Analysis of nitrogenous-disinfection byproduct (N-DBP) biotransformation products in human gastrointestinal tract culture

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Introduction

Disinfection by-products (DBPs) are ubiquitous tap water contaminants that are produced when disinfectant (e.g., chlorine, chloramine) reacts with organic matter. Over 700 DBPs have been identified in tap water, and these DBPs exhibit a spectrum of carcinogenic and toxic health effects. Nitrogenous-DBPs (N-DBPs) are unregulated DBPs, yet present heightened health hazards (e.g., cytotoxicity, genotoxicity) in comparison to many regulated DBPs. Nevertheless, little is known about N-DBP fate and mechanisms post-ingestion. Previous studies demonstrate that gastrointestinal tract microbiota have the potential to degrade xenobiotic compounds into biotransformation products with different toxic effects than the parent compound. Thus, defining the relationship between N-DBP ingestion and the gut microbiota may open new avenues to identify health consequences from exposure to this class of DBPs.

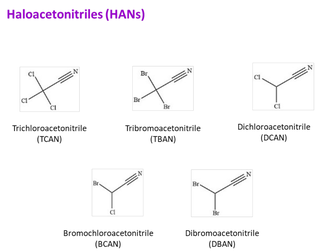
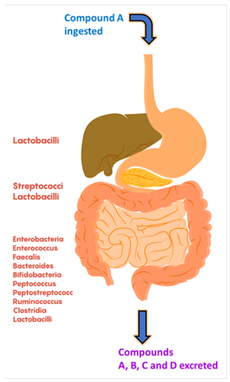
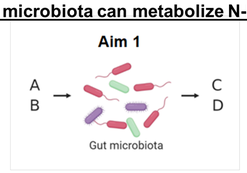


Figure 1. Nitrosamines (NAs) and haloacetonitriles (HANs) are two N-DBP chemical classes that are detected in water distribution networks, yet are unregulated by US EPA. Some water utilities monitor nitrosamines. However, little is known about HANs and health consequences of exposure. Literature suggests that HANs occur at similar concentrations as regulated DBPs, but are more toxic. This study will focus on investigating the chemical and biological interactions between five haloacetonitriles and the human gut microbiome.

Background and Project Aims



Gut microbiota can metabolize N-DBPs



N-DBPs can alter gut microbiome functional gene expression

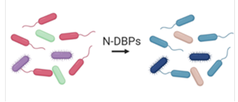
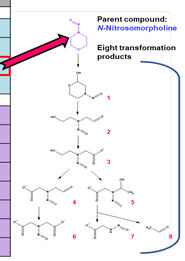


Figure 2. After ingestion, xenobiotic compounds, such as N-DBPs interact with gut microbiota. Aim 1 investigates gut microbiota degradation of N-DBPs. Specifically, the study will identify N-DBP biotransformation products in the presence of gut microbiota. Aim 2 investigates the influence of N-DBPs on the human gut microbiome. The project will determine microbial community gene expression changes in the gut following N-DBP exposure.

Table 1. Predicted biotransformation products for N-DBPs

Chemical compound (IUPAC or Canonical SMILES)	Molecular weight (g/mol)	Chemical structure
Parent N-DBP compounds		
N-nitrosodiethylamine	102.13	
N-nitrosodimethylamine	74.08	
N-nitrosomorpholine	116.12	
N-nitrosopyrrolidine	100.12	
NMOR Anticipated biotransformation products		
N-Nitroso-2-hydroxymorpholine	132.12	
N-(2-Hydroxyethyl)-*N*-(2-oxoethyl)nitrous amide	132.12	
N-(2-Hydroxyethyl)-*N*-carboxymethylnitrosamine	148.12	
[O-]C(=O)CN(CC=O)N=O	145.09	
CC(O)N(CC([O-])=O)N=O	147.11	
Nitrosoiminodiacetic acid	162.10	
2-(2-oxohydrazinyl)acetate	103.06	
Acetaldehyde	44.05	



