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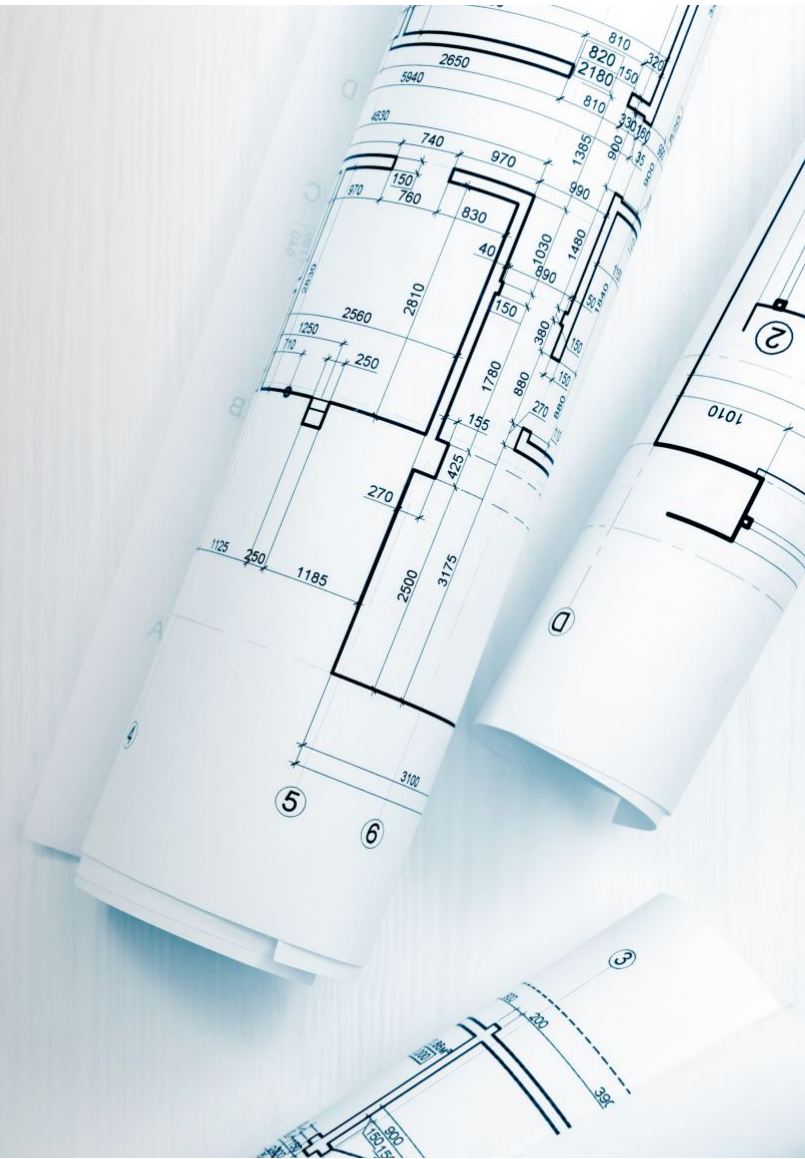
EXPOSURE TO PERSISTENT ORGANIC POLLUTANTS AND GLOBAL DNA METHYLATION IN WHALES

Research was supported by the National Institute Of Environmental Health Sciences of the National Institutes of Health under Award Number R25ES022866. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.



Purpose of Project

Development of epigenetic biomarkers for POPs exposure in marine mammals

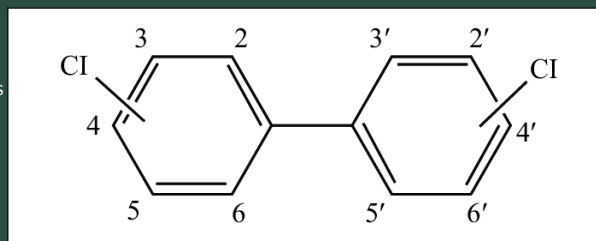


POPs

Chemical substances widely used but persist in environment.

