Assessment of Trace Metals in Tissues of Geoduck Clams from Eastern Puget Sound

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Abstract

The Suquamish Tribe and the Washington State Department of Health partnered to assess the concentrations of trace metals (As, Cd, Cr, Pb, and Hg) in the tissues of geoduck clams harvested from the Richmond Beach tract, located between Edmonds and Seattle, Washington in eastern Puget Sound. The purpose of this assessment was to evaluate the human health risks associated with consumption of geoducks from this area of Puget Sound. Such assessments require that samples be processed in a manner consistent with how they would be prepared by consumers. Accordingly, tissues from 60 geoducks collected from the Richmond Beach tract were segregated into three parts: viscera, siphon/mantle, and the outer skin of the siphon. Viscera and siphon/mantle tissues from each geoduck were separately analyzed for total As, Cd, Cr, Pb, and Hg. Composites of viscera and siphon/mantle tissues were analyzed for inorganic As. A subset of tissues from the outer skin of siphons was also analyzed. In general, trace metal concentrations were highest in the outer skin, and lowest in the siphon/mantle. The results from this study have helped to explain inconsistent results among past studies and suggest some considerations in future studies or health assessments of geoduck tissues.

Introduction

In 2006, the Suquamish Tribe and the Washington Department of Health collaborated to assess the status of trace metal contamination in geoducks from the Richmond Beach tract. The primary purpose of this assessment was to determine if, with respect to trace metal contaminants, geoducks from this tract are suitable for subsistence consumption and for commercial harvest.

The Richmond Beach tract of geoducks is located near the City of Seattle along the eastern shore of Puget Sound between Point Wells and Meadow Point (see figure 1). The adjacent shore is a highly urbanized environment characterized mostly by residential and commercial development. There are multiple potential sources of pollution in the surrounding area (e.g. stormwater, wastewater outfalls, marinas and other shore facilities). Because it was not known how current and past pollution may have impacted geoducks in this area, a necessary first step in the process of certifying this area for harvest was to determine whether concentrations of contaminants were at an acceptably low level for tribal subsistence and recreational consumption and commercial harvest.

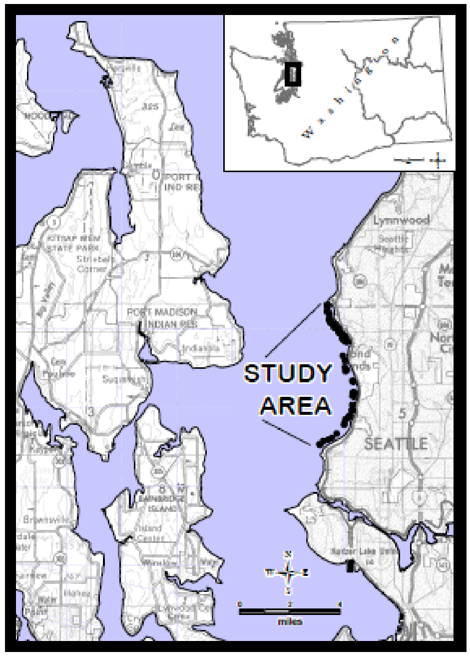

Figure 1: Map of study area with sample locations.

Contaminants of Concern

Relative to studies of intertidal bivalves, there are few studies of chemical contaminants in tissues of subtidal geoducks from Puget Sound. Consequently, little is known about how contaminant levels in geoducks vary by location, age, or tissue type. However, existing studies do reveal that organic contaminants are seldom detected or detected at very low concentrations in geoduck tissues (Barrick 2004; King County 2002; Kyte and Quiring 1999). This is true even for geoduck tissues sampled in areas that are known to have been impacted by organic contaminants by past industrial uses (Suquamish Tribe 2006; WDOH 2005). Conversely, metals have frequently been detected, and occasionally at concentrations that may be significant to human health. In particular, Pb and total As had been detected at relatively high concentrations in studies located near the Richmond Beach tract (Kyte and Quiring 1999; King County 2002; Suquamish Tribe 2006). We determined for this study that the contaminants most likely to cause concern for human health are the trace metals As, Cd, Cr, Pb, and Hg.

