Characterization of endocrine disrupting chemicals released into simulated gastric fluid utilizing non-targeted analysis coupled with in vitro bioassays

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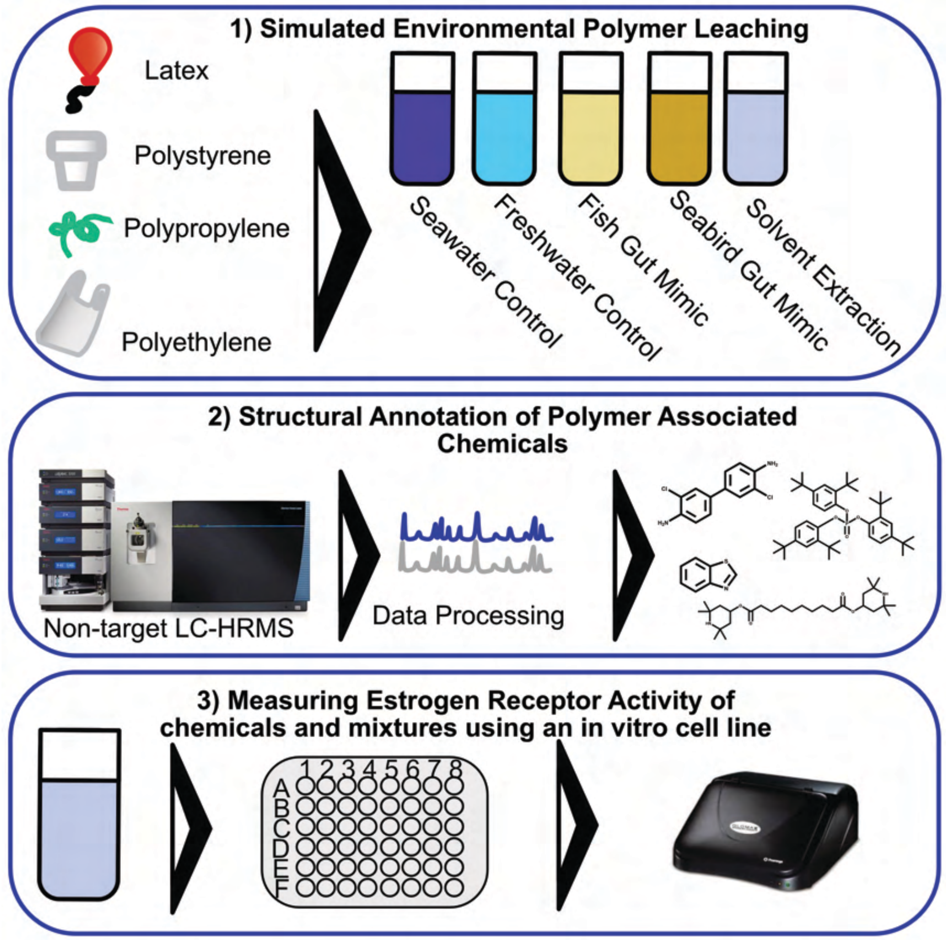
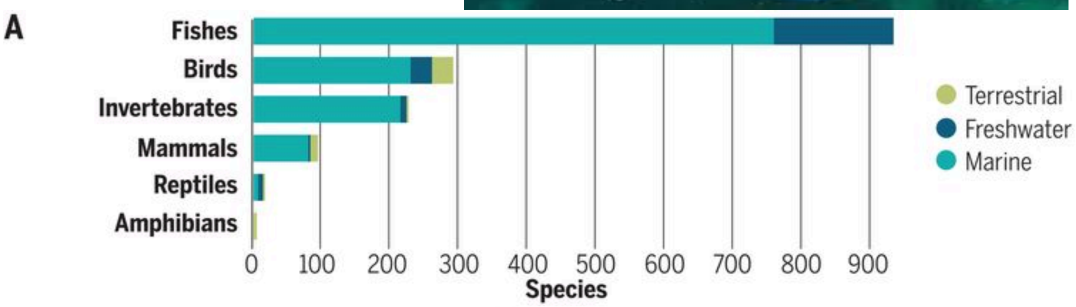


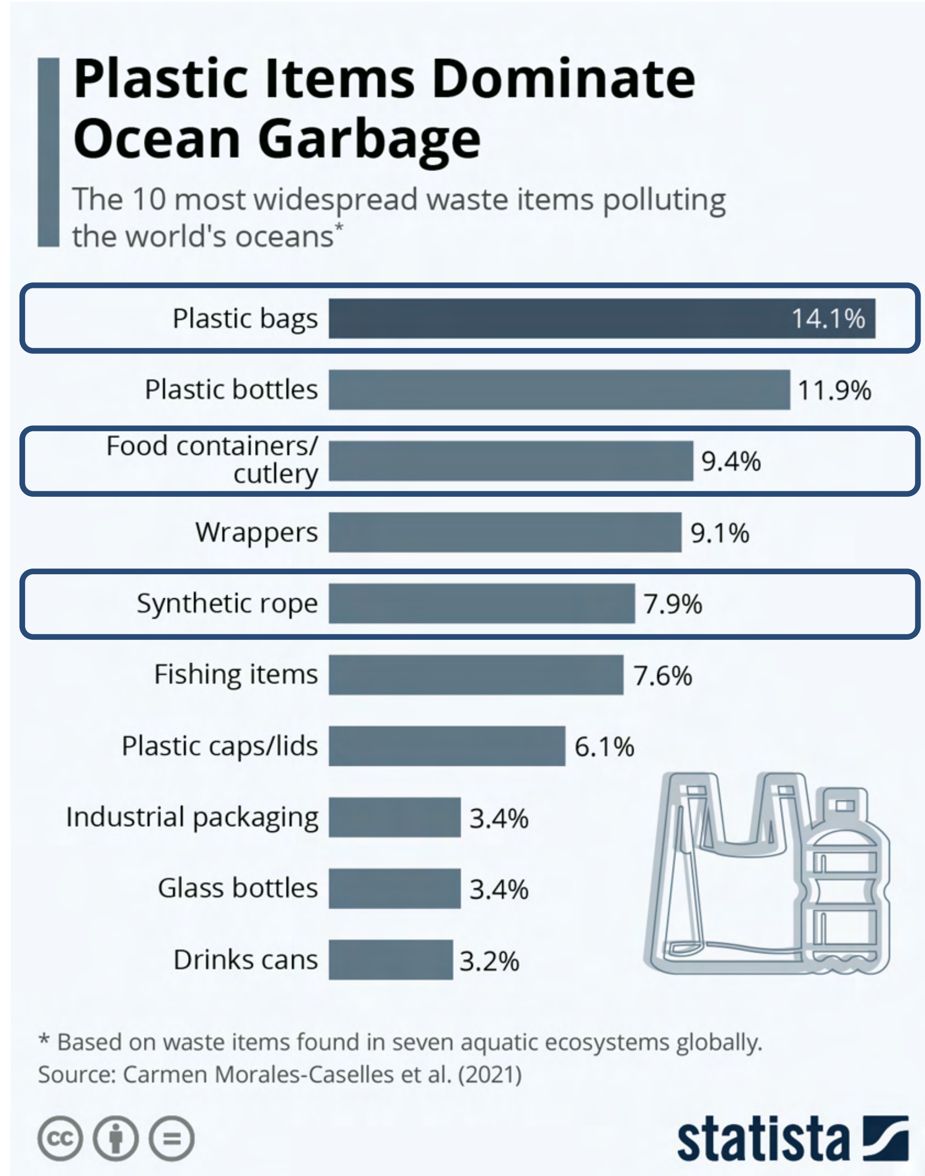
There are multiple sources and sinks for plastic debris

