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Are otters toxic? A trial in using enzyme-linked immunosorbent assays (ELISAs) to measure contaminants in sea and river otter diet and feces

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Are otters toxic?

A trial in using
enzyme-linked immunosorbent assays (ELISAs)
to measure contaminants
in captive sea and river otter diet and feces



SEATTLE AQUARIUM

Amy Olsen

Which toxic contaminants?

- PCBs

- Chlorinated hydrocarbon manufactured from 1929-1979.
- Used in hundreds of industrial and commercial applications.
- 209 congeners.
- Still measured to this day.

- PBDEs

- Flame retardant chemicals used in textiles, plastics, furniture and automobiles.
- Some forms banned (penta- and octaBDE)
- 209 congeners.

- Glyphosate

- Broad spectrum herbicide and desiccant
- Organophosphorus compound
- Earliest tradename Roundup™

- Pyrethroids

- Commonly used synthetic chemical insecticide
- Chemically similar to pyrethrins, an insecticide derived from chrysanthemum flowers
- Toxic to aquatic organisms



Why?

- Toxic contaminants have been measured in Puget Sound over decades.
- Water Source:
 - Sand filtered seawater from Elliott Bay, Puget Sound - sea otter exhibits
 - Sand and carbon filtered municipal water - river otter exhibit.
- Why otters?
 - Controlled exposure
 - Metabolism differences but similar gut transition time
 - Lack of blubber
 - Overlap in diet items between sea and river otters
 - Improve animal husbandry and care via diet composition
 - Captive animals are ideal for validation study



Why?

- Why ELISAs?
 - Current use for endocrine research
 - Cost and time effective
 - Relative trends rather than absolute values

Application of an ELISA for PCB 118 to the screening

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Received

Comparison of an enzyme-linked immunosorbent assay (ELISA) to gas chromatography (GC) – measurement of polychlorinated biphenyls (PCBs) in selected US fish extracts

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Development
ethers and ap

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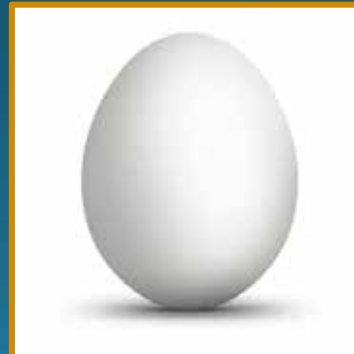
Pilot Survey for Determination of the Antifouling Agent Irgarol 1051 in Enclosed Seawater Samples by a Direct Enzyme-Linked Immunosorbent Assay and Solid-Phase Extraction Followed by Liquid Chromatography—Diode Array Detection

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Departments of Environmental Chemistry and Biological

Methods – Diet Samples

Sustainably harvested, restaurant quality seafood



River otters only

Methods – Diet Locations

Clams:

Butter – Washington, Oregon, Vancouver, B.C.,
Vancouver Island, B.C

Surf – Maine, Cape Cod, New Jersey, Virginia

Shrimp:

Port St. Joe, Florida (farm-raised), Texas

Mussels:

Whidbey Island, WA, Coupeville, WA

Fish:

Capelin – Canada (FAO 21),

Pollock – Alaska

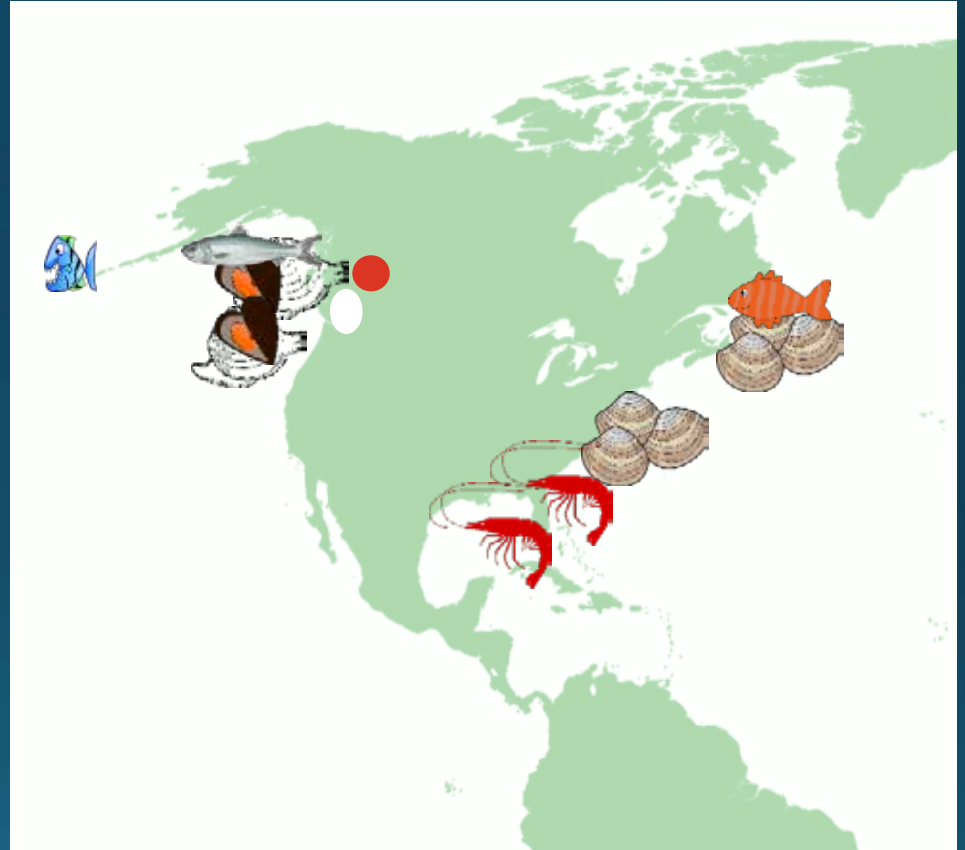
Herring: FAO 67 (Gulf of Alaska)