PCBs

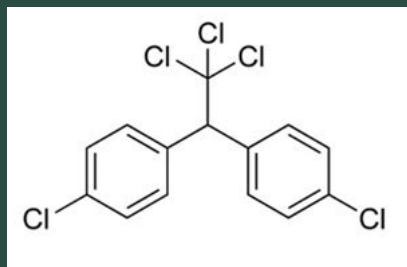
Polychlorinated biphenyls



Dioxin: slow to degrade

DDTs

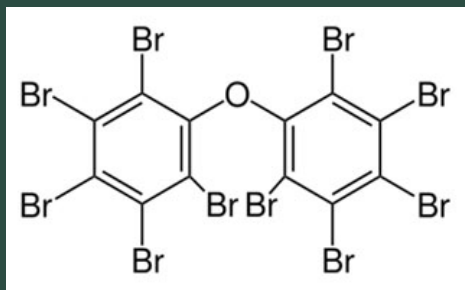
Dichlorodiphenyltrichloroethane



Insecticide: slow to degrade

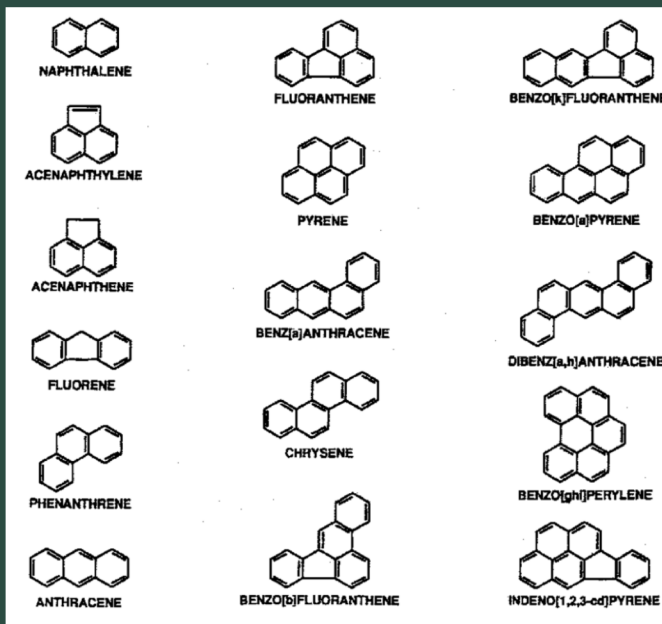
PBDEs

Polybrominated diphenyl ethers



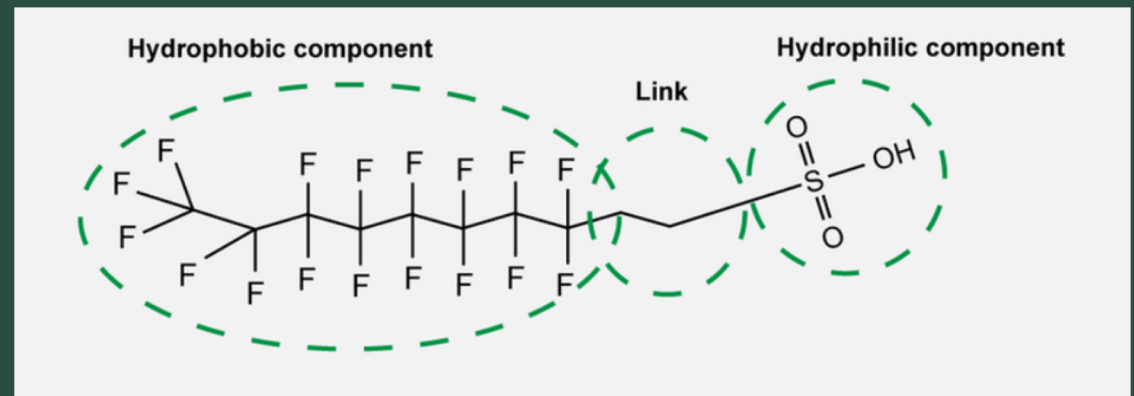
mixtures of several brominated substances

