

# Maternal Transfer of Metals in Steller Sea Lions (Eumetopias jubatus) from the Washington State Coast

FISH and WILDLIFE

Jacki Aitken, Elizabeth Madonick, Dyanna Lambourn, Annie Carter, Russ Gerads Brooks Applied Labs, 18804 Northcreek Parkway, Suite 100, Bothell, WA 98011 USA and Washington Department of Fish and Wildlife

# Objective

The objective of this study is to determine metal transfer relationships in twelve organs between four Steller sea lion mother-fetus pairs.

## Introduction

Metal bioaccumulation can cause deleterious effects to major organs and may be a contributing factor to death of Steller sea lions (*Eumatopias jubatas*). Due to their high position in the marine food web and relatively long life span, Steller sea lions can bioaccumulate trace elements to greater concentrations in comparison to other pinnipeds. Studies observing gestational transfer of metals have noted fetal development disruption, however, seal lion exposure to metals is not well understood. The relationships between maternal-fetal elemental concentrations may be an indicator of health concerns to fetuses. This study focuses on gestational transfer of 13 trace elements between four fetus-mother pairs of Steller sea lions from the Washington state coast.

# Design/Methods

Steller sea lions (SSL) used in this study were collected from the outer coast of Washington State from 2012-2013 to investigate their cause of death by the Washington Department of Fish and Wildlife – Marine Mammal Investigations (WDFW-MMI) in conjunction with Cascadia Research Collective, Hatfield Marine Science Center, and

arch concente, Flather Marine Science Center, and	Lung	1
Makah Marine Mammal Program. Organisms were in	Heart	
ng stages of decomposition at the time of necropsy,	Liver	
all numbers of tissues collected differ. After necropsy,	Muscle	
n samples were transferred from WDFW-MMI to ks Applied Labs, LLC.	Intestine	
R3 Applied Labs, LLC.	Bladder	
his study, metal concentrations in the brain, kidney,	Placenta	
heart, muscle, liver, intestine, pancreas, stomach,	Amniotic Fluid	
der, placenta, and amniotic fluid were quantified	Pancreas	
e 1). Overall, sixty-nine samples from four	Stomach	
ner-fetus pairs were analyzed for total elemental ent.	Table 1. Number of o	orga

analyzed from both mother and fetus SSL.

Organ

Kidney

**n**=

7

4

3

Samples were digested using EPA method 3050b (a nitric acid, hydrochloric acid, and hydrogen peroxide

combination) and were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) using a Perkin-Elmer DRC-e with interference reduction technology. These digests were analyzed for 37 elements including those with toxicological significance to the organism: arsenic, cadmium, cerium, cobalt, chromium, copper, manganese, molybdenum, nickel, lead, selenium, tellurium, and vanadium. For mercury, samples were digested with aqua regia and analyzed on a Perkin-Elmer DRC-e using cold vapor-inductively coupled plasma-mass spectrometry (CV-ICP-MS).

# Results/Discussion



Estimated method detection limits (eMDL) were calculated for each batch and for each analyte separately by multiplying the standard deviation value within 10x the eMDL for a given analyte was deemed a non-detect result (ND) due to the increased level of variability at those levels. Values above 10x the eMDL were considered significant. Relative percent deviation (RPD) was oncentrations of mother and fetus and to assign a relational value to the results. It was calculated

by taking the absolute value of the difference between the two concentrations and dividing by the average. The greater the RPD, the greater difference between the mother and fetus.

Mercury (Hg), arsenic (As), cadmium (Cd), copper (Cu), manganese (Mn), selenium (Se), and tellurium (TI) were the only metals found at significant levels in both mother and fetus samples. Significant concentrations are categorized in Table 2.

