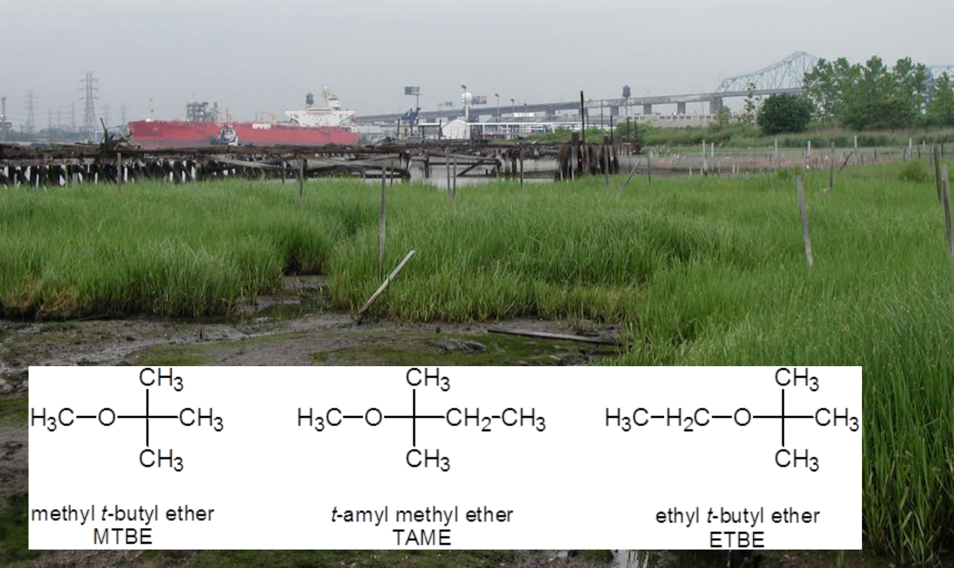
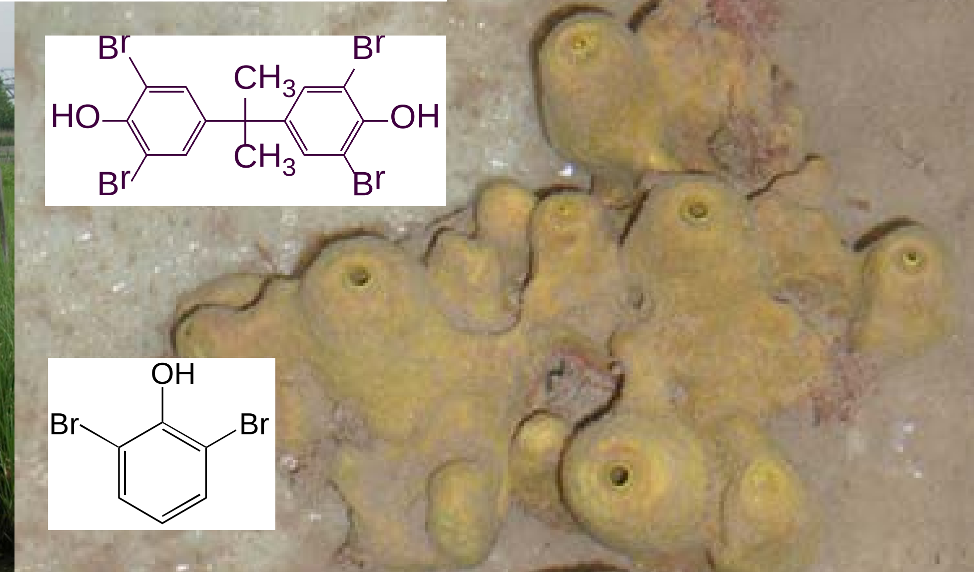
RUTGERS

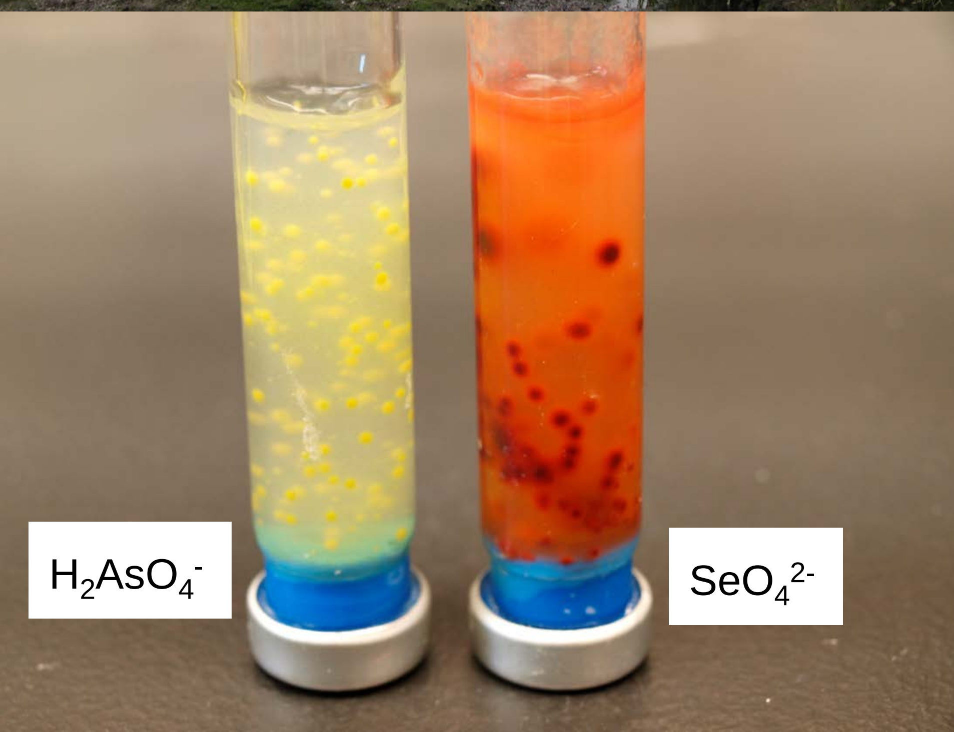
Bugs on Drugs:
Anaerobic Biodegradability of Pharmaceuticals and Personal Care Products

Max M. Häggblom
Rutgers University
Department of Biochemistry and Microbiology
School of Environmental and Biological Sciences
New Brunswick, New Jersey

“Unusual Appetites”







Pharmaceuticals and Personal Care Products

Pharmaceuticals and Personal Care Products

- PPCPs include a diverse array of thousands of chemical substances, including prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, and cosmetics.
- PPCPs are emerging environmental contaminants in watersheds around the world.
- PPCPs are released directly or indirectly into the environment, but their long-term fate is poorly understood
- Many PPCP compounds are biologically active and can thus pose adverse effects to aquatic biota.
- Thus, it is imperative to understand the ultimate fate of these compounds in the environment.

PPCPs as Contaminants of Aquatic Environments

TABLE 1. Summary of Analytical Results of Streams Sampled for 95 Organic Wastewater Contaminants[i]

chemical (method)	CASRN	*N*	RL (µg/L)	freq (%)	max (µg/L)	med (µg/L)	use	MCL or *HAL* (*23*) (µg/L)	lowest LC_{50} for the most sensitive indicator species (µg/L)/no. of aquatic studies identified (*24*)
Veterinary and Human Antibiotics									
carbodox (1)	6804-07-5	104	0.10	0	ND	ND	antibiotic	–	–/1
chlortetracycline (1)	57-62-5	115	0.05	0	ND	ND	antibiotic	–	88000[a]/3
chlortetracycline (2)	57-62-5	84	0.10	2.4	0.69	0.42	antibiotic	–	88000[a]/3
ciprofloxacin (1)	85721-33-1	115	0.02	2.6	0.03	0.02	antibiotic	–	–/0
doxycycline (1)	564-25-0	115	0.1	0	ND	ND	antibiotic	–	–/0
enrofloxacin (1)	93106-60-6	115	0.02	0	ND	ND	antibiotic	–	40[b]/29
erythromycin-H_2O (1)	114-07-8	104	0.05	21.5	1.7	0.1	erythromycin metabolite	–	665000[b]/35
lincomycin (1)	154-21-2	104	0.05	19.2	0.73	0.06	antibiotic	–	–/0
norfloxacin (1)	70458-96-7	115	0.02	0.9	0.12	0.12	antibiotic	–	–/6
oxytetracycline (1)	79-57-2	115	0.1	0	ND	ND	antibiotic	–	102000[a]/46
oxytetracycline (2)	79-57-2	84	0.10	1.2	0.34	0.34	antibiotic	–	102000[a]/46
roxithromycin (1)	80214-83-1	104	0.03	4.8	0.18	0.05	antibiotic	–	–/0
sarafloxacin (1)	98105-99-8	115	0.02	0	ND	ND	antibiotic	–	–/0
sulfachloropyridazine (2)	80-32-0	84	0.05	0	ND	ND	antibiotic	–	–/0
sulfadimethoxine (1)	122-11-2	104	0.05	0	ND	ND	antibiotic	–	–/5
sulfadimethoxine (2)	122-11-2	84	0.05	1.2	0.06	0.06	antibiotic	–	–/5
sulfamerazine (1)	127-79-7	104	0.05	0	ND	ND	antibiotic	–	100000[c]/17
sulfamerazine (2)	127-79-7	84	0.05	0	ND	ND	antibiotic	–	100000[c]/17
sulfamethazine (1)	57-68-1	104	0.05	4.8	0.12	0.02	antibiotic	–	100000[c] 17
sulfamethazine (2)	57-68-1	84	0.05	1.2	0.22	0.22	antibiotic	–	100000[c]/17
sulfamethizole (1)	144-82-1	104	0.05	1.0	0.13	0.13	antibiotic	–	–/0
sulfamethoxazole (1)	723-46-6	104	0.05	12.5	1.9	0.15	antibiotic	–	–/0
sulfamethoxazole (3)	723-46-6	84	0.023	19.0	0.52	0.066	antibiotic	–	–/0
sulfathiazole (1)	72-14-0	104	0.10	0	ND	ND	antibiotic	–	–/0
sulfathiazole (2)	72-14-0	84	0.05	0	ND	ND	antibiotic	–	–/0
tetracycline (1)	60-54-8	115	0.05	0	ND	ND	antibiotic	–	550000[b]/3
tetracycline (2)	60-54-8	84	0.10	1.2	0.11	0.11	antibiotic	–	550000[b]/3
trimethoprim (1)	738-70-5	104	0.03	12.5	0.71	0.15	antibiotic	–	3000[c]/4
trimethoprim (3)	738-70-5	84	0.014	27.4	0.30	0.013	antibiotic	–	3000[c]/4
tylosin (1)	1401-69-0	104	0.05	13.5	0.28	0.04	antibiotic	–	–/0
virginiamycin (1)	21411-53-0	104	0.10	0	ND	ND	antibiotic	–	–/0
Prescription Drugs									
albuterol (salbutamol) (3)	18559-94-9	84	0.029	0	ND	ND	antiasthmatic	–	–/0
cimetidine (3)	51481-61-9	84	0.007	9.5	0.58[d]	0.074[d]	antacid	–	–/0
codeine (3)	76-57-3	46	0.24	6.5	0.019	0.012	analgesic	–	–/0
codeine (4)	76-57-3	85	0.1	10.6	1.0[d]	0.2[d]	analgesic	–	–/0
dehydronifedipine (3)	67035-22-7	84	0.01	14.3	0.03	0.012	antianginal	–	–/0
digoxin (3)	20830-75-5	46	0.26	0	ND[d]	ND[d]	cardiac stimulant	–	10000000[a]/24
digoxigenin (3)	1672-46-4	84	0.008	0	ND	ND	digoxin metabolite	–	–/0
diltiazem (3)	42399-41-7	84	0.012	13.1	0.049	0.021	antihypertensive	–	–/0
enalaprilat (3)	76420-72-9	84	0.15	1.2	0.046[d]	0.046[d]	enalapril maleate (antihypertensive) metabolite	–	–/0
fluoxetine (3)	54910-89-3	84	0.018	1.2	0.012[d]	0.012[d]	antidepressant	–	–/0
gemfibrozil (3)	25812-30-0	84	0.015	3.6	0.79	0.048	antihyperlipidemic	–	–/0
metformin (3)	657-24-9	84	0.003	4.8	0.15[d]	0.11[d]	antidiabetic	–	–/0
paroxetine metabolite (3)	–	84	0.26	0	ND[d]	ND[d]	paroxetine (antidepressant) metabolite	–	–/0
ranitidine (3)	66357-35-5	84	0.01	1.2	0.01[d]	0.01[d]	antacid	–	–/0
warfarin (3)	81-81-2	84	0.001	0	ND	ND	anticoagulant	–	16000[c]/ 33
Nonprescription Drugs									
acetaminophen (3)	103-90-2	84	0.009	23.8	10	0.11	antipyretic	–	6000[a]/ 14
caffeine (3)	58-08-2	84	0.014	61.9	6.0	0.081	stimulant	–	40000[e]/ 77
caffeine (4)	58-08-2	85	0.08	70.6	5.7	0.1	stimulant	–	40000[e]/ 77
cotinine (3)	486-56-6	84	0.023	38.1	0.90	0.024	nicotine metabolite	–	–/0
cotinine (4)	486-56-6	54	0.04	31.5	0.57	0.05	nicotine metabolite	–	–/0
1,7-dimethylxanthine (3)	611-59-6	84	0.018	28.6	3.1[d]	0.11[d]	caffeine metabolite	–	–/0
ibuprofen (3)	15687-27-1	84	0.018	9.5	1.0	0.20	antiinflammatory	–	–/0
Other Wastewater-Related Compounds									
1,4-dichlorobenzene (4)	106-46-7	85	0.03	25.9	4.3	0.09	deodorizer	*75*	1100[c]/190
2,6-di-*tert*-butylphenol (4)	128-39-2	85	0.08	3.5	0.11[d]	0.06[d]	antioxidant	–	–/2
2,6-di-*tert*-butyl-1,4-benzoquinone (4)	719-22-2	85	0.10	9.4	0.46	0.13	antioxidant	–	–/0
5-methyl-1H-benzotriazole (4)	136-85-6	54	0.10	31.5	2.4	0.39	antiocorrosive	–	–/0
acetophenone (4)	98-86-2	85	0.15	9.4	0.41	0.15	fragrance	–	155000[e]/21
anthracene (4)	120-12-7	85	0.05	4.7	0.11	0.07	PAH	–	5.4[e]/188
benzo[*a*]pyrene (4)	50-32-8	85	0.05	9.4	0.24	0.04	PAH	0.2	1.5[a]/428
3-*tert*-butyl-4-hydroxy anisole (4)	25013-16-5	85	0.12	2.4	0.2[d]	0.1[d]	antioxidant	–	870[c]/14
butylated hydroxy toluene (4)	128-37-0	85	0.08	2.4	0.1[d]	0.1[d]	antioxidant	–	1440[a]/15
bis(2-ethylhexyl) adipate (4)	103-23-1	85	2.0	3.5	10[f]	3[f]	plasticizer	400	480[a]/9
bis(2-ethylhexyl) phthalate (4)	117-81-7	85	2.5	10.6	20[f]	7[f]	plasticizer	6	7500[a]/309