A challenge in reviewing and comparing the results of previous studies is the lack of a consistent approach in the sampling and analysis procedure for geoduck tissues. Existing Puget Sound protocols for determining contaminant concentrations in bivalve tissues were designed for smaller, shorter lived, intertidal species (PSAMP 1990). Past geoduck studies vary in their approach to compositing versus individual samples and the splitting of samples by tissue type (e.g. viscera and "edible" portions) and whole body samples. The example shown in Table 1 illustrates the challenge of comparing past studies. The first of the studies shown was performed as part of investigations for the siting and permitting of the Brightwater wastewater treatment plant. These geoduck samples were collected near Point Wells in Puget Sound (a sample area that overlaps the northern portion of the Richmond Beach tract). The second study was performed by the Suquamish Tribe on tracts adjacent to Eagle Harbor on the eastern shore of Bainbridge Island (Port Blakely and Tyee Shoals tracts; see WDFW 2002). Displayed are mean values of analytical results for As, Cd, Cr, and Pb. The studies differed in the 2 important respects. First, the King County study was an analysis of tissues from 18 individual clams whereas the Eagle Harbor study analyzed tissues from composite samples of 5 geoducks each and sampled from 6 locations. Second, King County analyzed 9 geoducks as whole body samples and separately analyzed only the "edible portion" of the other 9 geoduck samples. "Edible" portion samples consisted of just the siphon and mantle with the outer skin removed (King County did not analyze viscera separately). Whereas, in the Eagle Harbor study, samples were made up of composited viscera and siphon/mantle composited with skins included (the Suquamish Tribe did not analyze whole bodies).

King County was lead to conclude from their results that trace metals were more concentrated in the viscera tissues (King County 2002). However, we hypothesized that the inconsistent results suggested by the results of these 2 studies (especially for Cd, Cr, and Pb) were due to the differences in their design mentioned above. In particular, we were interested in whether including the outer skin in the homogenate of siphon/mantle tissues in the Eagle Harbor study could help explain the inconsistent results noted.

Table 1: Comparison of mean concentrations of select metals in geoduck tissues from 2 studies in Puget Sound (mg/Kg wet weight).

	King County (Brightwater)[1]		Eagle Harbor[2]	
	Whole	"Edible"	Viscera	Siphon/mantle
Arsenic	3.71	2.40	21.13	18.27
Cadmium	0.29	0.12	0.78	1.59
Chromium	0.41	0.18	3.34	7.06
Lead	2.62	0.05	1.19	4.73

[1]King County 2002, [2]Suquamish Tribe 2006

Human health comparison values

Comparison values were calculated in a manner consistent with EPA guidance (USEPA 2000). For the purpose of this assessment, comparison values were intended to represent the concentration of a contaminant that would result in a subsistence consumer's dose that is either equivalent to a non-cancer oral reference dose or increased cancer risk of 1×10^{-5}. The Suquamish Tribe 90th percentile (consumers only) all shellfish consumption rate (4.6 g/kg/day) was used to derive comparison values (Suquamish Tribe 2000). Contaminant levels exceeding comparison values indicate the need for a more thorough assessment to ensure public health is protected.

Inorganic Arsenic

Another objective of this study was to test a common assumption that inorganic As comprises about 10% of total As in fish and shellfish tissues in Puget Sound (e.g. Kyte and Quiring 1999). Arsenic toxicity is primarily associated with the inorganic species As^{+3} and As^{+5}, but for fish and shellfish in Puget Sound, inorganic species of As generally account for a small proportion of total As (Johnson and Roose 2002). Assuming that inorganic As is 10% of total As is thought to overestimate the actual concentration of inorganic As, and therefore be a conservative assumption when determining the potential health risks associated with fish or shellfish consumption.

Methods

The Richmond Beach tract was divided into 3 sections (north, south, and middle sections). Twenty locations (defined by their latitudes and longitudes) from each section were randomly generated using a Geographic Information System (GIS) software program for a total of 60 geoduck sample locations. Samples were located in the commercial harvest zone between depths of -18 and -70 MLLW.

Divers equipped with surface supplied air using standard harvesting methods collected geoducks at the 60 predetermined sampling locations in May of 2006. Tissue was separated from shells and then dissected to separate the viscera from the siphon/mantle. The outer skin on the siphon and mantle was removed by first dipping in near boiling water and then peeling away from the inner, fleshy tissue. Fourteen skin tissue samples were retained for trace metal analysis and the remainder were discarded.

