



Laboratory Analysis of Inorganic, Organic and Volatile Species of Selenium

Louis Wagner, National Technical Specialist (Inorganics)

Sept 28, 2023 SABCS conference



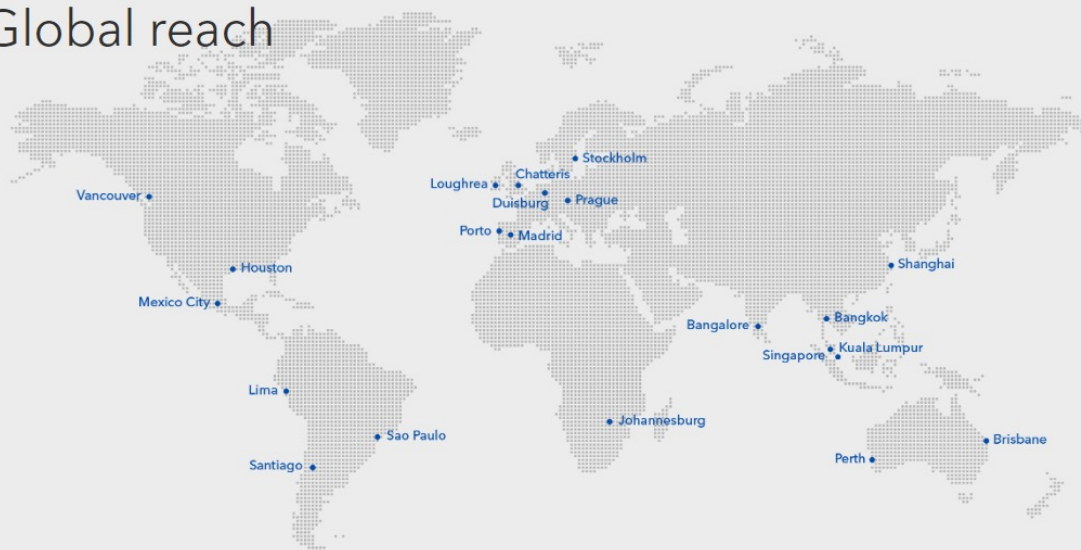
Right Solutions • Right Partner
www.alsglobal.com



Introduction (ALS)



Global reach



70+
Countries

350+
Offices

19K+
Staff

45
Industries



ALS Burnaby (Vancouver)

ALS Canada Technical Team

- R&D team across Canada with various fields of specialization.
- Collaborates with Laboratories within ALS globally across divisions.
- My specialization inorganic analysis with a recent focus on speciation and organometallic compounds.

What is Selenium?



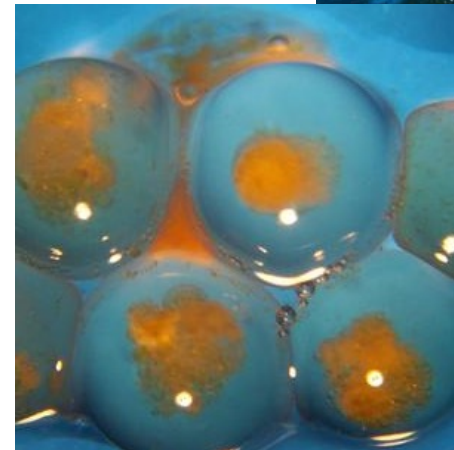
- Selenium
 - Often considered a metalloid. Properties between metals and non-metals
 - Chemically similar to Sulfur, particularly in biological processes.
 - Elevated Environmental Concentrations in BC/Western NA typically due to waste rock associated with mining activities



Why is Selenium Important?



- Selenium is an essential micro-nutrient required for various proteins essential for life
- Selenium is unique in that it has a narrow threshold between requirement and toxicity
- Selenium has emerged as an aquatic pollutant of global concern, although most global regulations involve human health and not ecological impact.
- Particularly detrimental to yolk-bearing vertebrates such as fishes, aquatic birds and amphibians.



Selenium in the News.



British Columbia

B.C. attempts to facilitate discussions with U.S. on selenium contamination in province's coal mines
International commission asks Canada to join probe of selenium flowing from B.C.

Ottawa has already rejected a similar request from the Ktunaxa First Nation

IN-DEPTH

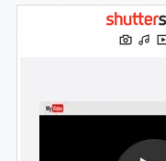
For decades B.C. failed to address selenium pollution in the Elk Valley. Now no one knows how to stop it.

There are no viable solutions: Canadian and U.S. water from mines owned and operated by mini-polluters. The potential fish population collapse signal more trouble to come

Tester asks Biden to tackle selenium pollution

By HAYDEN BLACKFORD
Daily Inter Lake | March 24, 2023 7:00 AM

Ahead of President Joe Biden's visit to Canada this week, Montana's U.S Sen. Jon Tester, Democrat, wrote to the president in order to voice several concerns the senator has about northwest Montana.