TABLE 1. (Continued)

chemical (method)	CASRN	*N*	RL (µg/L)	freq (%)	max (µg/L)	med (µg/L)	use	MCL or *HAL* (*23*) (µg/L)	lowest LC_{50} for the most sensitive indicator species (µg/L)/no. of aquatic studies identified (*24*)
Other Wastewater-Related Compounds									
bisphenol A (4)	80-05-7	85	0.09	41.2	12	0.14	plasticizer	–	3600[e]/26
carbaryl (4)	63-25-2	85	0.06	16.5	0.1[d]	0.04[d]	insecticide	*700*	0.4[a]/1541
***cis*-chlordane** (4)	5103-71-9	85	0.04	4.7	0.1	0.02	insecticide	2	7.4[b]/28
chlorpyrifos (4)	2921-88-2	85	0.02	15.3	0.31	0.06	insecticide	*20*	0.1[a]/1794
diazinon (4)	333-41-5	85	0.03	25.9	0.35	0.07	insecticide	*0.6*	0.56[a]/1040
dieldrin (4)	60-57-1	85	0.08	4.7	0.21	0.18	insecticide	0.2	2.6[c]/1540
diethylphthalate (4)	84-66-2	54	0.25	11.1	0.42	0.2	plasticizer	–	12000[c]/129
ethanol,2-butoxy-phosphate (4)	78-51-3	85	0.2	45.9	6.7	0.51	plasticizer	–	10400[e]/7
fluoranthene (4)	206-44-0	85	0.03	29.4	1.2	0.04	PAH	–	74[e]/216
lindane (4)	58-89-9	85	0.05	5.9	0.11	0.02	insecticide	0.2	30[c]/1979
methyl parathion (4)	298-00-0	85	0.06	1.2	0.01	0.01	insecticide	*2*	12[a]/888
4-methyl phenol (4)	106-44-5	85	0.04	24.7	0.54	0.05	disinfectant	–	1400[a]/74
naphthalene (4)	91-20-3	85	0.02	16.5	0.08	0.02	PAH	*20*	910[c]/519
N,N-diethyltoluamide (4)	134-62-3	54	0.04	74.1	1.1	0.06	insect repellant	–	71250[c]/9
4-nonylphenol (4)	251-545-23	85	0.50	50.6	40[g]	0.8[g]	nonionic detergent metabolite	–	130[e]/135
4-nonylphenol monoethoxylate (4)	–	85	1.0	45.9	20[g]	1[g]	nonionic detergent metabolite		14450[a]/4
4-nonylphenol diethoxylate (4)	–	85	1.1	36.5	9[g]	1[g]	nonionic detergent metabolite	–	5500[a]/6
4-octylphenol monoethoxylate (4)	–	85	0.1	43.5	2[g]	0.2[g]	nonionic detergent metabolite	–	–/0
4-octylphenol diethoxylate (4)	–	85	0.2	23.5	1[g]	0.1[g]	nonionic detergent metabolite	–	–/0
phenanthrene (4)	85-01-8	85	0.06	11.8	0.53	0.04	PAH	–	590[a]/192
phenol (4)	108-95-2	85	0.25	8.2	1.3[f]	0.7[f]	disinfectant	*400*	4000[c]/2085
phthalic anhydride (4)	85-44-9	85	0.25	17.6	1[f]	0.7[f]	plastic manufacturing	–	40400[c]/5
pyrene (4)	129-00-0	85	0.03	28.2	0.84	0.05	PAH	–	90.9[a]/112
tetrachloroethylene (4)	127-18-4	85	0.03	23.5	0.70[d]	0.07[d]	solvent, degreaser	5	4680[c]/147
triclosan (4)	3380-34-5	85	0.05	57.6	2.3	0.14	antimicrobial disinfectant	–	180[e]/3
tri(2-chloroethyl) phosphate (4)	115-96-8	85	0.04	57.6	0.54	0.1	fire retardant	–	66000[b]/8
tri(dichlorisopropyl) phosphate (4)	13674-87-8	85	0.1	12.9	0.16	0.1	fire retardant	–	3600[b]/9
triphenyl phosphate (4)	115-86-6	85	0.1	14.1	0.22	0.04	plasticizer	–	280[c]/66
Steroids and Hormones									
***cis*-androsterone** (5)	53-41-8	70	0.005	14.3	0.214	0.017	urinary steroid	–	–/0
cholesterol (4)	57-88-5	85	1.5	55.3	10[d]	1[d]	plant/animal steroid	–	–/0
cholesterol (5)	57-88-5	70	0.005	84.3	60[h]	0.83	plant/animal steroid	–	–/0
coprostanol (4)	360-68-9	85	0.6	35.3	9.8[d]	0.70[d]	fecal steroid	–	–/0
coprostanol (5)	360-68-9	70	0.005	85.7	150[h]	0.088	fecal steroid	–	–/0
equilenin (5)	517-09-9	70	0.005	2.8	0.278	0.14	estrogen replacement	–	–/0
equilin (5)	474-86-2	70	0.005	1.4	0.147	0.147	estrogen replacement	–	–/0
17α-ethynyl estradiol (5)	57-63-6	70	0.005	15.7	0.831	0.073	ovulation inhibitor	–	–/22
17α-estradiol (5)	57-91-0	70	0.005	5.7	0.074	0.03	reproductive hormone	–	–/0
17β-estradiol (4)	50-28-2	85	0.5	10.6	0.2[d]	0.16[d]	reproductive hormone	–	–/0
17β-estradiol (5)	50-28-2	70	0.005	10.0	0.093	0.009	reproductive hormone	–	–/0
estriol (5)	50-27-1	70	0.005	21.4	0.051	0.019	reproductive hormone	–	–/0
estrone (5)	53-16-7	70	0.005	7.1	0.112	0.027	reproductive hormone	–	–/11
mestranol (5)	72-33-3	70	0.005	10.0	0.407	0.074	ovulation inhibitor	–	–/0
19-norethisterone (5)	68-22-4	70	0.005	12.8	0.872	0.048	ovulation inhibitor	–	–/0
progesterone (5)	57-83-0	70	0.005	4.3	0.199	0.11	reproductive hormone	–	–/0
stigmastanol (4)	19466-47-8	54	2.0	5.6	4[d]	2[d]	plant steroid	–	–/0
testosterone (5)	58-22-0	70	0.005	2.8	0.214	0.116	reproductive hormone	–	–/4

[a] *Daphnia magna* (water flea) – 48 h exposure LC_{50}. [b] Other species and variable conditions. [c] *Oncorhynchus mykiss* (rainbow trout) – 96 h exposure LC_{50}. [d] Concentration estimated – average recovery <60%. [e] *Pimephales promelas* (fathead minnow) – 96 h exposure LC_{50}. [f] Concentration estimated – compound routinely detected in laboratory blanks. [g] Concentration estimated – reference standard prepared from a technical mixture. [h] Concentration estimated – value greater than highest point on calibration curve. [i] Compounds suspected of being hormonally active are in bold (*4, 22*). CASRN, Chemical Abstracts Service Registry Number; *N*, number of samples; RL, reporting level; freq, frequency of detection; max, maximum concentration; med, median detectable concentration; MCL, maximum contaminant level; HAL, health advisory level; LC_{50}, lethal concentration with 50% mortality; ND, not detected; –, not available; PAH, polycyclic aromatic hydrocarbon.

Kolpin et al. (2002) Pharmaceuticals, hormones and other organic wastewater contaminants in the US streams 1999-2000: a national reconnaissance. Environmental Science and Technology 36: 1202-1211.

Detection of Organic Wastewater Contaminants

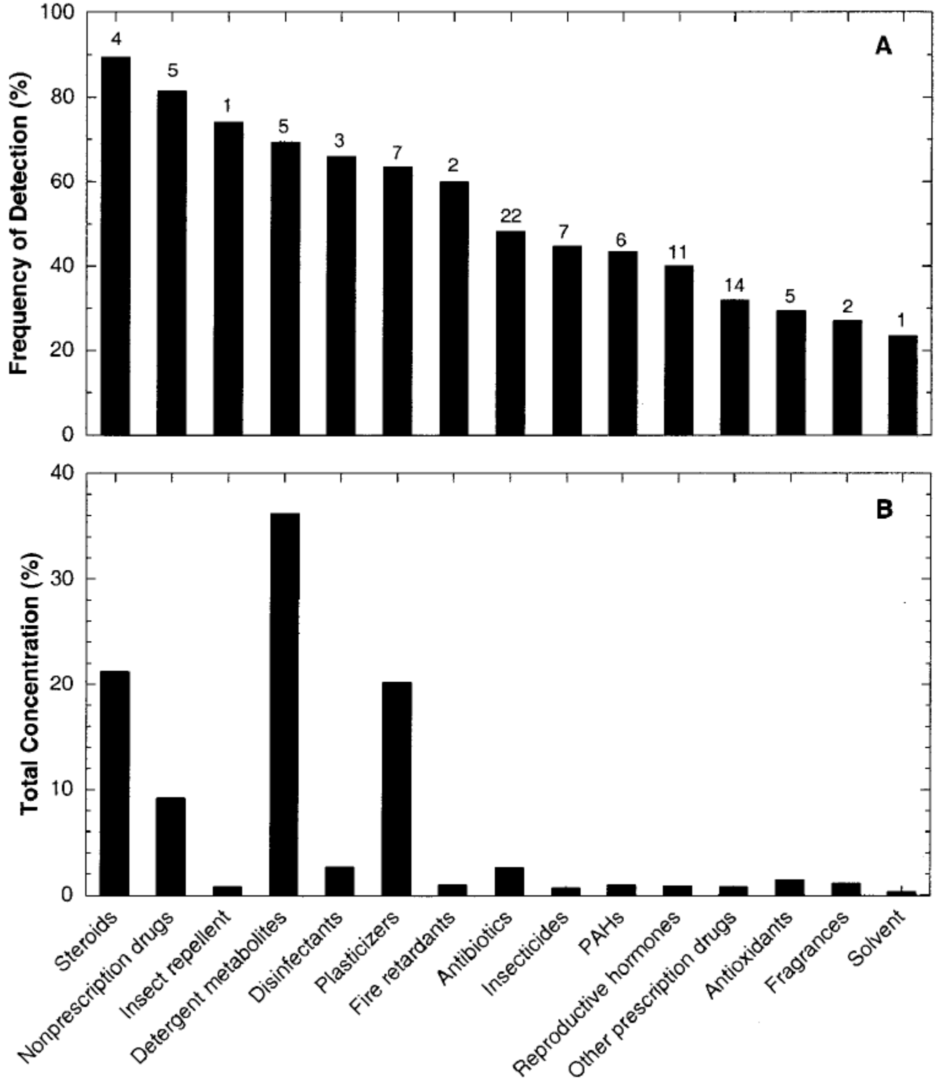
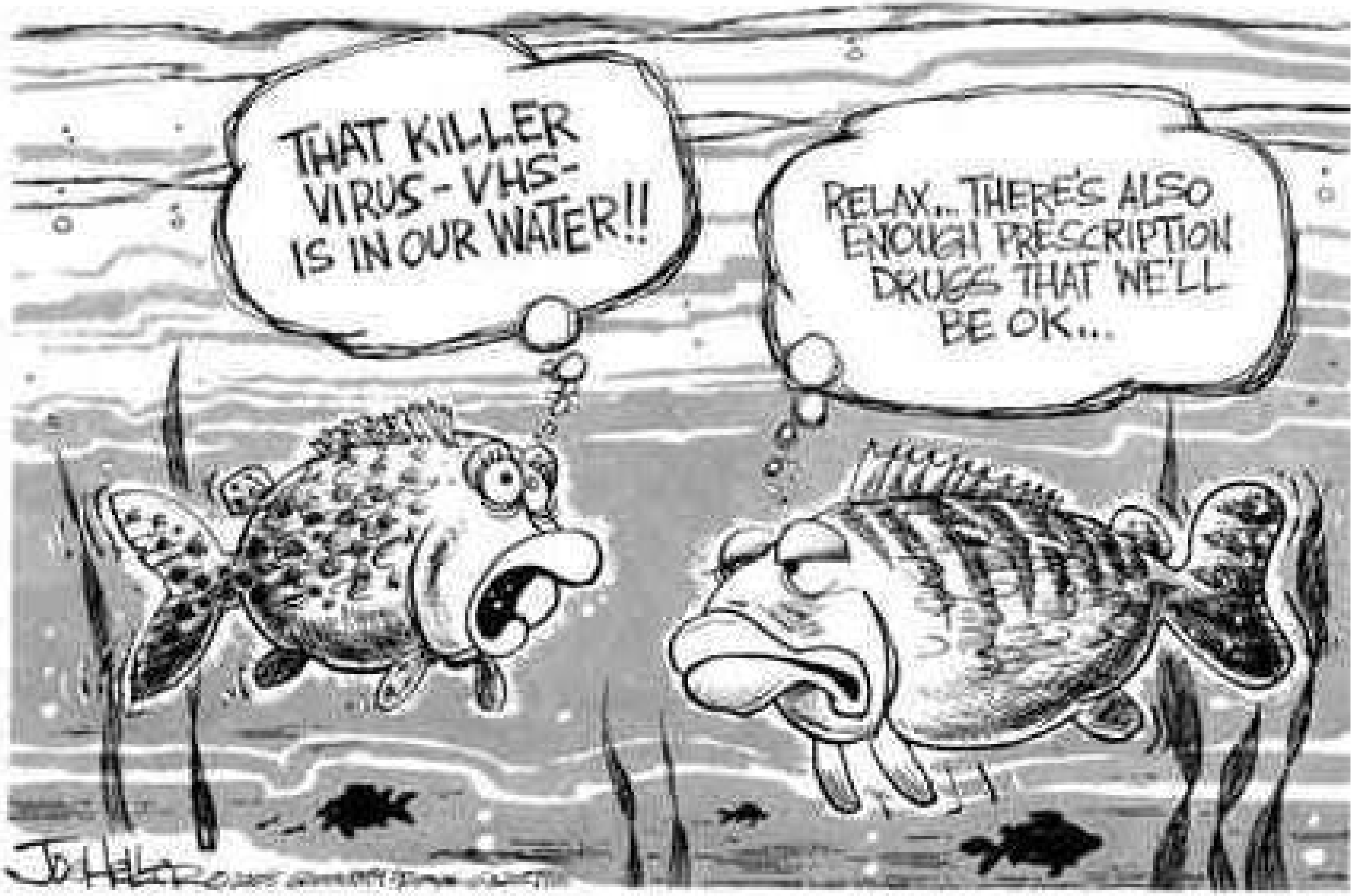
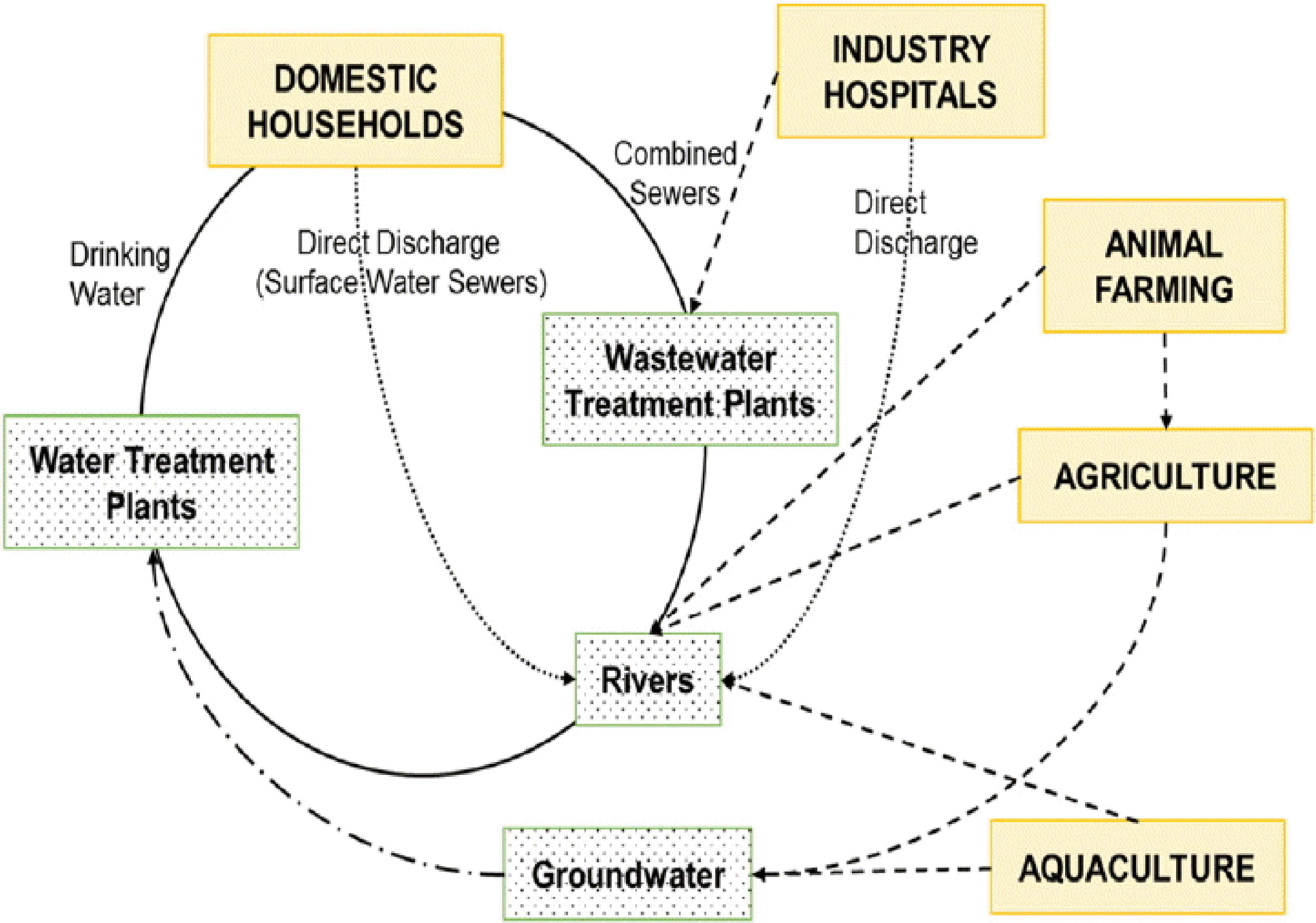


FIGURE 4. Frequency of detection of organic wastewater contaminants by general use category (4A), and percent of total measured concentration of organic wastewater contaminants by general use category (4B). Number of compounds in each category shown above bar.