- Land based sources contribute to 80% of marine debris (Li, 2016)
- 1.15 to 2.41 million tons of plastic enter oceans yearly from rivers (Lau, 2020)

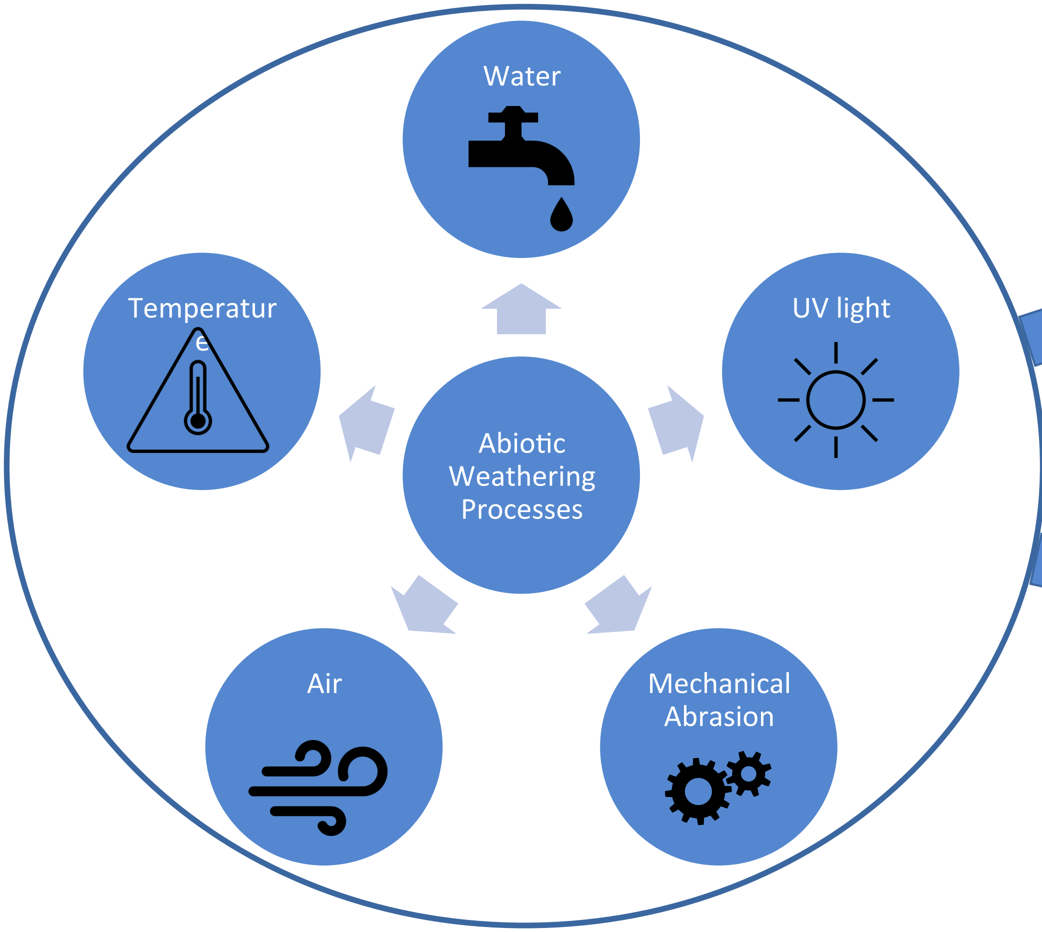
Plastic debris consumption is common within birds and fish



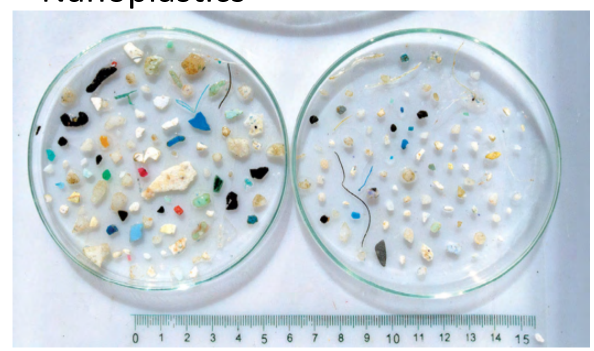
Robson G. Santos et al. Science 2021;373:56-60



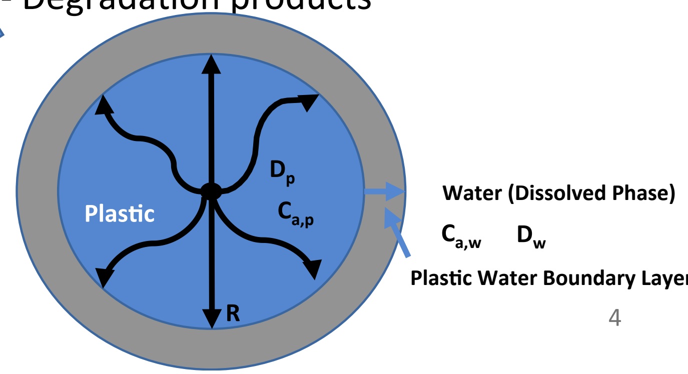
Environmental processes fragment plastic debris and release/transform polymer associated chemicals



- Fragments
- Microplastics
- Nanoplastics



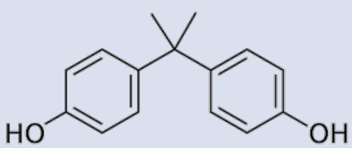
- Polymer associated chemicals
- Degradation products



Plastic contains thousands of known and unknown polymer associated chemicals (PACs)

Monomers

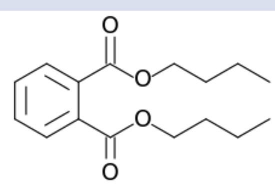
- Polymer building block
- Examples:
  - BPA
  - MDI
  - Styrene



Bisphenol A

Polymer additives and processing aids

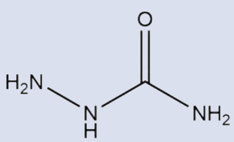
- Stabilizers
- Dyes and colorants
- UV inhibitors
- Flame retardants
- Plasticizers
- Antioxidants
- Biocides



Dibutyl Phthalate

Non-intentionally added substances

- Impurities
- Processing degradants



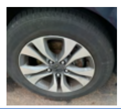
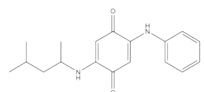
Semicarbazide

Polymer associated chemicals have the potential to be hazardous

Aquatic Toxicant

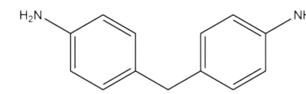
(Tian et. al, Science, 2021)