Organ	Sample Type	n=	Concentration (mg/kg)						
Organ	Sample Type	11—	Hg	As	Cd	Cu	Mn	Se	
	Mother	4	0.713	ND	ND	4.49	0.333	0.387	
Brain	Fetus	4	0.341	ND	ND	0.593	ND	ND	
	eMDL	_	0.020	0.014	0.0038	0.075	0.028	0.028	
	Mother	4	2.44	ND	13.3	5.03	1.00	1.75	
Kidney	Fetus	4	0.678	ND	ND	2.84	0.728	ND	
	eMDL	-	0.010	0.11	0.013	0.042	0.0093	0.082	
	Mother	4	0.686	ND	ND	ND	0.140	0.513	
Lung	Fetus	4	0.681	ND	ND	ND	0.200	ND	
	eMDL	-	0.049	0.022	0.010	0.26	0.013	0.042	
	Mother	3	0.692	ND	ND	ND	ND	2.65	
Heart	Fetus	4	0.839	ND	ND	ND	ND	2.48	
	eMDL	-	0.022	0.030	0.014	0.12	0.056	0.045	
	Mother	3	ND	ND	ND	1.06	ND	0.750	
Muscle	Fetus	4	ND	ND	ND	1.10	ND	ND	
	eMDL	-	1.2	0.10	0.0068	0.039	0.047	0.062	
	Mother	2	142	0.240	1.28	21.2	2.60	44.2	
Liver	Fetus	4	1.36	ND	ND	11.5	1.25	0.523	
	eMDL	-	0.14	0.019	0.0044	0.20	0.014	0.026	
	Mother	2	ND	ND	0.171	0.715	1.20	2.24	
Intestine	Fetus	4	ND	ND	0.107	3.87	3.84	3.08	
	eMDL	-	0.072	0.11	0.0054	0.063	0.049	0.20	
	Mother	2	0.512	0.114	ND	0.951	ND	0.419	
Bladder	Fetus	4	0.597	0.0650	ND	2.18	ND	0.228	
	eMDL	_	0.033	0.0063	0.013	0.030	0.023	0.016	
Placenta	Mother	4	ND	0.000	ND	1.61	ND	ND	
1 lacciita	eMDL	-	1.2	0.10	0.0068	0.039	0.047	0.062	
Amniotic Fluid	Mother	4	ND	ND	ND	0.725	ND	ND	
	eMDL	_	1.2	0.10	0.0068	0.039	0.047	0.062	

Table 2. Average tissue concentrations (mg/kg w/w) of fetus and mother SSL from the coast of Washington State. If less than 50% of the sample results were 10x greater than the eMDL, the average was deemed ND.