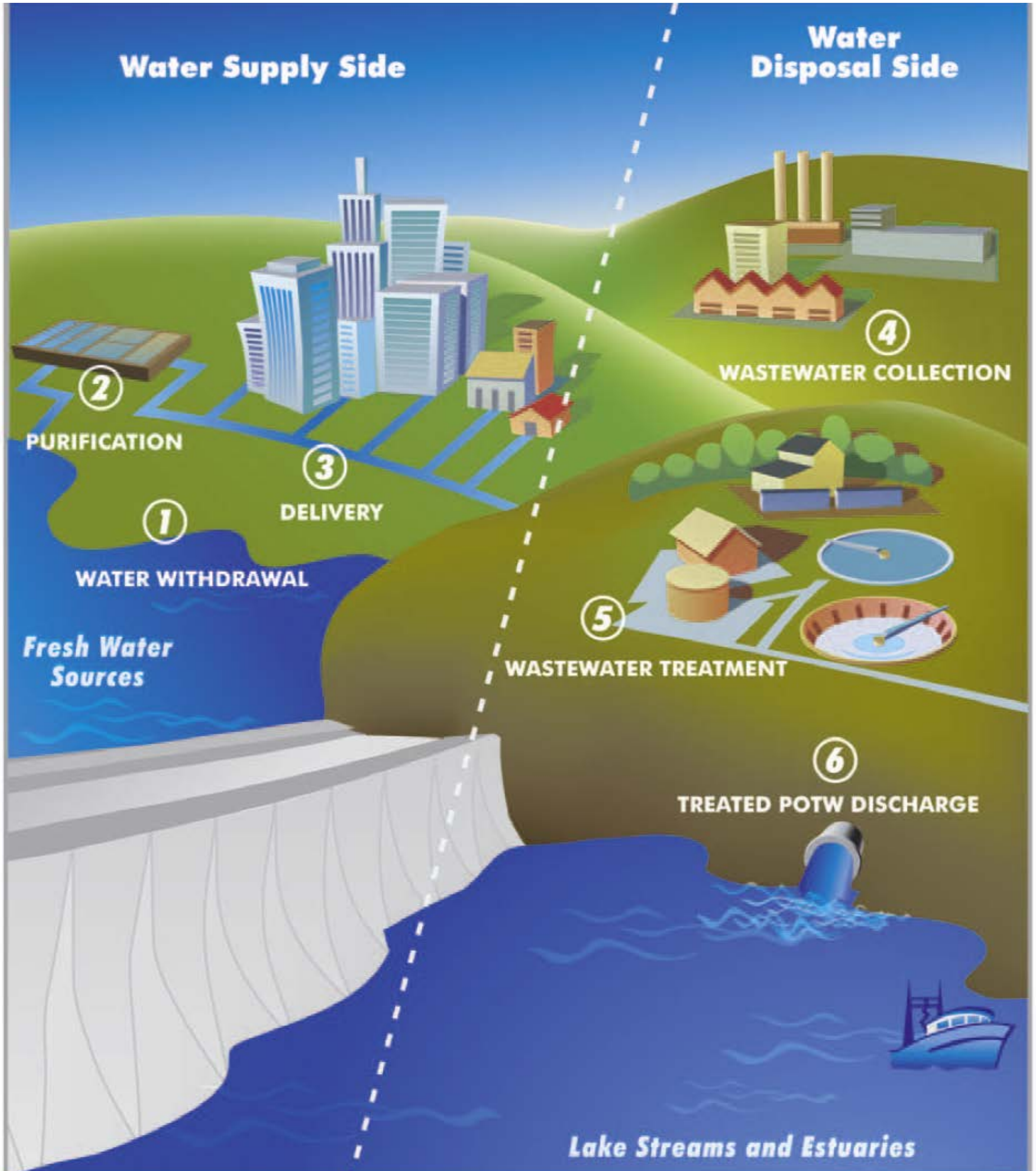
Kolpin et al. (2002) Pharmaceuticals, hormones and other organic wastewater contaminants in the US streams 1999-2000: a national reconnaissance. Environmental Science and Technology 36: 1202-1211.


THAT KILLER VIRUS - VHS - IS IN OUR WATER!!
RELAX... THERE'S ALSO ENOUGH PRESCRIPTION DRUGS THAT WE'LL BE OK...



Kim & Zoh (2016) Occurrence and removals of micropollutants in water environment. Environmental Engineering Research 21: 319-332. DOI: https://doi.org/10.4491/eer.2016.115

PPCPs and the Urban Water Cycle



Primer for Municipal Wastewater Treatment Systems.
EPA 832-R-04-001

Xenobiotics in the Epoch of the Anthropocene

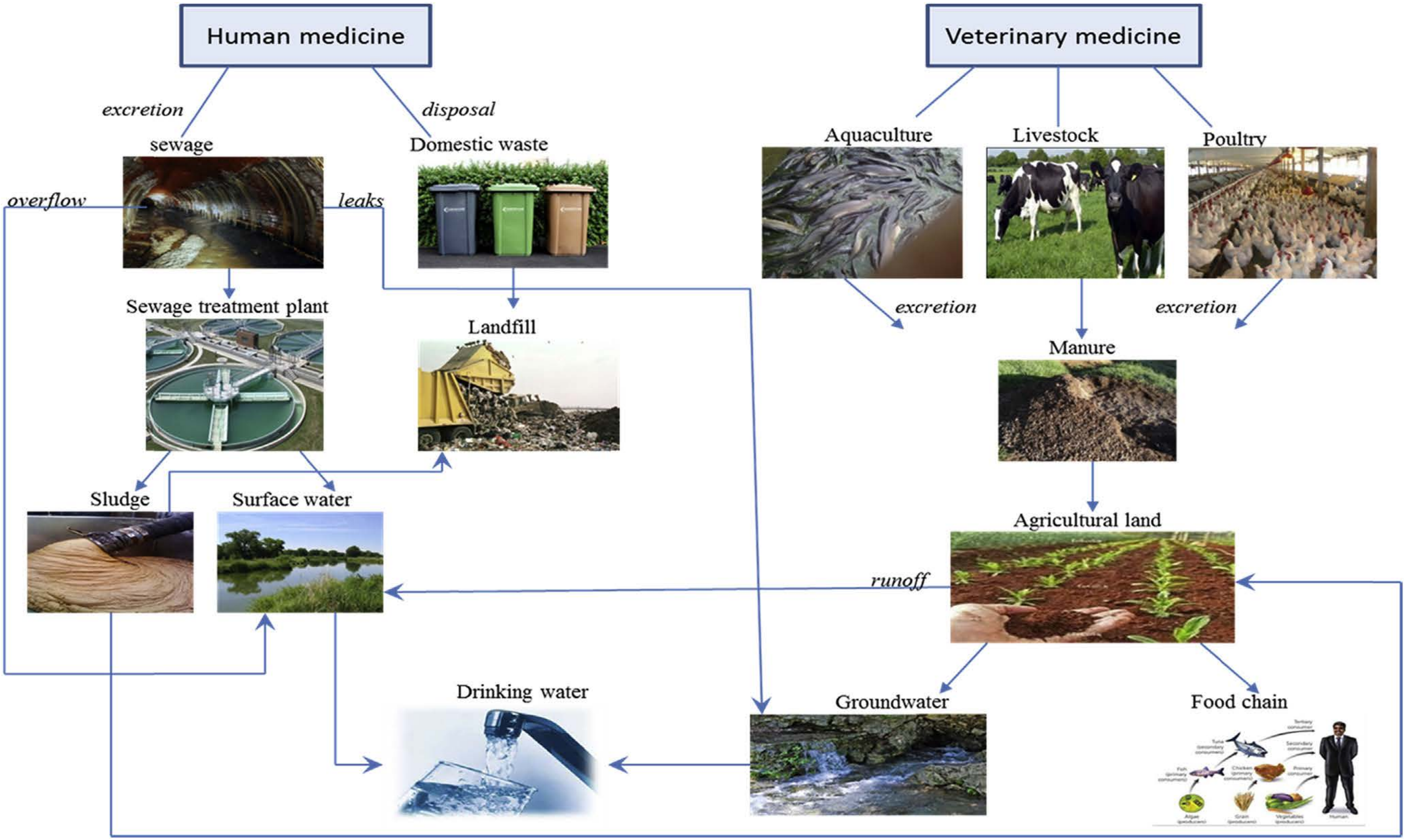
The continued introduction of new chemicals into the environment, such as the discharge of a plethora of PPCPs, requires new understanding of their behavior and environmental impact.

Microbial transformations of anthropogenic chemicals, such as PPCPs, can alter exposure and ecotoxicity, and result in increased risk to ecosystems.

Of potential concern are not only the original PPCP compounds released into the environment, but also their various metabolites.

A strong scientific understanding of the factors affecting the fate and biological effects of PPCPs is needed in order to inform policies aimed at protecting aquatic ecosystems.

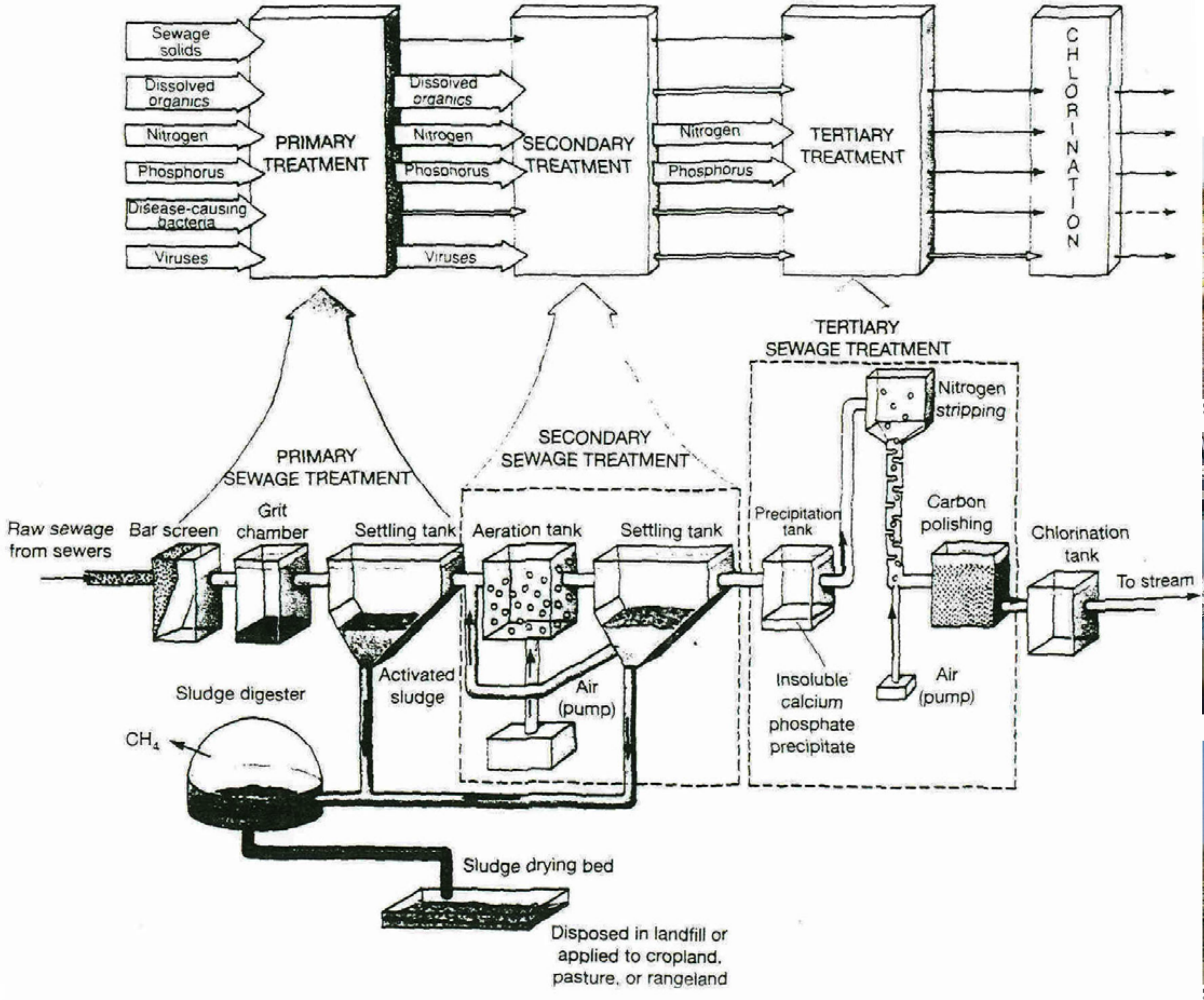
Sources and Sinks of PPCPs



Ebele et al. (2017) Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerging Contaminants 3: 1-16.

Sewage Treatment

FIGURE 26.20 An overview of a sewage treatment plant, showing primary, secondary, and tertiary treatment facilities.



John M. Martinko and Deborah O. Jung

Figure 28-6b Brock Biology of Microorganisms 11/e

T.D. Brock

Figure 28-5a Brock Biology of Microorganisms 11/e

"Microbial infallibility"

No natural compound is totally resistant to biodegradation provided that environmental conditions are favorable

- microbes have evolved that are capable of degrading most if not all naturally produced chemicals
- utilization of compound for growth: energy, carbon, N, P, S..
- biogeochemical cycles (Carbon, Nitrogen, Sulfur,...)

What about ***xenobiotic*** compounds?

xenos = foreign, Greek for *guest*, bios = Greek for *life*

Anthropogenic (industrial) chemicals foreign to the biosphere
Foreign molecular structures or chemical bonds

Environmental Fate

Organic compounds often classified as:

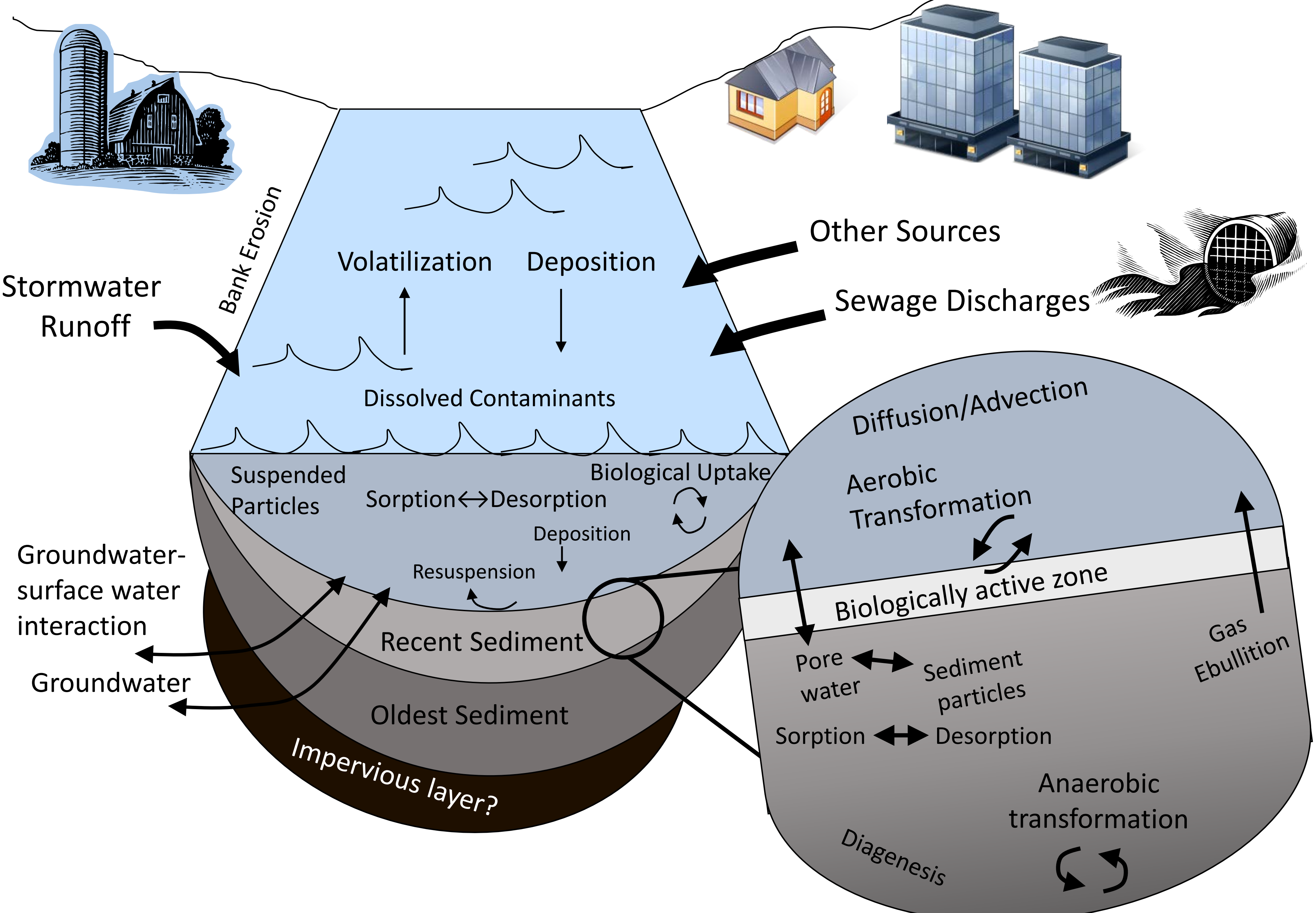
1) Biodegradable, 2) Persistent, or 3) Recalcitrant