- The EAWAG-BBD Pathway Prediction System was used to predict biotransformation product formation
- Potential biotransformation products were determined for each parent compound of interest

State of knowledge

Table 2. Toxicity analysis of regulated DBPs and unregulated N-DBPs

	Chemical compound	CAS	Human Health Effects: Acute Mammalian Toxicity: Oral	Acute Mammalian Toxicity: Inhalation	Acute Mammalian Toxicity: Dermal	Carcinogenicity	Genotoxicity Mutagenicity	Endocrine Disruption	Systemic Toxicity: Repeat Exposure	Systemic Toxicity: Single Exposure
Regulated	**Total trihalomethanes**									
	Bromoform	75-25-2	M	H		VH	VH	L	M	M
	Bromodichloromethane	75-27-4	M	M		VH	M	L	M	
	Chloroform	67-66-3	M	H	L	VH	H	H	H	H
	Dibromochloromethane	124-48-1	M			VH	VH	L	M	
	Five haloacetic acids									
	Dibromoacetic acid	631-64-1	H			VH	H	H		
	Dichloroacetic acid	79-43-6	L		VH	VH	VH	H	M	H
	Monochloroacetic acid	79-11-8	H	H	H		L	L	M	H
	Monobromoacetic acid	79-08-3	H	H	H		VH	L	H	M
	Trichloroacetic acid	76-03-9	M		L	VH	VH	H	L	
Unregulated	**Cyanogen halides**									
	Cyanogen bromide	506-68-3	VH	VH	VH		L	L	L	
	Cyanogen chloride	506-77-4	VH	VH			L	L	L	H
	Haloacetonitriles									
	Bromochloroacetonitrile	83463-62-1	H				VH	L		
	Dibromoacetonitrile	3252-43-5	H			VH	VH	H	H	
	Dichloroacetonitrile	3018-12-0	H				VH	L		
	Tribromoacetonitrile	75519-19-6	H				H	L		
	Trichloroacetonitrile	545-06-2	H	H	H		VH	L		
	Halonitromethanes									
	Bromodichloronitromethane	918-01-4	H				H	L		
	Dibromochloronitromethane	1184-89-0	H				H	L		
	Tribromonitromethane	0464-10-8	H				H	L		
	Trichloronitromethane	76-06-2	M	VH	VH		H	L	H	M
	Nitromethane	75-52-5	M	M	L	VH	L	L	L	H
	Haloacetamides									
	Dibromoacetamide	598-70-9					H	L		
	Dichloroacetamide	683-72-7	H				VH	L		
	Trichloroacetamide	594-65-0	M				VH	L		
	N-Nitrosamines									
	N-nitrosodiethylamine	55-18-5	H			VH	VH	L		
	N-nitrosodimethylamine	62-75-9	H	VH		VH	VH	L	H	H
	N-nitrosomorpholine	59-89-2	H			VH	H	L		
	N-nitrosopyrrolidine	930-55-2	H			VH	VH	L		

This table demonstrates that while toxicity data exist for many regulated DBPs, less data are available for N-DBPs. The DBPs listed in the left-most column include non-nitrogenous DBPs and N-DBPs. Toxicity parameters are included in the columns. Red, orange, yellow and green colors represent "very high", "high", "moderate", and "low" relative toxicities, respectively. White cells indicate that toxicity information is unavailable. These data were obtained from the US-EPA proof-of-concept "Hazard Comparison Dashboard, a derivative of the previously reported dashboard from Vegosen and Martin. Overall, this table demonstrates large knowledge gaps associated with toxicity characteristics for N-DBPs.