- 6PPD-quinone
  - Antioxidant degradant

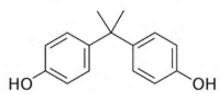
Carcinogenic

- 4,4-methylenedianiline
  - hardener/intermediate



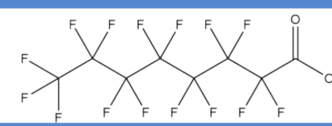
Endocrine Disruption

- Bisphenol A
  - Monomer



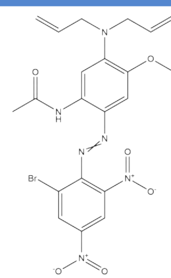
Persistent

- Perfluorooctanoic acid
  - Surfactant



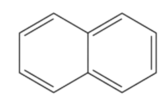
Mutagenic

- Disperse Blue 373
  - Disperse Dye



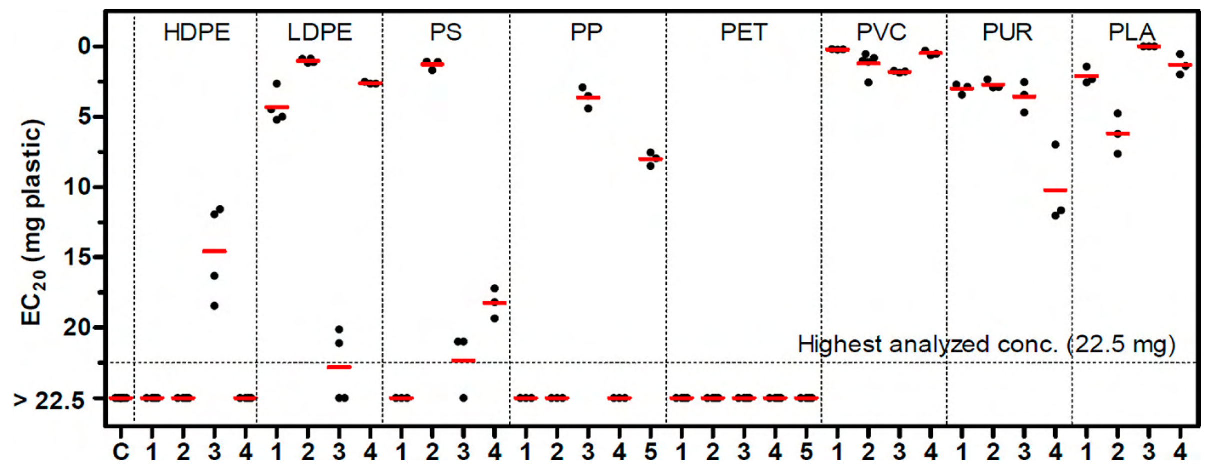
Bioaccumulative

- Naphthalene
  - Solvent/catalyst



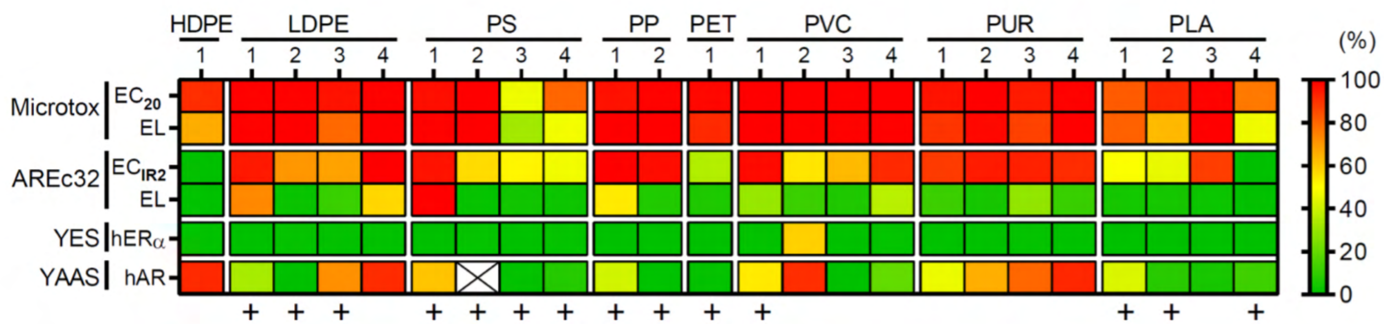
PAC Mixtures in the form of extracts and leachates have the potential to be toxic

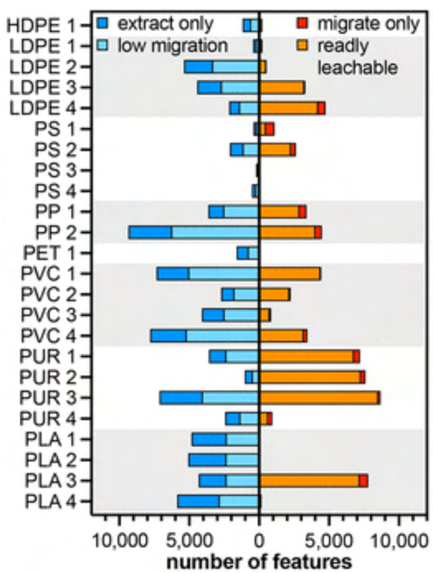
Extract



(Zimmerman et. Al., EST, 2019)

Leachates





(Zimmerman et. Al., EST, 2021)

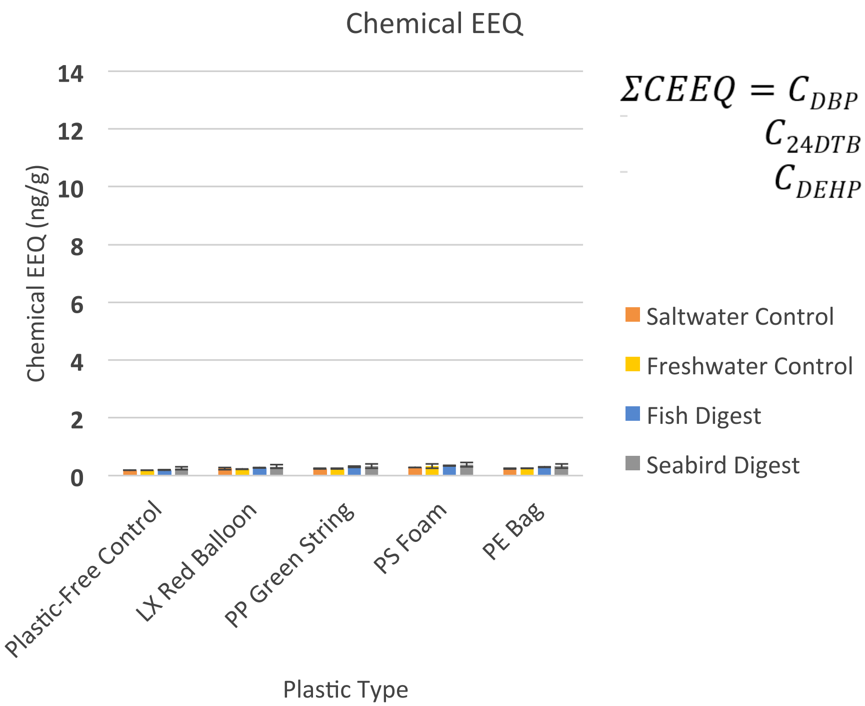
Motivation

16 Commonly-ingested plastic items

(Coffin et. al, 2019)