Selenium Regulations



- **Primary matrices**
 - Water Samples
 - Tissues (Fish & Wildlife)
 - Eggs/Ovaries (Fish/Amphibians)

Canadian Regulatory Snapshot

CCME, Freshwater Aquatic Life

1 ug/L

Federal Environmental Quality Guidelines, ECCC 2022

Fish Tissue Whole Body

6.7 ug/g (dry weight)

Fish Tissue Egg-ovary

14.7 ug/g (dry weight)

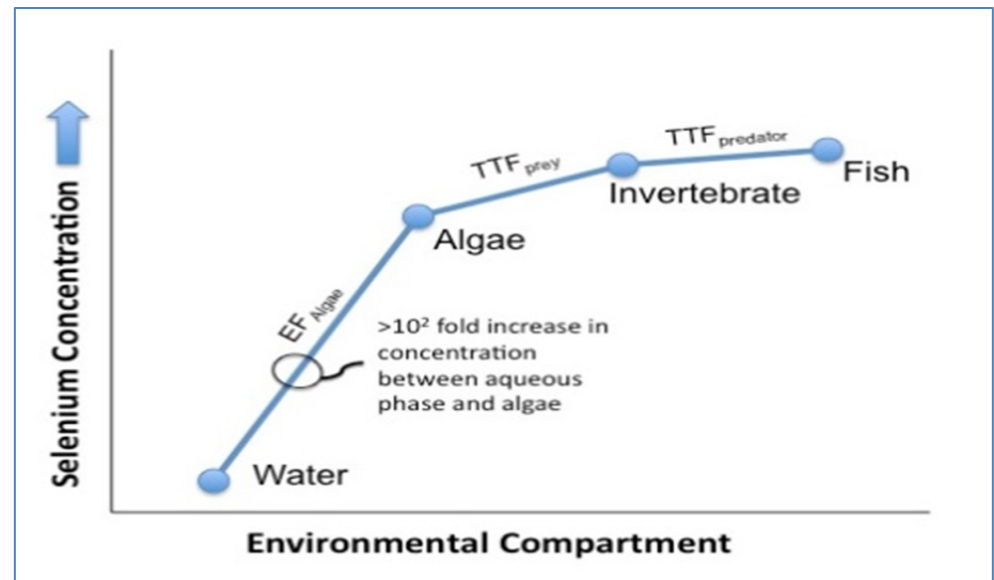
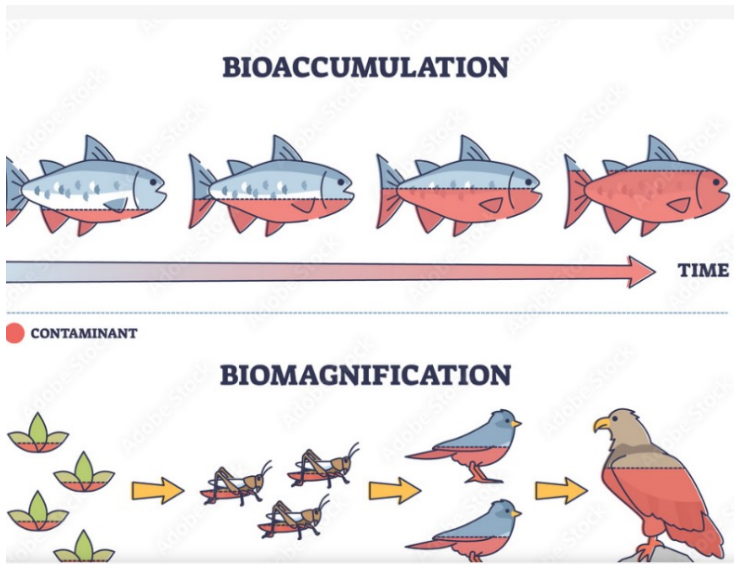
Bird Egg

11 ug/g (dry weight)

