (r =

0.699, $p<0.001$) and moisture ($r = 0.819$, $p<0.001$) contents were positively correlated with dietary SeMet concentrations.

3.4. Selenium Accumulation

Whole body Se accumulation was observed in a dose-dependent manner at lower dietary concentrations, but not as strongly at higher dietary concentrations (Table 2). Bioconcentration of Se above dietary concentrations was observed in sturgeon exposed to 0.4 and 9.6 µg Se/g diet. Selenium accumulated in all sampled tissues throughout the 8-wk dietary Se exposure with the highest concentrations generally found in the kidney tissue (Table 3). The linear correlation between tissue and dietary Se concentrations at 8 wks of dietary Se exposure was strongest in kidney tissue($r = 0.932$), followed by liver ($r = 0.900$), gill ($r = 0.8786$), muscle ($r = 0.8593$), and plasma ($r = 0.7779$) tissues. The positive Se accumulation rate during the last 4 wks of the growth trial in most tissues suggests that Se concentrations in these tissues had not reached an equilibrium state.

3.5. Histopathology

Histopathological lesions were observed in kidney and liver of white sturgeon exposed to 0.4-191 µg Se/g diet. No lesions were observed in gill and muscle. Significant histopathological lesions were observed in the liver and kidney of sturgeon exposed to 20.5-191.1 µg Se/g diet at 8 wks (Table 4). Liver lesions included glycogen depletion (GD), hepatocellular vacuolar degeneration and necrosis (VD), and hepatocellular and bile ductural hyperplasia (HP) (Figure 1). The frequency of liver lesion occurrence was positively correlated with dietary Se while the severity of damage was not. Lesions in the kidneys included tubular dilation (TBD), tubular cell

hydropic degeneration and necrosis (TBV), and tubular inclusions with either eosinophilic or basophilic cast materials in the lumen (TBI) (Figure 2). The frequency of TBD occurrence was significantly correlated with dietary Se while the frequency of TBV and TBI occurrence were not. The severity of TBD and TBV were significantly correlated with dietary Se concentrations while TBI severity was not.

4. Discussion:

Results of this study suggest that the white sturgeon is relatively less sensitive to Se toxicity in comparison to other fish species. White sturgeon exposed to dietary concentrations ranging between 0.4-191.1 µg Se/g diet as SeMet for 8 wks exhibited a mean survival rate of 99±0.43%, while bluegill and Chinook salmon experienced significant declines in survival when exposed to dietary Se within this range. Bluegill fed 6.5 µg Se/g diet as SeMet for 8.6 wks exhibited a significantly lower survival rate (77.5±2.5%) when compared to a 95±5% survival rate in bluegill fed 1.6 µg Se/g diet (Cleveland et al., 1993). Chinook salmon also exhibited a greater sensitivity to Se toxicity in comparison to white sturgeon where survival of salmon declined significantly to 62.5±14.0% when exposed to 35.4 µg Se/g diet as SeMet for 8.6 wks compared to a 100% survival in salmon exposed to 3.2 µg Se/g diet (Hamilton et al., 1990). Although white sturgeon survival was not adversely affected after a 8-wk exposure to SeMet, the significant negative growth rate observed in sturgeon exposed to 191.1 µg Se/g diet during the final 2 wks of the growth trial, suggests that prolonged exposure to Se would likely have adverse impacts on survival.

Studies that have documented modifications of fish behavior resulting from dietary Se exposure are also limited. Cleveland et al. (1993) reported that bluegills fed SeMet (≤ 25 µg Se/g diet) for 4 wks did not exhibit decreases in activity although condition factors were significantly reduced. Predator avoidance in Chinook salmon fed Se-contaminated mosquito fish (≤26 µg Se/g diet) for 4.2 wks was not adversely affected (Hamilton et al. 1986). Sturgeon in this study did not exhibit decreases in activity or condition factor when fed less than 41.7 µg Se/g diet for 4 wks. These studies suggest that sturgeon locomotive activity was less sensitive to Se toxicity when

compared to either bluegill or Chinook salmon. However, white sturgeon activity was impaired when fed between 41.7-191.1 µg Se/g diet. This decline in activity could have been due to a direct effect of Se toxicity on activity through either behavioral or physiological mechanisms. However, the observed activity effects could have due to an experimental artifact. For example, the smaller sized sturgeon fed higher dietary Se concentrations may have had the same activity level per unit of body length, but the overall distance traveled by smaller sized sturgeon would have been shorter. Although the smaller fork length in fish exposed to higher concentrations in Se may have been an influential factor in the decreased activity in white sturgeon fed over 20.5 µg Se/g, significant differences in activity among treatment groups was observed even when fork length was included as a covariate in the ANOVA model used to analyze the activity data.

