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Fish Toxicity Testing with Selenomethionine Spiked Feed – What's the Real Question Being Asked?

Mark C. Rigby[a,b], A. Dennis Lemly[c], Russ Gerads[d]

[a]Parsons, 10235 South Jordan Gateway, Suite 300, South Jordan, Utah 84095

[b]Marine Science Institute and the Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, California 93106

[c]USDA-Forest Service, Southern Research Station, Piedmont Aquatic Research Laboratory, Department of Biology, Wake Forest University, Winston-Salem, North Carolina 27109

[d]Applied Speciation and Consulting, 18804 Northcreek Parkway, Bothell, WA 98011

ABSTRACT: The US Environmental Protection Agency and several U.S. states and Canadian provinces are currently developing national water quality criteria for selenium that are based in part on toxicity tests performed by feeding freshwater fish a selenomethionine-spiked diet. Using only selenomethionine to examine the toxicity of selenium is based in part on the limitations of the analytical chemistry methods commonly used in the 1990s and 2000s to speciate selenium in freshwater biota. While these methods provided a good starting point, recent improvements in analytical chemistry methodology have demonstrated that selenium speciation in biota is far more complex than originally thought. Here, we review the recent literature that suggests that there are numerous additional selenium species present in freshwater food chains and that the toxicities of these other selenium species, both individually and in combination, have not been evaluated in freshwater fishes. Evidence from studies on birds and mammals suggests that the other selenium forms differ in their metabolic pathways and toxicity from selenomethionine. Therefore, we conclude that toxicity testing using selenomethionine-spiked feed is only partly addressing the question "what is the toxicity of selenium to freshwater fishes?" and that using the results of these experiments to derive freshwater quality criteria may lead to biased water quality criteria. We also discuss additional studies that are needed in order to derive a more ecologically relevant freshwater quality criterion for selenium.

1. Introduction

Due to its adverse effects on freshwater fishes and birds at relatively low concentrations, selenium has become one of the most widespread and vexing freshwater pollutants in modern times. Selenium is mobilized and released into freshwater habitats through a variety of common, large-scale activities such as agricultural irrigation, coal and phosphate mining, coal combustion, petrochemical operations, and natural gas extraction[1]. Once in freshwater habitats, selenium can quickly bioaccumulate to toxic levels with effects ranging from inconspicuous cellular and internal organ damage to outwardly visible physical deformities and reproductive failure in fish and birds[2,3].

Selenium toxicity occurs at much lower concentrations in freshwater fishes than in birds and mammals[4], with reduced reproductive success (e.g., lower egg hatching rates and increased larval deformity and mortality rates) occurring at the lowest reported exposure levels. The mechanistic causes of selenium toxicity have not been definitively demonstrated. There are, however, two main hypotheses: 1) biotransformation of selenium species generates reactive oxygen species (ROS; i.e. hydrogen peroxide and hydroxyl radicals) that overwhelm antioxidant

defenses and cause oxidative damage to proteins, lipids, and DNA[4,5] and 2) selenium incorporation into proteins instead of sulphur may lead to significant alterations in protein structure and consequently protein function[4]. However, it should be noted that to date neither mechanism has been shown to induce deformities, mortality, or edema in fish larvae.

Due to the greater sensitivity of freshwater fishes to selenium, the US Environmental Protection Agency (USEPA) established a national water quality criterion for selenium in 1987[6] based on fish mortality and reproductive failure documented at Belews Lake[7,8]. Since then, numerous toxicity tests have been performed aimed at answering one basic question: what is the toxicity of selenium to freshwater fishes? A reliable answer to this question has yet to be achieved.

Perhaps the major confounding factor is that many different selenium species occur in water, sediment, and biota. In freshwater and freshwater sediments, selenium is mostly present as the inorganic ions selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}). In biota, however, selenium is present not only as selenate and selenite but also as a variety of organic species that are subject to additional biotransformation(s) at each level of the food chain. The biotransformation of selenium and limitations in the available analytical chemistry methods means that our understanding of selenium speciation in biota is still evolving. In the 1990s, selenium speciation analyses were plagued by coelution of different seleno-amino acids, as well as limited resolution between seleno-proteins[9]. This allowed only a handful of selenium species to be identified and quantified. Molecular transformations during extraction, as well as the misapplication of enzymatic and other destructive extraction approaches, resulted in the identification of selenium species that were artifactually generated during preparation[10]. Newer methods have recently become available[11–13] that have added resolution to selenium speciation in biota, though they are still limited in their application. This more recent literature appears to indicate that the amount and types of organic selenium species may differ among fungi, plants, invertebrates, and mammals, and that the dominant species may differ among these biota[11–16].