Selenium in the Environment

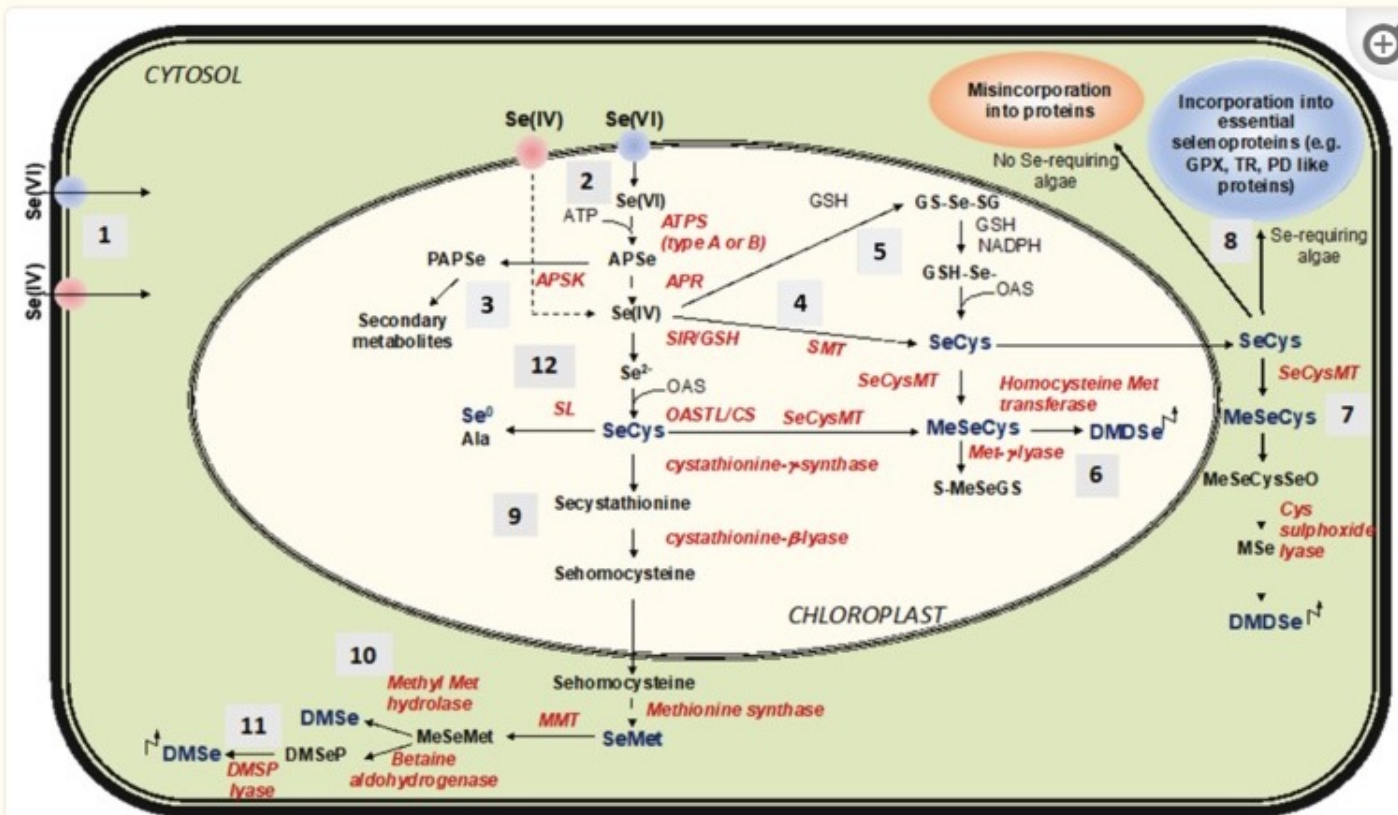


- Se (inorganic forms) enters the food chain and bioaccumulated and biomagnifies.
- Not unlike Hg and other persistent pollutants.
- Unlike these however Se is also an essential micronutrient. Pathways to this bioaccumulation more complex.



Selenium: It's complex

- Example algae metabolism pathways of selenium

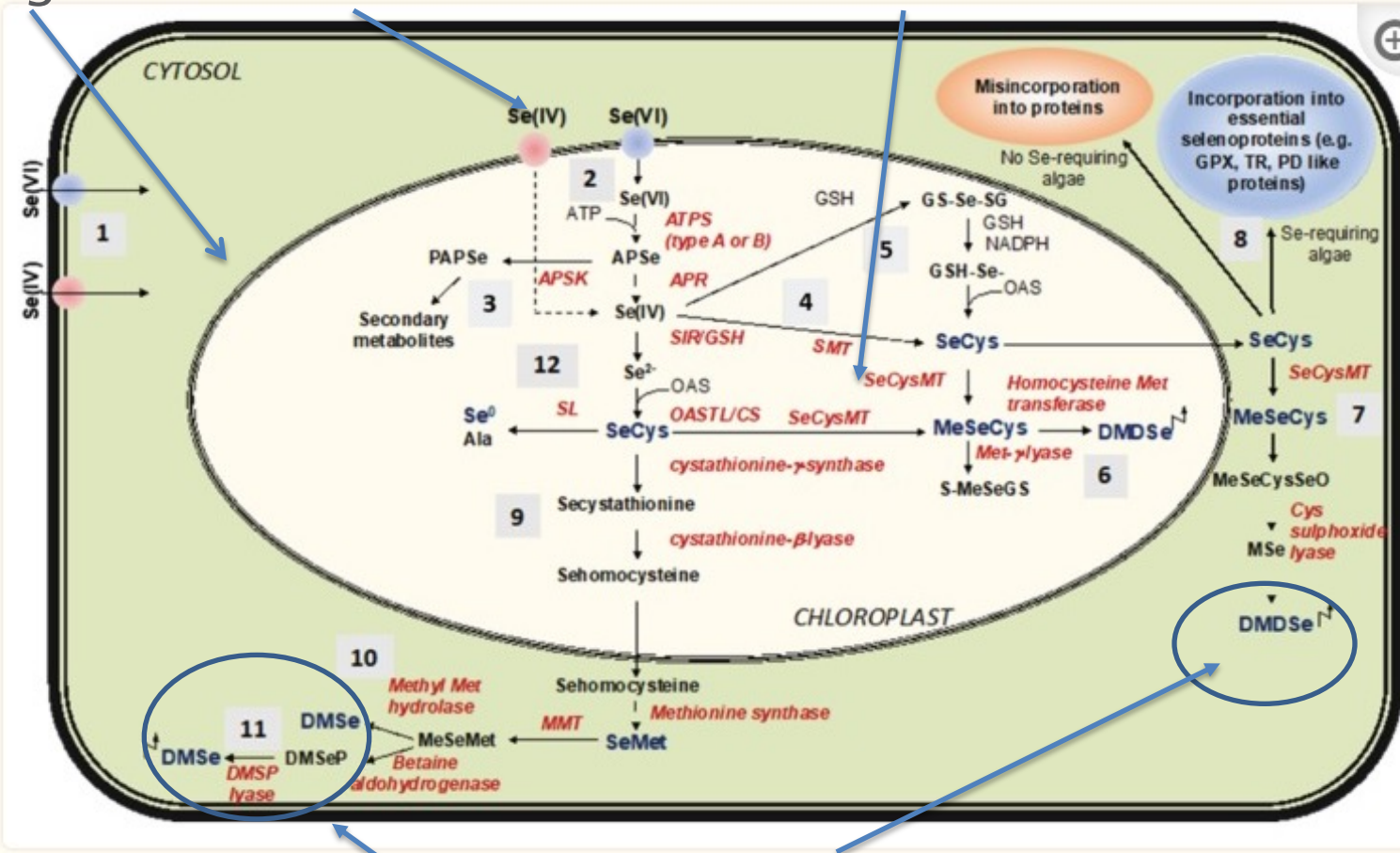


Ponton DE, Graves SD, Fortin C, Janz D, Amyot M, Schiavon M. Selenium Interactions with Algae: Chemical Processes at Biological Uptake Sites, Bioaccumulation, and Intracellular Metabolism. *Plants* (Basel). 2020 Apr 19;9(4):528.