Persistence = Compound + Environment + Time

- recalcitrance is a relative rather than absolute term
- if compound is recalcitrant → accumulates in the environment

POPs = Persistent Organic Pollutants

Input and Fate of PCPPs in the Watershed

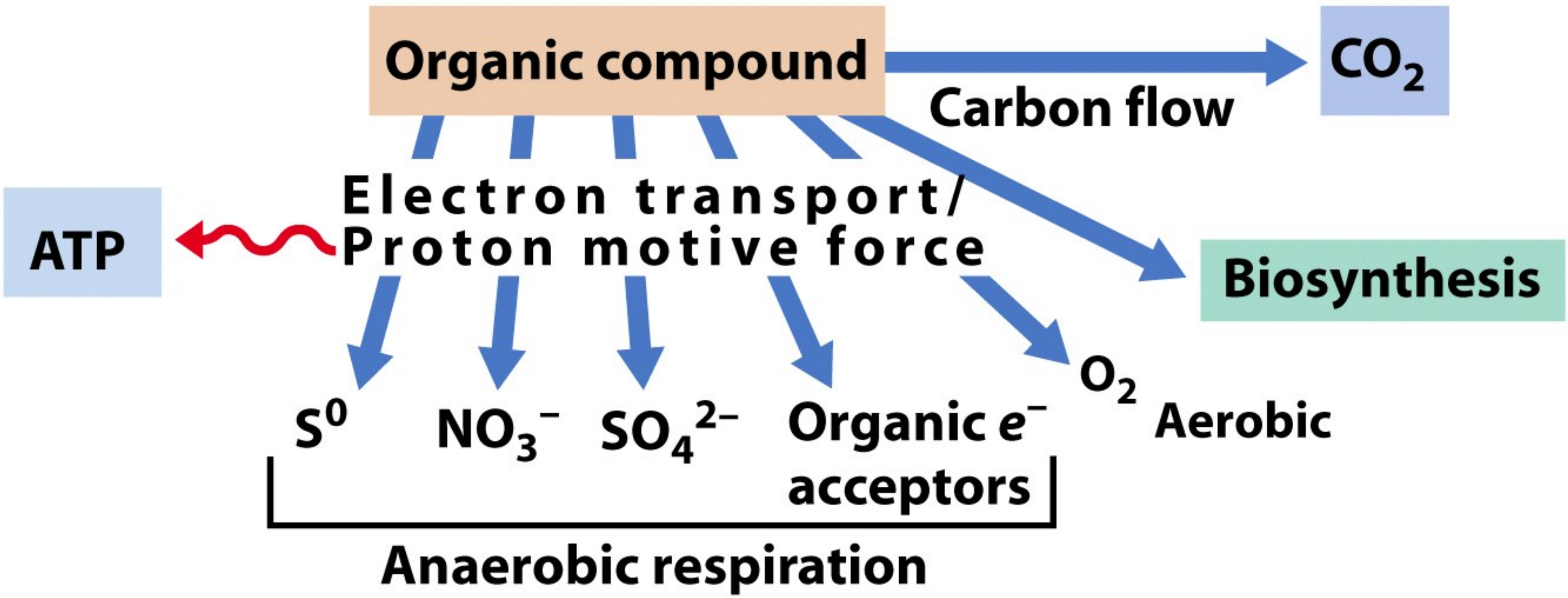


Hypotheses

PPCPs exit WWTPs largely untreated, but little is known about their fate in aquatic systems, in particular under anoxic conditions of the water column and sediment.

Our work addresses two main hypotheses:

1. Microbial biotransformation of PPCPs in natural aquatic ecosystems produces downstream metabolites that undergo further transformation by other community members.
2. Long-term PPCP contamination enriches for microbial communities capable of utilizing PPCPs as carbon and energy sources. The redox environment and the availability of alternate electron acceptors, such as sulfate in estuarine sediments, controls specific microbial activity mediating PPCP degradation.



Chemoorganotrophic metabolism

Figure 5-23a Brock Biology of Microorganisms 11/e

Respiration grouped by terminal electron acceptor

Oxidation:

$$C_2H_4O_2 + 2\ H_2O \rightarrow 2\ CO_2 + 8\ H^+ + 8\ e^-$$

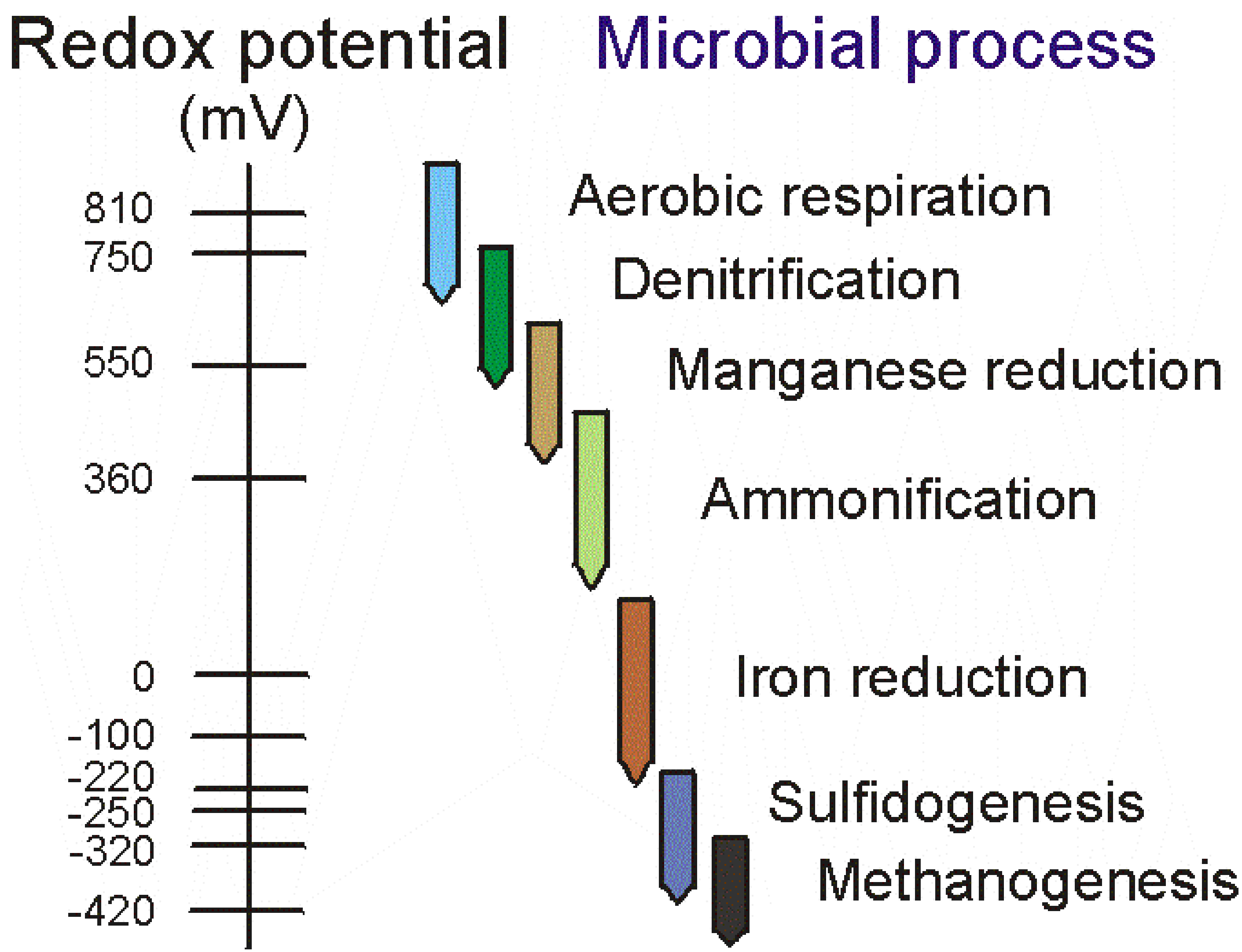
Reduction:

$$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$$
$$2\ NO_3^- + 12\ H^+ + 10\ e^- \rightarrow N_2 + 6\ H_2O$$
$$NO_3^- + 10\ H^+ + 8\ e^- \rightarrow NH_4 + {} + 3\ H_2O$$
$$MnO_2 + 4\ H^+ + 2\ e^- \rightarrow Mn^{2+} + 2\ H_2O$$
$$Fe^{3+} + e- \rightarrow Fe^{2+}$$
$$SO_4^{2-} + 10\ H^+ + 8\ e^- \rightarrow H_2S + 4\ H_2O$$
$$HCO_{3-} + 9\ H^+ + 8\ e^- \rightarrow CH4 + 3\ H_2O$$
$$2\ HCO_3^- + 10\ H^+ + 8\ e^- \rightarrow CH_3COOH + 4\ H_2O$$

Oxidation-Reduction Potential E_h

- measure of the tendency of a solution to donate or receive electrons
- useful scale for measuring anaerobiosis
- some stringent anaerobes will not initiate growth unless redox potential is sufficiently low (e.g. sulfidogens, acetogens, methanogens)

Stratification of Redox Processes



Objectives

- Determine how the redox environment impacts biodegradability of PPCPs in aquatic sediments over a freshwater-estuarine gradient with different historical exposures to PPCPs.
- Identify novel anaerobic bacteria and their functional genes encoding for the enzymes responsible for the transformation/degradation of specific PPCPs.

Specific tasks:

1. Determine how the redox environment impacts biodegradation of a suite of PPCP compounds in anoxic estuarine sediments.
2. Combine enrichment culture technique with molecular community analysis to identify the bacterial community members active in anaerobic degradation of PPCP compounds.
3. Apply compound specific isotope analysis to monitor biodegradation of select PPCP compounds.

Microcosms / Enrichment Cultures

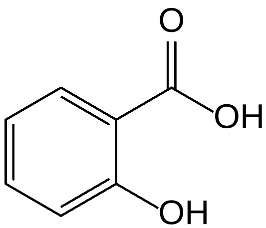
- enrichment culture concept developed by Sergei Winogradsky and Martinus Beijerink

- the microorganism that will grow best under the established conditions will grow faster and predominate

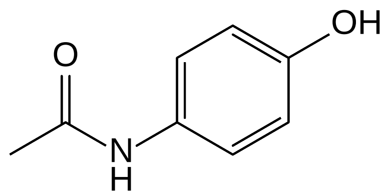
- every environmental niche is a selective enrichment for certain organisms

Everything is everywhere, the environment selects
(Baas Becking / Beijerink)

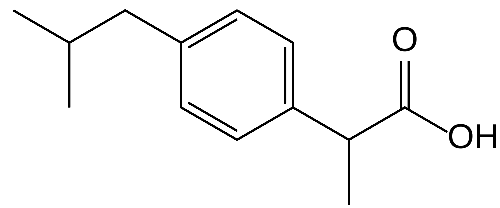
- enrichment selects for the microorganisms that grow best under the conditions applied



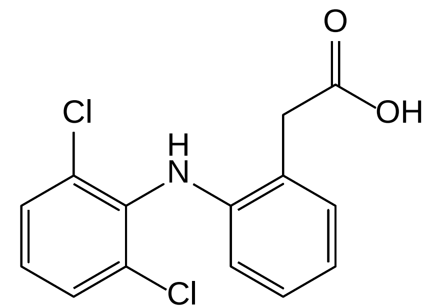
Aspirin



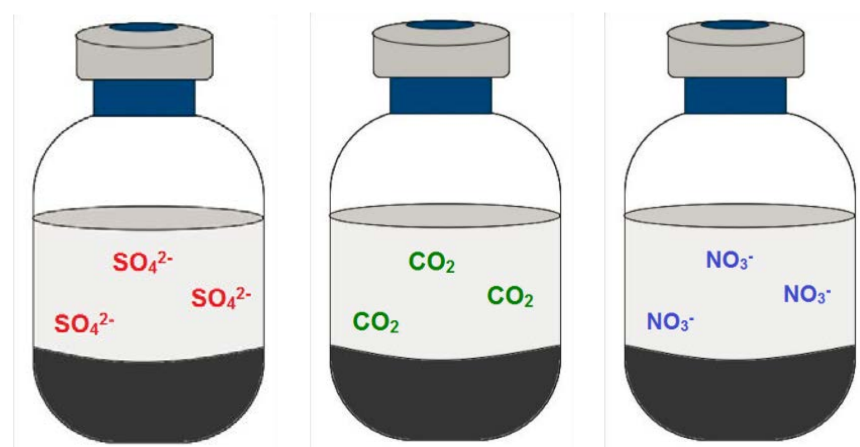
Acetaminophen



Ibuprofen



Diclofenac

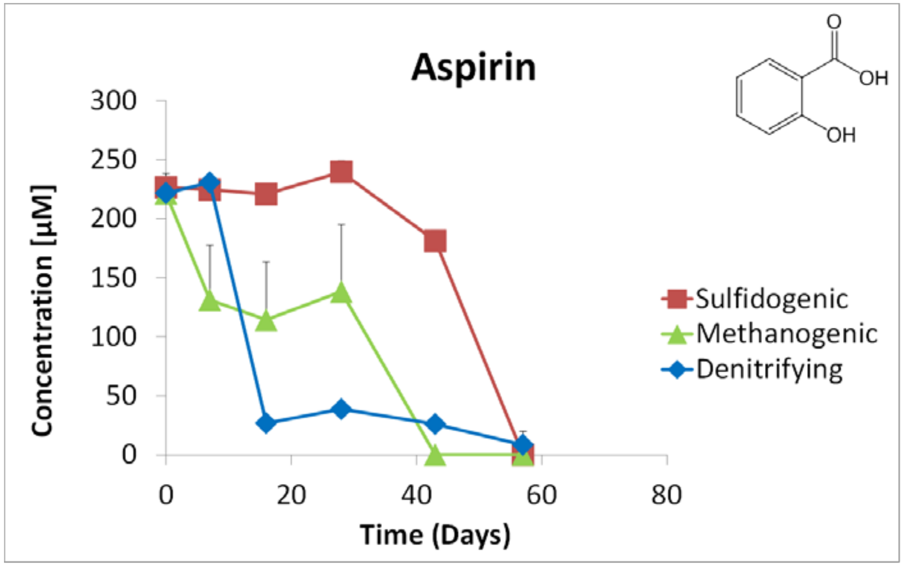


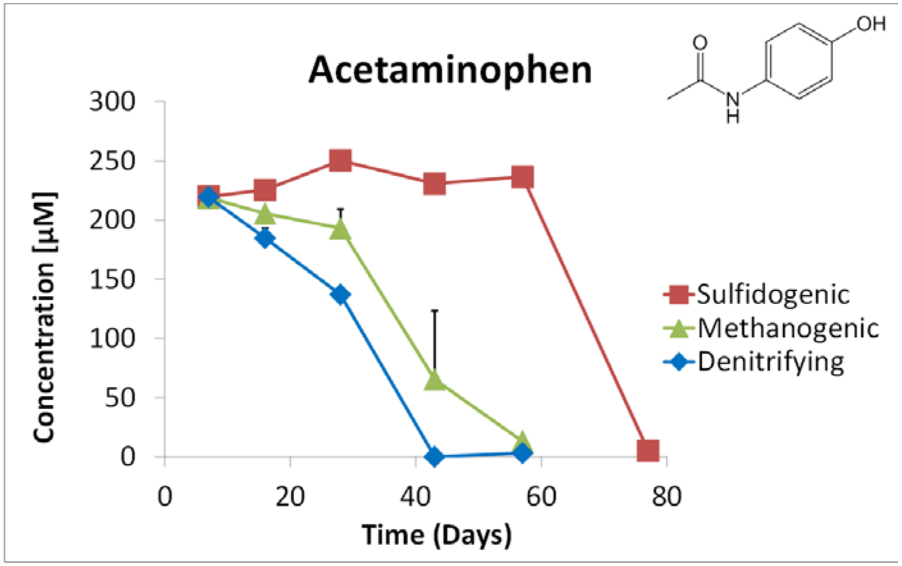
Sampling at 79th Street Boat Basin, New York