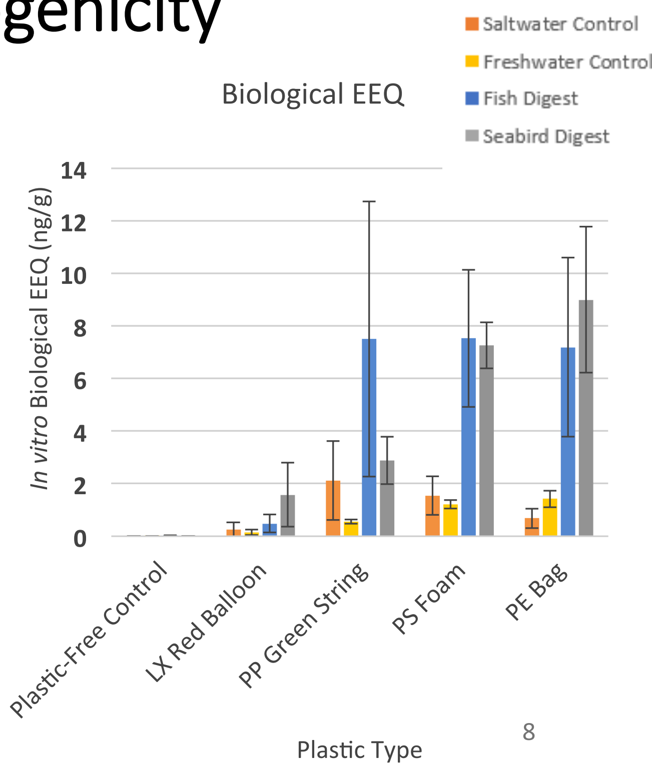
Treatments	Temp (˚C)	pH	Pepsin (g/L)
Saltwater Control	28	7	0
Freshwater Control	40	7	0
Fish Digest	28	2	2
Seabird Digest	40	2	10

ΣChemical Estrogenicity ≠ ΣBiological Estrogenicity



$$\Sigma CEEQ = C_{DBP} * EEF_{DBP} + C_{BPA} * EEF_{BPA} + C_{24DTB} * EEF_{24DTB} + C_{DEP} * EEF_{DEP} + C_{DEHP} * EEF_{DEHP} + C_{BPS} * EEF_{BPS}$$

CEEQ = Sum Chemical estrogenic response
C_x = Concentration of X
EEF_x = estradiol equivalency factor of X



Follow up study determined estrogenic fractions of PE bag in simulated Fish digests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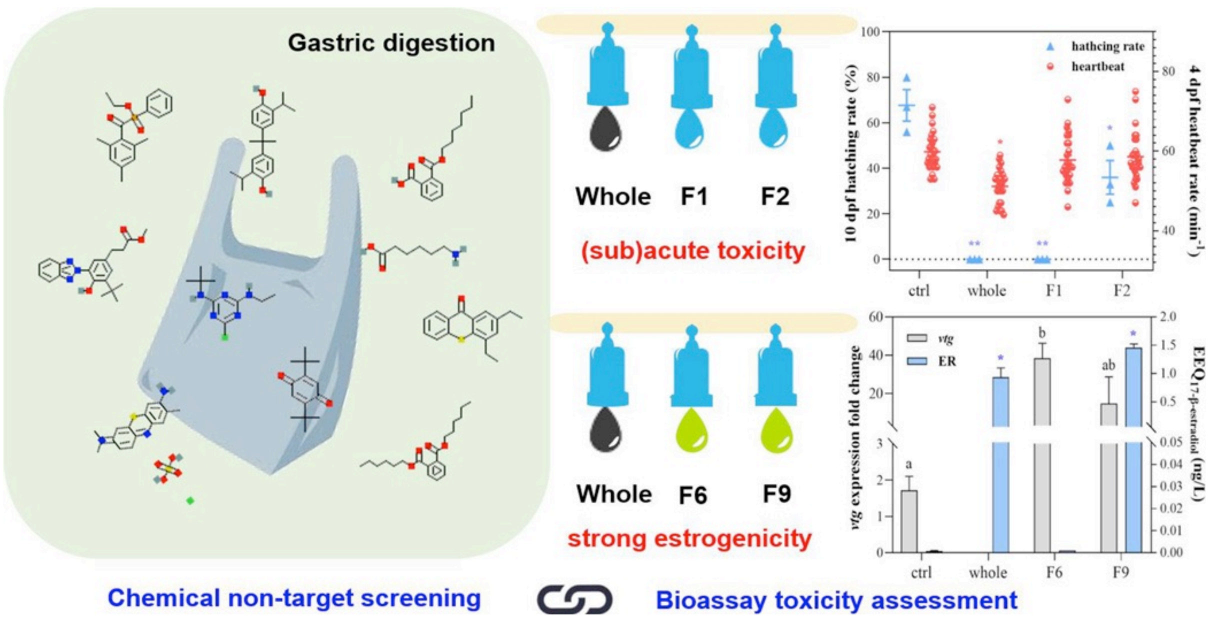
Bioassay guided analysis coupled with non-target chemical screening in polyethylene plastic shopping bag fragments after exposure to simulated gastric juice of Fish

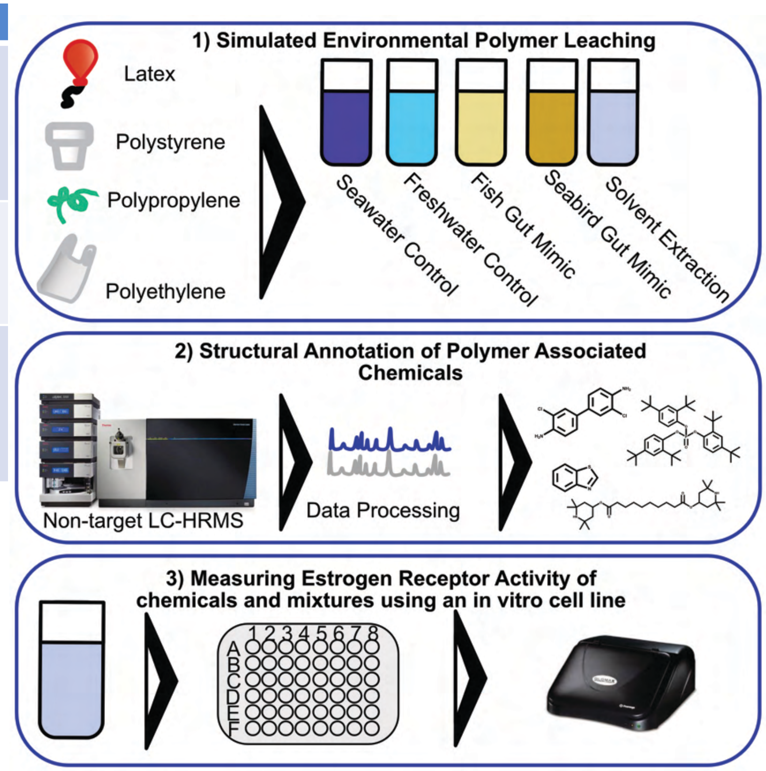
Qiqing Chen[a,*], Mauricius Marques dos Santos[b,c], Philip Tanabe[d], Gary T. Harraka[d], Jason T. Magnuson[d], Victoria McGruer[d], Wenhui Qiu[e], Huahong Shi[a], Shane A. Snyder[b,c], Daniel Schlenk[d,f,**]



- 103 compounds were structurally annotated
  - An additional 4 phthalates and 2 alkylphenols were tentatively identified in the most estrogenic PE bag fractions
- No actual chemicals were confirmed with standards or quantitatively determined to contribute to the estrogenicity of the samples.