Selenium: It's complex

- Inorganic Selenium IN

Lots going on



DMSe and DMDSe...More on these species later

Common Species of Selenium



- **Primary Inorganic Forms**
 - Selenite (IV) – SeO_3^{2-}
 - Selenate (VI) – SeO_4^{2-}
- **Other Inorganic Forms**
 - Selenocyanate (SeCN) and Selenosulfate (SeSO_3)
- **Biological Activity**
 - Selenocystein (SeCys)
 - Selenomethionine (SeMet)
 - Dimethyl Selenide (DMSe)
 - Dimethyl Diselenide (DMDSe)
- **Oxidation**
 - MethylSelenic acid (MeSe)
 - DiMethyl Selenoxide (DMSeO)
- **Others**
 - Elemental Se
 - ???

Why is Selenium Speciation important ?



- Form of Selenium present in the environment dictates the ecotoxicity.
- Organic Se forms can bioaccumulate at factors **100x** higher than inorganic forms.
- Water concentrations may not predict toxicity to aquatic organisms.

Why is Selenium Speciation important?



- Relationship between input water concentrations and ecotoxicity.
 - Relationship between water concentrations and fish tissue complex.
 - Water concentrations below current total selenium guidelines may not accurately predict tissue concentration
 - Forms of selenium
 - Receiving environment (lentic vs. Lotic)
 - Water concentrations are easier to treat than tissue concentrations.

- **Total Selenium – BC Lab Manual Digestion for Total Metals in Water**
 - Samples collected in 60mL HDPE bottles, preserved with HNO_3 to $< \text{pH } 2$. Samples digested with HNO_3/HCl .
 - Instrumentation: Collision/Reaction Cell ICP-MS (CCMS).
- **Dissolved Selenium**
 - Filtered using $0.45\mu\text{m}$ filter, preserved with HNO_3 , $< \text{pH } 2$.
 - Instrumentation method: Same as Total.



False Bias in Dissolved Selenium Results



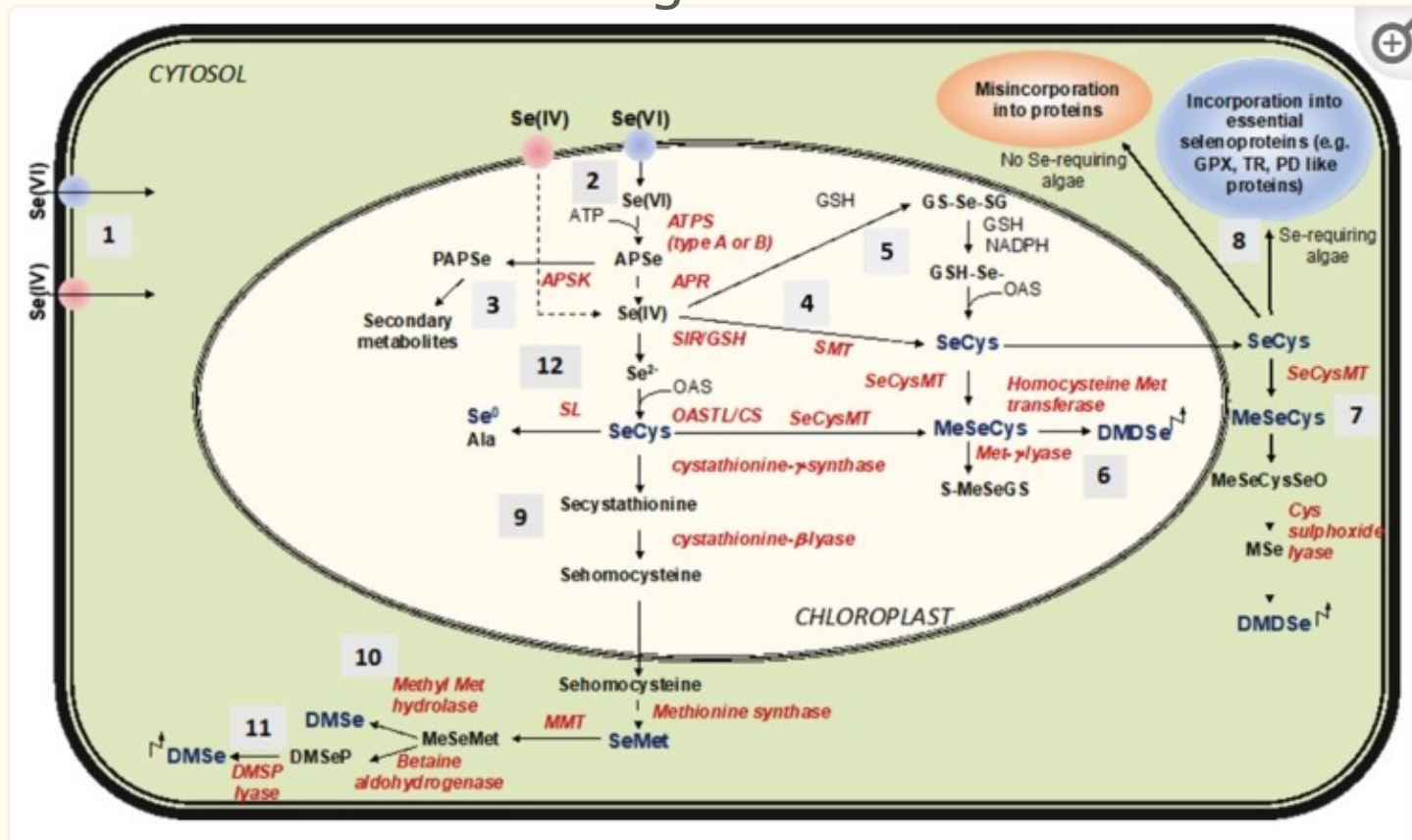
- Often Dissolved Selenium is observed to be > Total Selenium
 - Could be analytical variability if the results are similar and all the selenium is present in the dissolved form.
 - However sometimes Dissolved Selenium >>> Total Selenium.
- ALS observed this some time ago and has studied this apparent bias. Some initial observations initially observed:
 - Time to analysis seemed to reduce the D>T bias.
 - If we digested the Dissolved Sample, results were now in line with Total.