Chicken Egg:

Pike Place Market, WA

Meatball:

Canada



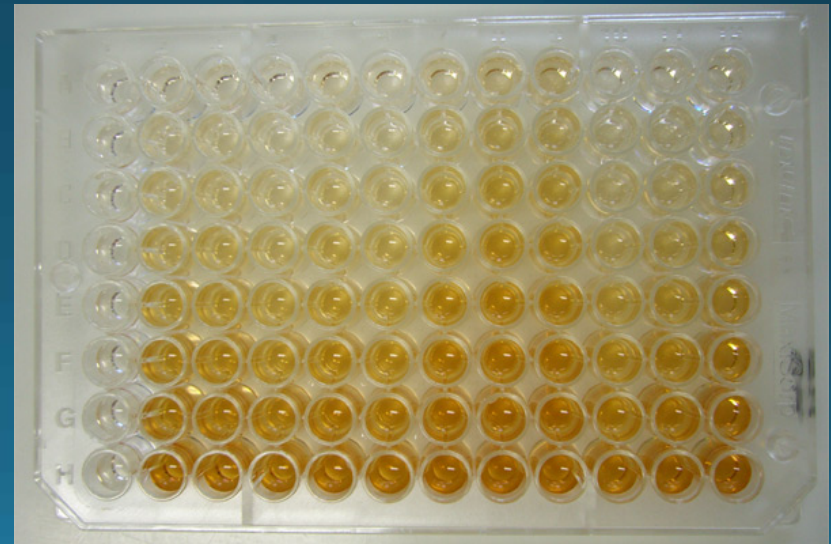
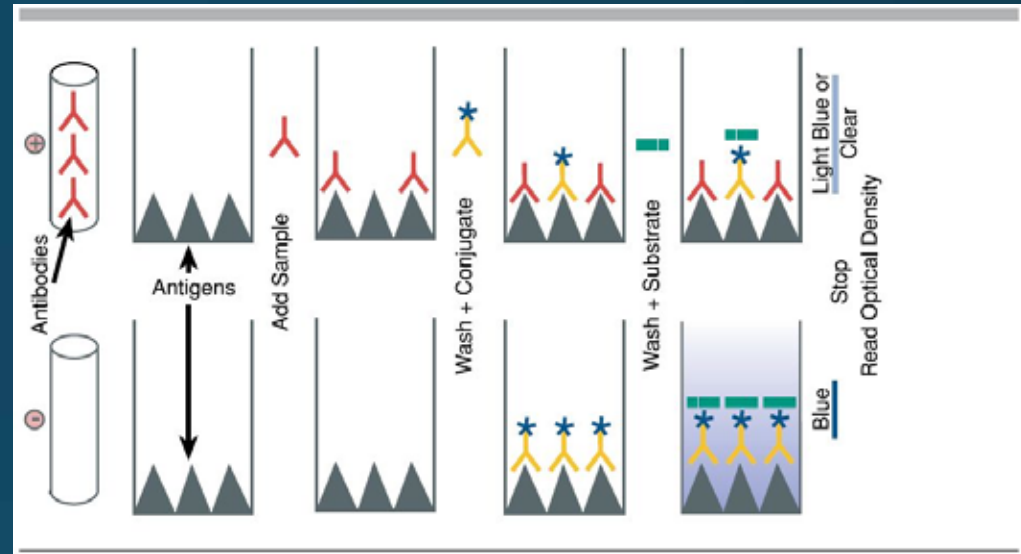
Methods – Fecal Samples