Objective: Identify endocrine disrupting chemicals and chemical mixtures

	(Coffin et al., 2019)	(Chen et al., 2021)	(Walker, 2022)
Plastics	16 plastics	PE bag	PE bag PS foam PP string LX balloon
Chemical Analysis	Chemical quantification (6 Phenols and phthalates)	NTA-structural annotation	NTA- structural annotation Chemical Quantification
Digest Conditions for chemical analysis	Freshwater Seawater Fish gastric Seabird gastric	Fish gastric	Freshwater Seawater Fish gastric Seabird gastric

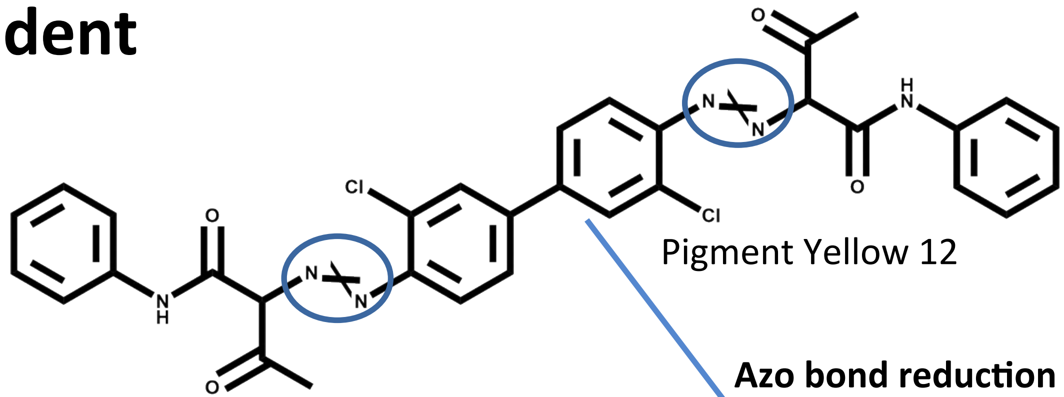


Compound structure annotation using mass spectral libraries and chemical reference standards for polymer associated chemicals in plastic digests and solvent extracts.

					Plastic Peak Area Percentile Rank High (100) - Low (0)					ERα Bioassay		TEST Model	
Name	CAS	MF	Ref. Std Match (Spectral Score)	Log K_{ow}	PE	PP	PS	LX	Structure Based Classification	AC_{50} (µM)	Toxcast Model (Williams 2017)	Dev. Toxin	LC_{50} (mg L^{-1})
4-Dodecylbenzenesulfonic acid	121-65-3	$C_{18}H_{30}O_3S$	Y (97)	5.92	98	97	96	91	Surfactant	NA	NA	DT	NA
Lauramidopropylbetaine	4292-10-8	$C_{19}H_{38}N_2O_3$	92	1.58	96	95	95	82	Surfactant	NA	NA	NT	NA
Nonapropylene glycol	2172326-56-4	$C_{27}H_{56}O_{10}$	77	0.89	89	62	70	70	NA	NA	NA	NT	109.63
Octapropylene glycol	45308-36-9	$C_{24}H_{50}O_9$	78	0.84	84	68	66	67	NA	NA	NA	NT	107.24
Tris(2,4-di-tert-butylphenyl) phosphate	95906-11-9	$C_{42}H_{63}O_4P$	Y	10.5	100	100	92	82	NA	NA	NA	NA	7.03E-04
Decylbenzenesulfonic acid	140-60-3	$C_{16}H_{26}O_3S$	89	4.97	96	95	93	83	Surfactant	NA	NA	DT	NA
3,3'-Dichlorobenzidine	91-94-1	$C_{12}H_{10}Cl_2N_2$	Y (95)	*3.31*	54	97	90	75	*Colorant*	14.47	Gain-Loss	DT	*1.05*
Tris(2-butoxyethyl) phosphate	78-51-3	$C_{18}H_{39}O_7P$	82	*3.75*	98	99	98	92	*Plasticizer*	Inactive	Constant	DT	9.52E-02
Palmitic acid	57-10-3	$C_{16}H_{32}O_2$	91	*7.17*	95	100	100	83	*Surfactant*	Inactive	Constant	NT	3.22
Dioctyl sulfosuccinate	10041-19-7	$C_{20}H_{38}O_7S$	94	5.28	85	85	89	71	Surfactant	NA	NA	*DT*	NA
2-(Dodecylsulfanyl)ethanol	1462-55-1	$C_{14}H_{30}OS$	91	5.73	50	39	99	67	Emulsion Stabilizer	NA	NA	NT	4.29
Benzothiazole	95-16-9	C_7H_5NS	Y (87)	*2.01*	92	91	99	82	Catalyst	Inactive	Constant	NT	28.07
Octadecenoic acid methyl ester	2733-86-0	$C_{19}H_{36}O_2$	87	7.68	27	64	98	74	Emollient	NA	NA	NT	0.88
3,5-Di-tert-butyl-4-hydroxymethylphenol	88-26-6	$C_{15}H_{22}O$	Y (91)	3.85	88	89	88	76	Antioxidant	Inactive	Constant	DT	1.54
2-Mercaptobenzothiazole	149-30-4	$C_7H_5NS_2$	96	2.42	30	27	28	26	Catalyst	Inactive	Constant	NT	*5.36*

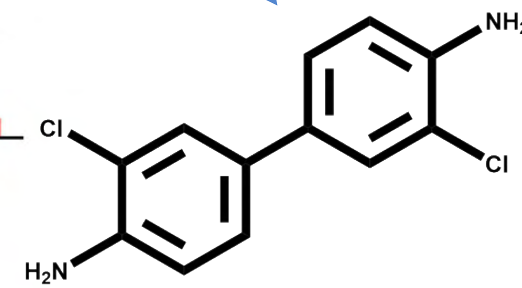
DCB release is treatment dependent

- DCB is colorant intermediate primarily released from green PP string.
- DCB estrogenic responses are significantly negative.