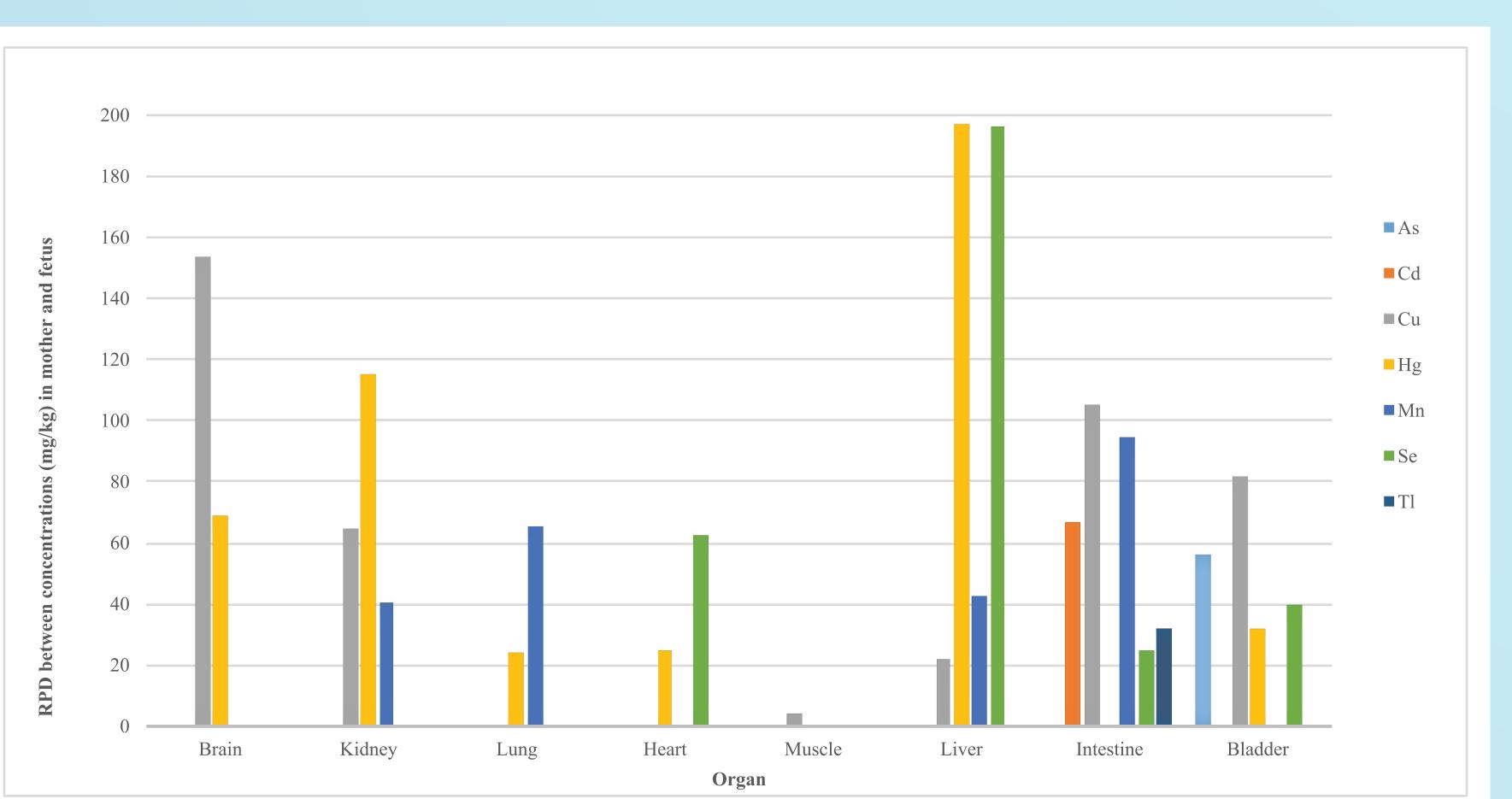


Figure 1. The average RPD of significant analyte concentrations in mother-fetus pairs of organs in Steller sea lions. ND results were excluded from this figure.

#### Brain

Fetal Hg concentrations in the brain of Steller sea lions from this study are roughly half of the corresponding mother's concentration (Table 2). Concentrations in the brain presented here raise concern over neurological development of the fetus. High blood Hg levels of pups relative to mothers were noted in Northern elephant seals at the beginni of lactation (Habran et al. 2011). In that study, Hg levels in pups declined as lactation progressed suggesting that gestational mercury transfer played a large role in the initial Hg concentrations found in the pups. The ratio of Hg transfer in the brain to offspring is far greater with Hg than in Cu, though the average concentrations of Hg were lower. Fetal brain Cu concentrations were almost 10x lower than their mothers yielding an RPD of 154 ± 12% between the four pairs (Table 3). These consistent results raise questions about transfer efficiency for Cu in the brain.

Омаан	Sample Type	n-	RPD (%)					
Organ	Sample Type	n=	Hg	As	Cd	Cu	Mn	Se
Duoin	Average RPD	4	69.0%	N/A	N/A	154%	N/A	N/A
Brain	stdev of RPD	-	22%	N/A	N/A	12%	N/A	N/A
Kidney	Average RPD	4	115%	N/A	N/A	65%	40%	N/A
Kidiley	stdev of RPD	-	29%	N/A	N/A	37%	28%	N/A
Lung	Average RPD	4	24%	N/A	N/A	NA	65%	N/A
Lung	stdev of RPD	-	29%	N/A	N/A	NA	61%	N/A
Heart	Average RPD	3	25%	N/A	N/A	N/A	N/A	63%
	stdev of RPD	-	22%	N/A	N/A	N/A	N/A	34%
Muscle	Average RPD	3	N/A	N/A	N/A	4%	N/A	N/A
Muscie	stdev of RPD	-	N/A	N/A	N/A	3.2%	N/A	N/A
Livon	Average RPD	2	197%	N/A	N/A	22%	43%	196%
Liver	stdev of RPD	-	2.4%	N/A	N/A	24%	6.0%	1.7%
Integrica	Average RPD	2	N/A	N/A	67%	106%	95%	25%
Intestine	stdev of RPD	-	N/A	N/A	39%	35%	36%	7.0%
D1a d d a ::	Average RPD	2	N/A	56%	N/A	82%	N/A	40%
Bladder	stdev of RPD	-	29%	54%	N/A	2.5%	N/A	1.6%

Table 3. Average tissue RPD (%) between fetus and mother Steller sea lions collected from the coast of Washington State. If less than 50% of the sample results were 10x greater than the eMDL, the average was deemed ND. ND concentrations resulted in N/A RPD's and stdev's.

#### Lung

Fetuses show less than 30% RPD of Hg concentration from their mothers in the lungs (Table 3). In this study, that ratio is similar to Hg patterns of maternal transfer in the heart and in the bladder. There is a wide range of RPD's of Mn in the lung (3%-150%) and concentrations in all lungs are below 0.45 mg/kg.

#### Heart

Fetuses also show less than 30% RPD of Hg concentration from their mothers in the heart (Table 3). In two of the three mother-fetus pairs, fetal Se in the heart is about 40% greater than in the mother. This is the only analyte that showed a pattern of concentrating higher in the fetus than the mother. Selenium is an essential nutrient important for protein synthesis that may be needed in larger concentrations in the heart for fetal development (NIH 2016).



