A Reproductive and Trophic Transfer Study Associated With Selenium Concentrations in the Mud River Watershed

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Abstract
Selenium in mining related discharges has created concern in the Appalachian Region where coal is a significant resource. In West Virginia, evaluation of streams receiving mining discharges focused attention on the Mud River watershed, where bioaccumulation of selenium was highest in preliminary surveys. Chronic exposure (mainly dietary) of mature female fish to selenium has the potential to cause developmental abnormalities in developing embryos due to the maternal transfer of selenium into the eggs. Literature suggests that factors affecting the bioaccumulation rate of selenium, and the concentration of selenium associated with the aforementioned effects are site-specific. The purpose of this study was to determine the whole-body selenium tissue concentration which is protective of aquatic life in the watershed as defined by the effective concentration resulting in greater than ten percent deformity (EC_{10}). Further, this study was undertaken to evaluate whether whole-body tissue concentrations in fish in the watershed are within an acceptable range and to test a trophic transfer model which would allow monitoring of selenium whole-body fish tissue concentrations via modeling using periphyton and water column selenium concentrations. By evaluating larval fish deformities within the Mud River watershed, it is demonstrated that a whole-body selenium value of 23.69 mg/kg dw selenium is the concentration shown to be protective of fish communities in this watershed. Whole-body fish tissue concentrations from streams sampled within the watershed generally show compliance with this safe level. Predicting the whole-body concentration using the trophic transfer model was successful for the streams evaluated except for Sites1 and 2 where variable interactions and site variability reduced the models predictive ability. This analysis confirms the trophic transfer model as a useful predictive tool in this watershed.

Key Words: aquatic, lotic, bioaccumulation, model, fish, deformity.

Introduction
Selenium in mining related discharges has created concern in the Appalachian Region where coal is a significant resource. In West Virginia, evaluation of streams receiving mining discharges focused attention on the Mud River watershed, where bioaccumulation of selenium was highest in preliminary surveys (WVDEP, 2009). Chronic exposure (mainly dietary) of mature female fish to selenium has the potential to cause developmental abnormalities in developing embryos due to the maternal transfer of selenium into the eggs (NAMC, 2008). Fish population effects can be seen when developmental abnormalities reach levels which impair recruitment to the population. The level at which populations may be affected has been suggested to be abnormality rates of ten percent or greater (EC_{10}) (NAMC, 2008). Factors affecting the bioaccumulation rate of selenium, and the concentration of selenium associated with the aforementioned effects are site-specific.

The purpose of this study was:

- to determine the whole-body selenium tissue concentration which is protective of aquatic life in the watershed as defined by the effective concentration resulting in greater than ten percent deformity (EC_{10});
- to evaluate whether whole body tissue concentrations in fish in the watershed are within an acceptable range based on the calculated EC_{10}; and

- to test a trophic transfer model which would allow monitoring of selenium whole-body fish tissue concentrations by modeling using periphyton and water column selenium concentrations.

Selenium induced deformities can include spinal curvatures, missing or deformed fins, craniofacial deformities, and edema. Swelling of the yolk sac, or yolk sac edema, is also associated with selenium, but may not result in permanent abnormalities (Chapman et al., 2009), therefore only the teratogenic deformities were used in the EC_{10} evaluation. An EC_{10} value, the value at which 10% of the fish larval population is deformed, is the point which is considered to be protective of the population (NAMC, 2008). DeForest et al., 2011 evaluated 22 studies involving 12 fish species across North America and concluded that an egg/ovary concentration of 20 mg Se/kg satisfied the EC_{10} criteria.

As selenium uptake is known to occur primarily through the dietary exposure route, biological characterization has included each trophic level from algae (primary producers) through the primary consumers (macroinvertebrates) through the top consumers, generally fish. Reproductive health was evaluated in streams with varying selenium conditions by evaluation of fish larval deformity rates, and selenium accumulation was weighed in each trophic level of the community.