In the laboratory, the question of selenium toxicity to freshwater fishes is usually evaluated by giving fish selenomethionine-spiked feed[17–22]. Although not usually explicitly stated, selenomethionine is used because it is 1) cheaper than the other commercially available species, 2) more readily absorbed by fishes from foods than either selenate or selenite[23], and 3) generally believed to be both the primary organic form in the aquatic food chain and the primary cause of ecotoxicity[3,24]. However, using only selenomethionine to evaluate the dietary toxicity of selenium to freshwater fishes incorporates three major assumptions: 1) other forms of selenium are either absent or present at insignificant concentrations, 2) if other forms of selenium are present in the diet of fishes, the other forms do not differ in their toxicity to fishes, and 3) if selenium speciation in fish tissues affects toxicity, dietary exposure to selenomethionine results in selenium speciation in fish tissues similar to natural exposures.

Although these assumptions are central to using the results of previous laboratory-based fish toxicity tests to derive water quality criteria, they have not been experimentally evaluated and were not considered when USEPA issued its draft criteria in 2004 and 2010, by Deforest et al[25], and by Kentucky DEP[26] in its recently approved chronic freshwater criterion. USEPA and several U.S. states and Canadian provinces are currently working on deriving water quality criteria. As the states and provinces are generally deriving selenium criteria based on selenium concentrations in fish ovaries and/or eggs, we thought it prudent to critically evaluate these assumptions.

2. Discussion

2.1. Other forms of selenium in fish diets.

In general, the diets of freshwater fishes in North America include detritus, plankton, invertebrates, and other fishes[27]. Therefore, selenium speciation in these types of biota should be evaluated before conducting dietary toxicity testing on fishes. Although Fan et al.[24] are often cited as showing that selenomethionine is the dominant selenium species in freshwater biota, the only selenium species that they attempted to identify was selenomethionine. Further, their results showed that while 18% of protein-bound selenium in macroinvertebrates was selenomethionine, 72% was in other form(s). However, Fan et al.'s[24] results should be considered semi-quantitative as the response for the selenomethionine spiked extracts differed by sample matrix (suggesting matrix interferences may have biased the results) and seleno-proteins were not spiked prior to the acetonitrile precipitation stage.

Many recent studies have used X-ray absorption spectroscopy (XAS) and X-ray absorption near-edge spectroscopy (XANES) to speciate selenium in aquatic biota[28–34]. However, XAS/XANES can only determine the class of selenium species and cannot distinguish between selenomethionine or any other C-Se-C selenium species (where C is a carbon atom)[16,35–37]. Some authors also do not distinguish between C-Se-C and C-Se-H[28,29,31,38,39]. While there are a great many different C-Se-C/ C-Se-H containing selenium species that have been reported from biota (Table 1), most of the studies to date on selenium speciation in freshwater biota that have used XAS/XANES[28–34] have assumed that the only C-Se-C present is selenomethionine.

In addition to C-Se-C/ C-Se-H, several recent XAS/XANES studies have shown that there are a number of other classes of selenium compounds present in freshwater biota (Figure 1). While those studies found that C-Se-C/ C-Se-H is generally the dominant class of selenium compounds, other types are present, including elemental selenium (Se^0), inorganic selenium (i.e., selenate, selenite, and iron bound selenium), selenoxides (C−Se(O)−C; e.g., dimethylselenoxide), diselenides (C-Se-Se-C; e.g., selenocystine and dimethyldiselenide), trimethyl selenonium (C_3-Se^+), and polyselenides (Se-Se-Se; e.g., seleno-homocysteinyl-diseleno-homocysteine)[12,28,29,31,32,39,40,41].