PAHs Polycyclic aromatic hydrocarbons



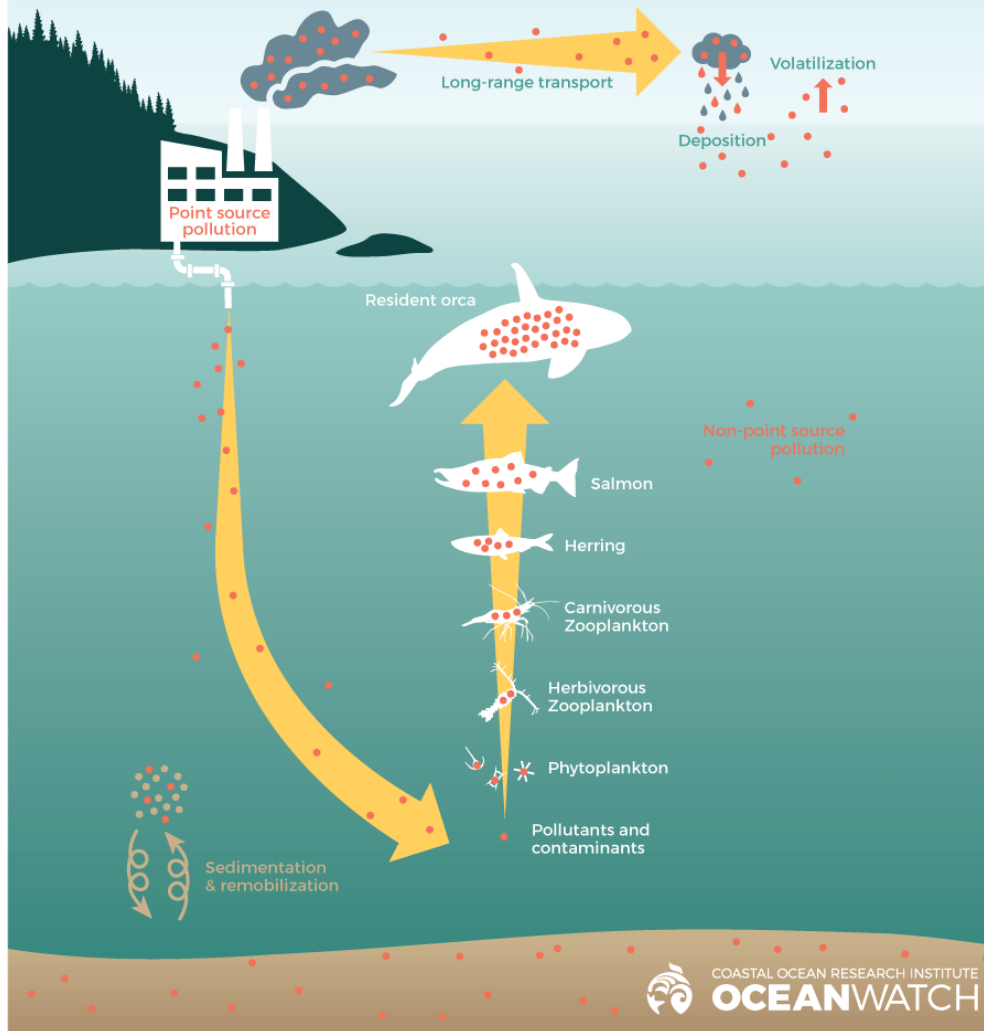
Low degradability and hydrophobic

PFAS polyfluoroalkyl substances



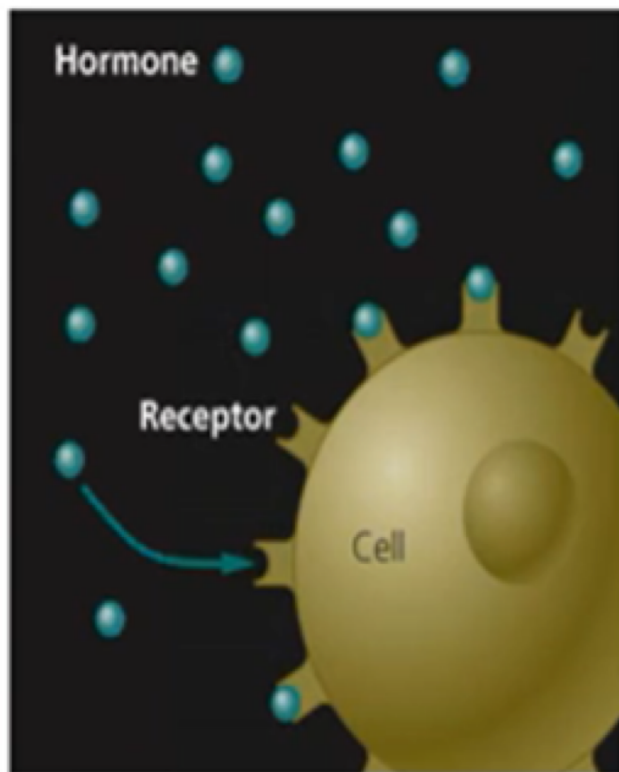
Carbon chains in which hydrogen atoms are replaced by fluorine atoms

Bioaccumulation in marine mammals

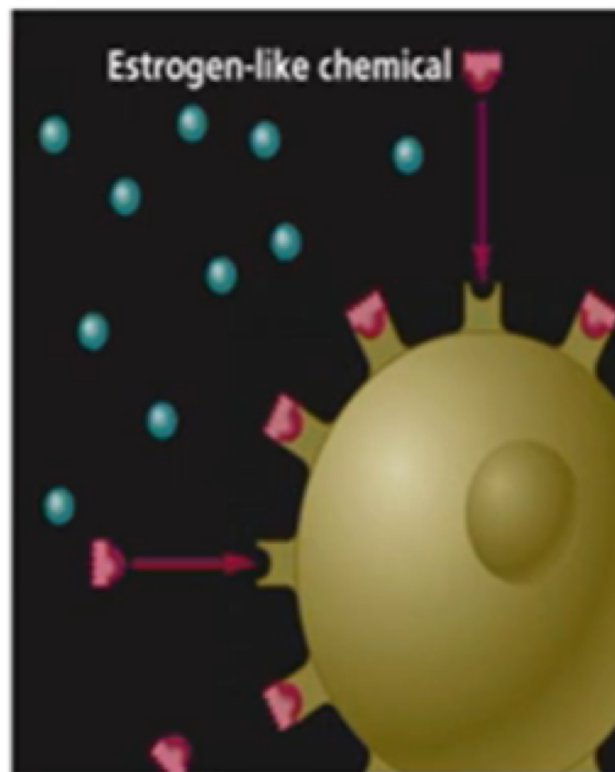


Bioaccumulate through the food chain and pose a risk of causing adverse effects to human health and the environment