Although the complete mechanistic understanding of how Se caused the observed decreases in white sturgeon activity cannot be determined with certainty, a number of hypotheses can be advanced based on the current understanding of Se toxicology. The non-selective substitution of methionine residues with their Se-containing analogous in proteins necessary formation for locomotion may have altered the physiochemical properties of proteins (Lemly, 2002a). A more indirect mechanism may involve cross-linking of actin filaments. Such cross-linking has been shown to occur when oxidative damage induced intermolecular disulfide bonds, resulting in the cross-linking of actin filaments (Dalle-Donne et al., 2001). Recent research has demonstrated that SeMet can induce oxidative stress in cells (Palace et al., 2004). Therefore, the induction of oxidative damage caused by the metabolism of SeMet in sturgeon would facilitate the cross-linking of actin filaments, causing impairment of muscle function necessary for locomotion.

Aside from the possible molecular level Se toxicity in muscle tissue, behavioral modifications to conserve energy for vital physiological activities may have led to the decreases in activity observed in this study. Conservation of energy through a decrease in activity may have been necessary if energy inputs were insufficient to meet the minimum energy requirements of sturgeon. Evidence for diminished energy resources in sturgeon fed above 20.5 µg Se/g diet was found in this study. Such evidence included slower growth rates, liver glycogen depletion, liver and kidney pathology, and drastic changes in the whole body proximate composition in sturgeon exposed to increasingly higher dietary Se concentrations.

The diminished energy reserves in sturgeon fed above 20.5 µg Se/g diet must have stemmed from causes other than an insufficient food supply because sturgeon were fed optimally. The decreases in locomotive activity mentioned previously may have prevented sturgeon from reaching the food before flowing out of the tanks. The duration that the food remained in the tanks varied greatly but usually did not extend longer than 10 min before flowing out of the tanks. Because the proportion of food uneaten was not quantified, the precise amount of food lost in each tank could not be determined but may have partly contributed to the slower growth rate observed in sturgeon exposed to higher concentrations of dietary Se. Aversion to the Se-laden diets may have also contributed to a decrease in energy intake, resulting in diminished energy reserves. Aversion to Se-ladened food has been shown to be a common response in animals fed high concentrations of selenium (Underwood, 1971). However, food aversion was likely to be a secondary factor because diet consumption in all treatment groups was observed throughout the growth trial.

Regardless of the cause, such pronounced decreases in activity level and energy reserves caused by exposure to dietary Se would likely have disastrous ecological consequences in the wild white sturgeon population in the S.F. Bay-Delta. Depletion in energy reserves may decrease the energy available for foraging, resulting in reduced foraging effort. The ability to swim away from predators or catch moving prey may also be impaired. The inability to move into critical environments during various life stages including seaward migration as juveniles and upstream movement during spawning may also be important adverse effects of an impaired inability to swim and/or decrease in energy reserves. Studies exploring the indirect effects of reduced activity by white sturgeon would prove useful to determine the ecologically-relevant effects of dietary Se in wild white sturgeon.

Sturgeon growth exhibited greater sensitivity to Se toxicity in comparison to survival, but to a much lesser degree in comparison to other fish species exposed to dietary SeMet. Hamilton et al. (1990) reported that Chinook salmon exposed to 18.2 and 35.4 µg Se/g diet as SeMet for 8.6 wks grew to only 78 and 37% of control fish weight. In contrast, sturgeon fed similar concentrations (20.5 and 41.7 µg Se/g diet) for 8 wks grew to 99 and 77% of control fish. Based on the present study, approximately 10 µg Se/g diet was the dietary Se threshold which failed to elicit any observable acute toxic effects in white sturgeon after an 8-wk exposure period, considerably higher than most proposed dietary threshold values (3-5 µg Se/g, Lemly, 2002a; Hamilton, 2004).

The dietary threshold from this study should be a tentative estimate until longer term toxicity studies are conducted and Se effects on more sensitive life stages are investigated. The earliest life stages are likely the most sensitive based on the well documented cases of Se embryo

toxicity and teratogenicity in fish and birds (Hoffman and Heinz, 1988; Lemly, 2002a; Teh et al., 2002). Such studies will help determine whether the dietary Se threshold proposed in this study should be modified in order to minimize Se toxicity to white sturgeon in the S.F. Bay-Delta.