Recent advances in analytical chemistry methods have permitted the definitive speciation of a much broader range of selenium compounds at much lower concentrations than were possible in the 1990s and 2000s[11-13]. Using the recently developed SAX-HPLC-ICP-MS (strong anion exchange-high performance liquid chromatography-inductively coupled plasma-mass spectrometry), Schmidt et al.[12] definitively speciated and quantified the selenium in several different types of aquatic biota. They found that selenomethionine was the dominant selenium species in brine shrimp and brine flies but inorganic selenium (i.e., selenite and selenate) was the dominant form in bacteria, diatoms, and green algae (Figure 2) in a brackish marsh. It should also be noted that ESI-Orbitrap-MS-MS (electrospray ionization-orbital electrostatic trap-tandem mass spectrometry) and RP-HPLC-ESI-Q-TOF-MS (reverse phase-high performance liquid chromatography-electrospray ionization-quadrupole-time of flight-mass spectrometry) have been used to definitively speciate selenium in yeast and terrestrial plants[12,41–46], resulting in the identification of an even wider diversity of organoselenium compounds than found by Schmidt et al.[12]. Thus, until more definitive selenium speciation studies are performed on aquatic biota, selenomethionine should not be assumed to be the primary selenium species present in the diet of all freshwater fishes. Furthermore, differentiation between free seleno-amino acids and those

incorporated into seleno-proteins may be necessary as not all seleno-proteins have been identified. Different enzymatic activities within organisms may induce different levels of denaturation and toxicological effects. Unfortunately, while XAS/XANES can identify the classes of selenium compounds in aquatic biota, these methods cannot definitively identify selenium species and, therefore, are of limited utility for selenium speciation.

2.2. Differential toxicity of selenium species.

To our knowledge, only one study has been published attempting to evaluate the dietary toxicity of different forms of selenium in fishes. Hamilton et al.[21] fed fingerling chinook salmon (*Oncorhynchus tshawytscha*) a mix of commercial fish food and freeze-dried mosquitofish (*Gambusia affinis*) in one of three diets as follows: 1) mosquitofish from a low-selenium site (control), 2) mosquitofish from a low-selenium site spiked with seleno-DL-methionine, and 3) mosquitofish from a high-selenium site. Commercial fish food and freeze-dried mosquitofish were mixed at different ratios to create several different total selenium concentrations in the fish feed. The authors found that reduced survival and growth occurred at lower total dietary selenium concentrations for feeds mixed with mosquitofish from the high-selenium site than feed mixed with seleno-DL-methionine. Although this appears to suggest that naturally bioaccumulated selenium may be more toxic to fishes than seleno-DL-methionine spiked feed, the feed mixed with mosquitofish also had elevated concentrations of boron, chromium, nickel, and strontium. Additionally, the mosquitofishes were collected from an agricultural drainage and, therefore, may have also had elevated levels of pesticides, which the authors didn't analyze. Therefore, Hamilton et al.'s[21] results cannot be used to evaluate the differential toxicity of naturally bioaccumulated selenium vs. feed spiked with selenomethionine.

Another differential toxicity study that is often cited as demonstrating that selenomethionine is more toxic than other selenium species to fishes is that of Niimi and LaHam[47]. By exposing newly hatched zebrafish (*Danio rerio*) to eight different dissolved selenium species, the authors observed the following toxicity hierarchy based on 5% mortality after 10 days of exposure: selenomethionine > sodium and potassium selenite > selenium dioxide > selenocystine > potassium and calcium selenate > sodium selenate. This would appear to indicate that selenomethionine is more toxic than inorganic selenium, which is in turn more toxic than selenocystine. However, since waterborne exposures were used to evaluate toxicity, and the larvae that they used were too young to feed, their results should not be assumed to represent chronic dietary toxicity. Rather, these results are more representative of the acute toxicity of waterborne releases from absorption across the skin and gills.