- Northern Sea Otter (*Enhydra lutris kenyoni*)
- Northern River Otter (*Lontra Canadensis*)
- Oven dried and extracted using matrix solid-phase dispersion method
- Indirect assessment of exposure (unassimilated portion of ingested prey)



Methods – ELISA (or EIA)

- Duplicate controls, standards and samples are loaded with a specific antibody to microtiter wells coated with Goat Anti-Rabbit Antibody.
- A conjugate is added to initiate competitive reaction.
- The presence is detected by adding an enzyme substrate and chromogen
- The color intensity is inversely proportional to the concentration in the sample



- ELISAs detect target molecule **and** related compounds, resulting in an overestimation of concentration

PBDE Compound	% React
PBDE Congener 47	100
PBDE Congener 99	31.9
PBDE Congener 28	8.1
PBDE Congener 100	2.0
PBDE Congener 153	0.0
5'methoxy-PBDE-47	159.7
5'methoxy-PBDE-99	1.1
3'OH-2,4,4'-PBDE	7.8
2'OH-2,4,4'-PBDE	3.7
5'OH-PBDE-47	14.2
Triclosan	1.1
PCB Arochlor 1254	0.1

Pyrethroid Compound	50% B/Bo (ppb)
Permethrin	4.25
Cypermethrin	100
Lambda (λ) Cyhalothrin	89.5
Bifenthrin	150
Resmethrin	2,400
Cyfluthrin	3,400
Tetramethrin	>10,000
3, PBA	1,700

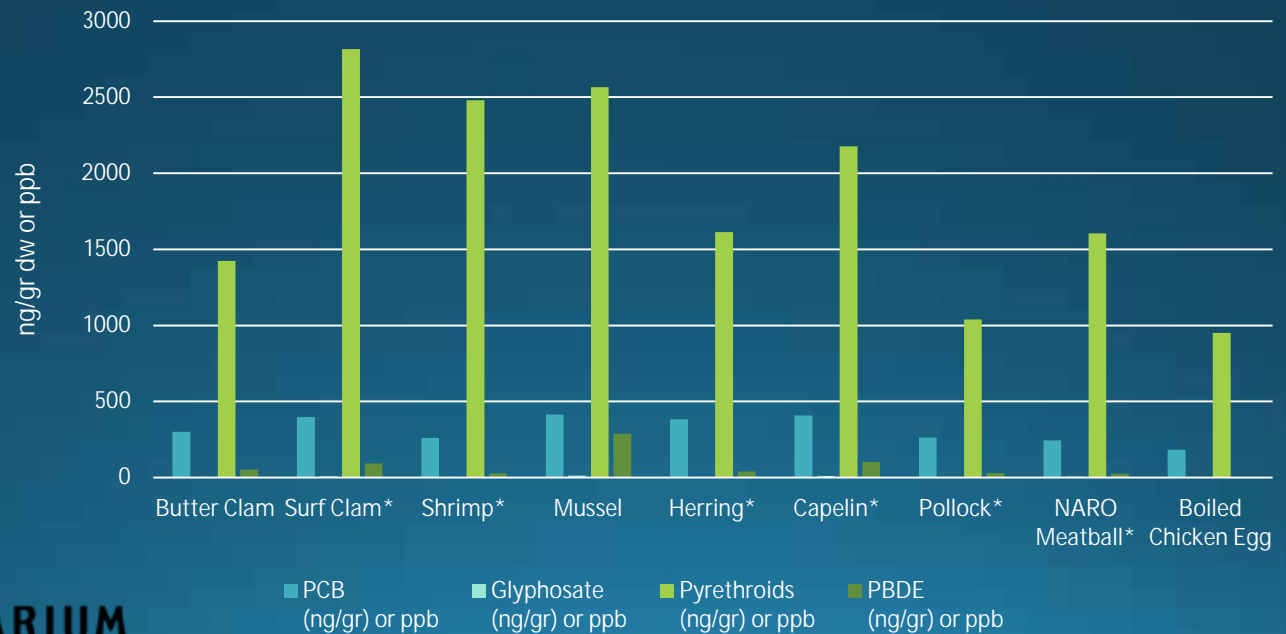
PCB Compound	% React
Arochlor 1254	100
Arochlor 1260	204
Arochlor 1248	50
Arochlor 1242	24
Arochlor 1262	225
Arochlor 1232	20
Arochlor 1268	22
Arochlor 1016	24
Arochlor 1221	1.6