	Total Selenium	Dissolved Selenium	Dissolved Selenium
Lab Treatment	Digested	Undigested	Digested
Influent	50 ug/L	45 ug/L	45 ug/L
Effluent	20 ug/L	100 ug/L	15 ug/L

Selenium results: influent and effluent from a Se treatment site.

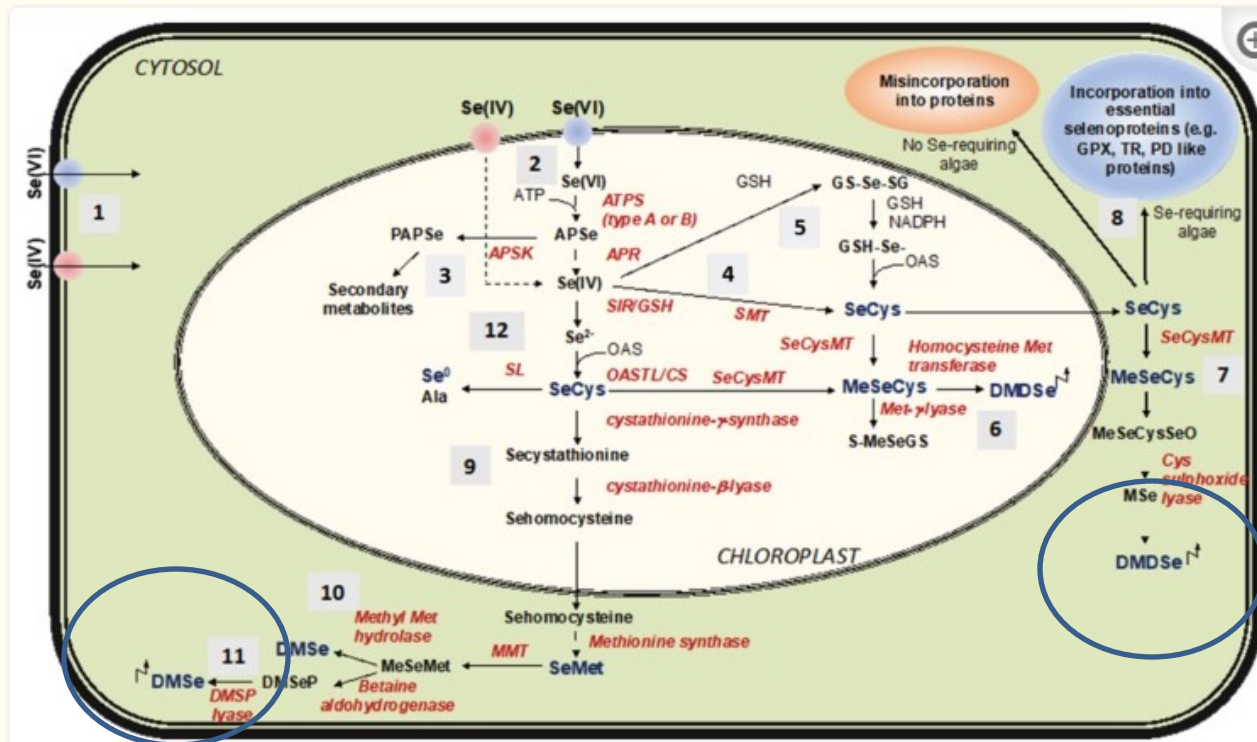
False Bias in Dissolved Selenium Results