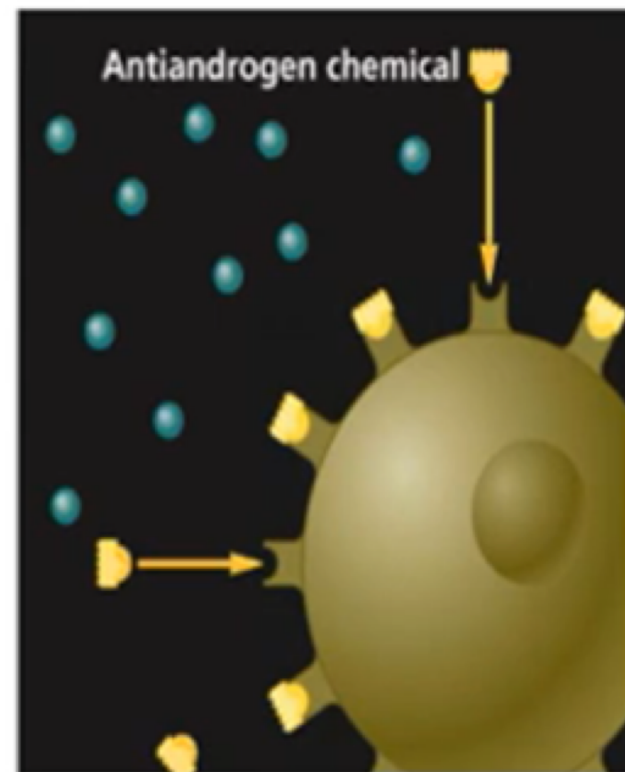
Endocrine Disruptor



Normal Hormone Process



Hormone Mimic



Hormone Blocker

What I am going to be working with

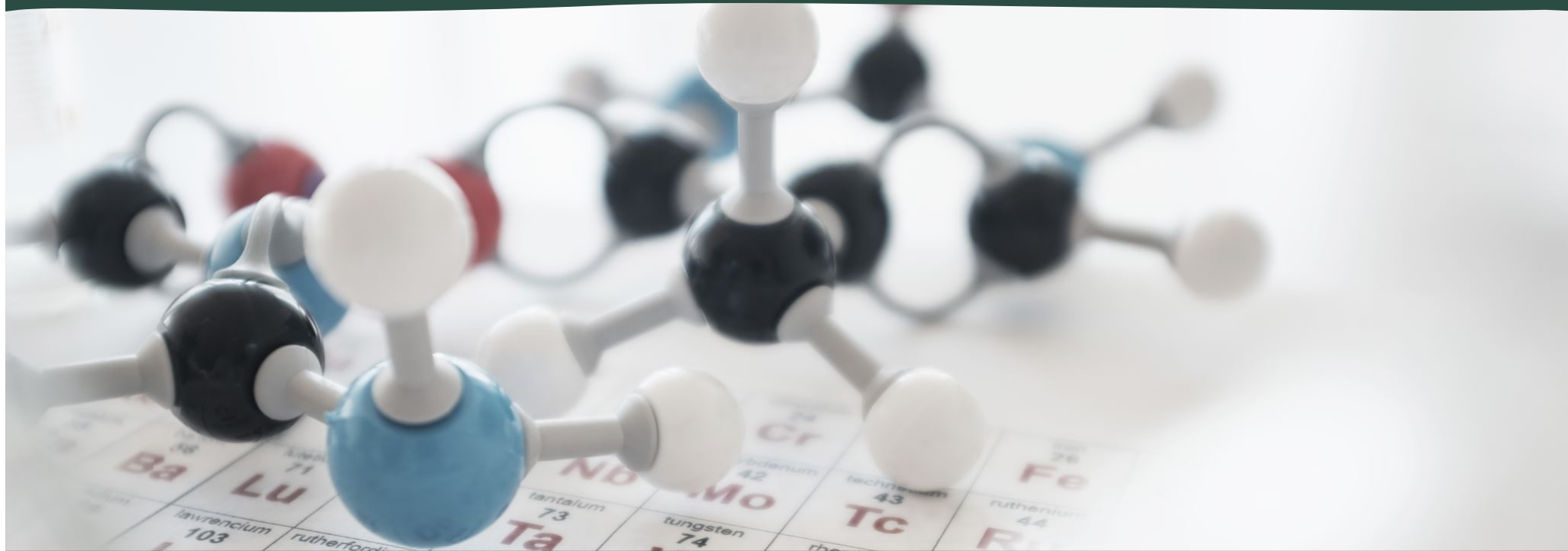
DNA from Dolphins
Delphinus capensis


- POP Levels




Code	PCBs	DDTs	PBDEs	PAHs	PFAS
		ΣDDTs	ΣPBDEs	ΣPAHs	
CMD1	4040.40	6630.10	824.59	166.62	195.21
CMD2	4101.76	5877.18	986.74	1251.70	156.60
CMD3	2184.53	3378.88	451.44	1.58	216.54
CMD4	3005.32	4480.00	642.80	1.18	501.04
CMD5	3311.02	4922.62	643.74	313.63	384.08
CMD6	3537.75	5466.78	825.94	120.03	69.80
CMD7	6645.05	10603.01	853.49	1.45	273.35
CMD8	3910.09	5853.41	691.01	2336.80	144.98
CMD9	3804.40	5843.39	658.05	194.09	211.66