The lower sensitivity of white sturgeon to Se toxicity in comparison to other fish species was not due to an inability to bioaccumulate Se. Whole body Se levels were observed to be as high as 34.4 µg Se/g dw in this study after the 8-wk exposure to dietary SeMet, well above whole body concentrations which were correlated with mortality in other species including the bluegill (4.3 µg Se/g dw, Cleveland et al., 1993) and Chinook salmon (5.4 µg Se/g dw, Hamilton et al., 1990). Furthermore, white sturgeon tissue Se concentrations were also above tissue Se concentrations toxic to other species (Lemly, 2002a). In this study, sturgeon accumulated Se very efficiently reaching levels above dietary concentrations in gill, kidney, liver, and muscle tissues in 8 wks (Table 4). Moreover, the positive Se accumulation rate observed in kidney, muscle, and liver tissues suggests that Se concentrations in these tissues had not reached equilibrium concentrations after the 8-wk exposure.

Liver and kidney Se concentrations exhibited a strong linear correlation with diet at concentrations up to 20.5 µg Se/g diet suggesting that Se concentrations in these tissues can serve as bioindicators of dietary Se exposure. However, no linear correlation was observed in these tissues when exposed to >20.5 µg Se/g diet. The cause of the non-linearity in these tissues may in part due to the rapid depuration of Se from the tissues after a possible decrease in Se uptake during the last 2 weeks of the feeding experiment due to decrease in feeding and/or activity as described previously. Alternatively, whole body, muscle and plasma Se concentrations in white sturgeon fed above 20.5 µg Se/g diet may have been near equilibrium

concentrations. If Se equilibrium concentrations within these tissues were similar in white sturgeon fed ≥40 µg Se/g diet, then approaching the equilibrium levels may be the reason for the non-linearity of the tissue-diet Se correlations.

Histopathological analysis of sturgeon exposed to dietary SeMet for 8 wks revealed significant histopathological lesions in the liver and kidneys of white sturgeon fed above 9.6 µg Se/g diet (Table 3). Certain types of histopathological lesions observed in white sturgeon such as hepatocellular vacuolar degeneration and kidney tubular epithelial cell necrosis were also found in other fish species exposed to waterborne or dietary Se (Lemly, 2002b; Teh et al., 2004). The dietary Se concentrations necessary to elicit pathological lesions in this study was consistent with the dietary Se concentrations required to elicit histopathological lesions in rainbow trout (Hilton et al., 1980) and Chinook salmon (Hamilton et al., 1986). Rainbow trout did not exhibit any histopathological lesions when fed up to 13 µg Se/g diet in the form of sodium selenite for 16 wks (Hilton et al., 1980). Contrary to this study, rainbow trout did experience mortality and slower growth although no histopathological lesions were observed, a phenomenon partially attributed to avoidance of food by trout fed 13 µg Se/g diet. Histopathological changes in the liver and kidneys of Chinook salmon were observed in fish fed 13 and 26 µg Se/g diet for 4.2 wks, a finding consistent with the Se concentrations needed to induce histopathological lesions in white sturgeon. Based on the severity of the lesions observed, 10 µg Se/g diet was determined to be the dietary Se threshold for white sturgeon.

In summary, this study demonstrated that dietary Se concentrations above 20.5 µg Se/g diet as SeMet for 8 wks adversely affected white sturgeon survival, activity, growth, and whole body proximate composition. Selenium bioaccumulated in all sampled tissues, with liver and

kidney pathology observed in white sturgeon exposed to dietary Se concentrations above 9.6 µg Se/g diet. The dietary Se threshold to prevent Se toxicity was estimated at 10 µg Se/g diet and liver tissue was suggested as the most accurate bioindicator of dietary Se concentration. Continued research on how dietary components, abiotic factors, and ecological interactions influence Se toxicity are needed to obtain a more complete understanding of the consequences of increased Se loading into the S.F. Bay-Delta.

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6. References

AOAC (Association of Official Analytical Chemists), 1984. S. Williams (Editor), Official Methods of Analysis of the Association of Official Analytical Chemists, 14th edition. Association of Analytical Chemists, Inc., VA, pp.152-160.

Billard, R., Lecointre, G., 2001. Biology and conservation of sturgeon and paddlefish. Rev. Fish Biol. Fish. 9, 355-392.

Brown, C.L., Luoma, S.N., 1995. Energy-related selenium and vanadium contamination in San Francisco Bay, California – Effects on biological resources. in: Energy and the Environment – Application of Geosciences to Decision-Making, Carter, L.M.H., ed., Tenth V.E. McKelvey Forum on Mineral and Energy Resources, p. 91-92.