- What is causing this apparent false bias??
- There is a clue in this diagram



False Bias in Dissolved Selenium Results

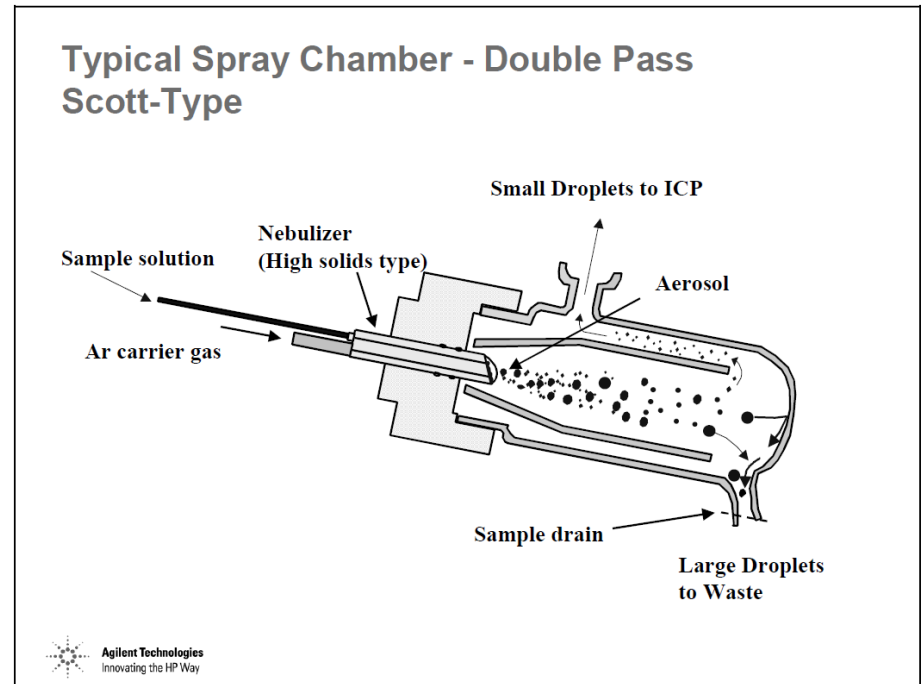
- Investigation of two of these compounds indicates these are volatile Se species.
- Could this be the cause?



Can Volatile Se Species cause this positive Bias?



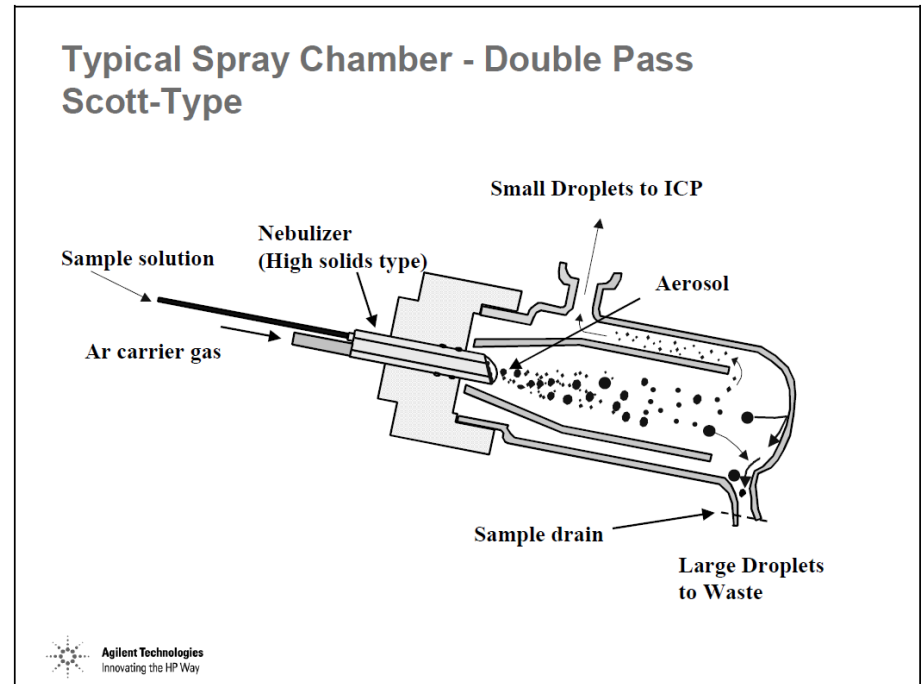
- Sample introduction of ICPMS involves the liquid sample being introduced through a nebulizer into the analyzer.
 - Nebulizer creates an aerosol spray
 - Spray transported into the plasma and analyzed by the instrument
 - Generally, transport efficiency (% of sample aspirated that reaches the plasma is 1-2%)



Can Volatile Se Species cause this positive Bias?



- Volatile Species however undergo “Vapour Enhancement” in the spraychamber.
- Volatilized into the gas phase in the spraychamber
- Transport efficiency is much higher
- Can be >50% efficient.
- Depends on exact instrument configuration and daily conditions

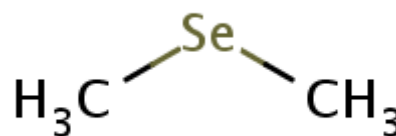


Q. Was this what we were observing??

Volatile Selenium Species (DMSe and DMDSe)

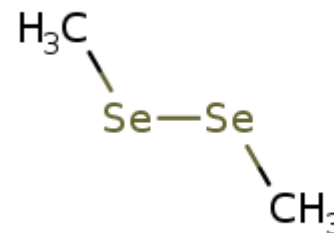


- 2 volatile species investigated in detail.
 - DMSe, DMDSe
- The smell of these standards reminded us of samples with particularly bad D > T Bias.
- Studies with these standards confirmed that these forms would have a biased high signal on ICPMS.