CMD10	4928.51	7668.64	874.81	2003.87	72.25
CMD11	4221.83	6495.58	694.71	324.86	92.61
CMD12	2505.32	3370.08	442.59	896.28	215.31
CMD14	2880.68	3852.51	491.80	759.01	267.06
CMD15	5157.87	8181.55	878.55	11.92	107.37
	3465.28	4255.05	518.95	6076.56	

Materials and Methods





PART 1
Nanodrop
and
DNA Dilution





Part 2

Luminometric Assay

Method to the analysis of
genomic DNA methylation

MMA

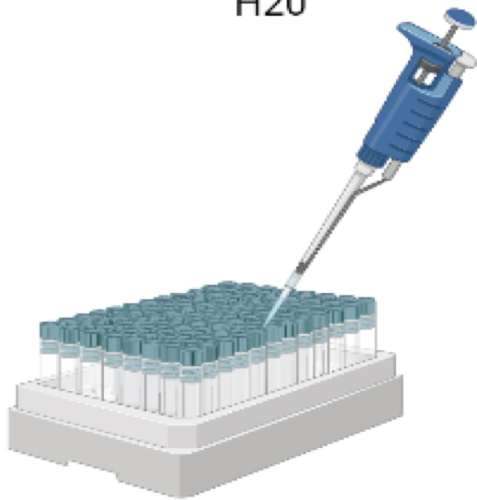
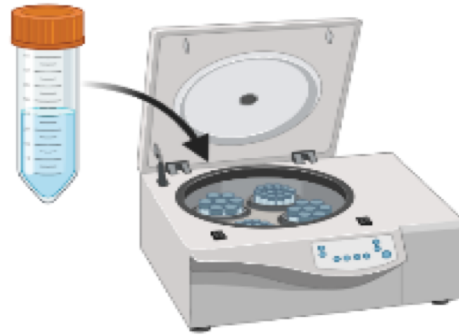


MMB

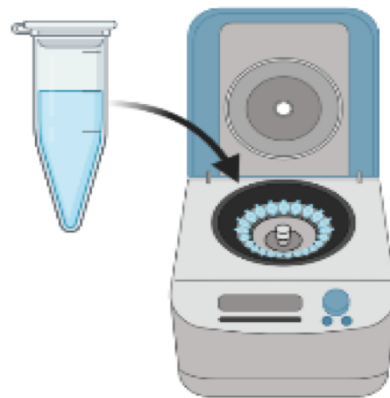


Tango Buffer
EcoRI
HpaII

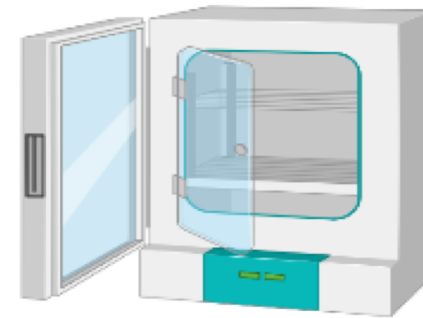
Tango Buffer
EcoRI
MspI
H₂O

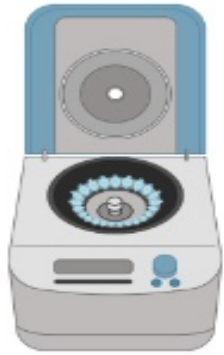


17.25 μ L DNA
2.75 μ L MMA/MMB



(No Air Bubbles)

