

### What are domestic wastewater tracers?

- Chemical or microbial markers that can be detected or measured to identify contamination
- Usually present in the initial wastewater source or chemical transformation products
- General Categories of commonly used:
  - Human/human-microbiome produced markers
    - e.g. E. Coli, enterococci, HF183, HumM2, steroids, steroid hormones (10.1021/acs.est.6b02564, [10.1016/j.watres.2020.116014](#), 10.1111/j.1462-2920.2004.00702.x, [10.3390/w9020143](#), 10.1021/es501944c, 10.1128/AEM.00028-17)
  - Organic contaminants of emerging concern
    - e.g. artificial sweeteners (sucralose, acesulfame-K, saccharin), over-the-counter pharmaceuticals (acetaminophen, ibuprofen), prescription pharmaceuticals (carbamazepine, sulfamethoxazole, diclofenac, atenolol, gemfibrozil), other metabolites (cotinine, caffeine, paraxanthine, theobromine), other chemicals in personal care products (triclosan, parabens)
    - Some review articles (though flawed)/other of value: ([10.1016/j.scitotenv.2016.06.043](#) , [10.1016/j.watres.2018.09.013](#), [10.3390/w9020143](#), [10.1016/j.scitotenv.2019.05.385](#))
  - Inorganic species
    - e.g. nitrogen, phosphorous, chloride, gadolinium, boron (10.1111/j.1745-6584.1998.tb01099.x, 10.1111/j.1745-6584.2005.00127.x, 10.1021/es990633h, 10.1021/es00060a030)
  - Isotopic signatures
    - e.g.  $\delta^{15}\text{N-NO}_3$ ,  $\delta^{11}\text{B}$ , radioisotope iodine-131 (10.1002/9780470691854, 10.1021/es00060a030, 10.1021/es800418c)
- There are different reasons for tracing domestic wastewater-overlapping concepts
  - Nutrients (nitrogen, phosphorous, carbon)
  - Ecological or human health related to chemicals in wastewater (e.g. toxicity, genotoxicity, etc.)
  - More acute pathogenic concern

### Why study tracers?

- While many wastewater tracers have been identified still some common issues and developing field
  - Some traditionally used domestic sewage indicators are not human specific
    - For example, for total coliform, coliform type bacteria are not fecal specific. Fecal coliform is more specific, but there are fecal coliform bacteria that thrive in the environment under conditions unfavorable to *Escherichia coli* (*E. coli*). *E. coli* is an even more specific measure of freshwater sewage impairment, but it also not human specific (10.2175/106143098X121752, 10.1021/es501944c). Similarly, enterococci, used in marine and freshwaters, is also not human specific. Water impairment indicated by fecal coliform and enterococci has in some cases been linked primarily to avian (gulls) (10.1021/acs.est.6b02564)

- notably “the first report of falconry used to address a bacteria TMDL”) or dog and avian ([10.1016/j.watres.2020.116014](#)) microbial sources.
- $\delta^{15}\text{N-NO}_3$ ;  $^{15}\text{N}$  enrichment in animal tissue and solid waste, because  $^{14}\text{N}$  is preferentially excreted in urine. Volatilization of  $^{15}\text{N}$  depleted ammonia = waste even heavier. So with oxidation, animal waste = isotopically heavy nitrate; but not human specific, also can just become isotopically heavy from denitrification ([10.1002/9780470691854](#))
  - Chloride by itself, or ratio with other halogens. In wastewater chloride is from personal care products, salt in diet, and in some cases from water softening systems. But also can be from agriculture, road salt application, geology. ([10.1111/j.1745-6584.1998.tb01099.x](#), [10.1111/j.1745-6584.2005.00127.x](#))
  - Still a lot of research needed on tracer behavior themselves – degradation pathways and sorption, persistence and characteristics of degradation products
  - Can be cost prohibitive
    - Often multiple types of tracers give the best conclusions about source and treatment, but \$\$\$
    - Detecting multiple specific human pathogens themselves would be very costly and laboratory intensive
    - Human-specific fecal indicators like *Bacteroides* HF183, *Escherichia coli* H8, HumM2 tend to be very cost prohibitive or only used in research and not available as offered analytical services
  - Can get more information using certain tracers
    - Age of wastewater source
      - Trends in product usage and approval – artificial sweeteners have been particularly used for this ([10.1016/j.watres.2015.10.051](#), [10.1016/j.envpol.2013.08.021](#), [10.1016/j.wasman.2019.06.010](#))
    - Source/treatment level
      - WWTP vs septic based on WWTP more likely to receive medical waste/waste from a larger population- e.g. gadolinium (used in MRI contrast agents) and sucralose ratios, ([10.1016/j.watres.2012.07.060](#)) iodinated contrast agents vs more household tracers
      - Level of treatment from more degradable vs more conservative tracers – e.g. caffeine and sucralose ratios for combined sewage overflow vs WWTP effluent ([10.1016/j.watres.2017.12.044](#)). Same concepts could extend to septic system vs WWTP effluent.