In contrast to the lack of comparative dietary toxicity studies in fishes, a limited number of such studies have been performed using birds and mammals. Heinz et al.[48] fed day old mallards (*Anas platyrhynchos*) three different forms of selenium (seleno-DL-methionine, seleno-L-methionine, and selenized yeast) added to a commercial duck food at two doses. The authors found that seleno-L-methionine reduced survival at the highest dose tested, whereas seleno-DL-methionine and selenized yeast did not. The authors also found that body weights differed among treatments after 2 weeks depending upon the dietary form of selenium as follows: control > selenized yeast > seleno-DL-methionine = seleno-L-methionine. In pigs, Kim and Mahan[49,50] demonstrated that feed spiked with sodium selenite caused a greater reduction in weight gain than feed mixed with selenized yeast at the same total selenium concentrations. In rats, Jia et al.[51] demonstrated that

reduced gain and enlarged livers and kidneys occurred at lower dietary total selenium intakes when the selenium was mixed in the feed as either "natural high selenium soybeans" or selenite vs. Se^0. Since the selenium in the seleninzed yeast[48-50] and high selenium soybeans[51] in the studies described above was not speciated, how closely the selenium speciation in the experimental diets reflects that in a natural diet cannot be determined. Nonetheless, there does appear to be some evidence to suggest that dietary toxicity differs among selenium species.

While the metabolic pathways for selenium have not been elucidated in fishes, it is presumably similar to that of mammals[5]. In mammals, the metabolism of 1) inorganic selenium (i.e., selenate and selenite), 2) selenomethionine, 3) selenocysteine, selenocystine, and 4) methylselenocysteine follow different pathways[52,53]. However, there appear to be multiple metabolic pathways for selenomethionine, at least one of which is common to the metabolic pathway for selenocysteine and selenocystine[53]. This is noteworthy as Barger et al.[54] showed that mice fed diets spiked with the same total selenium concentration as sodium selenite and selenized yeast had similar genetic expression profiles (in both the expression pattern of individual genes and gene functional categories) which were distinct from the genetic expression profile of mice fed a diet spiked with selenomethionine. Barger et al.[54] posited several potential explanations, including: 1) although selenomethionine may be the dominant selenium species in selenized yeast, it may be incorporated into larger proteins as opposed to the free selenomethionine in the selenomethionine spiked diet and 2) other selenium species may be present in selenized yeast, and 3) dietary selenomethionine may be "converted to a biologically less active derivative." Thus, the difference in metabolic pathways among selenium species, including naturally bioaccumulated selenium, may also translate to differential toxicity.

2.3. Selenium speciation in fish eggs, ovaries, and testes.

In fishes, reduced reproductive success is symptomatic of exposure to the lowest selenium concentrations. Thus, selenium speciation in eggs, ovaries, and testes are generally assumed to be of primary interest. While controlled experiments evaluating selenium speciation in fish reproductive organs are not available, three recent field studies have speciated selenium in fishes exposed to selenium. Nautilus Environmental[55] speciated selenium in the eggs of wild-caught Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) using ICP-MS and found that, on average, 58.2% of the selenium in eggs was incorporated into unspecified proteins, 11.7% selenite, 2.4% selenocyanate, 0.8% free selenomethionine, 0.3% methylsenic acid, and the remaining 26.6% being unaccounted for. Dreissenack et al.[38] speciated the selenium in fathead minnows (*Pimephales promelas*) caged in streams using XANES and found that the selenium present in ovaries, eggs, and larvae consisted of C-Se-C/ C-Se-H, diselenides (C-Se-Se-C), and selenite. Their results appear to show that while the fraction of C-Se-C/ C-Se-H decreases with development (i.e., ovaries > eggs > larvae), the fraction of diselenides and selenite increases with development. This may suggest that the selenium-containing compounds are being metabolized. Additionally, Hasegawa et al.[56] speciated the selenium in the cytoplasm of salmon (species not given) eggs purchased in a Japanese supermarket using HPLC-ICP-MS. They found seleno-proteins, some of which contained cysteine, and a seleno-amino acid (e.g., selenomethionine and selenocysteine). Thus, the available data indicate that there are likely to be multiple selenium species in fish eggs, ovaries, and testes. If selenium speciation in these tissues is affected by dietary selenium species, reproductive success may also be affected.