Methods

Site description

An evaluation of the condition of the biological communities and the extent to which elevated selenium levels may be affecting these communities was conducted in the Mud River watershed between 2009 and 2010. The watershed lies in the Cumberland Mountains of the Central Appalachian Plateau in West Virginia. Mining, forestry, and natural gas are the significant economic contributors in the watershed (USEPA, 2004 and Woods et al., 1999). Coal mining has been ongoing in the basin since the completion of the Norfolk and Western Railroad in the late 1800s. Large scale surface mining (known as mountaintop mining) began in the early 1980s in response to the increased demand for low sulfur coal (USEPA, 2004). In this watershed, there is a strip of land approximately 5 miles wide which lies in the primary mountaintop mining area as described by the West Virginia Geological and Economic Survey. Multiple coal seams are horizontally bedded and most mines extract five or more seams. The primary physiography is unglaciated divided hills and mountains with abrupt slopes and narrow ridges and the primary geology is Pennsylvania sandstone, siltstone, shale and coal of the Pottsville Group and the Allegheny Formation (Woods et al., 1999).

Fish egg collection was conducted in five streams in the Mud River watershed are shown in Table 1.

Table 1: Fish Egg Sampling Locations

Location	Latitude (°)	Longitude(°)
Mud River	38.09474 N	81.97635 W
Mud River Downstream Sugartree Branch	38.09284 N	81.96379 W
Ballard Branch	38.07332 N	81.94968 W
Sugartree Branch	38.09068 N	81.94989 W
Berry Branch	38.10087 N	81.98917 W
Upton Branch	38.13567 N	82.04774 W

Periphyton was collected at six locations (Table 2).

Table 2: Periphyton Sampling Locations

Site ID	Location	Latitude (°)	Longitude(°)
1	Mud River Upstream Ballard Branch	38.07103 N	81.95261 W
2	Ballard Branch	38.07261 N	81.94711 W
3	Stanley Fork	38.08472 N	81.95369 W
4	Sugartree Branch	38.09066 N	81.95040 W
5	Berry Branch	38.10496 N	81.97053 W
6	Mud River Downstream Berry Branch	38.09997 N	81.99063 W

Reproductive study methods

Eggs were collected in the watershed in spring 2009 and 2010 and each nest was reared in a laboratory with water from each site. After egg hatching in each tank, a subset of larvae was collected every 2 to 3 days until the majority became free swimming. Upon collection, larvae were transferred to labeled plastic jars with a small amount of water and placed in a freezer for 30-60 minutes to anesthetize prior to preserving them in a pre-buffered formalin solution (Formalin 10). After evaluating the larvae for deformities, they were transferred to a 70% ethanol solution for long term storage.

Preserved larval fish specimens were observed using a dissecting microscope and evaluated for deformities. Each specimen was viewed and the number of the following types of deformities were observed:

- Craniofacial – deformities that are associated with the head region (extension or reduction of jaw structure, malformations, eye diameter, etc.);
- Skeletal – deformities associated with the notochord or spine (severe bends or curvature along the notochord);
- Yolk Sac Edema – deformities associated in the yolk sac during larval development (accumulation of excess body fluid in the yolk sac);
- Finfold – deformities associated among the fins (absence or malformation associated with any developed / developing fins); and
- Teratogenic – the sum of permanent developmental deformities that are not reversible, which are craniofacial, skeletal and finfold deformities.

The larval specimens were identified down to the lowest practical taxon. The following literature was used for the identification and deformity evaluation of larval specimens: Auer's (1982) "Identification of Larval Fishes of the Great Lakes Basin with Emphasis on the Lake Michigan Drainage", Holm's (2003) "An Assessment of the Development and Survival of Wild Rainbow Trout (*Oncorhynchus mykiss*) and Brook Trout (*Salvelinus fontinalis*) Exposed to Elevated Selenium in an Area of Active Coal Mining", and Lemly's (1997) "A Teratogenic Deformity Index for Evaluating Impacts of Selenium on Fish Populations" along with other noted literature (Holm, 2005; Margulies, 1983).

At the time of egg collections, representative species of fish were collected in the aforementioned streams by use of an electro-backpack shocker. The fish were stored in ice and transported to Biochem Testing Laboratories for whole-body selenium tissue analysis.