Pigment Yellow 12

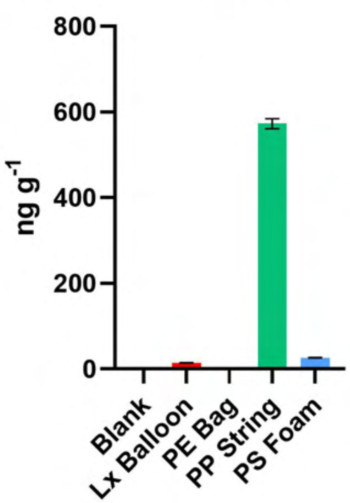
Azo bond reduction



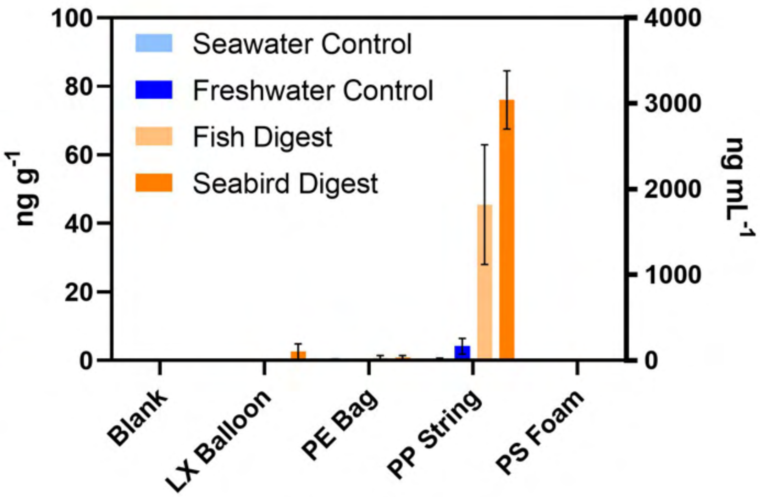
3,3'-Dichlorobenzidine

3,3-Dichlorobenzidine (DCB)

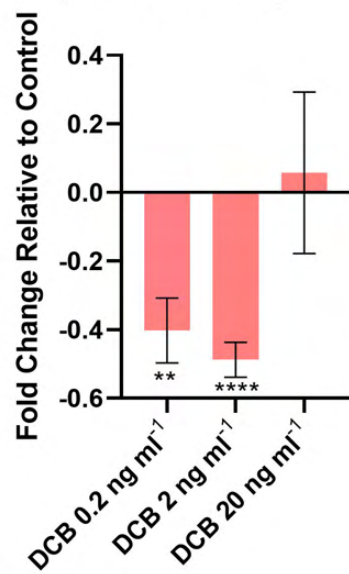
Extract



Leachate

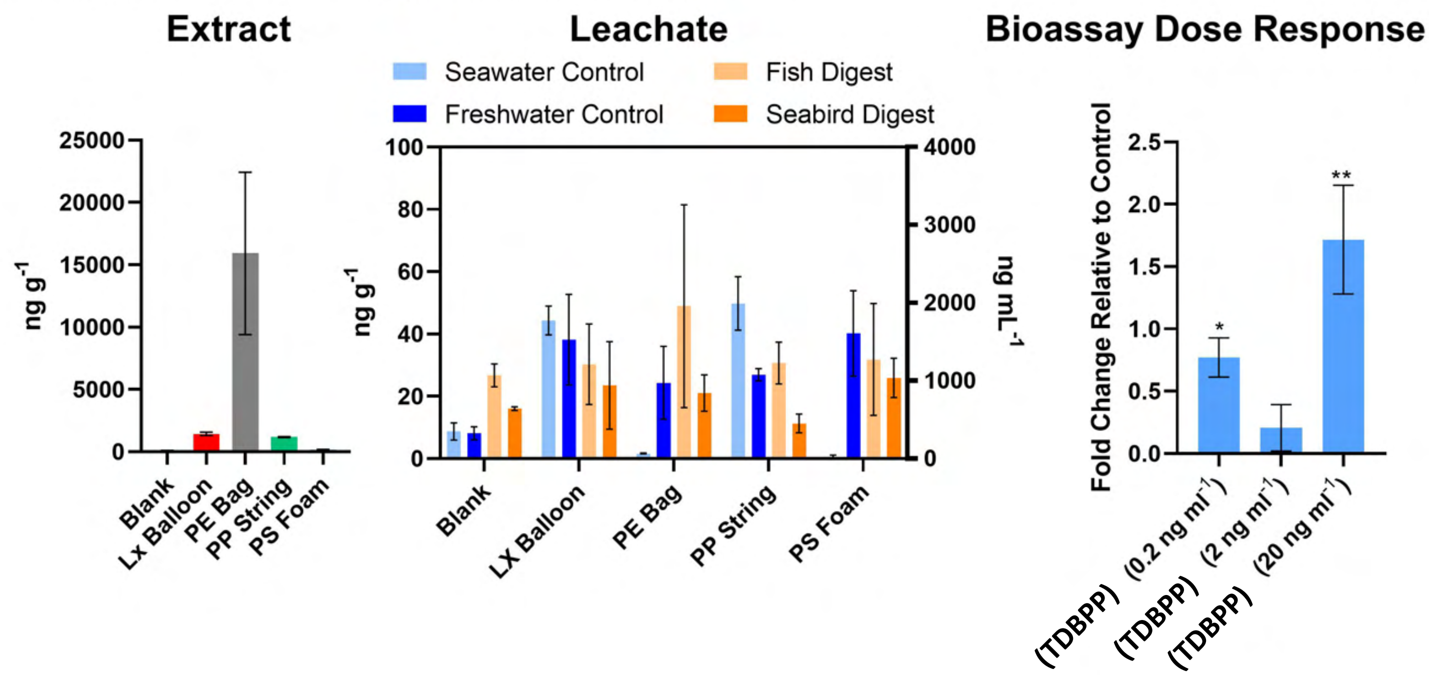


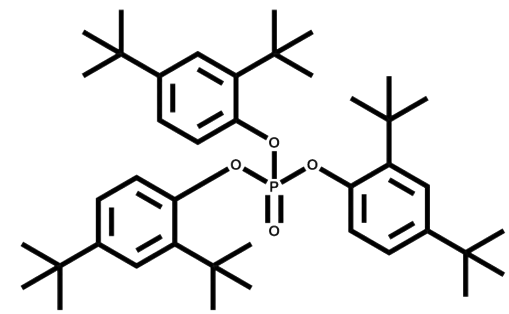
Bioassay Dose Response



TDBPP has significant estrogenic activity

Tris(2,4-ditert-butylphenyl)phosphate (TDBPP)