Cleveland, L., Little, E.E., Buckler, D.R., Wiedmeyer, R.H., 1993. Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill (*Lepomis macrochirus*). Aquat. Toxicol. 27, 265-280.

Cui, Y.B., Hung, S.S.O., 1995. A prototype feeding-growth table for white sturgeon. J. Appl. Aquacult. 5, 25-34.

Cui, Y.B., Hung, S.S.O., Deng, D.F., Yang, Y., 1997. Growth of white sturgeon as affected by feeding regimen. Prog. Fish-Cult. 59, 31-35.

Dalle-Donne, I., Rossi, R., Milzani, A., Di Simplicio, P., Columbo, R., 2001. The actin cytoskeleton response to oxidants: from small heat shock protein phosphorylation to changes in the redox state of actin itself. Free Radic. Biol. Med. 31, 1624-1632.

Deforest, D.K., Brix, K.V., Adams, W.J., 1999. Critical review of proposed residue-based selenium toxicity thresholds for freshwater fish. Hum. Ecol. Risk Assess. 5, 1187-1228.

Fan, T.W.-M., Higashi, R.M., Lane, A.N., 1998. Biotransformations of Selenium Oxyanion by Filamentous Cyanophyte-Dominated Mat Cultured from Agricultural Drainage Waters", Environ. Sci. Technol. 32, 3185-3193.

Fan, T.W.M., Teh, S.J., Hinton, D.E., Higashi, R.M., 2002. Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. Aquat. Toxicol. 57(1-2), 65-84.

Hamilton, S.J., 2004. Review of selenium toxicity in the aquatic food chain. Sci. Total Environ. 326, 1-31.

Hamilton, S.J., Buhl, K.J., Faerber, N.L., Wiedmeyer, R.H., Bullard, F.A., 1990. Toxicity of organic selenium in the diet of chinook salmon. Environ. Toxicol. Chem. 9(3), 347-358.

Hamilton, S.J., Palmisano, A.N., Wedemeyer, G.A., Yasutake, W.T., 1986. Impacts of selenium on early life stages and smoltification of fall Chinook salmon. Transactions of the North American Wildlife and Natural Resources Conference 51, 343-356.

Hilton, J.W., Hodson, P.V., Slinger, S.J., 1980. The Requirement and Toxicity of Selenium in Rainbow Trout (*Salmo gairdneri*). J. Nutr. 110, 2527-2535.

Hoffman, D J., Heinz, G.H., 1988. Embryotoxic and teratogenic effects of selenium in the diet of mallards. J. Toxicol. Environ. Health 24(4), 477-490.

Hug, P., Fletcher, B.B., Trott, S.S., 2000. Opinion of Judge Hug and Judge Trott (dissent) in appeal of Firebaugh Canal v USA 9516641. Office of the Circuit Executive, U.S. Court of Appeals for the Ninth Circuit. Fresno, California. 15p.

Hung, S.S.O., Lutes. P.B., 1987. Optimum feeding rate of juvenile white sturgeon (*Acipenser transmontanus*): at 20°C. Aquaculture 65, 307-317.

Hung, S.S.O., B.J. Moore, C.E. Bordner, F.S. Conte. 1987. Growth of juvenile white sturgeon (*Acipenser transmontanus*) fed different purified diets. Journal of Nutrition 117:328-334.

Kroll, K.J., Doroshov, S.I., 1991. Vitellogenin: Potential vehicle for selenium bioaccumulation in oocytes of the white sturgeon (*Acipenser transmontanus*). In: Acipenser, P. Williot, ed., CEMAGREF publ., pp. 99-106.

Lemly, A.D., 2002a. Selenium assessment in aquatic ecosystems: a guide for hazard evaluation and water quality criteria. Springer-Verlag, New York. pp. 161.

Lemly, A.D., 2002b. Symptoms and implications of selenium toxicity in fish: The Belews Lake case example. Aquat. Toxicol. 57(1-2), 39-49.

Linville, R.G., Luoma, S.N., Cutter, L., Cutter, G.A., 2002. Increased selenium threat as a result of invasion of the exotic bivalve *Potamocorbula amurensis* into the San Francisco Bay-Delta. Aquat. Toxicol. 57(1-2), 51-64.

Luoma, S.N., Presser, T.S., 2000. Forecasting selenium discharges to the San Francisco Bay-delta estuary: ecological effects of a proposed San Luis Drain extension. U.S. Geological Survey. Open-file report 00-416.