Approach

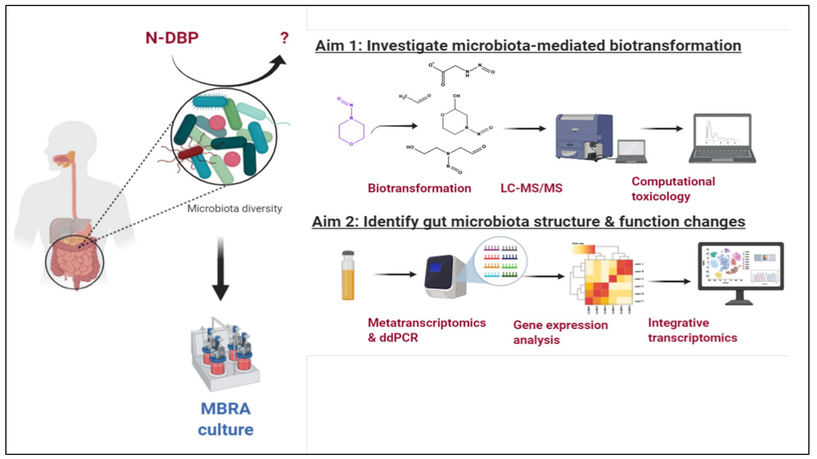
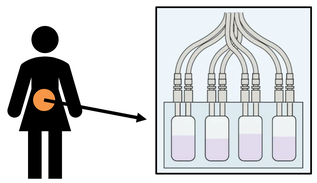


Figure 3. To assess microbially-mediated biotransformation and dynamic transcriptional changes in the human gut, a MiniBioreactor Array (MBRA) will be used to simulate transformation conditions that are representative of human microbial communities. The experimental procedures to be deployed per research aims are shown.

Materials and Methods

MiniBioreactor Array.



- Fecal samples obtained from five healthy adults; samples cultivated separately
- 15 replicates run simultaneously; 25 mL reactor volumes
- Bioreactor operated at 37 °C and pH 7; anaerobic conditions; eight-hour retention time
- Two-week bioreactor stabilization prior to DBP spike
- Reactor fed with defined medium representing nutrients in human gut

Figure 4. The MiniBioReactor Array will be used to recapitulate the human gastrointestinal tract microbiome

Materials and Methods (cont.)

Experimental Layout.

Characterization of biological matrix effects

- Negative pressure
- Reverse phase SPE
  - Recover HANs and transformation products
  - Remove background matrix

Quantification of HANs in biological culture

Thermo Scientific ISQ 7000 Single Quadrupole GC-MS system

- GC-MS methods will be used to quantify five HANs in culture over time.
- Target compounds include: bromochloroacetonitrile (BCAN), dibromoacetonitrile (DBAN), dichloroacetonitrile (DCAN) , tribromoacetonitrile (TBAN), and trichloroacetonitrile (TCAN)

Identification of HANs in biological culture

Thermo Scientific LC (Equan MAX system) – MS (Exactive Plus Orbitrap)

- LC-MS/MS will be applied to identify the biotransformation products of interest
- Fragmentation patterns will be used to determine the presence/absence of non-target analytes

Figure 5. Analytical methods consist of SPE, GC-MS, and LC-MS/MS.

Conclusions

- We predicted N-DBP anaerobic biotransformation products that would be generated in the human gut culture.
- We predicted toxicity outcomes for several regulated and unregulated DBPs, and determined that several knowledge gaps exist for HANs.

Future Work

- Finalize analytical protocols for HAN sample processing during MBRA fate experiments.
- Determine the analytical protocol performance in complex biomatrices based on spike matrix comparisons.
- Develop comprehensive strategies for mitigating signal interferences for HANs and biotransformation products.

References

[1] Auchtung et al. (2016). *Methods Mol Biol, 1476*, 235-258. [2] Krauss & Hollender. (2008). *Analytical Chemistry, 80*(3), 834-842. [3] Richardson et al. (2007). *Reviews in Mutation Research, 636*, 178-242. [4] Gao et al. (2010), *Nucleic Acids Res, 38*. [5] Vegosen & Martin. (2020). *Clean Technologies and Environmental Policy*, *22*, 441–458

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