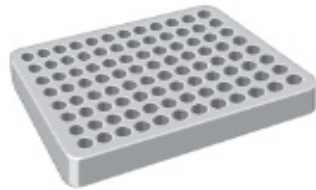




Annealing Buffer



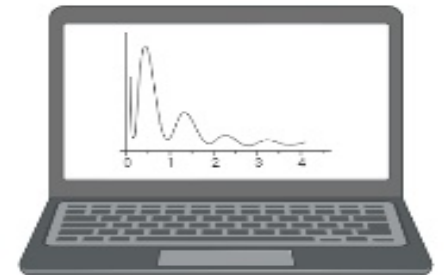
15 μ L



30 μ L Mixture



Pyrosequencing Plate
Nucleotides
Enzyme Mix
Substrate Mix





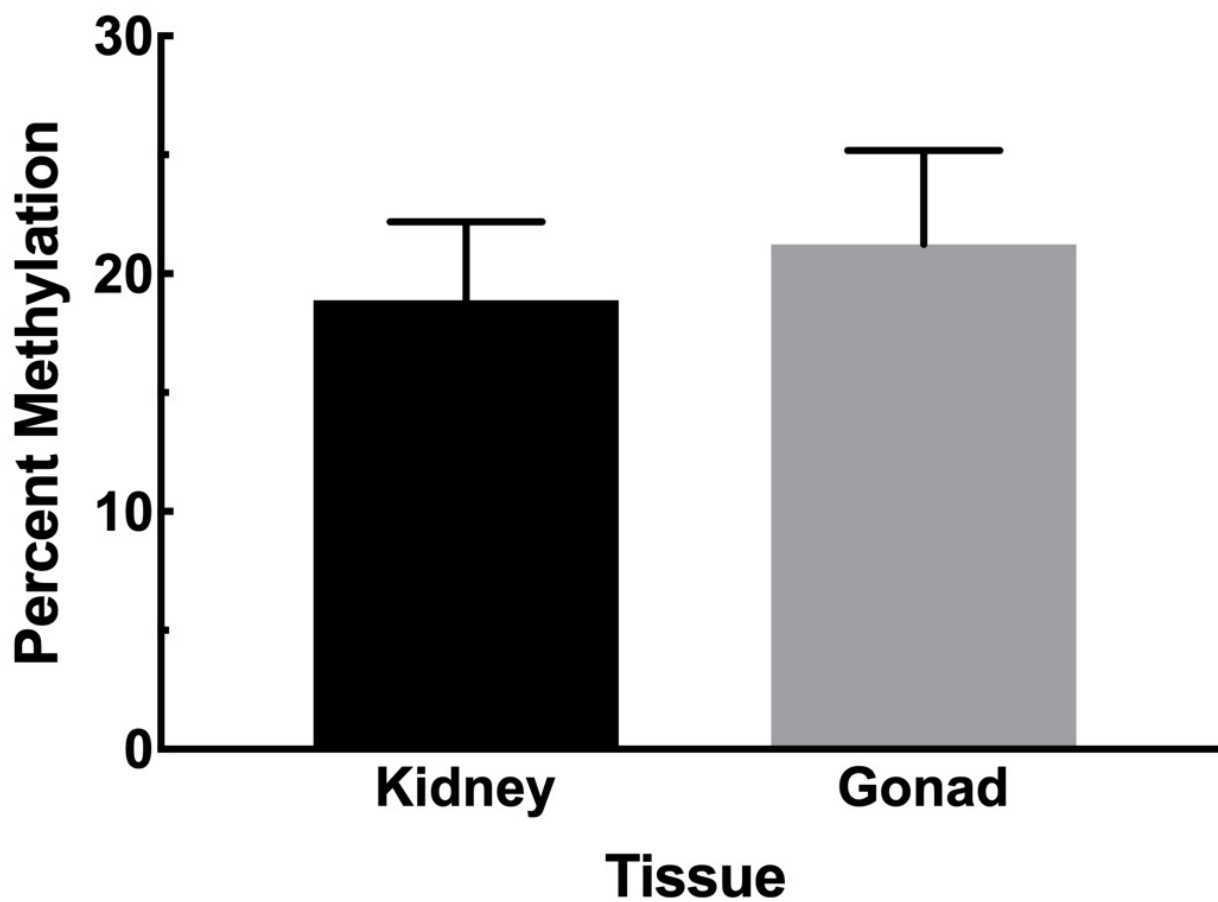
DATA

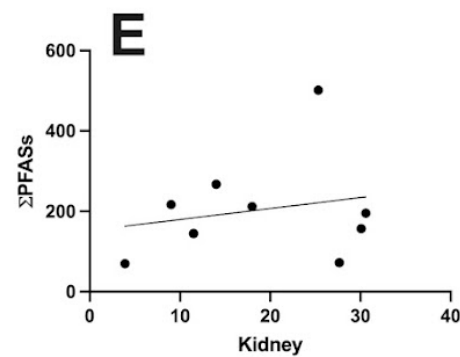
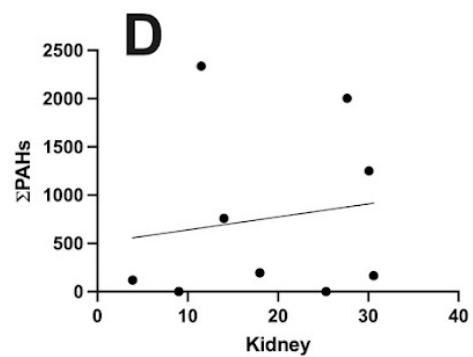
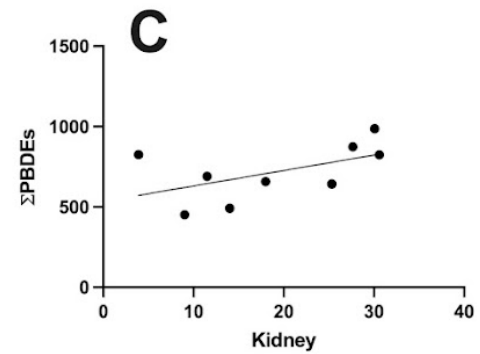
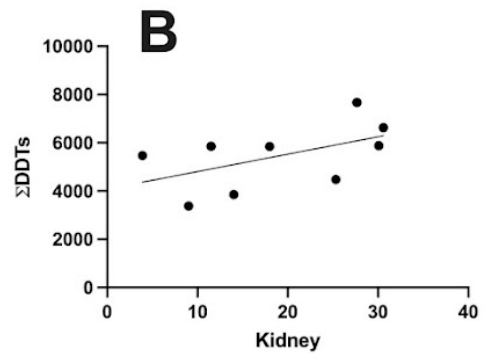
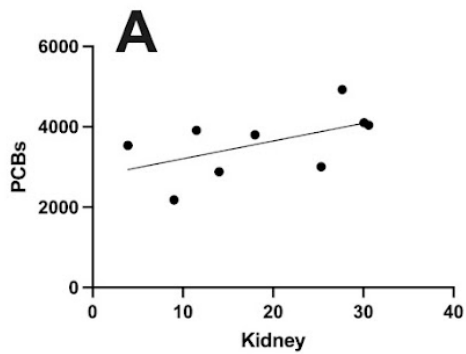
Calculation of Percent of DNA Methylation

Entry ID: LUMA Jess		EcoRI HpaII(MMA) or MspI(MMB)													
Disp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Well	G	T	G	T	C	A	C	A	T	G	T	G	T	G	
1G MMA	A1	59.77	59.29	10.8	5.9	90.96	66.81	18.43	5.83	58.88	43.97	20.19	5.12	4.7	1.76 1GA G/T= 0.746773
5G MMA	A2	47.89	23.21	6.62	3.98	61.92	29.87	12.16	3.74	27.78	28.14	11.36	4.58	3.44	1.72 5GA G/T= 1.012959