McKechnie, R.J., Fenner, R.B., 1971. Food habits of white sturgeon, *Acipenser transmontanus*, in San Pablo and Suisun Bays, California. Calif. Fish and Game 57, 209-212.

Palace, V.P., Spallholz, J.E., Holm, J., Wautier, K., Evans, R.E., Baron, C.L., 2004. Metabolism of selenomethionine by rainbow trout (*Oncorhynchus mykiss*) embryos can generate oxidative stress. Ecotoxicol. Environ. Saf. 58(1), 17-21.

State Water Resources Control Board, 1987-1991. Selenium verification study. Reports to the State Water Resources Control Board, Sacramento, 1987, 79 p.9 appendices, 1988, 60 p. & 8 appendices, 1989 81p & 11 appendices.

Teh, S.J., Deng, X., Teh, F.C., Hung, S.S.O., 2002. Selenium-induced teratogenicity in Sacramento Splittail (*Pogonichthys macrolepidotus*). Mar. Environ. Res. 54: 605-608.

Teh, S.J., Deng, X., Deng, D.F., Teh, F.C., Hung, S.S.O., Fan, W-M.T, Liu, J., and Higashi., 2004. Chronic Effects of Dietary Selenium on Juvenile Sacramento Splittail (*Pogonichthys Macrolepidotus*). Environ. Sci. Technol.; 38(22) pp 6085 - 6093.

Underwood, E.J. 1971. Selenium. In: Underwood, E.J. (Ed.), Trace elements in animal and human nutrition, 3rd ed., Academic Press, p. 291-324.

United States Environmental Protection Agency, 1992. Rulemaking: water quality standards: establishment of numeric criteria for priority toxic pollutants: States' compliance: Final Rule, 57 FR 60848.

Urquhart, K.A.F., Regalado, K., 1991. Selenium Verification Study, 1988-1990: California State Water Resources Control Board Report 91-2-WQWR. Sacramento, CA. 94p.

Table 1. Mean (± SE) white sturgeon swimming activity (SA), body weight increase (BWI), fork length (FL), condition factor (CF), and hepatosomatic index (HSI) after exposure to dietary SeMet for 4 and 8 wks. Letters denote statistical groupings among treatment groups within each exposure period ($p<0.05$).

	Treatment group (µg Se/g diet)	Exposure Period 4 wks	Exposure Period 8 wks
SA (sq/min)	0.4	32.0 ± 3.4 [ab]	43.9 ± 2.9 [a]
	9.6	36.4 ± 3.6 [a]	44.9 ± 3.4 [a]
	20.5	37.2 ± 1.9 [a]	44.5 ± 4.0 [a]
	41.7	27.2 ± 2.7 [ab]	26.4 ± 3.0 [b]
	89.8	27.8 ± 2.9 [ab]	25.2 ± 4.8 [b]
	191.1	22.0 ± 3.1 [b]	15.2 ± 3.4 [b]
BWI[1] (%)	0.4	119.1 ± 1.8 [a]	282.9 ± 4.6 [a]
	9.6	121.4 ± 2.7 [a]	285.5 ± 9.9 [a]
	20.5	114.0 ± 6.2 [a]	277.7 ± 6.1 [a]
	41.7	88.8 ± 6.3 [b]	191.0 ± 12.6 [b]
	89.8	62.2 ± 4.0 [c]	106.5 ± 5.8 [c]
	191.1	27.7 ± 0.9 [d]	28.6 ± 3.6 [d]
FL (cm)	0.4	17.9 ± 0.1 [a]	21.8 ± 0.3 [a]
	9.6	17.8 ± 0.3 [a]	21.6 ± 0.2 [a]
	20.5	18.0 ± 0.1 [a]	21.8 ± 0.2 [a]
	41.7	17.6 ± 0.3 [ab]	20.6 ± 0.5 [a]
	89.8	17.0 ± 0.6 [ab]	19.1 ± 0.2 [b]
	191.1	16.3 ± 0.2 [b]	17.1 ± 0.3 [c]
CF[2] (g/cm^3)	0.4	0.98 ± 0.02 [a]	1.06 ± 0.02 [ab]
	9.6	0.98 ± 0.01 [a]	1.07 ± 0.02 [ab]
	20.5	0.95 ± 0.03 [ab]	1.03 ± 0.03 [abc]
	41.7	0.97 ± 0.02 [a]	1.01 ± 0.03 [abc]
	89.8	0.84 ± 0.05 [b]	0.96 ± 0.02 [bc]
	191.1	0.84 ± 0.01 [b]	0.85 ± 0.02 [d]
HSI[3] (%)	0.4	2.24 ± 0.10 [abcd]	2.20 ± 0.08 [a]
	9.6	2.60 ± 0.14 [ab]	2.45 ± 0.09 [a]
	20.5	2.44 ± 0.01 [abc]	2.23 ± 0.08 [a]
	41.7	2.66 ± 0.13 [ab]	2.47 ± 0.09 [a]
	89.8	2.11 ± 0.07 [bcd]	2.22 ± 0.11 [a]
	191.1	1.88 ± 0.11 [cd]	1.66 ± 0.08 [b]