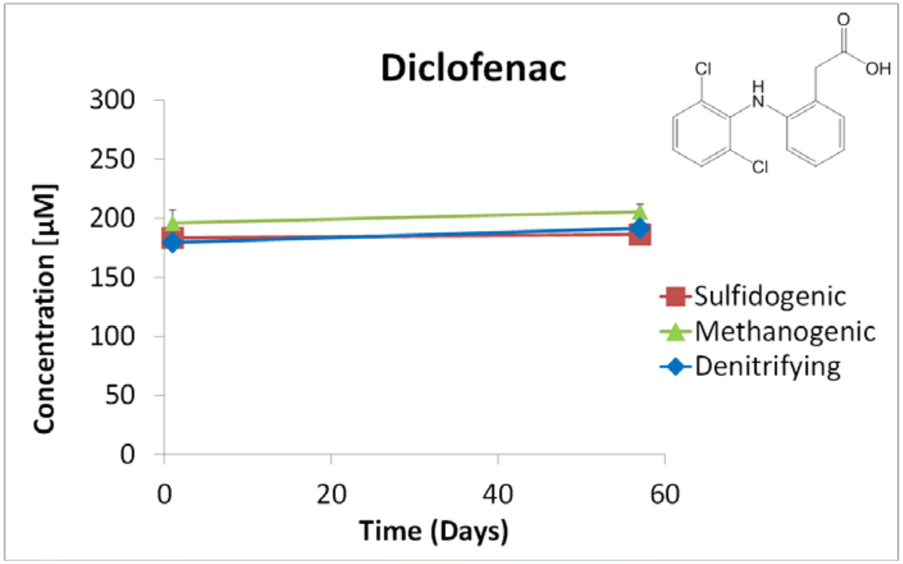
Seal under a headspace of N_2 with Teflon coated stoppers and aluminum crimps

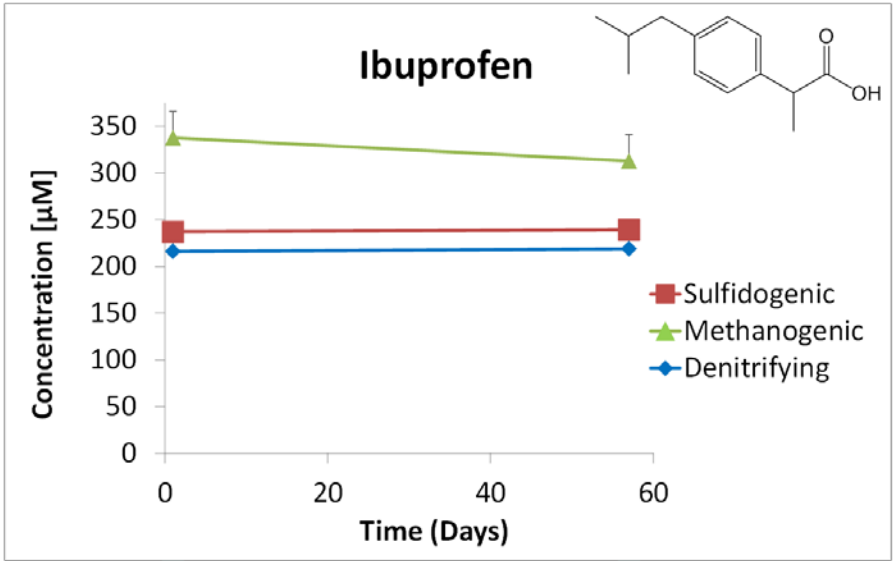
Sampling using strict anaerobic technique

Degradation of PPCPs in Arthur Kill Sediment Microcosms under Denitrifying, Methanogenic, and Sulfidogenic Conditions

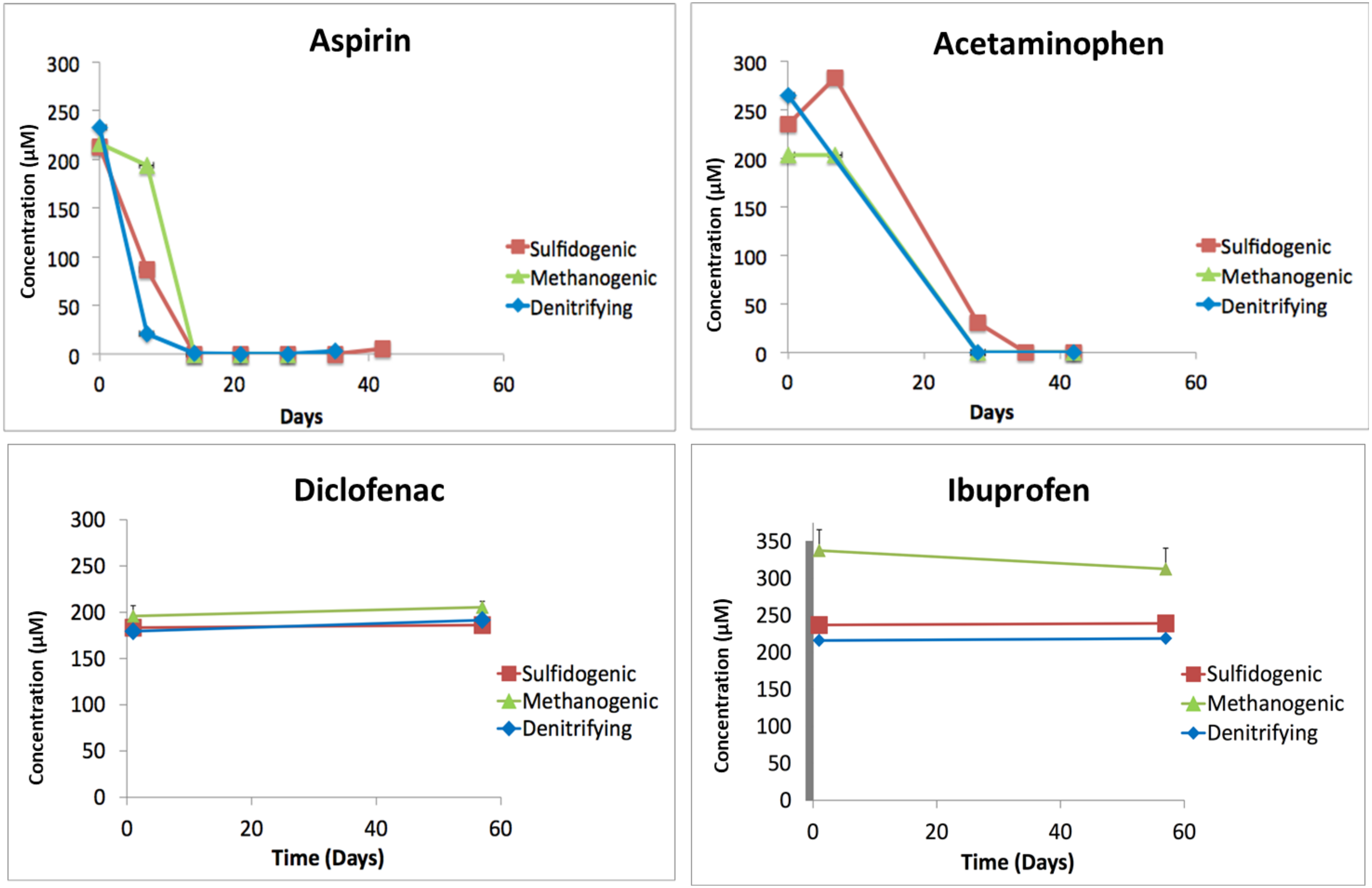




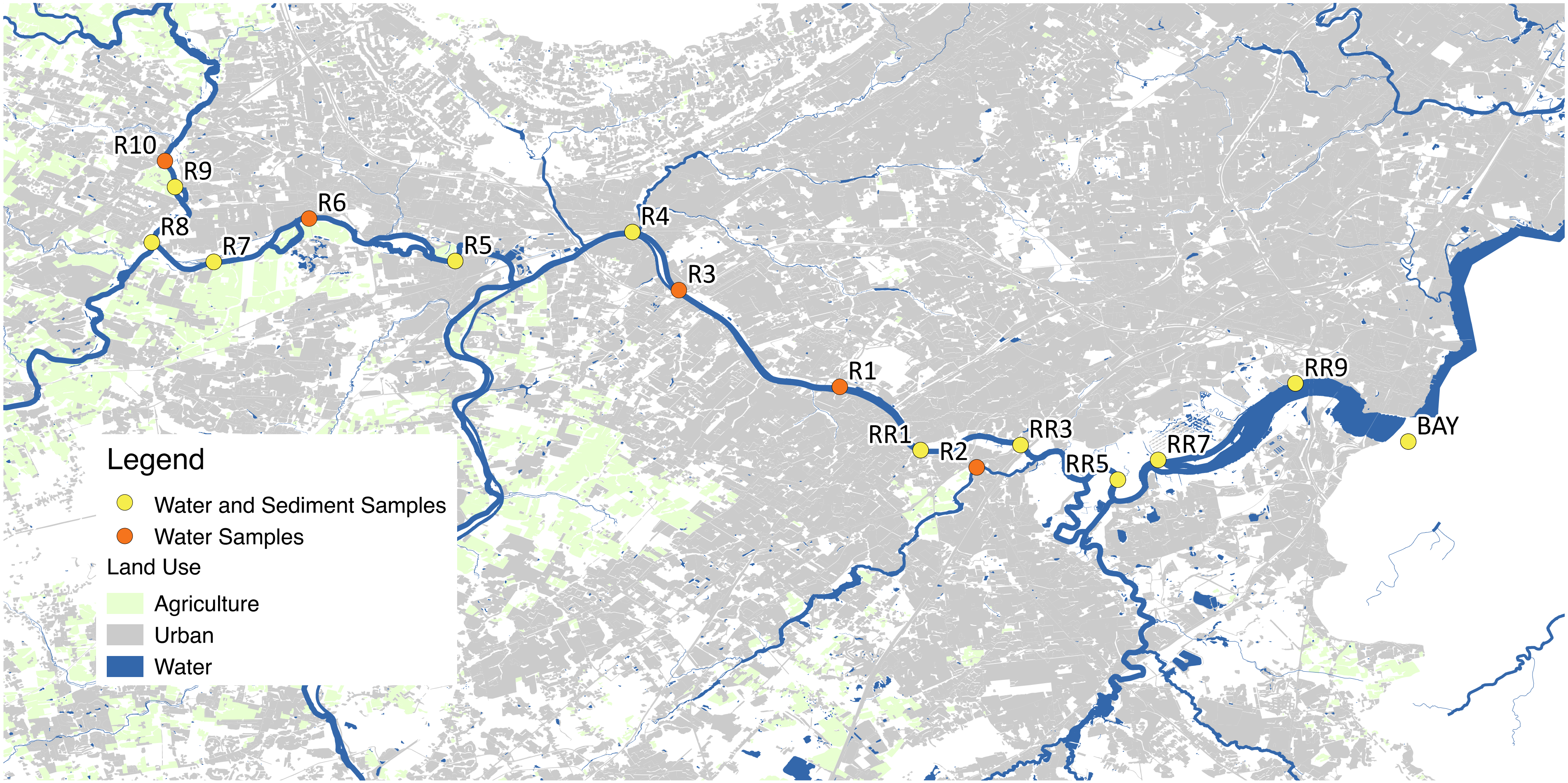




Degradation of PPCPs in Hudson River Sediment Microcosms under Denitrifying, Methanogenic, and Sulfidogenic Conditions

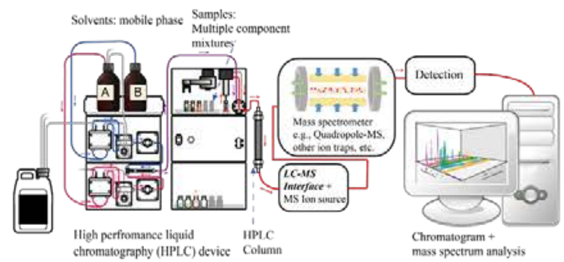


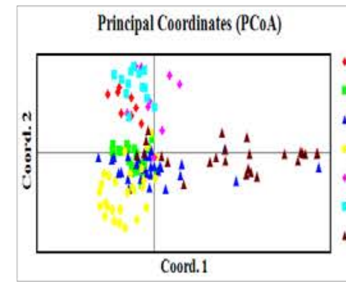
Raritan River Watershed as a Model



Limited Land Use, Sampling Region
Map credits: Alex Mossawir


RUTGERS





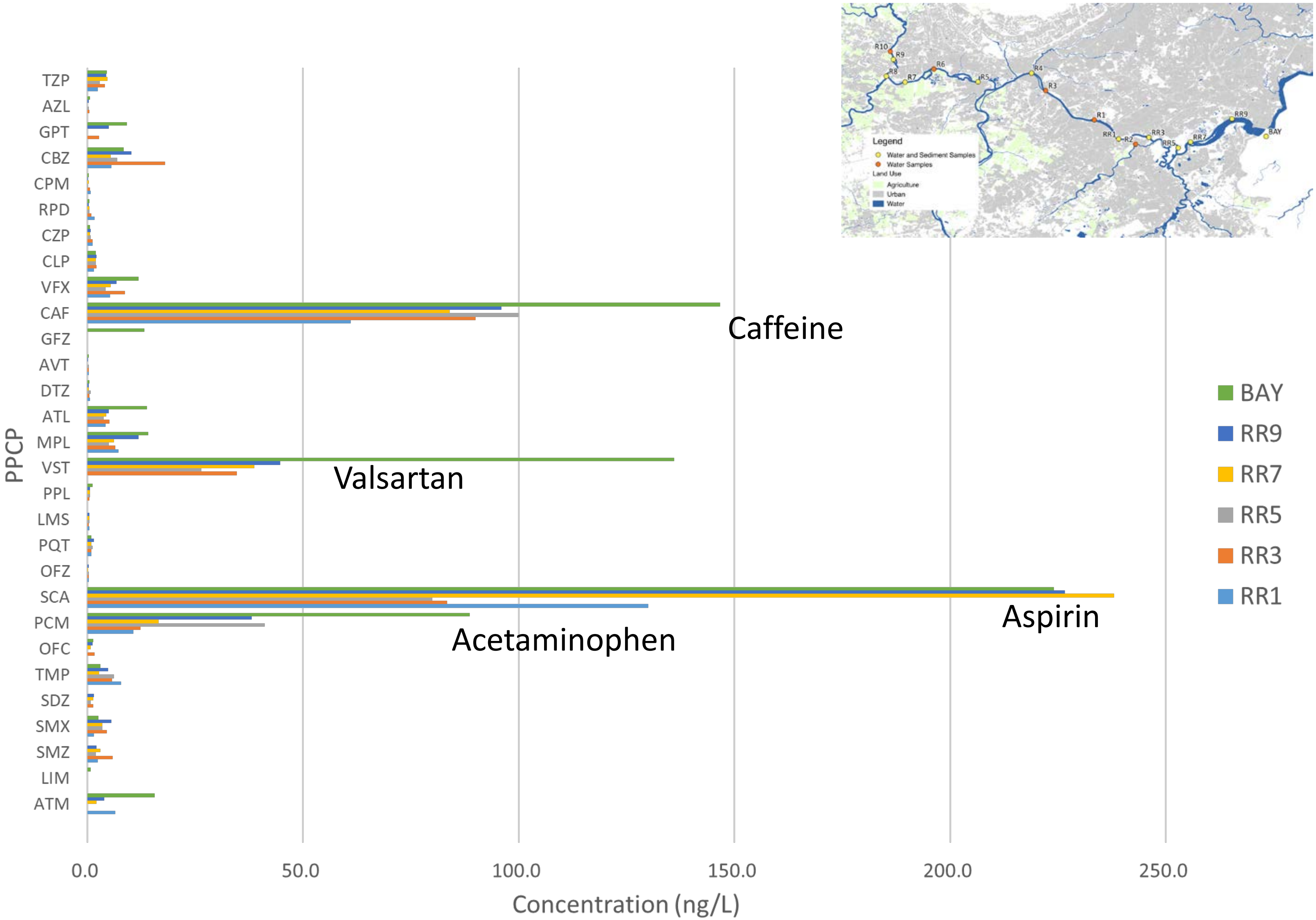
Collect samples from Raritan River, and WWTP influent and effluent

Concentrate and purify PPCPs from water by Solid Phase Extraction (SPE)