Modeling methods

Periphyton sampling was conducted from summer 2009 through spring 2010, quarterly, in six mine-influenced streams. Unglazed 1-inch x 1-inch tiles were placed in sampling sites and periphyton was allowed to colonize. Four tiles were randomly collected at two week intervals for a total of four samples per season. Periphyton was transported on ice and in dark containers and analyzed for selenium concentration ($\mu g/m^2$), ash free dry weight (AFDW) (g/m^2), and chlorophyll-a (mg/m^2) using laboratory methods EPA 6020, SM10300C.5, and SM10200-H, respectively. Any periphyton or water selenium measurements which were below the detection limit were not used in the modeling. The detection limit for water concentration was 0.001 mg/L. The detection limit for periphyton concentration is based on the sample size and variable dilution volumes used in sample processing. The detection limit for periphyton concentration ranged from 3.3 µg/m^2 to 17.2 µg/m^2. Due to laboratory malfunction, stolen/washed out tiles at various data points for all three parameters were missing throughout summer, winter and spring.

To calculate the particulate selenium concentration (µg/g) for a particular sampling event, the average periphyton selenium concentration (µg/m^2 dw) from the four tiles was divided by the average dry weight (g/m^3) (cf. AFDW) of the four tiles. In order to translate water-column selenium concentration to whole-body fish tissue concentration, several factors in each modeling event had to be selected. Selenium water column concentrations, which were sampled from summer 2009 through spring 2010, were used in the modeling. Both selenium water column and periphyton concentrations are shown in Table 3.

Table 3: Model Input

Se Water Column Concentrations (µg/L)				
Site ID	**Summer 2009**	**Fall 2009**	**Winter 2010**	**Spring 2010**
1	6.2	3.3	nd	nd
2	1.7	nd	nd	nd
3	9.1	8.1	10.3	8.8
4	15.7	14.2	19.7	18.9
5	20.2	5.3	8.0	5.3
6	19.9	14.0	10.5	6.1
Average Se Periphyton (Particulate) Concentration Ranges (µg/g)				
Site ID	**Summer 2009**	**Fall 2009**	**Winter 2010**	**Spring 2010**
1	2.8-25.6	nd - 5.3	nd - 7.8	nd - 1.6
2	4.7-5.8	nd	nd	0.6-0.8
3	nd - 3.2	1.2-1.4	0.4-1.6	nd - 0.5
4	11.2-11.7	nd - 10.3	2.6-3.6	missing tiles
5	1.5-12.1	2.0-4.2	nd	0.8-2.3
6	nd - 0.3	2.5-8.8	1.8-2.6	nd - 0.8

nd=non-detect

Fish species, predator food web, trophic transfer functions for fish and invertebrates, and the operationally defined distribution coefficient (K_d) were all independently selected for each modeling event. Additionally, selenium uptake was presumed to be seasonal due to seasonal periphyton productivity. The equation used for modeling whole-body selenium fish tissue concentration via periphyton selenium concentration is as follows:

$$C_{water} = (C_{fish}) \div (TTF_{fish})\ (K_d)\ (TTF_{invertebrate}) \qquad \text{(Equation1)}$$

K_d was calculated as the ratio of the particulate concentration to the water-column concentration. The trophic transfer functions (TTFs) were selected from a summary of TTFs derived from field averages of multiple matched data sets from sites with similar food webs or regressions for a series of individual sites with similar food webs (Presser and Luoma, 2010). If an invertebrate-to-fish TTF was not available, a TTF_{fish} of 1.1 was used, which is a mean value based on a study of 25 fish species. Most fish species consume a mixed diet, with an inclination towards certain types of food. When selecting TTFs for the food of individual fish species, the preferred foods and the available foods for that particular location and season were taken into account. In order to have the most accurate $TTF_{invertebrate}$, prey fractions for each species' foods were incorporated in the equation. Species designations were found in the USEPA's Rapid Bioassessment Protocols for Streams and Rivers (Barbour et al., 1999). Common foods for each species were found in the Fish and Wildlife Service's Habitat Suitability Index Models. The prey fractions that were chosen take into account, not only the preferred foods for each species, but the available foods in the Mud River watershed, the change in eating habits of each species as they mature, and the time for each species to mature. The United States Geological Survey's Habitat Suitability Index Models were referenced when choosing prey fractions for each of the modeled species. Prey fractions selected are shown in Table 4.