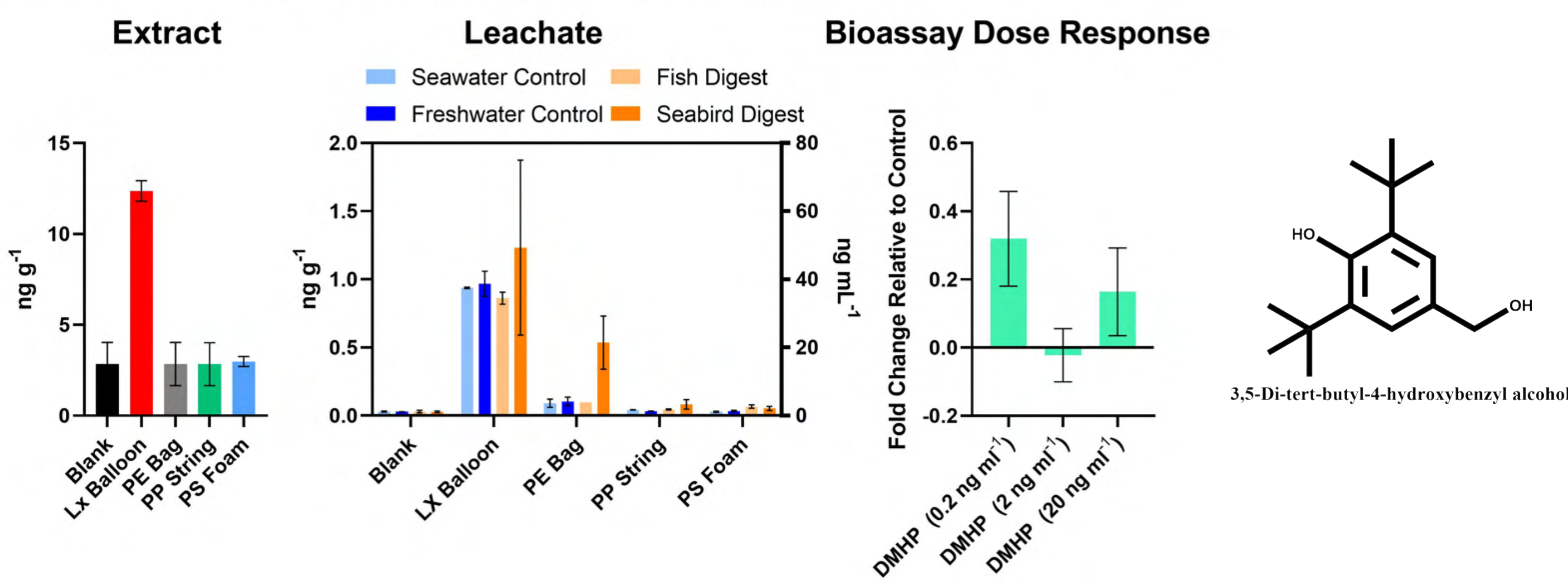


Tris(2,4-ditert-butylphenyl)phosphate

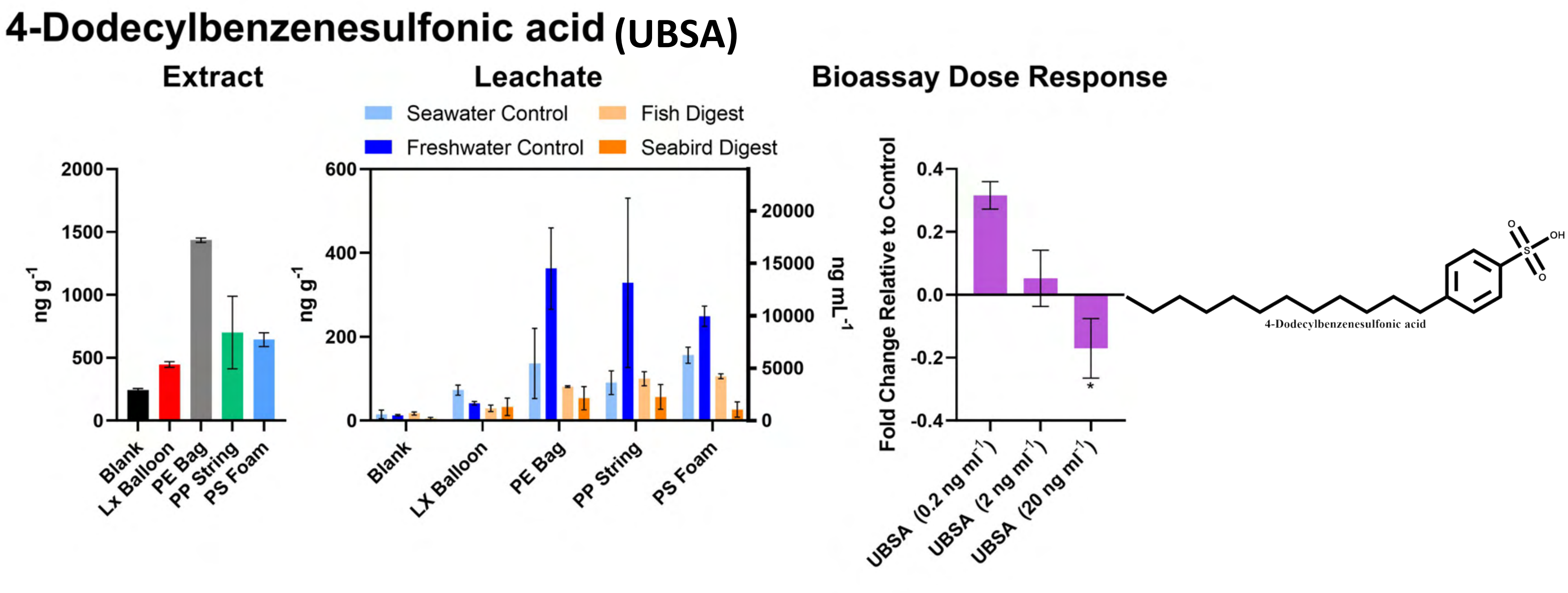
- TDBPP is primarily extracted from the PE bag.
- TDBPP release is NOT treatment dependent

DMHP release is NOT treatment dependent and is primarily released from the balloon.

3,5-Di-tert-butyl-4-hydroxybenzyl alcohol



UBSA release is NOT treatment dependent and is primarily released from the bag.

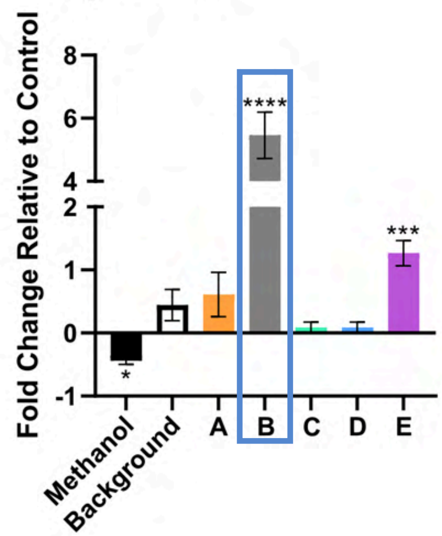


There are significant *In vitro* estrogenic activity responses for the mixture chemically representing the PE bag

A) Table of Chemical Mixtures

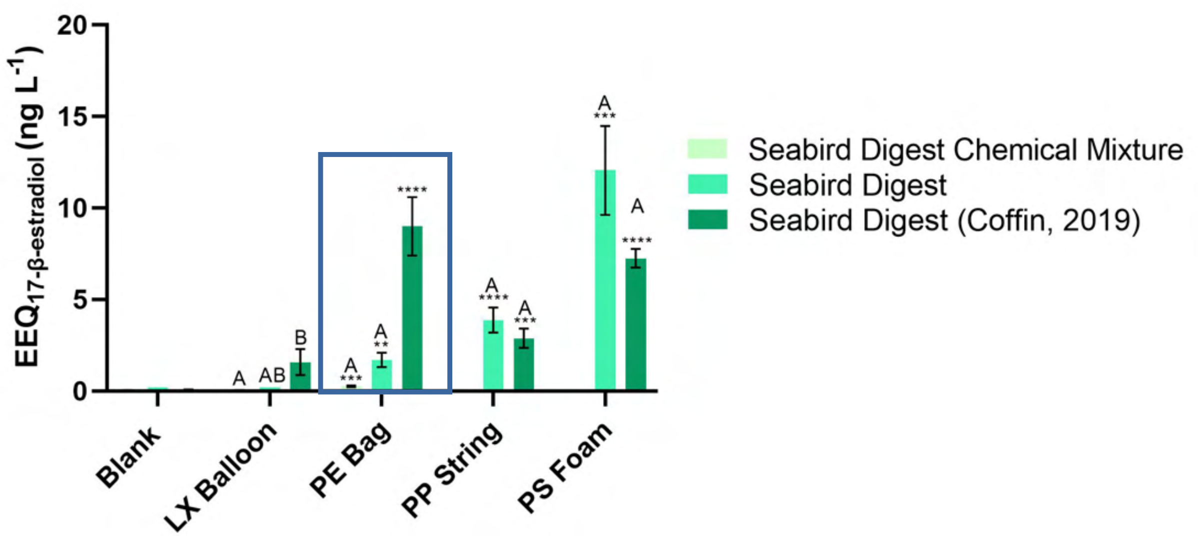
Graph Labels	Background	A	B	C	D	E
Chemical Conc. (ng/mL^{-1})	Background Contam. Control	LX Balloon	PE Bag	PP String	PS Foam	Equal Mix
2,4-DTB	0.0	1.6	1.6	1.2	1.5	10
DBP	3.3	3.6	20	3.8	3.2	10
DEHP	0.0	0	13	0	0	10
DEP	0.53	1.5	1.5	0	0	10
BPS	0.0	0	0.090	0.090	0.090	10
BPA	0.0	1.3	1.3	1.1	0.4	10
SUM	**3.830**	**8.000**	**37.29**	**5.490**	**5.190**	**60.00**