3. Conclusions

The standard practice for laboratory selenium toxicity tests is to feed fish diets spiked only with selenomethionine. However, freshwater fishes in the wild are likely exposed to large variety of dietary selenium species, with metabolic pathways differing from that of selenomethionine that likely also lead to differences in toxicity. Our review of the literature suggests that current standard laboratory tests for selenium toxicity in freshwater fishes may only partly answer the question "what is the toxicity of dietary *selenium* exposures to freshwater fishes?" but rather the more specific question "what is the dietary toxicity of *selenomethionine* to fishes?" Additionally, we caution against constructing a species sensitivity distribution (SSD) or deriving an ambient water quality criterion using the results of both 1) field toxicity tests where fishes are exposed to a large variety of selenium species and 2) laboratory toxicity tests conducted using a single selenium species.

We see a need for performing 1) evaluating the effect of excluding toxicity tests in which fish are fed a selenomethionine spiked diet in SSDs, which we plan to do shortly, 2) additional definitive selenium speciation studies, like that of Schmidt et al.[12], to determine whether selenomethionine is the primary dietary form of selenium for freshwater fishes from multiple trophic levels and types of freshwater bodies and 3) comparative studies on the effects of dietary selenium speciation in fish eggs, ovaries, and testes using several species from multiple trophic levels and different families would provide some insight into these assumptions. To evaluate the assumptions discussed here, performing dietary toxicity studies with fish comparing the effects of using a) selenomethionine-spiked feed to b) a diet naturally rich in selenium is needed. Any such experiment, however, should strive eliminate other contaminants into the feed, as was the case for Hamilton et al.[21]. One way to accomplish this is to use mesocosms where selenate and selenite are introduced into the water column and allowed to bioaccumulate in a range of aquatic biota that are subsequently fed upon by fishes[57–59].

Disclaimer

The views expressed in this review do not reflect the views and policies of the US government, United States Forest Service, Parsons, UCSB, or Applied Speciation. Mention of trade, product, or company names does not constitute endorsement.

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Figure Captions

Figure 1. Result of recent aquatic biota speciation studies using XAS/XANES. Selenium classes shown include elemental selenium (Se^0, black), selenate/selenite (SeO_4^{2-}/SeO_3^{2-}, white), iron bound selenium (Fe-Se-X, red), selenides/selenols (C-Se-C/C-Se-H, blue), selenoxides (C−Se(O)−C, green), diselenides (C-Se-Se-C, purple), and trimethyl selenonium (C_3-Se^+, yellow). Note that even though selenides/selenols are the dominant form, the XAS/XANES cannot distinguish among the species of selenides/selenols. Where results for more than one species, field location or treatment were provided, the average is shown.

Figure 2. Speciation of aquatic biota using SAX-HPLC-ICP-MS[12]. Selenium species shown include selenate/selenite (SeO_4^{2-}/SeO_3^{2-}, white), selenomethionine (blue), selenocytine (purple),

selenocysteine (red), methionine selenoxide (green), selenocystathionine (yellow), and γ-glutamyl-methyl-selenocysteine (black). Where results for more than one species or life stage were provided, the average is shown. The totals for bacteria/diatoms/algae and brine shrimp have been scaled up to 100% here for illustrative purposes.