Glyphosate Compound	50% B/Bo (ppb)
Glyphosate	0.5
Glyphosine	3000
Glufosinate	70,000
AMPA	>1,000,000
Glycine	>1,000,000

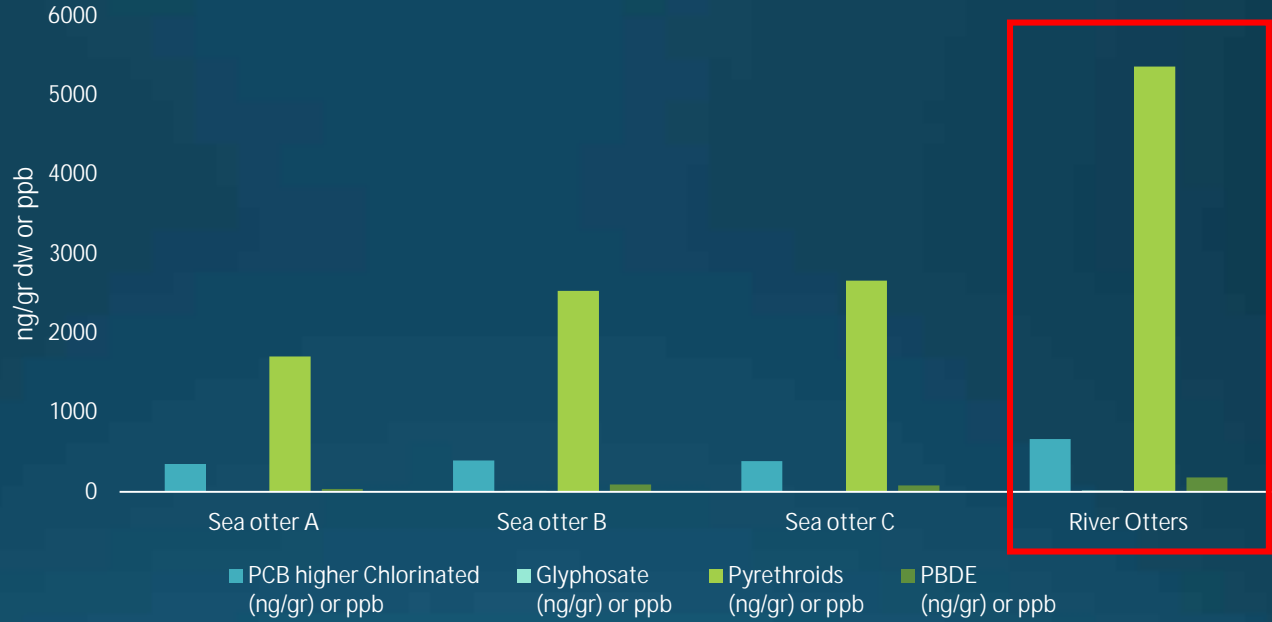


Averages at a glance:	PCB (pg/gr dw) or ppb	Glyphosate (pg/gr dw) or ppb	Pyrethroids (pg/gr dw) or ppb	PBDE (pg/gr dw) or ppb
Boiled Chicken Egg*	184	2	952	<0.03
Butter Clam	301	4	1424	53
Capelin*	408	11	2178	104
Herring*	383	4	1613	46
Meatball*	245	7	1605	26
Mussel	414	12	2565	288
Pollock*	263	5	1038	29
Shrimp*	261	5	2480	27
Surf Clam*	398	9	2815	92