Table 4: Prey fractions (%) used in trophic transfer modeling

Fish Species	Aquatic Insect	Crayfish	Fish	Zooplankton	Amphipod
Green sunfish	35	25	25	15	---
Creek chub (fall, winter & spring)	25	---	50	---	25
Creek chub (summer)	---	---	100	---	---
Blacknose dace	85	---	---	15	---

Results

Reproductive study results

From the evaluation of the larval deformities and the whole-body selenium fish tissue concentrations in the Mud River watershed the EC_{10} was found to be 23.69 mg/kg dw. The regression coefficient (r^2 =0.7427) generated from this relationship is significant (Figure 1).

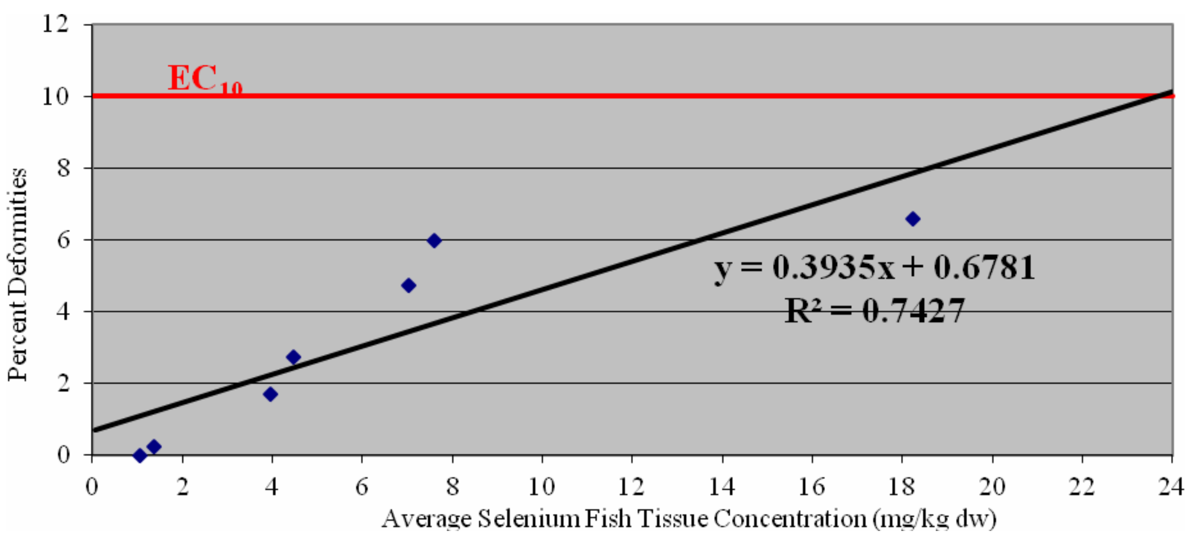

Figure 1: Percent teratogenic deformities of larval fish in relation to average selenium whole-body fish tissue concentrations

Fish were not corrected for age, but all were adults of reproductive age. Whole-body tissue concentrations ranged from 3.51 to 25.54 mg/kg dw in fish collected from the five sites in the watershed. These concentrations were generally below the projected EC_{10} with the exception of the one creek chub concentration of 25.54 mg/kg dw.

Modeling results

Modeled whole-body fish tissue concentrations of creek chubs, green sunfish, and blacknose dace were compared to measured whole-body fish tissue concentrations of the same species collected contemporaneously with the periphyton collection in 2009. In general, modeled and measured values showed good agreement. As Figures 2-4 illustrate, the majority of the modeled whole-body selenium fish tissue concentrations follow the same trends for each sampling site as the measured data.