[1] % BWI=100*(BW_f-BW_i) / (BW_i) where BW_i and BW_f represent the average initial and final body weights of sturgeon in each treatment group respectively. [2]CF=Fish weight*100 / Fork Length^3 [3]HSI=100*Liver Weight/Body weight

Table 2. Mean (± SE) white sturgeon moisture, lipid, crude protein, ash content, and whole body Se after 8 wk exposure to dietary SeMet. Letters denote statistical groupings among treatment groups ($p<0.05$).

Teatment group	Moisture	Lipid	Crude Protein	Ash	Whole Body Se
(µg Se/g diet)	% wet weight				µg Se/g dw
0.4	76.8 ± 0.5 [b]	9.5 ± 0.4 [abc]	12.5 ± 0.1	2.2 ± 0.1 [b]	5.2 ± 0.4 [c]
9.6	77.0 ± 0.7 [b]	9.5 ± 0.9 [abc]	12.5 ± 0.2	2.2 ± 0.0 [b]	11.8 ± 0.9 [b]
20.5	76.8 ± 0.3 [b]	10.1 ± 0.4 [ab]	12.1 ± 0.2	2.3 ± 0.1 [ab]	14.7 ± 0.8 [b]
41.7	77.3 ± 0.5 [b]	9.6 ± 0.7 [abc]	12.4 ± 0.2	2.3 ± 0.0 [ab]	22.5 ± 1.4 [a]
89.8	78.5 ± 0.3 [ab]	7.6 ± 0.4 [bcd]	12.4 ± 0.3	2.5 ± 0.1 [ab]	34.4 ± 2.3 [a]
191.1	80.0 ± 0.4 [a]	6.1 ± 0.4 [cd]	11.5 ± 0.3	2.6 ± 0.1 [a]	27.5 ± 4.4 [a]

[1] White sturgeon moisture, lipid, crude protein, ash content, and whole body Se prior to selenium exposure (wk 0) were 78.0±0.4%, 8.5±0.2%, 11.7±0.3%, 2.4±0.1%, and 6.7±0.8 µg Se/g dw, respectively.

Table 3. Mean (±SE) tissue Se concentrations (µg Se/g dry weight) in white sturgeon exposed to dietary SeMet for 8 wks.[1] Letters denote statistical groupings among Se concentrations within each tissue type and exposure period ($p<0.05$).