*River otter diet items

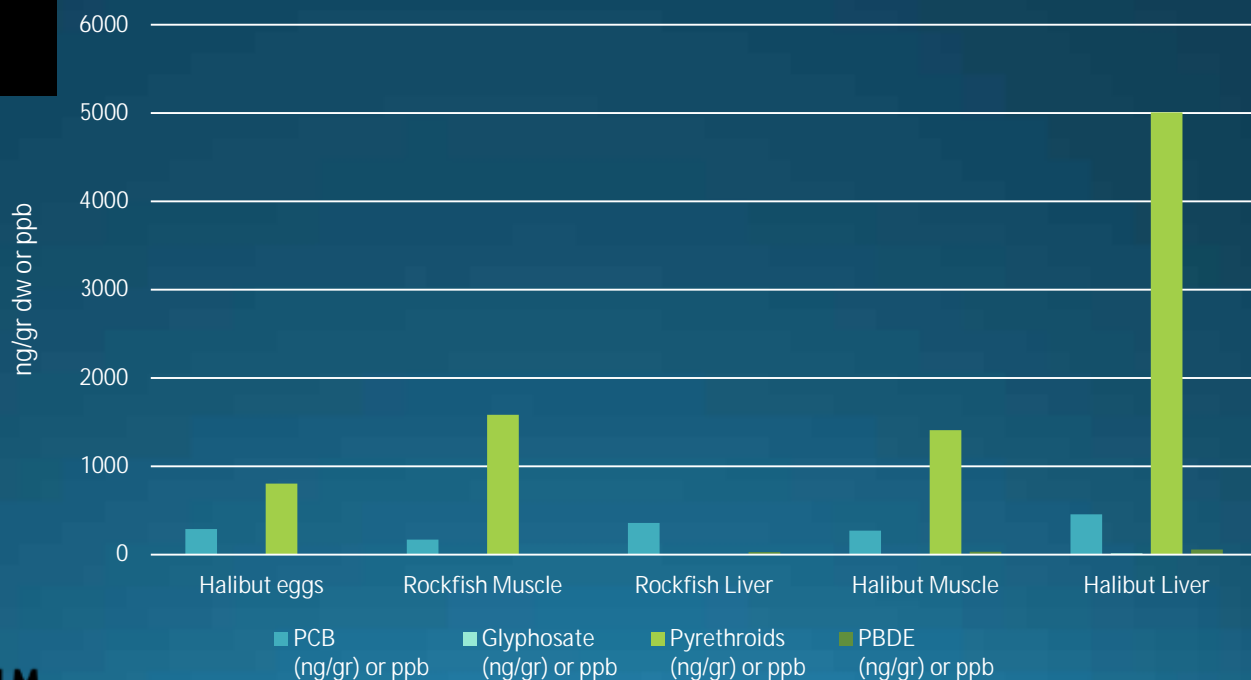


Averages at a glance:	PCB (pg/gr dw) or ppb	Glyphosate (pg/gr dw) or ppb	Pyrethroids (pg/gr dw) or ppb	PBDE (pg/gr dw) or ppb
Female Sea Otter Feces (A)	349	5	1708	34
Female Sea Otter Feces (B)	387	7	2665	81
Female Sea Otter Feces (C)	397	10	2532	92
Male River Otters Feces	666	17	5361	179



- River otters
 - Eat 15-20% total body weight/day
 - Metabolic rate 50% higher than similarly sized land mammal
- Sea otters
 - Eat 25-30% total body weight/day
 - Metabolic rate 25x higher than similarly sized land mammal

Averages at a glance:	PCB (pg/gr dw) or ppb	Glyphosate (pg/gr dw) or ppb	Pyrethroids (pg/gr dw) or ppb	PBDE (pg/gr dw) or ppb
Halibut Eggs	291	<0.05	805	<0.03
Halibut Liver	456	13	5007	56
Halibut Muscle	273	6	1408	33
Rockfish Liver	360	4	<0.75	30
Rockfish Muscle	170	<0.05	1581	<0.03



Comparison to GC/MS

- Ran a subset of river otter fecal samples on GC/MS
- PBDEs in ELISAs were underestimated 10 times lower than absolute value of specific congeners and sum
- PCBs in ELISAs were overestimated 2 times higher than absolute value of aroclors and sum

???



What's going on?

- Oven drying the samples may cause volatilization of lower weight compounds
- We used methanol as a solvent, where GC/MS uses dichloromethane
- Matrix effect of methanol (dilution not high enough?)
- % Binding?



Next steps...

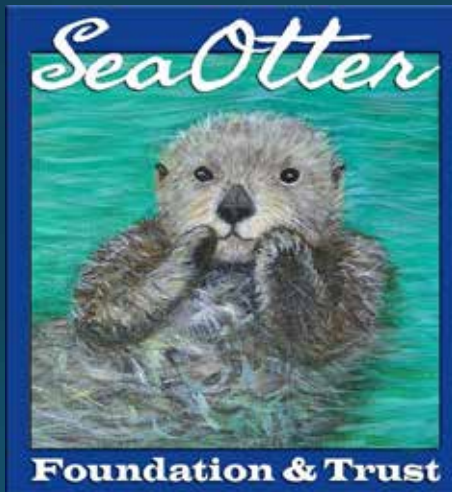
- In endocrinology, validation tests include parallelism and accuracy testing – is this relevant?
- Extract using dichloromethane as a solvent, dry down and reconstitute with methanol?
- Lipid normalize
- Look into freeze drying to avoid losing volatile compounds?
- Collect all excrement including saliva, urine, etc?
- Run matched poop and blood samples from exams



Thank you!

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