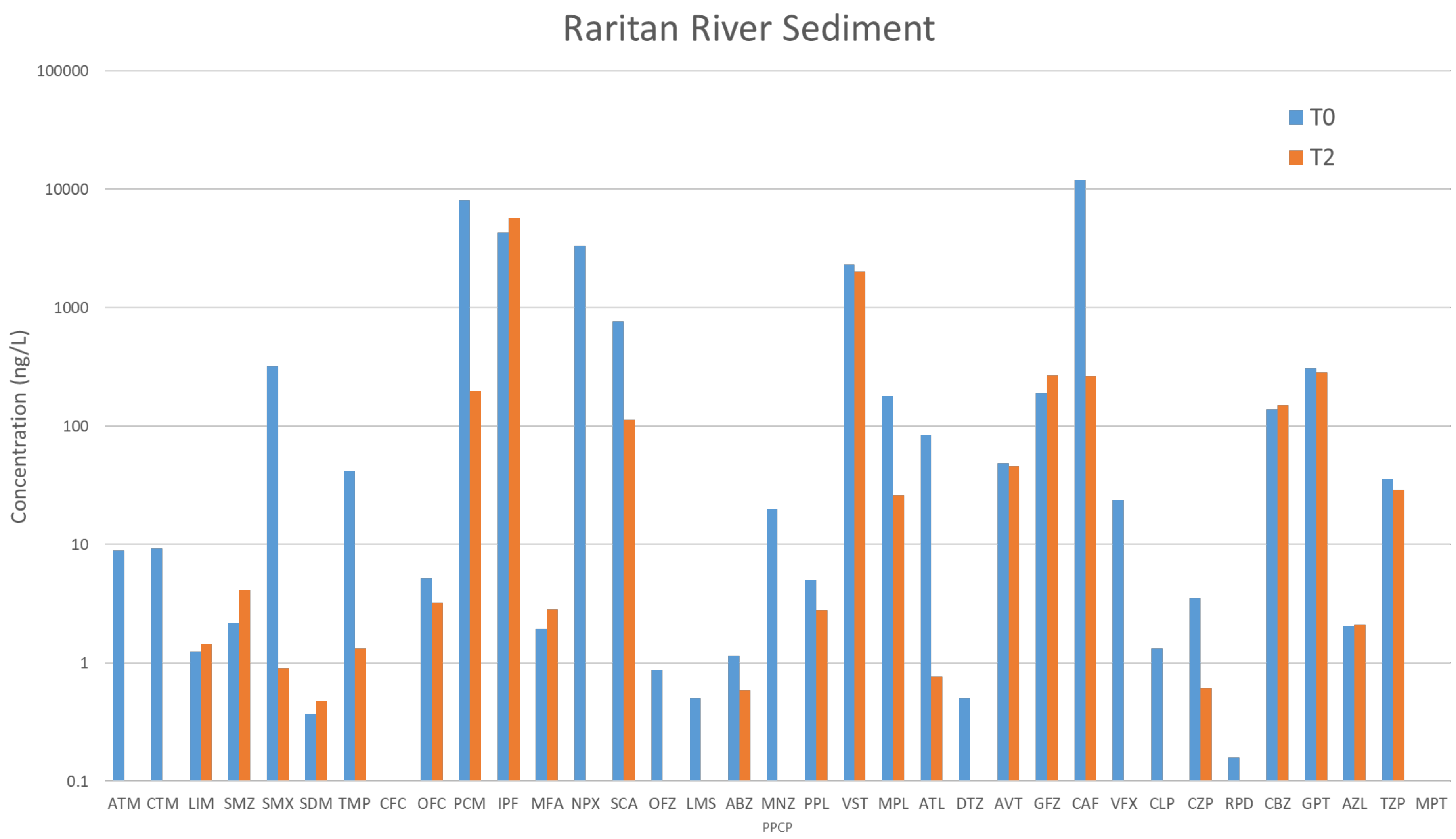
Identify and quantify PPCPs by LC-MS/MS

Analyze the fate and distribution of PPCPs in watersheds

PPCPs in the Lower Raritan River

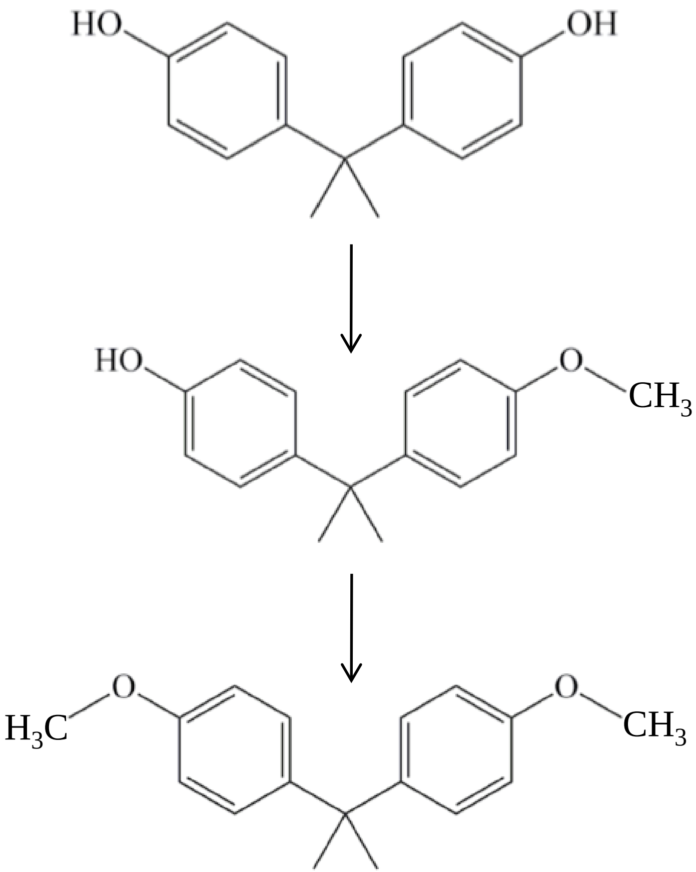


Degradation of PPCPs in Sediment Microcosms



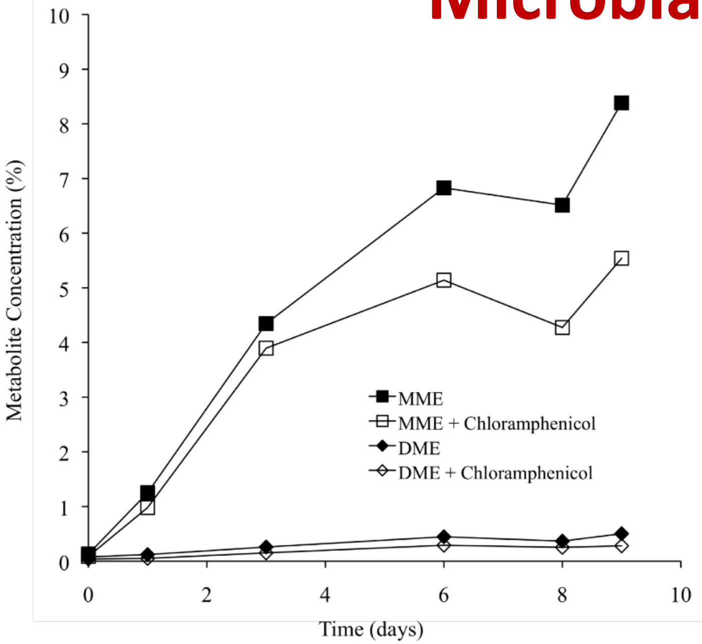
Sediment microcosm was composed of 10% Raritan River sediment from Johnson Park, New Brunswick mixed with in RTMUA Influent to mimic raw sewage overflow

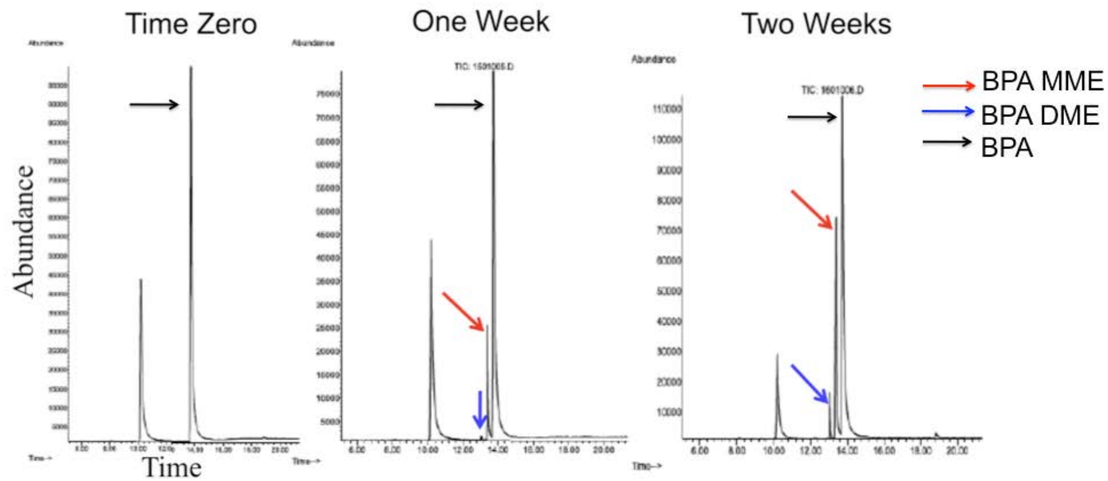
O-Methylation of Bisphenol A

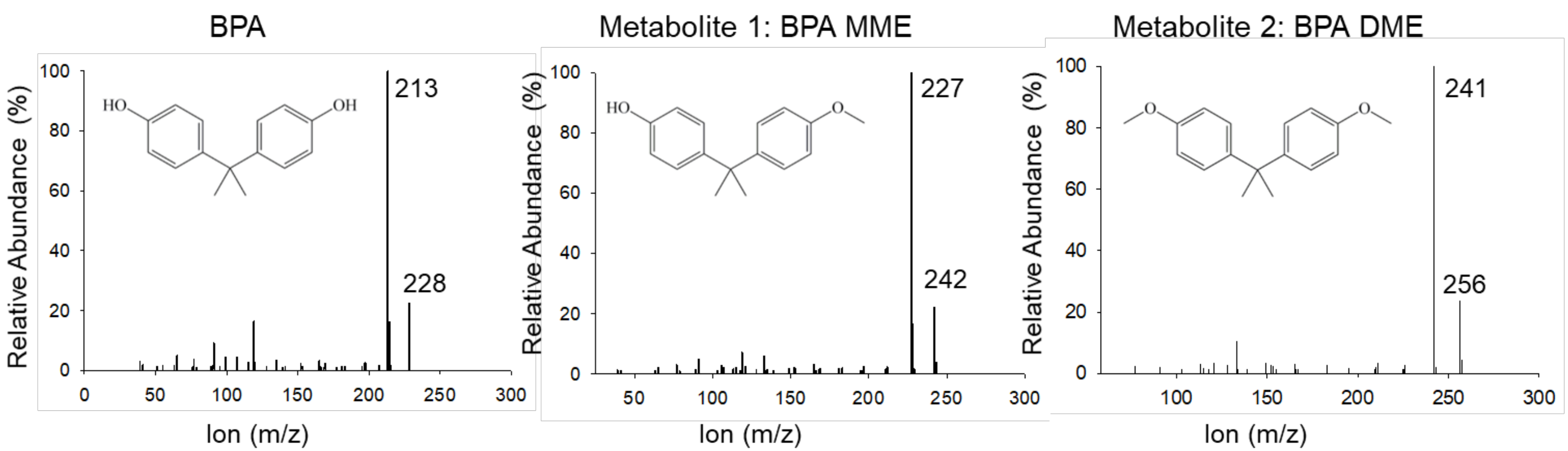


- novel microbial metabolites of BPA are more toxic to the developing zebrafish embryo than the parent compound
- microbial transformations of BPA and other pharmaceutical and manufacturing compounds can have far-reaching impact on aquatic and terrestrial systems.

Microbial O-methylation of BPA



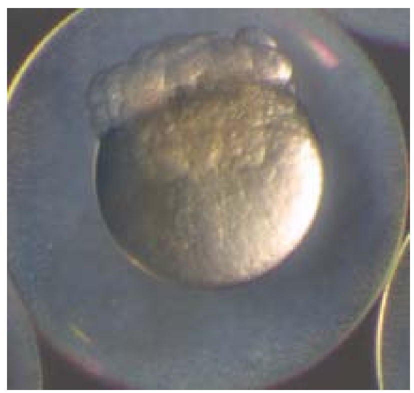




McCormick et al. (2011) Microbially mediated O-methylation of bisphenol A and the toxicity of bisphenol A, and bisphenol A monomethyl ether and dimethyl ethers in the developing zebrafish (*Danio rerio*) embryo. Environ. Sci. Technol. 45:6567-6574.

Use of the Zebrafish *(Danio rerio)* as a Model Organism

- *Ex utero* fertilization
- Transparent chorion - allows for visualization throughout development
- Sensitive to many compounds - great for toxicological studies
- Results can be extrapolated to humans and environmental health

Collaborators: Keith R. Cooper & Lori A. White

O-methylation of BPA results in metabolites with increased toxicity to the developing zebrafish embryo

Short term vs. long term LC50 values and Lowest Observed Adverse Effect Levels (LOAEL)

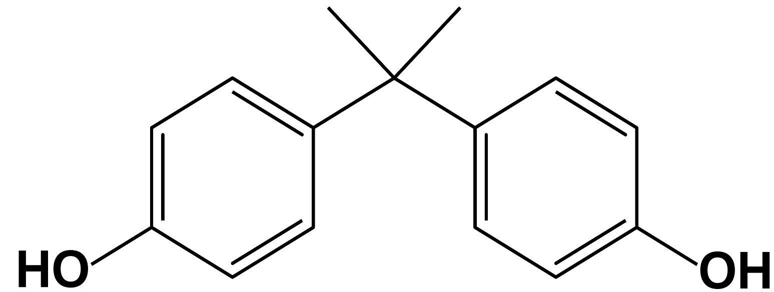
	5 dpf			28 dpf		
	BPA	MME	DME	BPA	MME	DME
LC50	17.5 μM	2.7 μM	4.7 μM	7.9 μM	1.6 μM	< 2 μM
LOAEL	22 μM	2.1 μM	2.0 μM	---	---	---

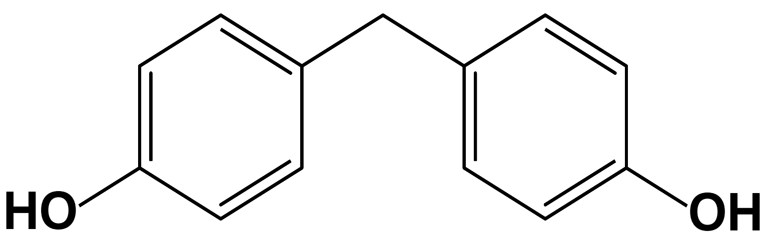
- BPA MME and BPA DME have at least a 3 fold lower LC50 values than BPA at 5 and 28 dpf
- BPA MME and BPA DME have LOAEL values approximately 10 fold lower than BPA at 5 and 28 dpf
- The O-methylated metabolites are more toxic that the parent compound, BPA

Representative Lesions in the Developing Zebrafish

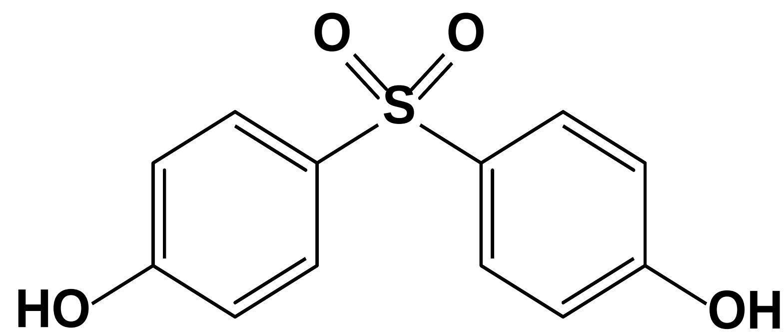
Biotransformation of Bisphenol A

- BPA can be O-methylated by *Mycobacterium* spp. to the corresponding BPA mono- and dimethyl ethers.
- O-methylation of BPA results in metabolites that are more toxic than BPA.
- Microbial transformations of BPA and other pharmaceutical and manufacturing compounds can have far-reaching impact on aquatic and terrestrial systems.
- Future work needs to examine the molecular mechanism of toxicity of these O-methylated metabolites, as well as their distribution and fate in the environment.