Viscera, siphon/mantle, and outer skin tissue samples were separately homogenized in a blender fitted with a titanium blade. Tissue homogenates and samples of the water used to remove the outer skin were analyzed for total As, Cd, Cr, and Pb by ICP-MS and for Hg by CVAA (PSEP 1996). Twenty-four composite samples (6 composites from each of the 3 sampling sections) were formed from the 120 (60 siphon/mantle and 60 viscera) tissue homogenate samples and analyzed for organic and inorganic As species by ion-chromatography/ICP-MS (EPA 2006). Arsenic in the outer skin tissues was not speciated (Suquamish Tribe and WDOH 2006).

Shells were retained and later used to determine the age of individual geoducks. This is accomplished by counting the growth annuli observed in a thin cross-section taken from the umbo of one valve (Honeycutt and Valero 2003).

Results

Geoduck ages ranged from 8 to 106 years with a mean age of 45 and median of 44 years. The ages of 4 samples could not be determined.

Trace metals

In general, trace metals were present at detectable concentrations in the three tissues types analyzed. The only exceptions were for Cr, which was not detected in 10 of 60 siphon/mantle tissue samples, and for Pb, which was not detected in 13 of 60 siphon/mantle tissue samples. All metal analytes except Cr were detected in at least 1 sample of water used to remove the outer skin. As and Cd were detected in all water samples. Pb was detected in all except 2 water samples, and Hg was detected in all except 1 water sample.

Results by tissue type and analyte are summarized in Table 2 together with human health comparison values (CVs). Mean concentrations in edible tissues (siphon/mantle tissue and viscera tissue) for Cd, Cr, Pb, and Hg were mostly below CVs, whereas the mean concentration in the outer skin tissues (considered a non-consumed tissue) exceeded the health comparison value for Cd, Cr, Pb, and Hg (outer skins were not analyzed for inorganic As).

Table 2: Mean and median trace metal concentrations by tissue type, and health comparison values (mg/Kg wet weight).

	Siphon/Mantle		Viscera		Outer Skin		Health Comparison value[2]
	Mean	Median	Mean	Median	Mean	Median	
Total As	2.60	2.70	3.74	3.35	7.34	4.42	*
Cd	0.10	0.10	0.11	0.10	1.62	1.61	0.2
Cr	0.07	0.06	0.29	0.24	1.10	0.88	0.7
Pb	0.01	0.02	0.11	0.09	24.9	22.7	0.07
Hg	0.015	0.015	0.022	0.015	NA[1]	NA[1]	0.02

[1]interference suppressed analytical results for Hg in skin tissue
[2] Cd, Cr, Hg comparison values based on non-cancer reference doses; Pb comparison value based on IEUBK model (child consumption rate of 50 g/day); Cr comparison value assumes all Cr present as Cr^{+6}
*Toxicity is primarily associated with inorganic As. A comparison value for inorganic As of 0.002 mg/Kg wet weight was based on cancer risk of 1×10^{-5}. Inorganic As results are discussed below.

Inorganic As

Inorganic As (As^{+3} + As^{+5}) was not detected (method detection limit = 0.082 mg/Kg dry weight) in any composite samples of siphon/mantle tissues and was present at detectable concentrations in only in 2 of

12 composite samples of viscera tissue. The wet weight detection limit in siphon/mantle tissues (computed based on percent solids in the sample) ranged from 0.0165 mg/Kg to 0.0196 mg/Kg inorganic As. Similarly, the wet weight detection limit for viscera tissues range from 0.0102 mg/Kg to 0.0146 mg/Kg inorganic As.

Trace metal concentration by tissue type

In general, trace metal concentrations were greatest in the outer skin tissue and lowest in the siphon/mantle tissues (see Table 2 and Figure 2). For a statistical comparison of trace metal concentrations between tissues types we used a non-parametric test (2-tailed Wilcoxon signed rank test). Differences were significant ($p < 0.05$) for all paired tissue types except for 2 combinations: 1) [As] between skin and viscera and 2) [Cd] between viscera and siphon/mantle.[1] This result is apparent in plots of trace metal concentration by tissue type versus age (see Figure 2). The differences are most marked for Cr and Pb where, in each geoduck, the concentration in the outer skin was greater than that in the viscera tissue, which in turn exceeded the concentration in the siphon/mantle tissue. Cadmium concentration in the outer skin of each geoduck sampled similarly exceeded the concentration found in either the viscera or siphon/mantle tissue.