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Figure 1

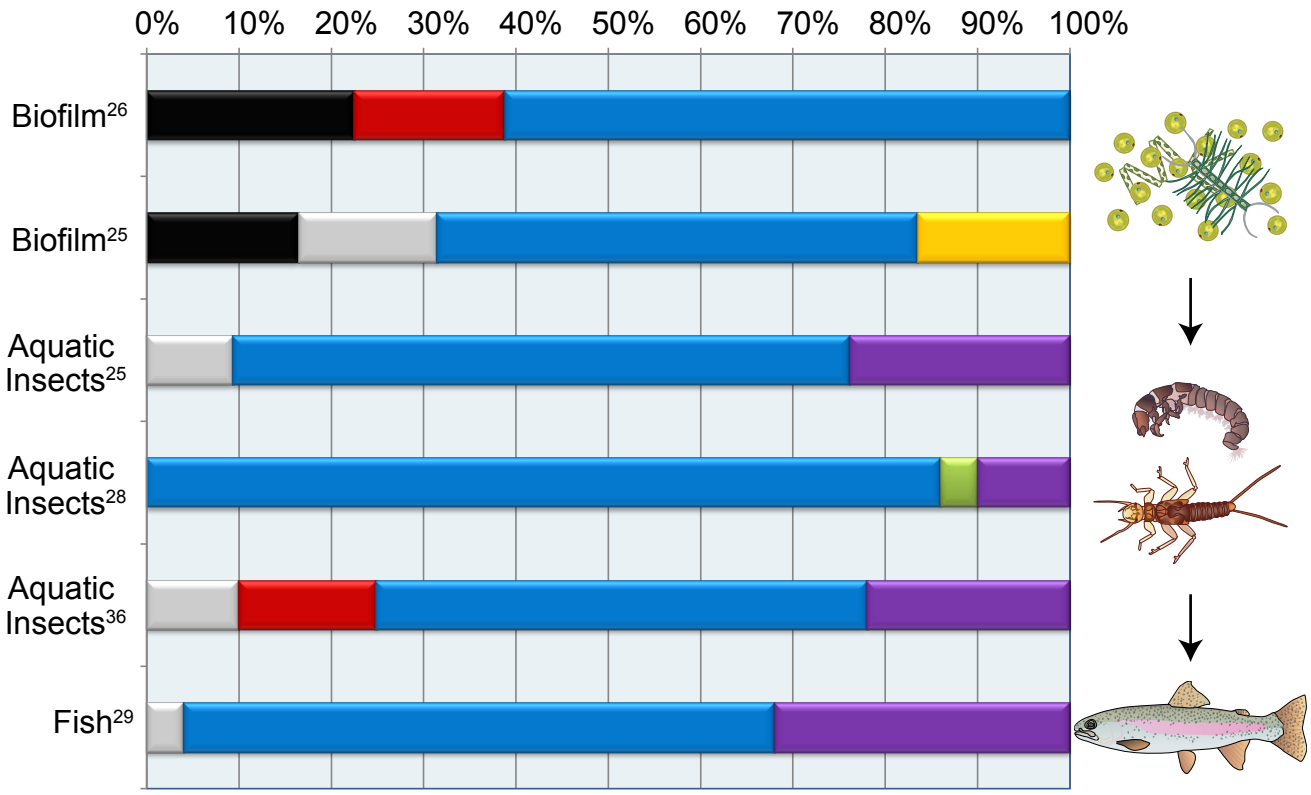

Figure 2

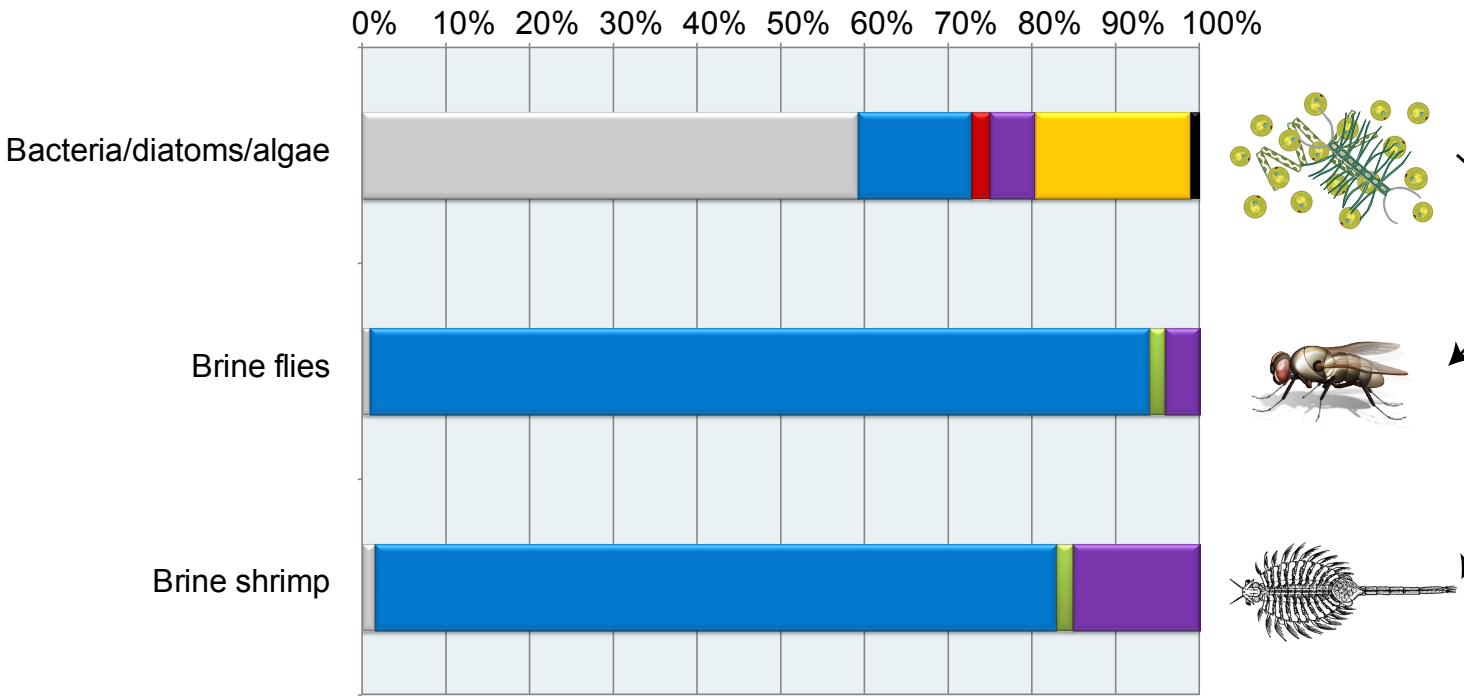

Table 1. Partial list of C-Se-C and C-Se-H containing chemicals found in biota. The observed mass to charge ratio (m/z) is also given for use by analytical chemists. Note that there are more than are shown here and the number of known compounds is continually expanding.