Dimethyl Selenide (DMSe)
b.p. 55°C

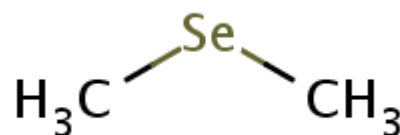
Dimethyl Diselenide
(DMDSe)
b.p. 155-157°C



Volatile Selenium Species (DMSe and DMDSe)

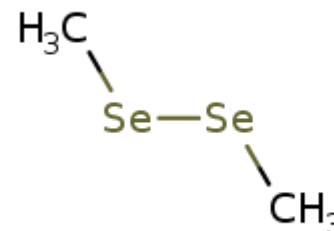


- What about digesting these samples?
- Enhancement was eliminated.
- Se was recovered. No significant losses.
- Indicates digestion procedure rapidly oxidizes these species to non-volatile forms of Se
- Conversion/not evaporative loss



Dimethyl Selenide (DMSe)
b.p. 55°C

Dimethyl Diselenide
(DMDSe)
b.p. 155-157°C

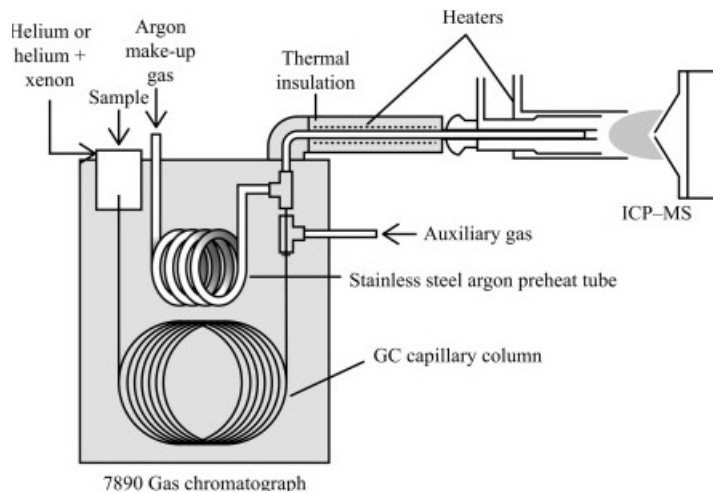


- Can we analyze for these species directly?
- ALS has been analyzing for DMSe and DMDS_e for some time by GCMS based on routine VOC methods. Sensitivity ppb level
- Good if samples will be very high in volatile species, perhaps directly from bioreactor or treatment system.
 - Enhancement can be seen in samples with far lower concentrations.
- We need more sensitivity to provide meaningful data. What are we missing using GCMS?
- New Ultra Trace method for ppt level detection of Volatile Selenium.

New Method for Ultra-Trace Analysis of Volatile Selenium Species



GC-ICPMS (Gas Chromatography - Inductively Coupled Plasma Mass Spectrometry)



- Use of GC-ICPMS in environmental laboratories is extremely rare.
- Unmatched sensitivity for organo-metallic compounds
- ALS method analyzes for volatile selenium by static-headspace followed by GC-ICPMS
- Limit of Reporting (2 ng/L !!! For both DMSe and DMDS_e). 250x more sensitive than GCMS for DMSe and 2500x more sensitive for DMDS_e. 25x more sensitive than routine Total and Dissolved Selenium analysis.
 - Able to now detect these species to a level that may impact trace level dissolved Se analysis.
 - Can detect and quantify other species if observed.

Resolving D > T Selenium Bias



- Digestion of Dissolved fraction will eliminate this bias.
- What about full accounting of volatile species that may be present in a sample?
 - Zero headspace sampling?
 - False biases for dissolved selenium due to enhancements from volatile species are frequently observed due to very trace relative concentrations of volatile selenium species/ e.g. Volatiles present as only 1-2% of the total dissolved selenium concentration can exhibit a significant bias in undigested dissolved analysis
 - For majority of environmental samples, the relative proportion is insignificant compared to the more prevalent species. Industry standard sampling protocols are sufficient.
- Not all samples may contain only trace volatile species.
- For samples where a significant bias of D>T selenium is observed, ALS recommends direct measurement of the concentrations of volatile selenium species to ensure that significant levels of selenium have not been missed with routine analysis.