Table 3: Regression analysis of trace metal concentration – age data.

	Siphon/mantle n=56			Viscera n=56			Outer skin[2] n=14		
	r^2	F-ratio	*P*-value	r^2	F-ratio	*P*-value	r^2	F-ratio	*P*-value
As	0.66	103	<0.001	0.29	22.2	<0.001	0.71	28.8	<0.001
Cd	0.43	41.5	<0.001	0.03	1.38	0.245	0.64	21.5	0.001
Cr	0.86	337	<0.001	0.23	16.4	<0.001	0.55	14.7	0.002
Pb	0.24	17.0	<0.001	0.03	1.82	0.183	0.85	65.9	<0.001
Hg	0.78	189	<0.001	0.52	58.1	<0.001	NA[1]	NA[1]	NA[1]

[1]interference suppressed analytical results for Hg in skin tissue
[2]Metals concentrations in outer skin tissue were log transformed for regression

Age – trace metal concentration relationship

We used a linear regression model to examine the relationship between age and metal concentrations in each of the tissue types (see Table 3; concentrations in outer skin tissue were log transformed). Linear regression against age was significant ($p < 0.05$) for all metals concentrations in siphon/mantle tissues and all of the outer skin tissues. Linear regression of trace metal concentrations in viscera with age was significant only for As, Cr, and Hg. In general, geoduck age had the highest coefficients of determination (r^2) with [Cr], [Hg], and [As] in siphon/mantle and visceral tissue. Regression of the log concentrations in outer skin tissues was significant for As, Cd, Cr, and Pb (Hg results were not available).

[1]Due to interference that likely caused suppression of Hg results for outer skin tissues, no comparisons were made to [Hg] in outer skin tissue.