#### Muscle

Copper concentrations in the fetus muscle samples are consistently equivalent to their mothers, RPD is below 8% for each pair. Caspian seals have a maternal-fetal RPD of Cu in muscle of about 52% (Table 5) (Watanabe et al. 2002). This is suggestive that there may be a threshold for Cu accumulation in muscle of both mother and fetus in both

Study	Sample Type	n=	Cu (mg/kg)
Aitken et al.	Mother	4	1.06
(2016)	Fetus	3	1.10
	RPD	-	4.4%
Watanabe et al.	Mother	6	1.0
	Fetus	1	1.7
(2002)	RPD	-	52%

Table 5. Mother and fetus Cu concentrations (mg/kg) in muscle of SSL from the current study and Caspian seals from

In kidneys, fetal concentrations are lower than the respective mother for all analytes (Table 2). A similar study investigating mother and fetus pairs of Caspian seals found RPD's that were consistent between mother and fetus pairs but varied by analyte for Hg and Mn. (Figure 2) (Watanabe et al. 2002). This suggests that neonate pups of both Caspian seals and SSL may have similar metabolic pathways for Hg and Mn in the kidney. On average, Cu concentrations of the mother Steller sea lion kidneys were higher, than in the Caspian seals, with a fetal concentration about equivalent (Table 4). This may account for the greater RPD in the current study (Table 3).

Study	Sample Type	n=	Concentration (mg/kg)			
Study	Sample Type		Hg	Cu	Mn	
Aitken et al. (2016)	Mother	4	2.44	5.03	1.00	
	Fetus	4	0.678	2.84	0.728	
Watanabe et al.	Mother	6	1.4	2.9	1.0	
(2002)	Fetus	1	0.3	2.8	0.4	

Table 4. Average mother and fetus metal concentrations (mg/kg) in kidney of SSL from the current study and Caspian seals from Watanabe et al. 2002.