Potential best options considering costs:

- While LC-MS/MS method startup costs are high, it can detect a couple to dozens of nonvolatile, organic wastewater tracers at same time with little increase in labor or preparation. Through this a suite of tracers that have complementing factors to each other can be selected.
  - Sucralose – an artificial sweetener, sold under the brand name Splenda, used in wide variety of products including food, beverages, toothpaste, mouthwash, among other uses.

- Pros:
  - Has very low degradability, hydrophilic, essentially conservative tracer, also widespread and high usage in the United States ([10.1016/j.watres.2011.05.014](#))
  - In the U.S., presence can be used as an age marker for wastewater from after the year 2000 ([10.1016/j.watres.2015.10.051](#))
- Cons:
  - Lower ionizing so can have higher detection limits than other organic wastewater tracers
  - Very persistent, will still be high even after other chemicals from effluent source have degraded, nitrogen removed, any bacterial background has been eliminated
- Acesulfame-K – an artificial sweetener, part of the formulation for the brand name sweetener Equal, used in wide variety of products including food, beverages, toothpaste, mouthwash, among other uses.
  - Pros:
    - Low degradability, though more degradable than sucralose ([10.1021/es900126x](#))
    - Essentially ubiquitous and high use in the United States ([10.1016/j.watres.2012.07.060](#))
    - In the U.S., presence can be used as an age marker for wastewater from after the year 1988 ([10.1016/j.watres.2015.10.051](#))
  - Cons:
    - Our experience has shown it to require more specialized sample extraction than other popular organic tracers
    - Persistent, can still be present after removal of other tracers of concern
- Carbamazepine - prescription anticonvulsant, used for epilepsy, schizophrenia, bipolar disorder
  - Pros:
    - Persistent, low degradation during wastewater treatment, ([10.1016/j.watres.2003.10.058](#), [10.1016/j.chemosphere.2008.07.086](#)) though not as persistent as sucralose
  - Cons:
    - While it is a widely used prescription pharmaceutical, may perform poorly as a wastewater tracer in small watersheds if not in use among smaller effluent population source
- Caffeine/Paraxanthine – caffeine is stimulant in coffee, tea, soft drinks, chocolate; paraxanthine is the major human metabolite of caffeine
  - Pros: Appear to readily degrade aerobically so can suggest a more recent/untreated wastewater source ([10.1016/j.watres.2016.05.046](#))
  - Cons:
    - Paraxanthine is not a human exclusive metabolite
    - May not work in areas with native caffeine containing plants