Tissue	Treatment (µg Se/g diet)	Selenium Concentration Exposure period (wks)	
		4	8
Kidney	0.4	6.6 ± 0.3 ^{d}	7.5 ± 0.3 ^{e}
	9.6	18.0 ± 1.2 ^{c}	20.0 ± 1.2 ^{d}
	20.5	23.8 ± 0.5 ^{c}	30.9 ± 2.0 ^{c}
	41.7	41.3 ± 3.2 ^{b}	51.7 ± 2.7 ^{b}
	89.8	59.8 ± 6.4 ^{ab}	107.9 ± 5.7 ^{a}
	191.1	98.7 ± 13.4 ^{ab}	128.1 ± 6.6 ^{a}
Liver	0.4	7.0 ± 0.7 ^{e}	15.7 ± 0.7 ^{ef}
	9.6	11.4 ± 0.8 ^{d}	18.8 ± 1.2 ^{def}
	20.5	15.4 ± 0.7 ^{d}	22.0 ± 1.2 ^{de}
	41.7	26.2 ± 3.5 ^{bc}	37.4 ± 1.7 ^{bc}
	89.8	34.3 ± 0.7 ^{abc}	53.1 ± 8.3 ^{abc}
	191.1	42.2 ± 3.4 ^{ab}	82.7 ± 12.7 ^{ab}
Gill	0.4	4.7 ± 0.2 ^{f}	5.7 ± 0.2 ^{de}
	9.6	13.5 ± 0.2 ^{e}	13.9 ± 0.5 ^{cde}
	20.5	21.2 ± 0.5 ^{d}	19.1 ± 1.5 ^{cd}
	41.7	31.7 ± 2.1 ^{c}	36.0 ± 1.9 ^{b}
	89.8	42.8 ± 1.4 ^{b}	50.1 ± 2.0 ^{a}
	191.1	52.2 ± 3.0 ^{a}	54.9 ± 4.8 ^{a}
Muscle	0.4	7.8 ± 0.3 ^{d}	8.2 ± 0.6 ^{e}
	9.6	13.3 ± 0.4 ^{c}	17.2 ± 0.7 ^{d}
	20.5	18.0 ± 1.2 ^{b}	22.9 ± 1.5 ^{c}
	41.7	30.0 ± 1.2 ^{a}	36.8 ± 1.8 ^{b}
	89.8	32.8 ± 2.5 ^{a}	52.9 ± 3.2 ^{a}
	191.1	37.1 ± 1.7 ^{a}	54.8 ± 2.8 ^{a}
Plasma	0.4	5.8 ± 0.2 ^{d}	5.8 ± 0.4 ^{d}
	9.6	15.4 ± 0.5 ^{c}	17.8 ± 0.6 ^{c}
	20.5	22.3 ± 0.6 ^{b}	21.1 ± 1.2 ^{c}
	41.7	38.3 ± 4.4 ^{a}	35.8 ± 1.0 ^{b}
	89.8	39.2 ± 3.8 ^{a}	50.0 ± 2.1 ^{a}
	191.1	42.8 ± 2.9 ^{a}	48.4 ± 2.5 ^{a}

[1]Selenium concentrations in white sturgeon tissues prior to dietary SeMet exposure were 7.3±0.4, 9.0±0.8, 5.2±0.2, 14.8±2.9, and 0.3±0.0 µg Se/g dw, in the kidney, liver, gill, muscle, and plasma, respectively.

Table 4. Mean (± SE) occurrence frequency and severity of liver and kidney histopathological lesions in white sturgeon exposed to dietary SeMet for 8 wks. Letters denote statistical groupings among treatment groups. Blank cells represent treatments in which histopathological lesions were not observed. Significance of correlations between dietary Se and either occurrence frequency or severity are provided in parenthesis adjacent to the correlation coefficients (r).