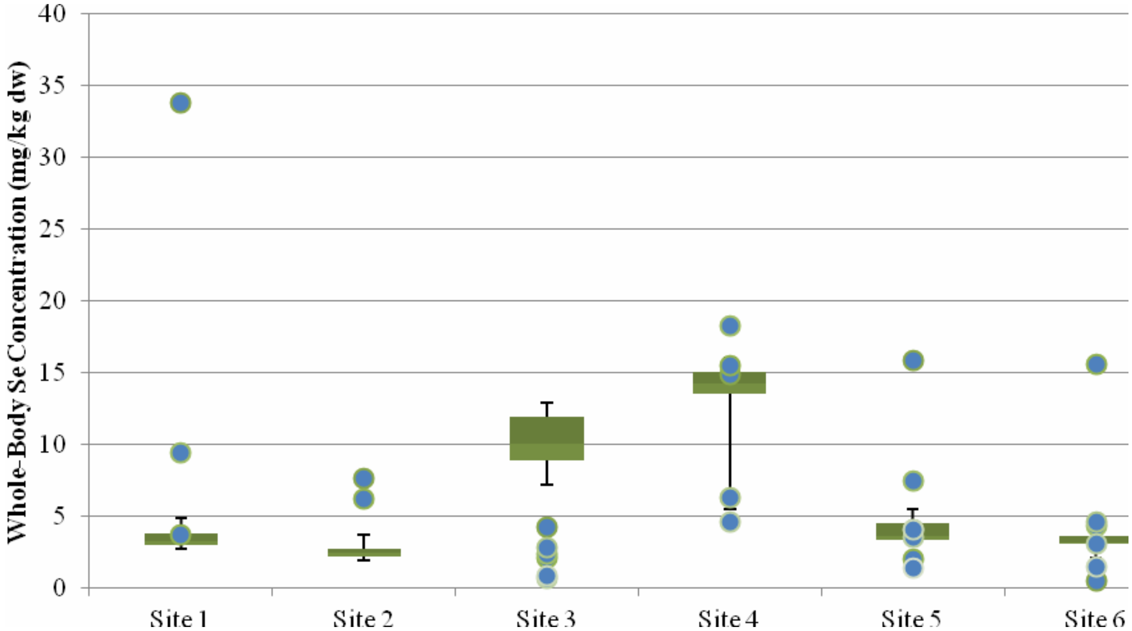

Figure 2: Whole-Body creek chub selenium concentrations (green) plotted with modeled concentrations (blue).