1G MMB	A3	158.53	58.99	14.22	5.29	177.53	66.17	17.9	5.77	58.15	47.25	19.46	4.01	3.69	2.1 1GB G/T= 0.812554
5G MMB	A4	117.08	25.23	9.53	4.38	129.6	32.07	14.73	4.88	31.05	33.43	13.38	3.55	3.35	1.47 5GB G/T= 1.076651
H2O	A5	0.3	2.13	1.48	1.27	1.16	1.82	2.16	0.55	1.59	1.82	0.81	0.74	1.36	1.56 H2O G/T= 1.144654
1G MMA	B1	66.76	68.62	11.26	5.74	96.37	72.57	18.85	7.14	60.85	45.47	20.61	5.29	3.96	1.8 1GA G/T= 0.747247
5G MMA	B2	47.46	25.59	7.25	3.27	65.77	32.73	12	4.7	27.21	29.26	12.27	3.67	2.34	2.32 5GA G/T= 1.07534
1G MMB	B3	166.11	64.27	16.55	7.03	186	67.17	21.1	5.93	64.01	53.55	22.59	5.87	4.73	1.58 1GB G/T= 0.836588
5G MMB	B4	108.58	23.45	7.94	4.58	117.17	30.72	12.97	4.58	29.78	31.16	10.88	3.16	2.88	1.46 5GB G/T= 1.04634
H2O	B5	0.79	5.89	4.45	4.07	2.56	1.6	3.71	0.69	4.62	1.43	4.94	0.77	2.39	1.63 H2O G/T= 0.309524
2G MMA	C1	75.88	56.06	13.06	7.05	94.64	66.34	18.88	5.34	54.76	49.25	21.24	6.69	4.13	1.86 2GA G/T= 0.899379