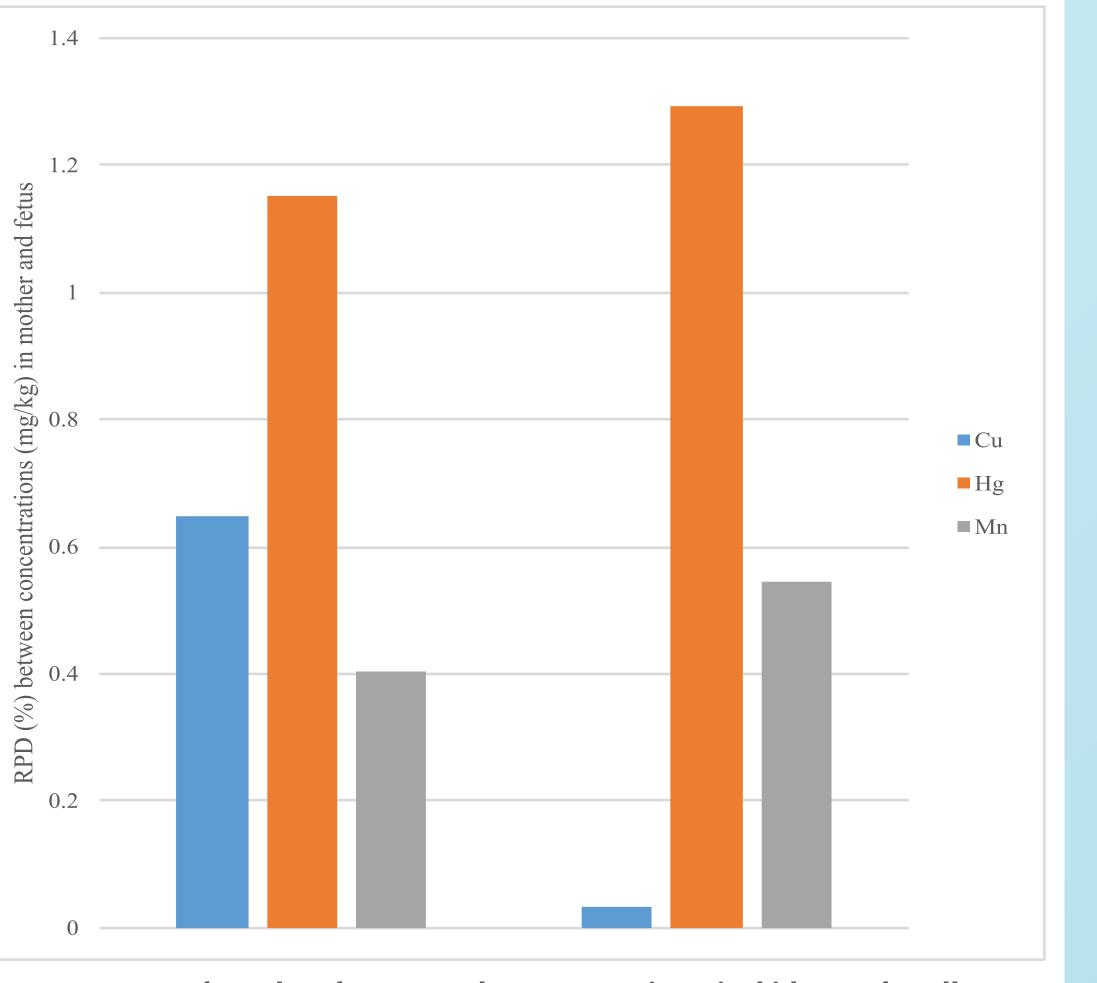


Figure 2. RPD of mother-fetus metal concentrations in kidney of Steller sea lions and Caspian seals.

Mercury concentrations in the livers of the mothers were as high as 247 mg/kg wet weight. Sydeman and Jarman (1998) investigated metals in livers of Steller sea lion pups from the California coast. The levels of Hg in the pups in the 1998 study, 4.8-73 mg/kg, were higher than were seen in the fetuses of the current study, 1.36 mg/kg) (Table 6). Similarly, the pups in the 1998 study also had higher Cu and Se concentrations.

Study	Comple Type		Concentration (mg/kg)					
	Sample Type	n=	Hg As	As	Cd	Cu	Se	
Aitken et al. (2016)	Mother	2	142	0.240	1.28	21.2	44.2	
	Fetus	4	1.36	ND	ND	11.5	0.523	
Sydeman and Jarman (1998)	Pup	Variable	19.0	0.4	15.0	91.0	4.1	

Table 6. Average metal concentrations (mg/kg) in liver of different age classes of Steller sea lions from the current study and from Sydeman and Jarman

Comparing the values obtained by Sydeman and Jarman (1998) to the current study suggests potential bioaccumulation of Hg and Se as the pup grows (ages are not specified). It is also unclear as to why the number of samples varies between analyte in the 1998 study. Mercury and Se, from the current study, show similar RPD's (197% and 196%, respectively) in the liver and have significantly higher concentrations than any other analyte or tissue analyzed (Tables 3 and 2, respectively). The fetal liver concentrations of Hg and Se are consistently lower than their mothers (Table 2).

## Conclusion

To the best of the authors' knowledge, this is the most comprehensive elemental study of maternal-fetal concentrations in Steller sea lions to date. Elements analyzed in this study showed that each organ exhibits distinct gestational transfer patterns for each analyte. This may suggest that some analytes may be incorporated into the fetus because they are essential nutrients. Other analytes with high RPD's suggest that there may be a limit to the amount of each analyte that is transferred to the fetus. The patterns presented here are comparable to previous studies showing that other pinniped species may exhibit similar gestational transfer properties. Due to the small sample size of this study, it is difficult to draw conclusions of gestational transfer from mother to fetus. To increase confidence in the findings, it is recommended that future investigations expand the scope to generate greater statistical significance. Further expansion of the current study will also investigate compounding effects of elements on the health of the

# Acknowledgements

The authors would like to thank the team at Brooks Applied Labs, specifically Hakan Gürleyük, Tamas Ugari, Aubrey Ernst, and Patrick Nye for their assistance with analysis and sample homogenization. A huge thank you is also given Brooks Applied Labs as a whole for resources and sample storage space. We would also like to thank the interns and employees at WDFW-MMI as well as Jessie Higgins at CRC for collecting the

## Works Cited

- Habran, S. et al. 2011. Blood dynamics of mercury and selenium in Northern elephant seals during the lactation period. Environmental Pollution. 159:
- National Institutes of Health. "Dietary Supplement Fact Sheet: Selenium Health Professional Fact Sheet." U.S National Library of Medicine. U.S. National Library of
- https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/.
- Sydeman, W. and Jarman, W. 1998. Trace metals in seabirds, stellar sea lion, and forage fish and zooplankton from Central California. Marine Pollution Bulletin. 36 (4): (828-832).
- Watanabe, I. et al. 2002. Accumulation of heavy metals in Caspian seals. (Phoca caspica). Archives of Environmental Contaminations and Toxicology. 43: (109-120)