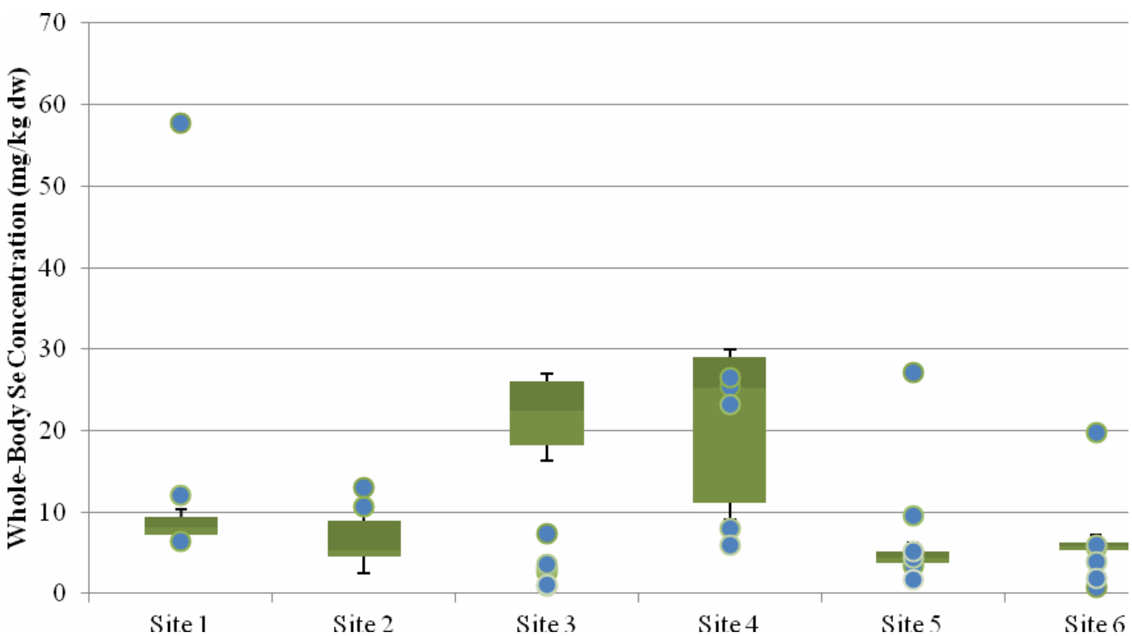

Figure 3: Whole-Body green sunfish selenium concentrations (green) plotted with modeled concentrations (blue).

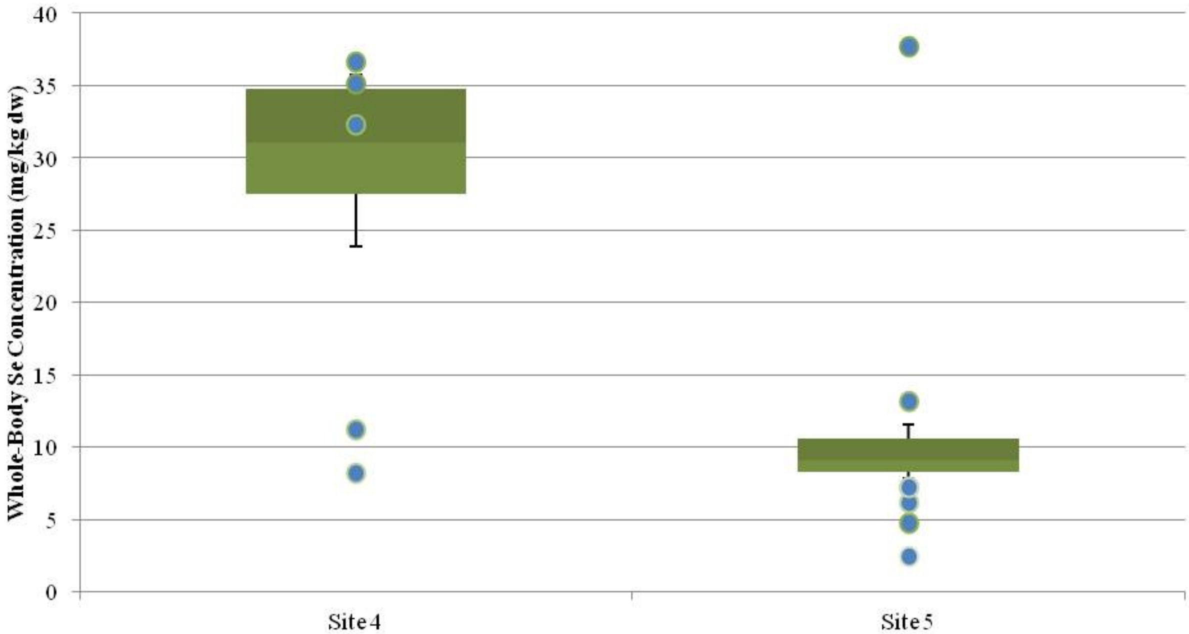

Figure 4: Whole-Body blacknose dace selenium concentrations (green) plotted with modeled concentrations (blue).

To evaluate modeling accuracy, modeled whole-body fish tissue concentrations of creek chubs, green sunfish, and blacknose dace were statistically compared to measured whole-body fish tissue concentrations of the same species. For each fish species and site, the modeled and measured data were ranked and a General Linear Model two-way analysis of variance (ANOVA) was utilized to compare the data. Homogeneity of variance was evaluated.

As shown in Table 5, there was no significant difference in the measured and modeled data for all three fish species. As expected, however, there was a significant difference between whole-body selenium fish tissue concentrations and the sampling sites. While this data demonstrates the accuracy of the modeling, the interaction probability levels for creek chubs and green sunfish conveys that there is an interference between the sampling site and the measured versus modeled data. That is to say, there is a difference in the predictability of the model at the different sites, or some sites are more accurately modeled than others.