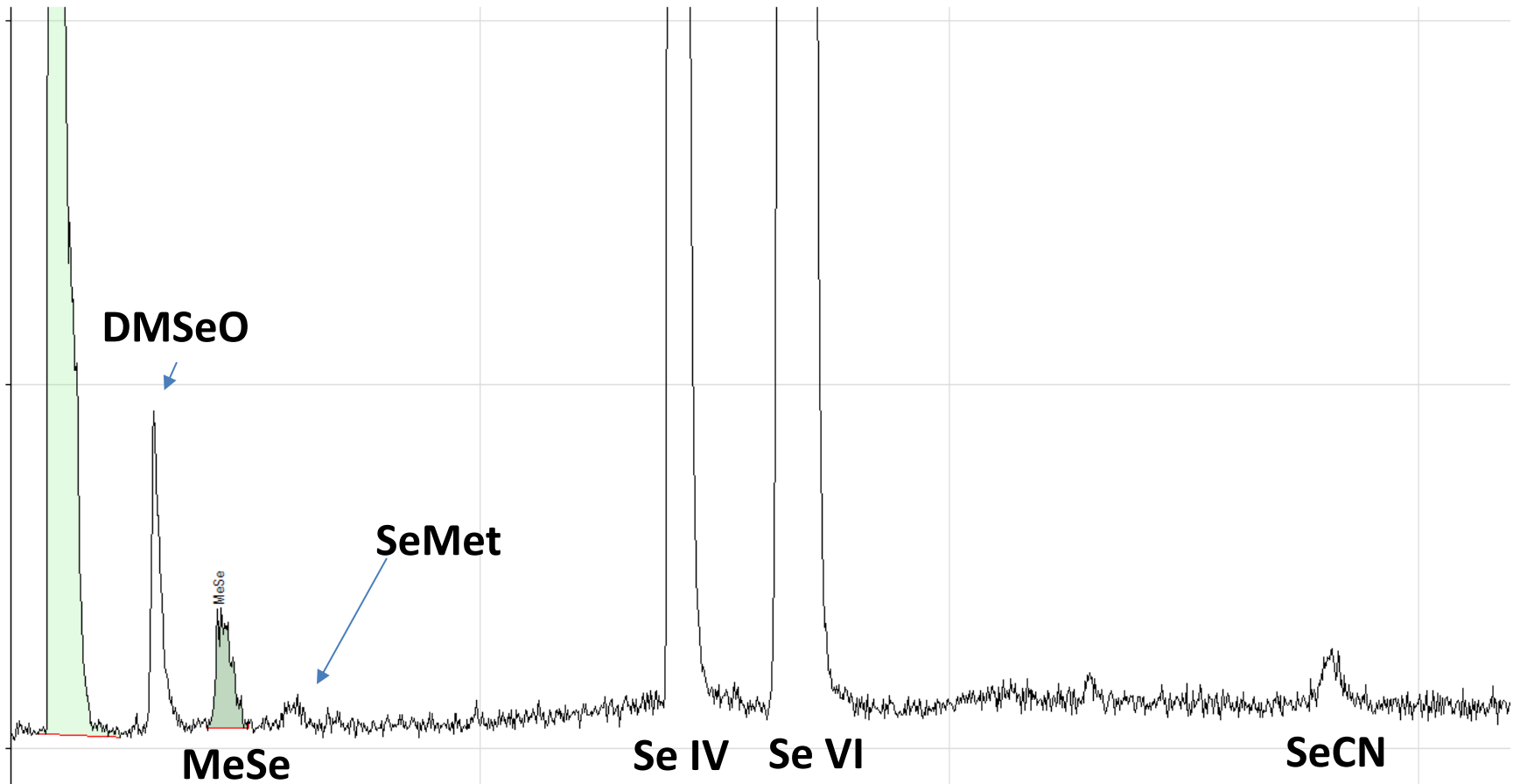
What about other species?



- ALS also performs HPLC-ICPMS analysis for other inorganic and organic selenium species



HPLC-ICP-CCMS Chromatograms



- Se in the environment is complicated
- Many possible forms with implications for ecotoxicity and analysis.

	Total Selenium	Dissolved Selenium	Digested Dissolved Selenium
Test Method	ICPMS	ICPMS	ICPMS
ALS Test Code	E420	E421	E423A
Sample Amount	60 mL	60 mL	60 mL
LOR (Limit of Reporting)	0.050 µg/L	0.050 µg/L	0.050 µg/L
Sample Containers & Preservation	60 mL HDPE, HNO ₃ , chill (≤ 6°C)	60 mL HDPE, Field filtered, HNO ₃ , chill (≤ 6°C)	60 mL HDPE, Field filtered, HNO ₃ , chill (≤ 6°C)
Recommended Holding Time	6 months	6 months	6 months

Se Speciation Tests: Sampling & Analysis Details



<i>Volatile Selenium Species</i>	
Dimethyl Selenide (as Se)	0.0020 µg/L
Dimethyl Diselenide (as Se)	0.0020 µg/L
Unidentified Volatile Se Species (as Se)	0.0020 µg/L
<i>Sampling Details</i>	
Test Method	GC-ICPMS
ALS Test Code	E546
Sample Amount	40 mL
Sample Containers & Preservation	40 mL amber glass, zero headspace, HNO ₃ , chill (≤ 6°C)
Recommended Holding Time	7 days

ALS Canada Selenium Speciation by HPLC-ICPMS Tests	Biota	Water
<i>Inorganic Selenium Species</i>		
Selenite (Se IV)	✓	✓
Selenate (Se VI)	✓	✓
<i>Organic Selenium Species</i>		
Selenocyanate (SeCN)	–	✓
Selenomethionine (SeMet)	✓	✓
Methylseleninic (IV) Acid (MeSe)	–	✓
Other Organoselenium Compounds	✓	✓
<i>Sampling Details</i>		
Sample Amount	≥ 10 g (wet)	60 mL
Sample Containers & Preservation	125 mL glass jar, freeze (-18°C)	60 mL opaque plastic bottle, field filter 0.2 µm, chill (≤ 6°C)
Recommended Holding Time	2 years	30 days

- Lack of consensus methods for Se speciation
- Few laboratories perform, labs may all be different
- Need for industry/regulator partnerships
 - Other labs, regulators, government agencies
 - Should we report more species, and required LORs?
 - Determine industry standards and best practices
- ALS is offering soon lower LORs and more Species.

Thank you and any Questions?



Louis Wagner, BSc
ALS Vancouver, Burnaby, BC
+1 604 253 4188
Louis.Wagner@alsglobal.com

Acknowledgements

MaryAnne Vrbaneck: ALS Burnaby Organics Technical Specialist
Mark Hugdahl: ALS Canada Technical Director