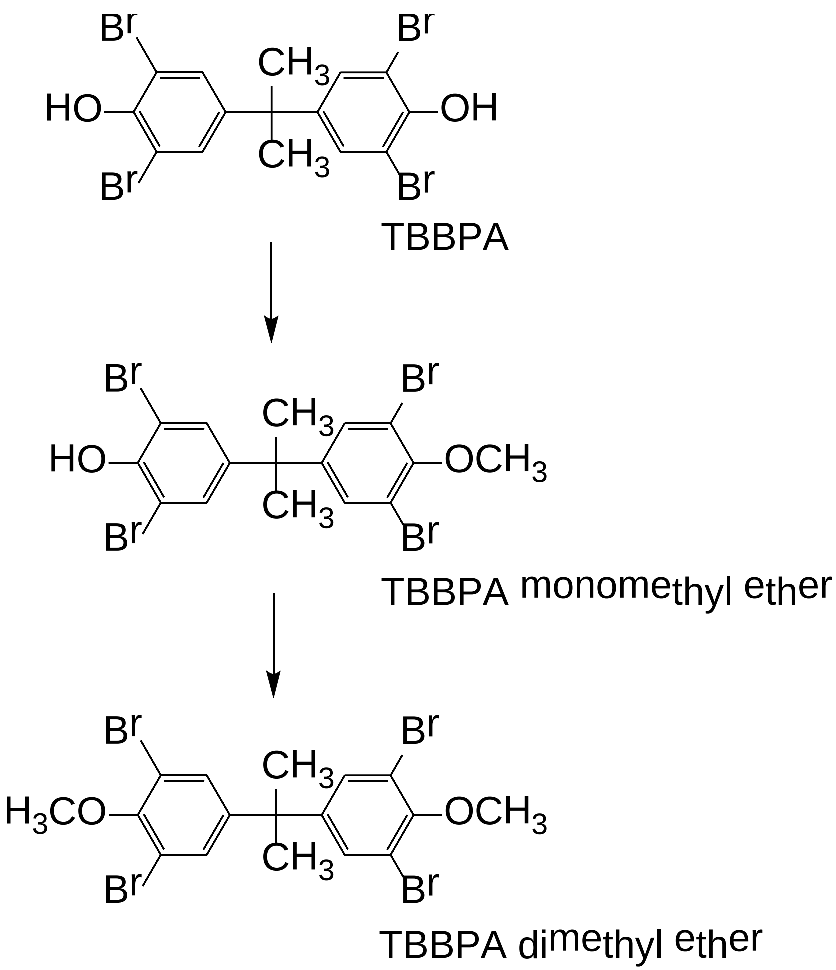


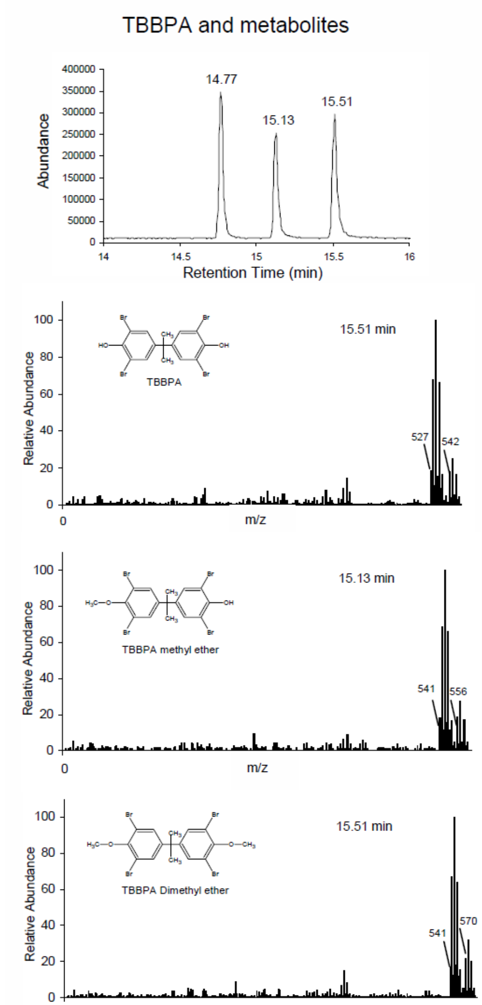
Bisphenol F



Bisphenol S

O-Methylation of Tetrabromobisphenol A

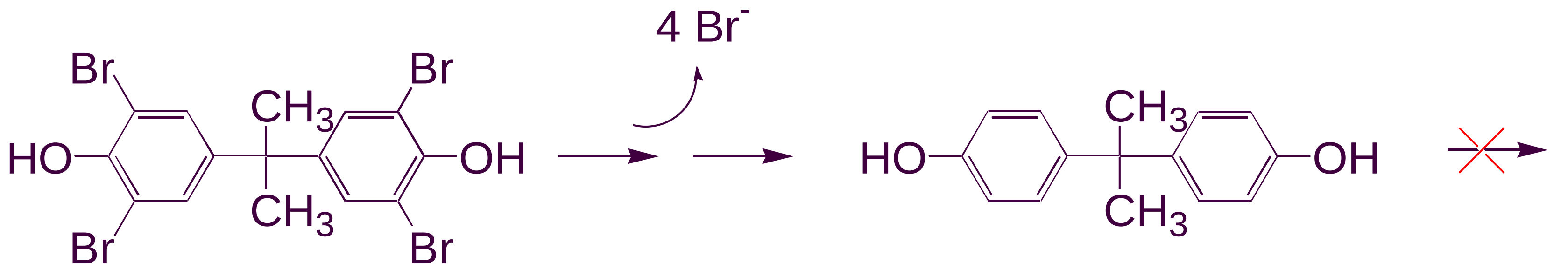




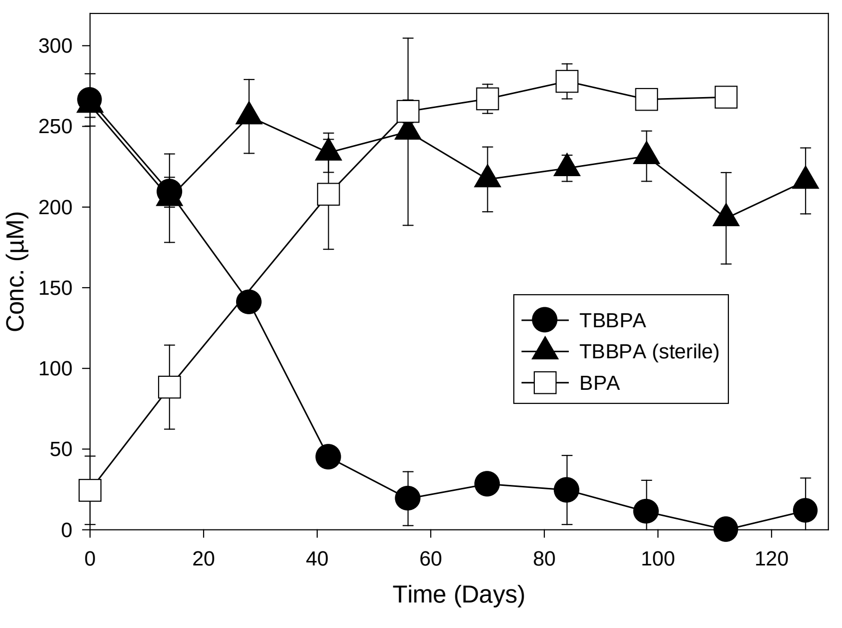
- TBBPA is not always degraded aerobically but can be biotransformed to its corresponding mono- and dimethyl derivatives
- microbial transformation of TBBPA results in metabolites with different chemical and toxicological properties than the parent compound

George & Häggblom *(2008)* Environ. Sci. Technol.

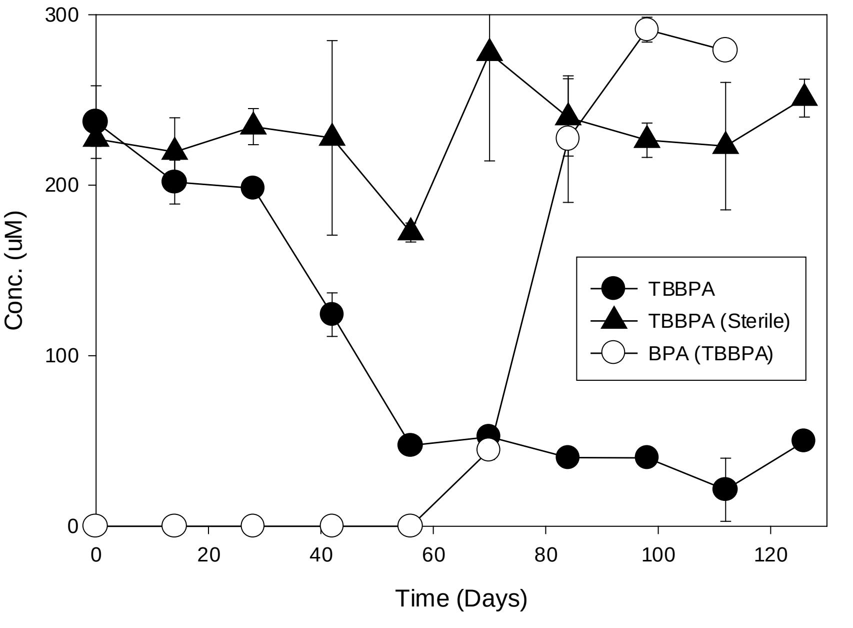
Dehalogenation of Tetrabromobisphenol A



Methanogenic



Sulfidogenic



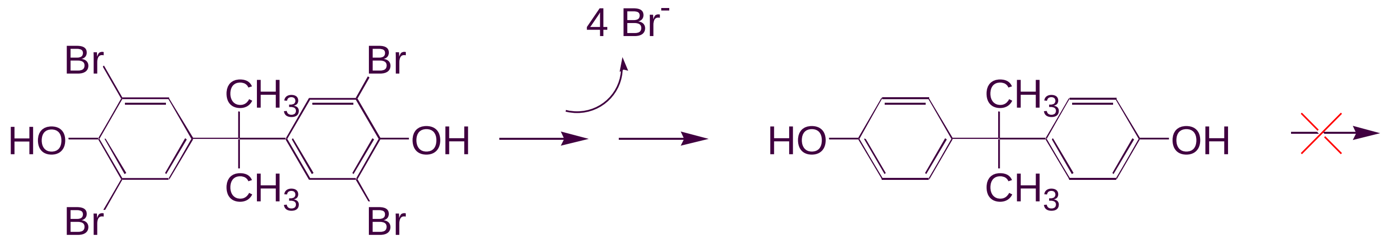
Arthur Kill sediment

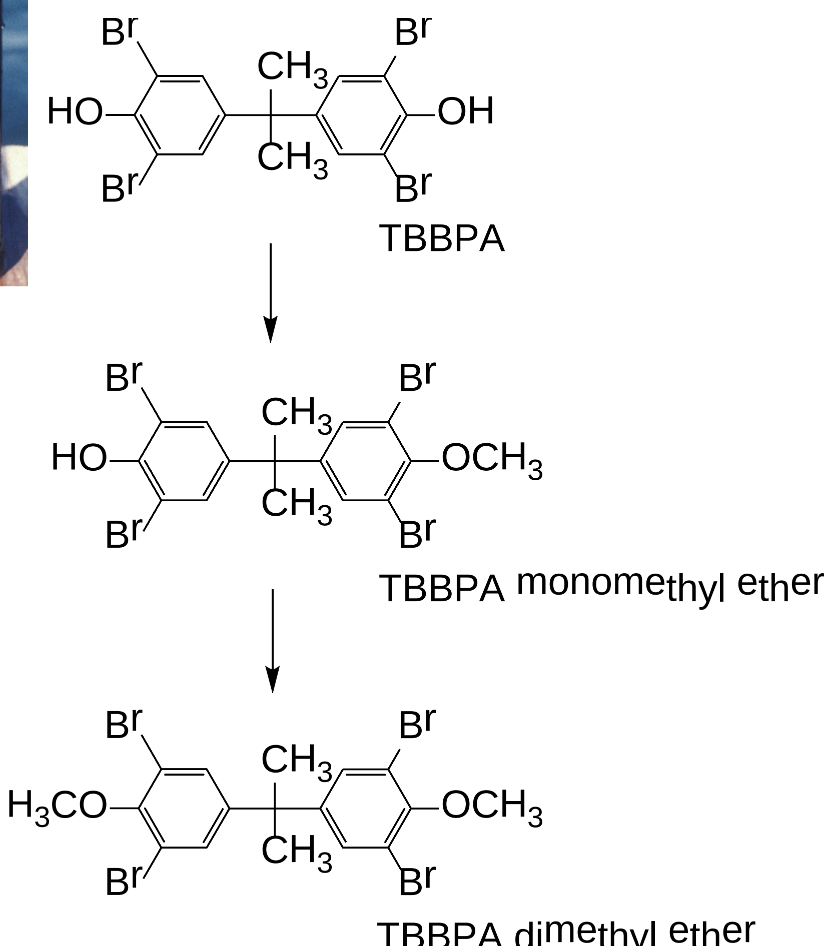
Voordeckers *et al.* 2002 Env. Sci. Technol

Fate of Tetrabromobisphenol A

Anaerobic

Aerobic





- TBBP is readily dehalogenated to bisphenol A in anaerobic sediments which persists
- TBBPA is not degraded aerobically, but is biotransformed to its corresponding mono- and dimethyl derivatives
- microbial transformation of TBBPA results in metabolites with different chemical and toxicological properties than the parent compound

Conclusions

Some PPCPs are biodegraded to various degrees in river and estuarine sediment by native microorganisms, others are not.

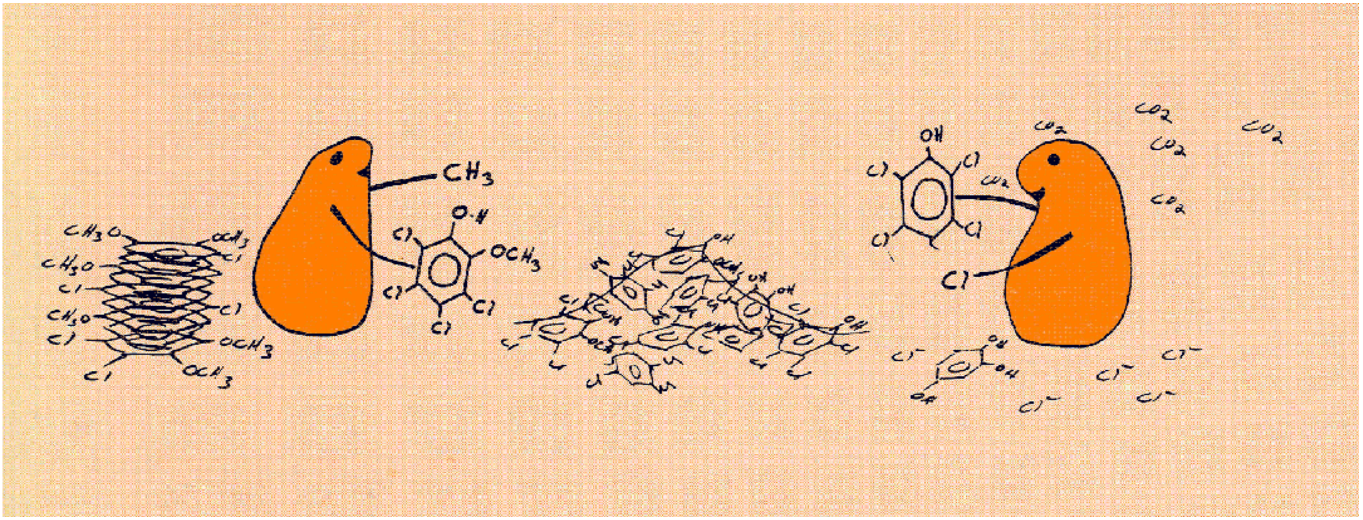
Depending on the dominant redox condition of the sediments, PPCP compounds may be recalcitrant in the natural environment.

Similar PPCP degradation profiles have been observed at differnet sites and PPCP-degrading bacteria appear to be globally distributed.

Some PPCPs are transformed to new metabolites with potential adverse biotic effects.

There is much that we still do not know and understand.