- Our experience has shown seasonal signal for caffeine and paraxanthine, unclear if this is because a larger wastewater impact to streams occurs during Spring/Summer or if some other seasonal removal process is present, but suggests could perform less well as a source tracer in Fall/Winter
- Ibuprofen - NSAID, sold by brand name Advil
  - Pros:
    - Readily degraded, likely aerobically ([10.1021/es981014w](#), [10.1016/j.scitotenv.2012.08.073](#)), may suggest a more recent/untreated wastewater
  - Cons:
    - Has potential to be more degradable than signal of interests (nitrogen, bacteria)
- Acetaminophen - NSAID, aka paracetamol, sold by brand name Tylenol
  - Pros:
    - Readily degraded aerobically, may suggest a more recent/untreated wastewater, ([10.1016/j.chemosphere.2019.124391](#)) possibly even more so than ibuprofen
  - Cons:
    - In our experience appears to be more degradable than ibuprofen, which may be of benefit for an even more recent/untreated signal but also binds to soils ([10.1016/j.chemosphere.2019.124391](#)) and may be too removable to where it is not being observed when bacteria are still present
- Sulfamethoxazole – prescription antibiotic
  - Pros:
    - Very low sorption to soil ([10.1016/j.watres.2016.09.034](#))
    - Of concern for promoting antibiotic resistance
  - Cons:
    - Some studies have found highly degradable, some found persists for decades. Has complicated degradation process ([10.1016/j.watres.2016.09.034](#), [10.1016/j.trac.2008.10.001](#))
    - Our experience in Maryland streams is that it was rarely detected, but in single stream where was consistently detected it correlated well with total dissolved nitrogen. This low presence may be due to complex degradation controls.
- These are some of the most recommended organic tracers in wastewater tracing. Additionally, in a study of organic tracers versus fecal coliform, James et al. ([10.1016/j.watres.2016.05.046](#)) found acetaminophen, caffeine, paraxanthine, theobromine, sulfamethoxazole, and sucralose to have the best correlations with fecal coliform. This study included ibuprofen, but not acesulfame-K.
- While not human specific, in combination with more human-specific organic chemicals, E Coli. Or Enterococci, and traditional TDN, NO<sub>3</sub>, NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub>, Cl would provide valued additional information and are all cost effective
  - e.g. high e coli. but no sucralose would be good indicator for non-human animal waste

- $\delta^{15}\text{N-NO}_3$  also could be cost effective and provide valued information for fecal vs fertilizer nitrogen sources, most informative in regions without higher tropic level animal waste
- In situations where still unclear possible fecal indicator bacteria origin after use of cheaper tracers, could then use expensive genetic microbial source tracer markers

**Recent developments relating to chemical wastewater tracers to be aware of:**

- **Acesulfame-K** was historically considered excellent wastewater tracer because of very limited degradability, but bacterial metabolic pathways to degrade are believed to have evolved around 2013. (10.3389/fmicb.2019.02606, 10.1021/acs.est.7b05619, 10.1016/j.watres.2016.11.041) Now considered degradable in WWTPs, unknown how evolved microbial enzymes are present in environment at large. Acesulfame-K is still considered persistent.
- Recent research has shown **sucralose** instability at temperatures previously considered stable. (10.1038/srep09598, 10.1016/j.foodchem.2020.126700) This has relevance to sucralose use in baking, e-cigarette liquid, and product formulations that require heating, and could lead to changes in regulation.
- Recent research has shown **gadolinium** accumulating in the brain and other tissues from gadolinium-based contrast agents. (10.1016/j.mri.2016.08.024, 10.1016/S1474-4422(17)30158-8) This is likely changing use, and regulation surrounding gadolinium contrast agents is changing. (<https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-fda-warns-gadolinium-based-contrast-agents-gbcas-are-retained-body>)
- **Sulfamethoxazole** has been shown to likely have a more complicated degradation pathway than previously known, with cyclic degradation (i.e. degradation products revert back into sulfamethoxazole) under some conditions. (10.1016/j.watres.2016.09.034, 10.1016/j.trac.2008.10.001) This may explain discrepancies seen in persistence.
- **Triclosan** was banned in household antiseptic wash products in 2017, though it is still in use in other household products and medical settings. (<https://www.federalregister.gov/documents/2016/06/30/2016-15410/safety-and-effectiveness-of-consumer-antiseptics-topical-antimicrobial-drug-products-for>)
- **Diclofenac** received over-the-counter use approval in February 2020, (<https://www.fda.gov/news-events/press-announcements/fda-approves-three-drugs-nonprescription-use-through-rx-otc-switch-process>) so it may become a more effective wastewater tracer in the coming years. (10.1016/j.watres.2017.05.055)
- The generally considered human-specific, **HF183**, was, surprisingly, measured in gull feces and cloacae. Thought to be from gulls travelling to landfills and wastewater treatment plant sites. (10.1016/j.scitotenv.2017.09.232)