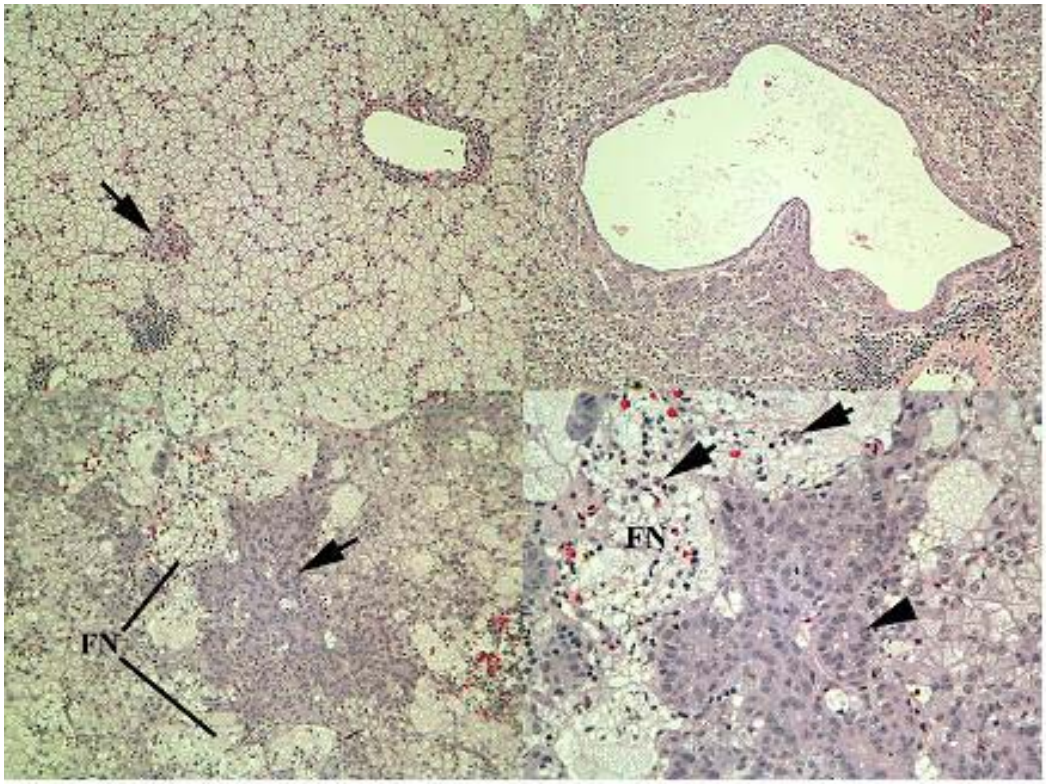
	Dietary Se	Liver			Kidney		
	(µg Se/g diet)	GD[1]	HP[2]	VD[3]	TBD[4]	TBV[5]	TBI[6]
Occurrence frequency (%)	0.4	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0
	9.6	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0 [b]	0.0 ± 0.0
	20.5	12.5 ± 12.5 [b]	12.5 ± 12.5 [ab]	12.5 ± 12.5 [b]	0.0 ± 0.0 [b]	37.5 ± 16.1 [ab]	0.0 ± 0.0
	41.7	6.3 ± 6.3 [b]	18.8 ± 12.0 [ab]	43.8 ± 15.7 [ab]	18.8 ± 6.3 [ab]	81.3 ± 6.3 [a]	31.3 ± 23.7
	89.8	68.8 ± 12.0 [a]	56.3 ± 12.0 [a]	87.5 ± 12.5 [a]	50.0 ± 14.4 [a]	68.8 ± 12.0 [a]	31.3 ± 23.7
	191.1	68.8 ± 18.8 [a]	62.5 ± 21.7 [a]	68.8 ± 12.0 [a]	37.5 ± 12.5 [a]	37.5 ± 16.1 [b]	12.5 ± 7.2
	r	0.753 (<0.001)	0.704 (<0.001)	0.689 (<0.001)	0.640 (<0.001)	0.298 (0.157)	0.188 (0.377)
Severity[7]	0.4	*	*	*	*	*	*
	9.6	*	*	*	*	*	*
	20.5	1.0 ± 0.0	1.0 ± 0.0	1.5 ± 0.5	*	1.0 ± 0.0 [a]	*
	41.7	1.0 ± 0.0	2.0 ± 0.6	2.1 ± 0.4	1.0 ± 0.0	1.0 ± 0.0 [ab]	1.0 ± 0.0
	89.8	1.5 ± 0.2	1.7 ± 0.3	1.7 ± 0.2	1.1 ± 0.1	1.2 ± 0.1 [b]	1.2 ± 0.2
	191.1	1.8 ± 0.3	1.7 ± 0.3	2.3 ± 0.3	1.7 ± 0.3	1.5 ± 0.2 [b]	1.0 ± 0.0
	r	0.347 (0.090)	0.064 (0.766)	0.174 (0.326)	0.484 (0.049)	0.517 (0.001)	0.018 (0.955)

[1]GD=glycogen depletion, [2]HP=hyperplasia, [3]VD=hepatocellular vacuolar degeneration including cell necrosis, [4]TBD=tubular dilation, [5]TBV=tubular cell hydropic degeneration and necrosis, [6]TBI = Tubular inclusions where eosinophilic or basophilic cast materials observed in the lumen, [7]1=Mild severity, 2=Moderate severity, 3=High severity

Figure captions

Figure 1. (Top left) Liver of white sturgeon exposed to 0.4 µg Se/g diet for 8 wks. Arrow is pointing to the bile duct. (Top right) Polycystic dilation of bile duct in white sturgeon exposed to 89.8 µg Se/g diet for 8 wks. (Bottom left) Focal necrosis (FN) and bile pre-ductular and ductular hyperplasia (arrow) in a white sturgeon exposed to 41.7 µg Se/g diet for 8 wks. (Bottom right) A magnified view of the focal necrosis (FN), necrotic hepatocytes (arrows), and bile ductular hyperplasia (arrowhead) in the same sturgeon.

Figure 2. (Top left) Kidney of white sturgeon exposed to 0.4 µg Se/g diet for 8 wks. (Top right) Tubular necrosis (arrow) and cast material (arrowhead) in the kidney of sturgeon exposed to 41.7 µg Se/g diet for 8 wks. (Bottom left) Polycystic dilation of kidney tubules of a white sturgeon exposed to 191.1 µg Se/g diet for 8 wks. Rightmost arrow is pointing to a macrophage aggregate. Two arrowheads on the left are pointing to polycystic dilation of kidney tubules. (Bottom right) A magnified view of the polycystic dilation of kidney tubules in the same sturgeon.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