Biodegradation vs. Biotransformation



- microbial degradation of toxic chemicals can ultimately lead to their complete degradation (mineralization) and detoxification

- microbial transformations of chemicals (such as pharmaceuticals and personal care products pharmaceutical and personal health care products) can lead to formation of compounds that are more problematic than the starting compounds

The “Consortium”

Aamani Rupakula
Michelle Zeliph
Jessica McCormick

Alex Mossawir, Albert Enriquez, Sam Wieczerzak, Shivani Patel

Lee Kerkhof
Donna Fennell
Lori White
Keith Cooper

Shen Yu & Bing Hong (Chinese Academy of Sciences)
Ivonne Nijenhuis & Hans Richnow (UFZ Leipzig)

Hudson River Foundation
Raritan River Consortium
Rutgers Centers for Global Advancement and International Affairs

RUTGERS School of Environmental and Biological Sciences

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

RUTGERS Raritan River Consortium

HUDSON RIVER FOUNDATION for Science & Environmental Research

RUTGERS Centers for Global Advancement and International Affairs

PPCPs in the Lower Raritan River

Class	Name	Abbr.	Influent Flemington WWTP	Effluent Flemington WWTP	RR1	RR3	RR5	RR7	RR9	BAY
Anti-inflammatory drug	Paracetamol crs (Acetaminophen)	PCM	22320.00	53.60	10.67	12.27	41.07	16.53	38.07	88.67
	Salicylic acid (Aspirin)	SCA	705.60	96.40	130.00	83.33	80.00	238.00	226.67	224.00
Antibiotic	Lincomycin hydrochloride-see-leaflet-	LIM	6.62	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Sulfamethoxazole	SMX	1064.00	5.32	1.50	4.50	3.40	3.40	5.50	2.60
	Ofloxacin	OFC	292.00	9.64	0.00	1.60	BDL	0.80	1.20	1.30
	Ciprofloxacin crs	CFC	233.60	2.22	BDL	BDL	BDL	BDL	BDL	BDL
	Azithromycin crs-see-leaflet-	ATM	169.60	9.84	6.40	0.00	BDL	2.10	3.90	15.70
	Clarithromycin	CTM	101.60	4.88	BDL	BDL	BDL	BDL	BDL	BDL
	Trimethoprim	TMP	435.20	1.61	7.90	5.70	6.10	2.60	4.70	3.00
	Sulfadiazine	SDZ	42.72	BDL	BDL	1.40	0.80	1.40	1.50	BDL
Antiparasitic drug	Albendazole	ABZ	3.38	BDL	BDL	BDL	BDL	BDL	BDL	BDL
	Oxfendazole	OFZ	BDL	BDL	0.29	0.34	0.37	0.17	0.30	BDL
	Levamisole hydrochloride	LMS	3.90	BDL	0.40	0.24	0.38	0.40	0.49	BDL
	Metronidazole	MNZ	33.28	5.84	BDL	BDL	BDL	BDL	BDL	BDL
	Praziquantel	PQT	BDL	BDL	0.84	0.86	1.15	0.95	1.57	0.95
Cardiovascular drug	Gemfibrozil	GFZ	BDL	BDL	BDL	BDL	BDL	BDL	BDL	13.20
	Valsartan crs	VST	3656.00	112.40	0.00	34.60	26.33	38.73	44.67	136.00
	Atorvastatin calcium	AVT	279.20	4.84	0.30	0.30	0.30	0.00	0.18	0.32
	Atenolol	ATL	402.40	67.60	4.20	5.04	3.69	4.31	4.99	13.73
	(±)-Metoprolol (□)-tartrate salt	MPL	783.20	333.20	7.20	6.47	4.94	6.09	11.80	14.07
	Diltiazem hydrochloride	DTZ	236.80	2.41	0.52	0.48	0.67	0.23	0.25	0.47
	(±)-Propranolol hydrochloride	PPL	62.08	2.44	0.00	0.50	0.54	0.57	0.58	1.17
Central nervous system drug	Caffeine	CAF	43360.00	163.20	61.07	90.00	100.00	84.00	96.00	146.67
	Gabapentin	GPT	BDL	BDL	BDL	2.74	BDL	BDL	4.93	9.13
	Carbamazepine	CBZ	310.40	304.40	5.57	18.00	6.87	5.37	10.20	8.33
	Venlafaxine hydrochloride	VFX	158.40	3.68	5.21	8.67	4.15	5.35	6.80	11.87
	Temazepam	TZP	66.64	70.00	2.34	4.06	2.87	4.66	4.36	4.51
	Clozapine crs	CZP	BDL	BDL	1.15	1.21	0.71	0.58	0.67	0.53
	Citalopram hydrobromide	CLP	97.60	3.60	1.56	2.10	2.01	1.97	2.10	1.92
	Risperidone	RPD	0.00	0.00	1.59	0.95	0.50	0.39	0.36	0.39
	Chlorpromazine hydrochloride	CPM	BDL	BDL	0.70	0.60	0.10	0.30	0.20	0.30
	Alprazolam	AZL	4.30	6.04	0.00	0.46	0.29	0.00	0.31	0.59
	Flunitrazepam	FZP	5.18	BDL	BDL	BDL	BDL	BDL	BDL	BDL

PPCP Concentrations in Raritan River Samples and Influent and Effluent of a Sewage Treatment Plant

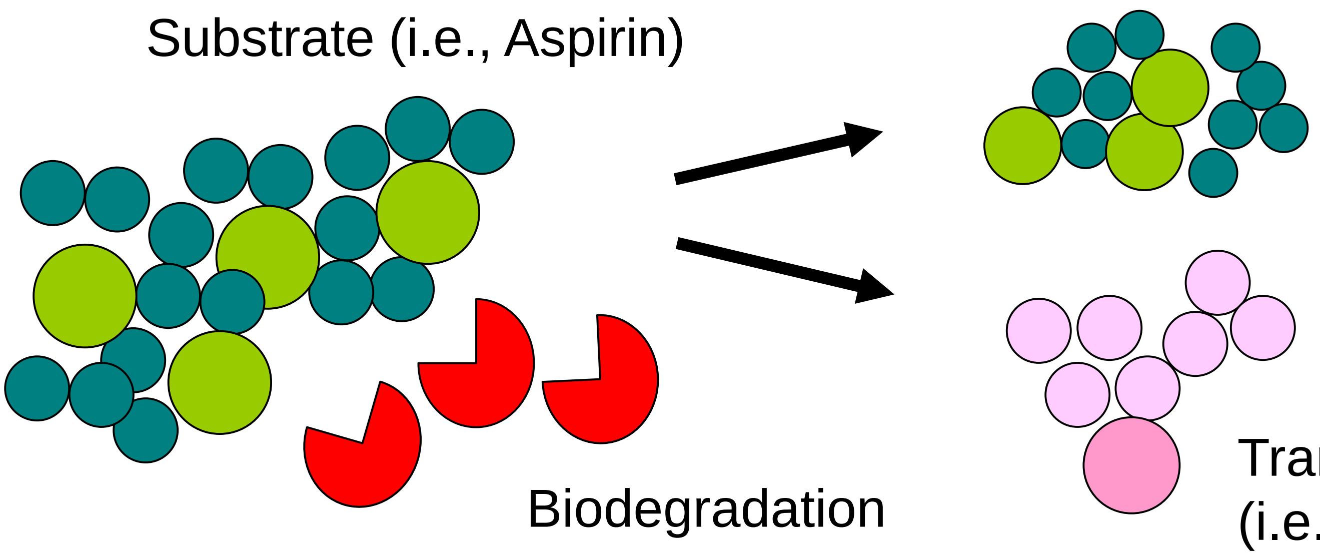
Compound Specific Isotope Analysis (CSIA)

^{13}C substrate

- Bonds with heavy isotopes have higher activation energies and are more stable.

^{12}C substrate

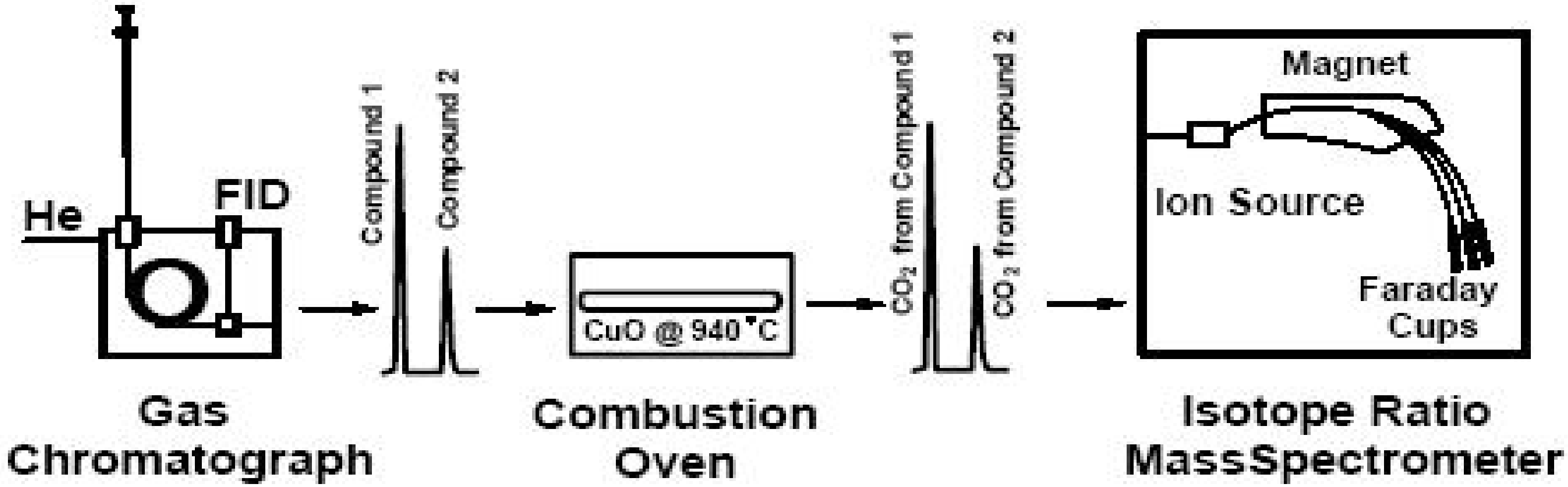
- Bonds with light isotopes broken more easily



Stable Isotope Ratio Analysis

Precise analysis of concentration ratio of two stable isotopes of an element

GC-IRMS



separates compounds

combusts compounds oxidizes hydrocarbons

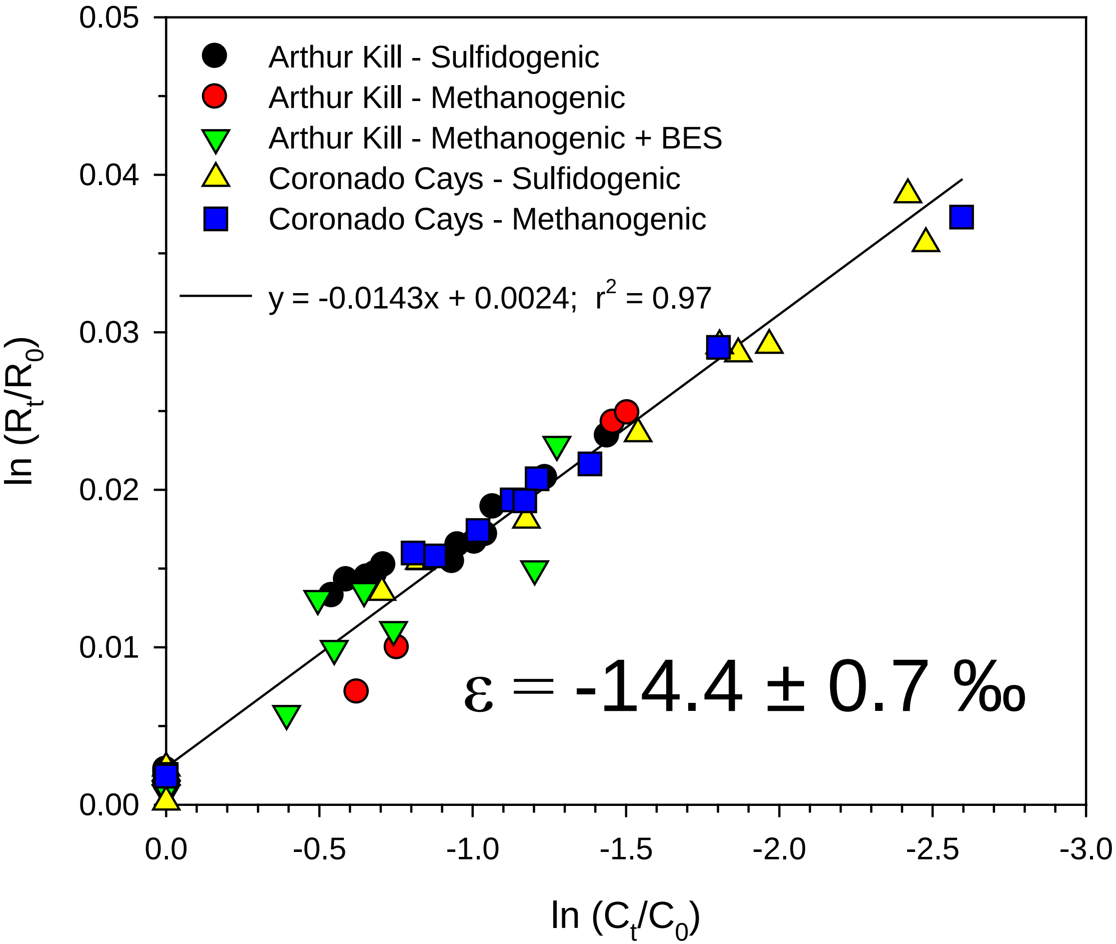
separates $^{13/12}$-CO_2 by mass in a magnetic field, collects & measures specific masses

Can determine stable isotope ratio of a specific compound in a complex mixture

image from microseeps.com

Isotopic Composition vs. Residual Substrate Concentration

Double Logarithmic Plot of Rayleigh Equation



Somsamak et al. Environ. Sci. Technol. 2005
Somsamak et al. Appl. Environ Microbiol. 2006
Youngster et al. Appl. Microbiol. Biotechnol. 2010
Häggblom et al. Adv. Appl. Microbiol. 2007

Rayleigh equation for closed system:

$$R_t / R_0 = (c_t / c_0)^{(1/\alpha - 1)}$$

R = isotope ratio
C = concentration
α = kinetic isotope fractionation factor

α = kinetic isotope fractionation factor determined from slope of curve; $b = 1/\alpha - 1$

ε = isotopic enrichment factor
$\varepsilon = 1000 \times (1/\alpha - 1)$

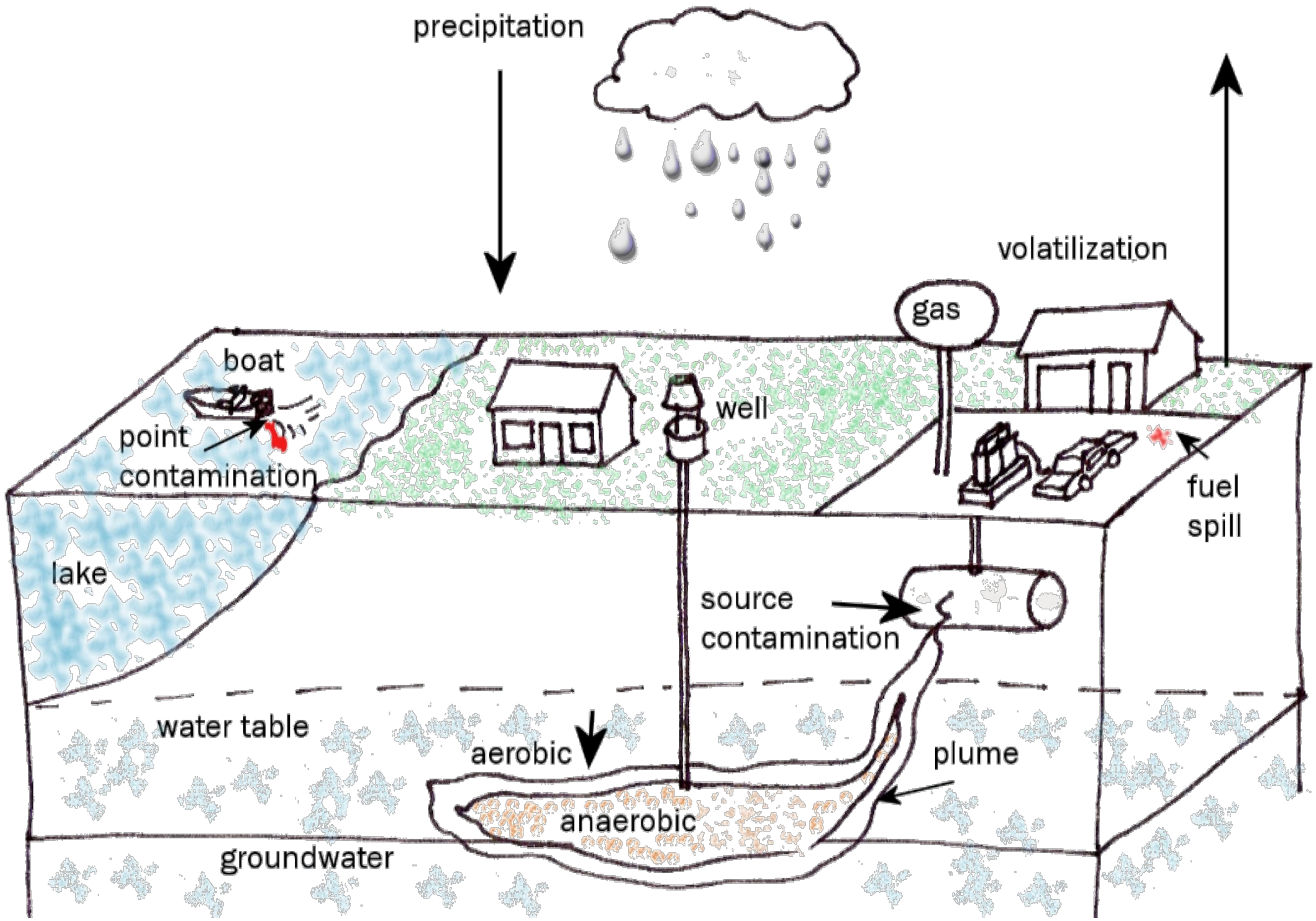
- Anaerobic of MTBE and TAME results in significant C isotope fractionation
- Useful tool for monitoring anaerobic biodegradation *in situ*

$$R_t / R_0 = (c_t / c_0)^{(1/\alpha - 1)}$$

R = isotope ratio

C = concentration

α = kinetic isotope fractionation factor



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Bugs on Drugs:
Anaerobic Biodegradability of Pharmaceuticals and Personal Care Products

Max M. Häggblom
Rutgers University
Department of Biochemistry and Microbiology
School of Environmental and Biological Sciences
New Brunswick, New Jersey