Table 5: Results of statistical comparisons of measured and modeled selenium concentrations in sampling sites in the Mud River watershed.

Fish Species	**Creek Chubs**		**Green Sunfish**		**Blacknose Dace**	
Statistic	F-Ratio	Prob. Level	F-Ratio	Prob. Level	F-Ratio	Prob. Level
Sampling Site	9.52	0.000001*	8.49	0.000004*	10.46	0.004878*
Measured vs. Modeled	1.63	0.206502	1.12	0.293155	1.20	0.288110
Interaction	11.48	0.000000*	8.43	0.000004*	0.00	0.961575
**Term significant at alpha=0.05*						

Discussion

The reproductive health of the streams was evaluated by comparing the percentage of deformed fish larvae to the whole-body selenium concentration (mg/kg dw) in each stream. Despite having sampled in streams substantially influenced by mining, efforts did not generate deformity rates higher than the 10% which is considered to be protective of fish communities (NAMC, 2008). As shown in Table 6, the deformity rates (teratogenic only - not including edema) were generally lower than the EC_{10} despite tissue concentrations greater than the whole-body screening level of 7.9 mg/kg dw (USEPA 2004).

Table 6: Lotic deformity statistics from larval fish from the Mud River watershed

Collection location and date*	Taxa represented	Average Se fish tissue (mg/kg dw)	Total # of fry	% Yolk sac edema	Total % teratogenic deformities
Sugartree Branch	creek chub	18.24	577	0.17	6.59
Mud River	creek chub & striped shiner	7.61	335	0.60	5.97
Upton Branch	creek chub	7.03	1039	1.35	4.72
Berry Branch	creek chub	4.46	476	2.52	2.73
Berry Branch	white sucker **	1.36	407	0.25	0.25
Upton Branch	white sucker **	1.05	130	0.00	0.00
Berry Branch	creek chub	3.96	295	0.34	1.69

**From 2009 and 2010 reproductive studies (POTESTA a, 2011).*

***White suckers were not collected during the fish tissue sample collections but were present when spawning. Tissue concentrations for this species are represented by an average of all species for which data were available.*

While no deformity rates greater than 10% were measured, a fairly strong regression was generated by the data providing as site-specific screening value. The projected EC_{10} for whole-body fish tissue concentration, 23.69 mg/kg dw, was greater than tissue concentrations measured in most streams in the watershed and population level effects from selenium are not generally expected in lotic systems in the watershed. In one stream individual fish tissue concentrations slightly exceeded this number.

These findings are consistent with a recent publication (Deforest et al., 2011) from Canada which summarized available data for developing selenium thresholds based on selenium egg/ovary concentrations. Deforest suggested that sufficient data were available to support a threshold for toxicity and finds a conservative egg/ovary guideline of 20 mg/kg dw. This value is conservative as it represents the 5th percentile of the species sensitivity distribution and no species mean toxicity thresholds lower than this have been identified. When tissue concentrations exceed the threshold, site-specific studies should be conducted to evaluate potential risks (Deforest et al., 2011). Using a site-specific whole-body to egg/ovary selenium concentration conversion factor developed for the Mud River watershed (POTESTA a, 2011), the 23.69 mg/kg dw selenium whole-body concentration converts to a selenium egg/ovary concentration of 26.15 mg/kg dw.

Additional details from the study described herein can be found in "An Evaluation of the Effects of Selenium on Reproductive Success of Fish in Streams Receiving Coal Mining Discharges – 2010 (POTESTA b, 2011).

An ecosystem-scale model was developed to conceptualize and quantify the process of selenium moving through media in the food web of the Mud River watershed. By employing this type of modeling, dissolved selenium is related to bioaccumulated selenium by systematically quantifying each of the influential processes (Presser and Luoma, 2010). Translating selenium whole-body fish tissue concentrations to a dissolved selenium water column concentration can facilitate site-specific regulation, or show general comparisons among ecosystems (Presser and Luoma, 2010). Additionally, depending on the ecological pathways and biogeochemical conditions in the system, safe levels of dissolved selenium will vary among ecosystems (NAMC, 2008). Ecosystem-scale modeling was utilized to predict whole-body selenium fish tissue concentrations from water column and periphyton in lotic systems, as shown in Tables 7-9.