Chemical	Found in	m/z	Formula
Selenides (C-Se-C)			
Allyl-N-hydroxy-selenourea	Plants	181	$C_4H_9N_2OSe$
Dimethylselenide	Plants, animals	110	C_2H_6Se
Methyl seleno-adenosine	Yeast	346	$C_{11}H_{15}N_5O_3Se$
Ethyl seleno-adenosine	Yeast	360	$C_{12}H_{18}O_3N_5Se^+$
Methyl seleno-glutathione	Yeast	370	$C_{11}H_{20}O_6N_3Se^+$
Methyl-selenocysteine	Yeast, plants	184	$C_4H_{10}NO_2Se$
Deamino methyl-selenocysteine	Plants	167	$C_4H_7O_2Se$
y-glutamyl-methyl-seleno-cysteine	Yeast, plants	313	$C_9H_{17}N_2O_5Se$
2,3-Dihydroxy-propionyl-methyl-selenocysteine	Yeast	272	$C_7H_{14}NO_5Se$
Seleno-adenosyl-homocysteine	Yeast, plants	433	$C_{14}H_{21}O_5N_6Se^+$
Allyl-seleno-adenosyl-homocysteine	Yeast	431	$C_{14}H_{19}O_5N_6Se^+$
Methyl-seleno-dehydro-homocysteine	Yeast	196	$C_5H_{10}NO_2Se^+$
Seleno-hydroxy-adenosyl-homocysteine	Yeast	449	$C_{14}H_{21}O_6N_6Se^+$
N-3-Hydroxy-propionyl-seleno-adenosyl-homocysteine	Yeast	505	$C_{17}H_{24}N_6O_7Se$
N-2,3-Dihydroxy-propionyl-seleno-adenosyl-homocysteine	Yeast	521	$C_{17}H_{24}N_6O_8Se$
N-2,3-Dihydroxy-propionyl-seleno-adenosyl-2,3-dihydroxy-propionyl-homocysteine	Yeast	595	$C_{20}H_{29}N_6O_{10}Se$
Seleno-biotin-sulfoxide	Yeast	377	$C_{13}H_{20}N_4O_2Se$
Seleno-cystathionine	Yeast, plants	271	$C_7H_{14}N_2O_4Se$
y-glutamyl-seleno-cystathionine	Yeast, plants	400	$C_{12}H_{22}O_7N_3Se^+$
N-acetyl-seleno-cystathionine	Yeast	313	$C_9H_{16}N_2O_5Se$
N-propionyl-seleno-cystathionine	Yeast	357	$C_{10}H_{17}O_7N_2Se^+$
N-2,3-dihydroxy-propionyl-seleno-cystathionine	Yeast	359	$C_{10}H_{18}N_2O_7Se$
Seleno-homolanthionine	Yeast, plants	285	$C_8H_{17}O_4N_2Se^+$
2,3-dihydroxy-propionyl-seleno-homolanthionine	Yeast	373	$C_{11}H_{21}N_2O_7Se$
Deamino-hydroxy-seleno-homolanthionine	Plants	286	$C_8H_{16}NO_5Se$
2,3-dihydroxy-propionyl-seleno-lanthionine	Yeast, plants	345	$C_9H_{17}N_2O_7Se$
Seleno-methionine	Yeast, plants, animals	198	$C_5H_{11}NO_2Se$
y-glutamyl-seleno-methionine	Plants	326	$C_{10}H_{18}O_5N_2Se$
Adenosyl-seleno-methionine	Plants	432	$C_{15}H_{23}N_6O_5Se^+$
Selenosugars and carbohydrates (C-Se-C)			
Deamino selenocysteine-hexose	Plants	317	$C_9H_{17}O_7Se$
Methyl-seleno-pentose-hexose	Plants	407	$C_{12}H_{23}O_{10}Se$
Methyl-seleno-deoxypentose-hexose	Plants	408	$C_{12}H_{26}NO_9Se$
Selenohomocysteine-ribofuranose	Yeast	316	$C_9H_{17}NO_6Se$
Selenols (C-Se-H)			
Methylselenol	Animals	96	CH_4Se
Seleno-adenosine	Yeast	332	$C_{10}H_{13}N_5O_3Se$
Seleno-cysteine	Plants, animals	169	$C_3H_7NO_2Se$
Seleno-glutathione	Yeast	356	$C_{10}H_{18}O_6N_3Se$
Seleno-homocysteine	Plants, animals	364	$C_4H_9NO_2Se$
Seleno-neine	Animals	553	$C_{18}H_{29}N_6O_4Se_2$
Acylated choline related compounds (C-Se-C)			
Methyl-seleno-acetyl-choline	Plants	240	$C_8H_{18}NO_2Se$
Methyl-seleno-butyryl-choline	Plants	268	$C_{10}H_{20}NO_2Se$
Glucosinolates (C-Se-C)			
Gluco-seleno-erucin	Plants	468	$C_{12}H_{22}NO_9S_2Se$
Gluco-seleno-iberverin	Plants	454	$C_{11}H_{20}NO_9S_2Se$
Methyl-seleno-acetyl-gluconapin	Plants	508	$C_{14}H_{22}NO_{10}S_2Se$
Methyl-seleno-acetyl-sinigrin	Plants	494	$C_{13}H_{20}NO_{10}S_2Se$
Methyl-seleno-hydroxy-gluco-brassicin	Plants	559	$C_{17}H_{23}N_2O_{10}S_2Se$
Methyl-seleno-sinapoyl-gluconapin	Plants	674	$C_{23}H_{32}NO_{13}S_2Se$
Methylselenosinapoylsinigrin	Plants	660	$C_{22}H_{30}NO_{13}S_2Se$

Chemical	Found in	m/z	Formula
Sinapine related compounds (C-Se-C)			
Methylselenosinapine	Plants	404	$C_{18}H_{30}NO_4Se$
N-2,3-dihydroxyl-propionyl-selenocysteine-sinapine	Plants	567	$C_{22}H_{35}N_2O_{10}Se$

Environmental impact statement

The standard laboratory toxicity test that is used to evaluate the ecotoxicity of selenium to fishes is to spike the fishes' food with selenomethionine. However, using selenomethionine spiked feed to evaluate the ecotoxicity of selenium in general makes several assumptions. In the paper that my co-authors and I have prepared, we evaluate the current scientific literature and we believe that we have shown that those assumptions are not supported. Thus, we believe that using the results of toxicity tests in which fish are fed a selenomethionine spiked diet to develop water quality criteria may lead to biased results.