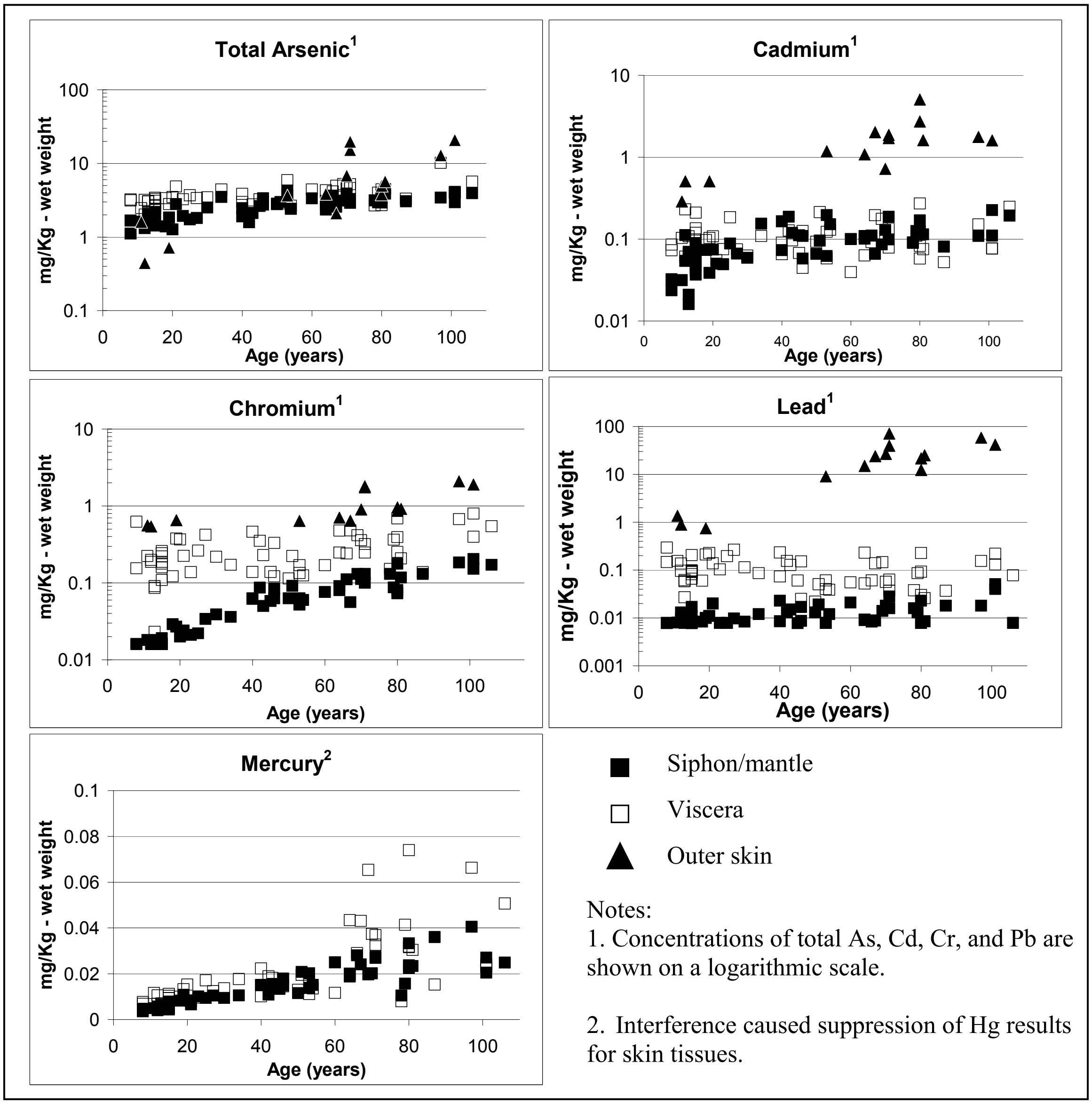

Figure 2: Geoduck age and tissue concentrations of As, Cd, Cr, Pb, and Hg.

Discussion

Geoducks are harvested in Puget Sound for both commercial and subsistence purposes. Most geoducks that enter the commercial market are exported for consumption in East Asia where they command a high price and are considered a delicacy. Most of the soft tissues are consumed. Siphon and mantle tissues (with outer skin removed) may be consumed raw in sushi or salads or cooked in soups or stir-fried. Viscera tissues are sometimes used as a base in soups or breaded and fried. We have noted the lack of a consistent approach in the processing of geoduck tissue samples for the purposes of human health assessment. Because proper preparation for consumption requires removal of the outer skin and separation of the viscera and siphon, we believe that separately analyzing the siphon and viscera has merit. With the exception of Cd, we found significant differences between trace metal concentrations in the siphon/mantle and the viscera. This additional information may be important to communities and individuals who are frequent and high consumers of geoduck clams.

We also found that trace metals concentrations were significantly higher in the outer skin than the siphon/mantle or the viscera. This is particularly true of Pb. [Pb] was 3 orders of magnitude higher in the outer skin than the siphon. Accounting for the weights of the tissue samples by tissue type and converting concentrations to absolute quantities, we found that, by weight, 98.5% of the Pb body burden of Richmond Beach geoducks is carried in the outer skin tissue. This result appears to explain the apparent inconsistency between the Eagle Harbor (Suquamish 2006) and Brightwater (King County 2002) studies. The proportion of overall body burden carried by skin tissue was also high for Cd (55%) and Cr (37%). Detection of analytes in the water used to remove skins indicates some loss of metals and perhaps some tissue from the outer skin during its removal from the siphon/mantle.

We found that inorganic As is less than 1% of total As in geoduck tissues from Richmond Beach. This seems to confirm that the use of 10% of total As is a conservative proxy for inorganic As in health risk assessments when speciated As data is not available.

Geoducks are a very long-lived animal (individuals have been aged at >140 years; Harbo et al. 1983) and therefore provide a unique record of long-term exposure to trace metals in the marine environment. Our results showed a strong correlation between the concentrations of these trace metals in the skin and siphon/mantle and the age of the geoduck (see Table 3). The observed increase in metals concentrations with age implies that geoducks are accumulating these metals over the course of their lives. Because Geoducks achieve adult size within the first few years of life with their size changing very little after about 10 years (Goodwin and Pease 1989), we conclude that the rate of uptake of these metals must exceed the rate of their excretion resulting in accumulation. We observed the highest concentrations in outer skin and viscera tissues and therefore speculate multiple pathways (e.g. outer skin contact with sediments and ingestion of food, suspended sediments, and waters containing these metals) for the uptake of metals.