1GA G/T= 0.746773	1G A/B= 0.919045	(1-A/B)X100=	0.080955	X100=	8%
5GA G/T= 1.012959	5G A/B= 0.940842	(1-A/B)X100=	0.059158	X100=	6%
1GB G/T= 0.812554					
5GB G/T= 1.076651					
H2O G/T= 1.144654					
1GA G/T= 0.747247	1G A/B= 0.893208				
5GA G/T= 1.07534	5G A/B= 1.027716				
1GB G/T= 0.836588					
5GB G/T= 1.04634					
H2O G/T= 0.309524					
2GA G/T= 0.899379	2G A/B= 0.889111				

Comparison of Whale Tissues



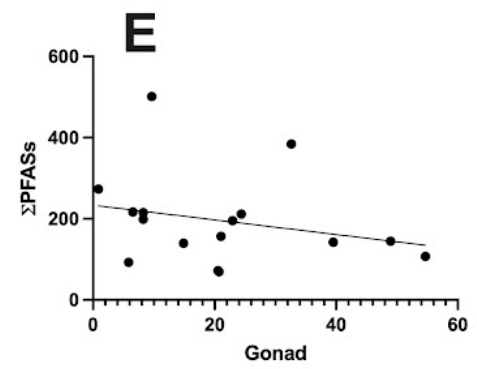
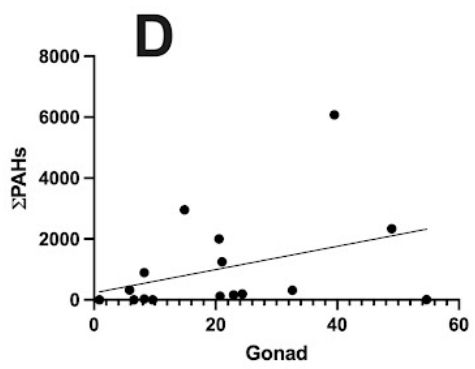
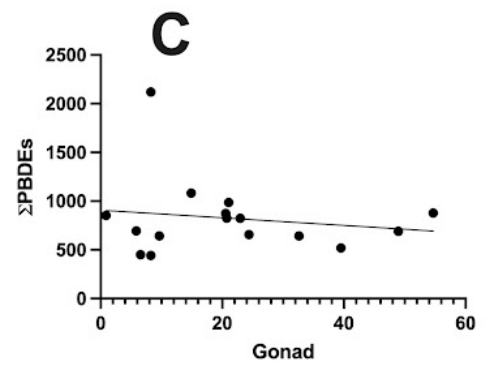
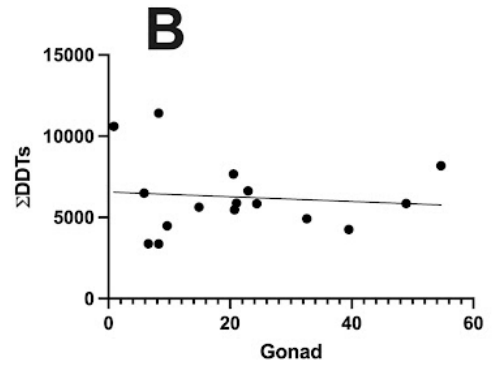
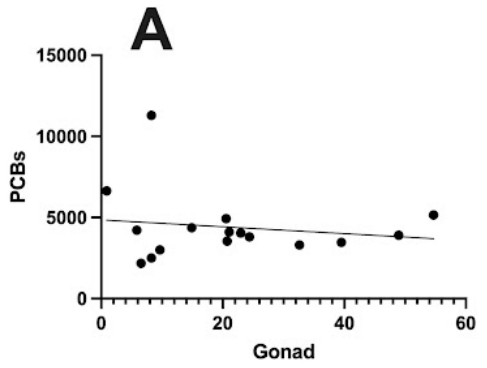


Pearson's r

PCBs	ΣDDTs	ΣPBDEs	ΣPAHs	ΣPFASs
0.540	0.528	0.535	0.148	0.209

P -value

PCBs	ΣDDTs	ΣPBDEs	ΣPAHs	ΣPFASs
0.133	0.144	0.138	0.705	0.590



Pearson's *r*

PCBs	ΣDDTs	ΣPBDEs	ΣPAHs	ΣPFASs
-0.157	-0.101	-0.158	0.368	-0.250

P-value

PCBs	ΣDDTs	ΣPBDEs	ΣPAHs	ΣPFASs
0.561	0.711	0.558	0.161	0.351

Conclusions

- Genomic DNA methylation in Kidney is lower than that in gonad, but it was not significant.
- No significant correlation between genomic DNA methylation levels and POPs-exposed levels in whales.

Acknowledgements

- Drs. Yoon Hee Cho, Andrij Holian, and Chris Migliaccio
- Special thanks to Minsun Koo and Paulette Jones at University of Montana
- Special thanks to my parents

- NIH (R25ESES022866-07) - Opportunity to learn new laboratory techniques in the field of Epigenetics.