Table 7: Creek chub whole-body selenium tissue concentrations (mg/kg dw)

Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
Model	**Measure**	**Model**	**Measure**	**Model**	**Measure**	**Model**	**Measure**	**Model**	**Measure**	**Model**	**Measure**
33.79	2.79	7.66	2.77	4.28	13.26	14.84	13.19	15.91	4.99	0.46	3.05
3.72	3.84	6.22	2.74	2.07	10.48	15.48	13.69	1.99	4.41	15.57	3.03
9.45	2.76		2.55	2.41	9.08	18.26	5.52	7.47	3.50	4.37	2.10
	3.46		2.30	2.83	7.22	6.33	14.45	4.07	3.40	4.64	3.81
	3.84		2.28	0.70	11.63	4.62	14.14	3.51	3.76	3.10	3.56
	3.15		2.02	0.87	9.58		15.37	4.11	3.36	1.44	3.85
	3.89		2.74		12.83		15.04	1.38	4.92		3.24
	3.05		1.89		8.40		15.04		3.32		3.18

Table 8: Green sunfish whole-body selenium tissue concentrations (mg/kg dw)

Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
Model	**Measure**	**Model**	**Measure**	**Model**	**Measure**	**Model**	**Measure**	**Model**	**Measure**	**Model**	**Measure**
57.75	8.17	13.09	5.60	7.32	25.57	25.37	28.99	27.19	5.15	0.79	6.29
6.36	6.23	10.62	6.86	2.64	16.46	26.45	25.24	3.41	5.52	19.84	4.68
12.04	12.77		3.57	3.08	18.88	23.28	11.10	9.52	4.82	5.57	3.76
	8.20		5.19	3.61	22.21	8.07	9.15	5.19	4.06	5.91	6.20
	9.30		4.87	0.89	22.51	5.89	31.12	4.47	3.64	3.96	5.68
	7.40		17.64	1.11	27.29			5.23	3.88	1.84	6.30
	9.55		15.24		33.38			1.76	2.68		5.48
	6.84		2.52		16.39				12.06		5.92

Table 9: Blacknose dace whole-body selenium tissue concentrations (mg/kg dw)

Site 4		Site 5	
Model	**Measure**	**Model**	**Measure**
35.15	38.51	37.67	12.64
36.65	23.90	4.72	10.85
32.25	31.06	13.20	7.85
11.18		7.19	8.48
8.16		6.19	8.20
		7.25	9.70
		2.44	

These results were then statistically compared to actual whole-body fish tissue concentrations. The modeled fish tissues were similar to measured values with no significant differences between in green sunfish, blacknose dace or creek chubs. As expected, due to the differences in selenium exposures, significant differences were noted between the sites. The significant interactions indicate additional evaluations would be beneficial to determine factors not included in the model which may be influencing selenium bioaccumulation.

Additionally, variation in measured and modeled data could be attributed to non-detect levels of selenium in both the periphyton and the water column. Non-detect values were not used in the calculations to possible erroneous assumptions skewing the data set. If the actual non-detect values were known and utilized in the modeling, there would be additional modeled data points with lower values and the statistical analyses would show more of a similarity between the measured and modeled data.

The trophic-transfer modeling of selenium was found to successfully predict measured concentrations at Sites 4-6 and may be a useful tool in selenium regulation and monitoring. Additional evaluations to determine factors which may be influencing variability in the model, and overall selenium bioaccumulation, are needed to gain better fitness at the range of site conditions. Similarly, while the strong correlation indicates that the EC_{10} for the lotic environment in the Mud River watershed is in the vicinity of 23.69 mg/kg dw, this estimate is based on a low number of data points. More data are necessary and would provide confidence in the relationship between selenium whole-body concentrations and teratogenic deformities.

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Acronyms

ANOVA	General Linear Model analysis of variance