Conclusion

The observed concentrations of Cd, Cr, Pb, Hg and inorganic As in tissues consumed by humans were mostly below conservative comparison values for human health protection. Thus, chemical contaminants in geoduck tissues from this urbanized area do not appear to be significant to human health. Demonstrating sanitary water quality conditions in tracts near urban areas and addressing the closures associated with WWTP outfalls will likely continue to be the greatest challenges in certifying these tracts for commercial harvest.

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References

Barrick, R. 2004. Summary of geoduck and subtidal sediment conditions – Point Wells oil spill from Barge 242-P2. Memorandum to Foss Barge 248-P2 Oil Spill Shellfish and Sediment Committees. March 31, 2004. Entrix, Inc.

Goodwin, C.L., and B. Pease. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest)--Pacific geoduck clam. U.S. Fish. Wildl. Serv. Biol. Rep. 82(11.120). U.S. Army Corps of Engineers, TR EL82-4.

Harbo, R., B. Adkins, P. Breen, and K.Hobbs. 1983. Age and size in market samples of geoduck clams (Panope generosa). Can. Manuscr. Rep. Fish. Aquat. Sci. No. 1714.

Honeycutt, H. and J. Valero. 2003. A guide to geoduck processing. School of Aquatic and Fishery Sciences. University of Washington.

Johnson, A. and M. Roose. 2002. Inorganic arsenic levels in Puget Sound fish and shellfish from 303(d) listed waterbodies and other areas. Washington State Department of Ecology, Olympia, Washington. Ecology Publication No. 02-03-057

King County. 2002. Brightwater marine outfall geoduck tissue study. Final Report. November, 2002. Marine and Sediment Assessment Group. King County Department of Natural Resources and Parks, Wastewater Treatment Division.

Kyte, M. and S. Quiring. 1999. A preliminary investigation of geoduck (*Panope abrupta*) tissue chemistry for the Kingston wastewater treatment plant outfall project. Final Report . URS Greiner Woodward Clyde. Prepared for CH2M HILL and Kitsap County.

PSAMP, 1990. Puget Sound Ambient Monitoring Program (PSAMP). Shellfish Programs Implementation Plan. February 1990.

PSEP, 1996. Puget Sound Estuary Program (PSEP). Recommended Protocols for measuring metals in Puget Sound Marine Water, Sediment, and Tissue Samples. Prepared for the Puget Sound Estuary Program (U.S. Environmental Protection Agency 10) by the King County Environmental Laboratory. Seattle, Washington.

Suquamish Tribe. 2000. Fish consumption survey of the Suquamish Indian Tribe of the Port Madison Indian Reservation, Puget Sound Region.

Suquamish Tribe. 2006a. Wyckoff/Eagle Harbor geoduck tissue and sediment field sampling report.

Suquamish Tribe and Washington Department of Health. 2006. Final Quality Assurance Project Plan. Richmond Beach tract geoduck tissue sampling and analysis plan. May 2006.

WDFW, 2002. Washington Department of Fish and Wildlife (WDFW). 2002 Geoduck Atlas, Atlas of Major Geoduck Tracts of Puget Sound. Annual Report Number FPA02-05. May 2002.

WDOH. 2005. Washington Department of Health (WDOH). Evaluation of dioxins in crab and geoduck tissue from a Lower Elwha Klallam fishing area near Port Angeles, WA. Health Consulation. Rayonier mill site exposure investigation. EPA Facility ID: WAD000490169.

USEPA. 2000. U.S. Environmental Protection Agency (USEPA). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 2 – Risk Assessment and Fish Consumption Limits, Third Edition. U.S. Environmental Protection Agency, Office of Water. November 2000. EPA 823-B-00-008.

USEPA. 2006. U.S. Environmental Protection Agency (USEPA). Draft method for determining arsenic species in seafood using ion chromatography/inductively coupled plasma – mass spectrometry. USEPA Manchester Laboratory SOP method for inorganic arsenic, tissues.