B) Chemical Mixture Response

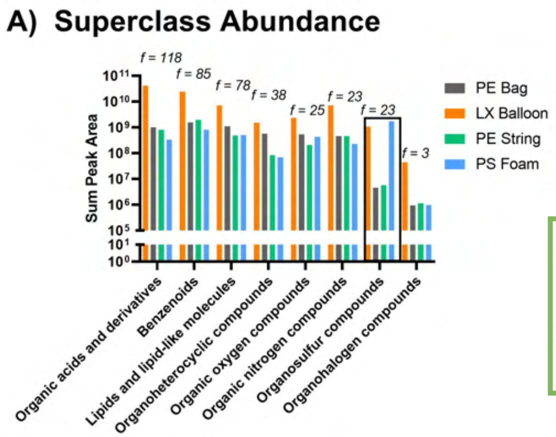


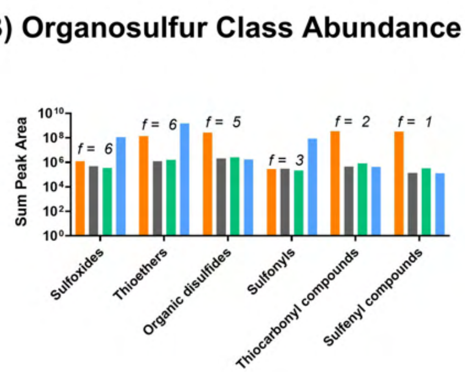
There are significant *In vitro* estrogenic responses for the seabird digested plastic

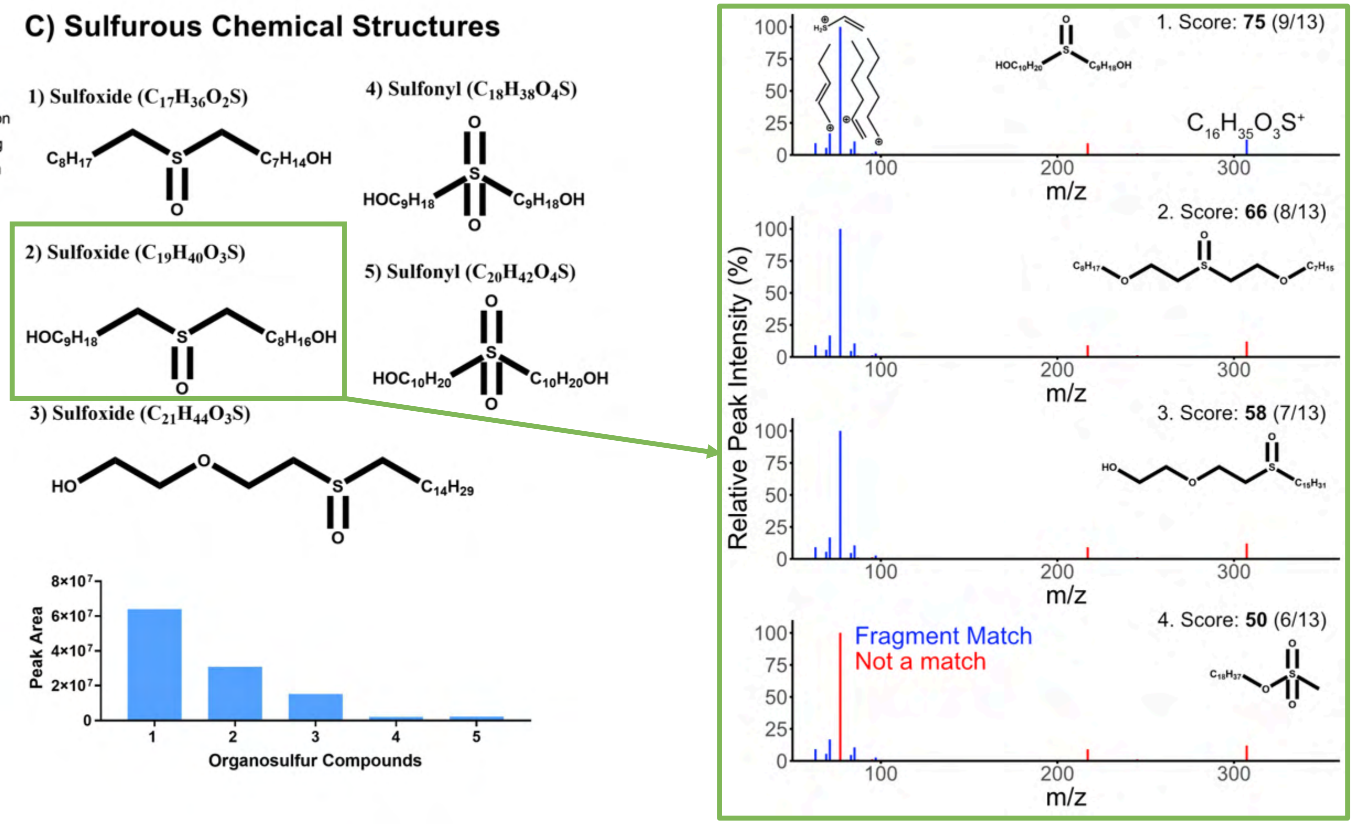
- Chemical mixtures account for 20% of the estrogenic response for the PE bag digest.
- There are significant differences in the digest response for the PE bag indicating possible chemical degradation (2019 vs 2021)
- Estrogenic activity is not explained by the chemically representative mixtures for the PP string and foam



Organosulfur based compounds are dominant to the PS foam seabird digest samples.







Summary

- Fifteen extractable and leachable polymer associated chemicals were structurally annotated and functioned as catalysts, colorants, intermediates, antioxidants, plasticizers, and lubricants.
- Further structural annotation and chemical classification highlights organosulfur-based compounds as a dominant chemical class released from PS foams.
- Mixtures of phenols and phthalates representing the chemical composition of the PE bag after simulated seabird digestion demonstrated significant estrogenic response that were not significantly different from the biological activity of the gastric digest.
- Estrogenic responses were not significantly explained for the PP string and PS foam.

Questions? Happy to chat!

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