Advancing the Science for Drinking Water Chemical Exposure Assessment and Health Research
15-16 September 2022

Hunting for Aquatic Bladder Carcinogens

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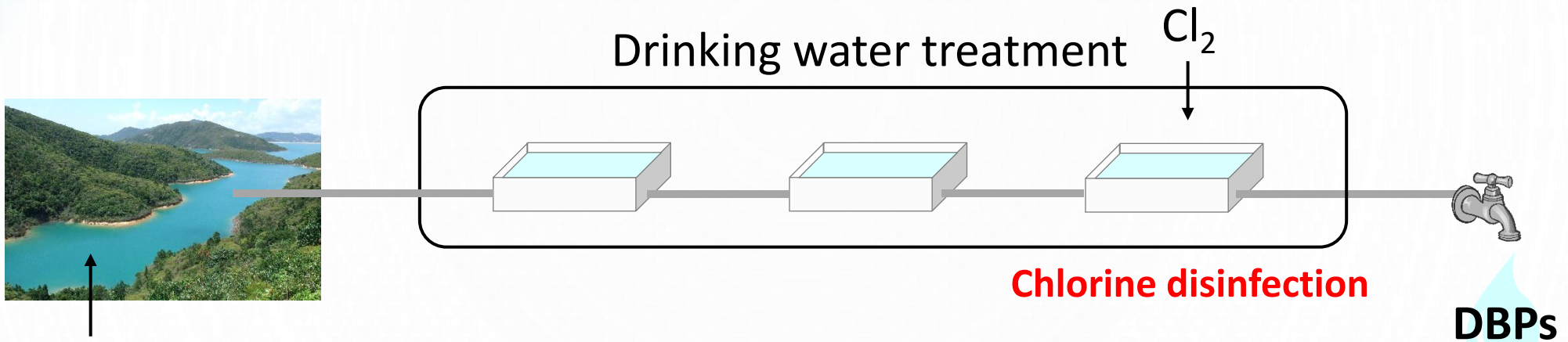
FORMATION OF DISINFECTION BYPRODUCTS

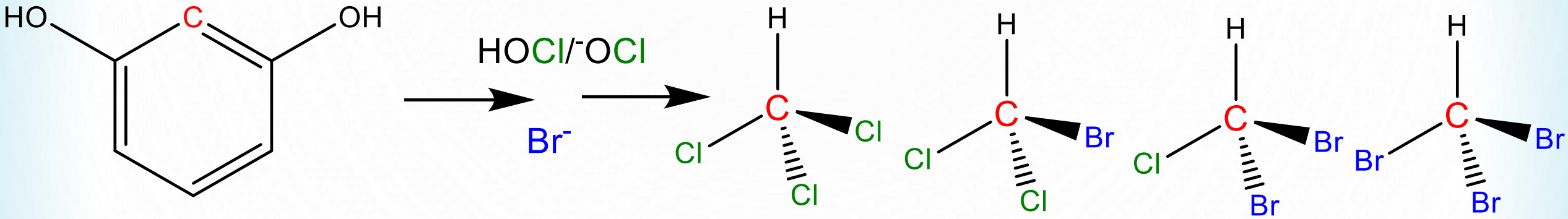


NOM + Cl_2 → Disinfection by-products (DBPs)

	U.S. (µg/L)	E.U. (µg/L)
Sum of 4 trihalomethanes (THMs)	80	100
Sum of 5 haloacetic acids (HAAs)	60	60
Bromate (BrO_3^-)	10	10
Chlorite (ClO_2^-)	1,000	700*/250
Chlorate (ClO_3^-)		700*/250

*When ClO_2 used as disinfectant

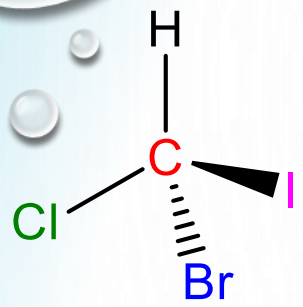
FORMATION OF CHLORINATION BYPRODUCTS

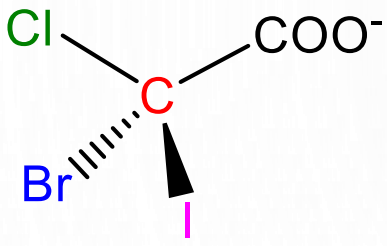


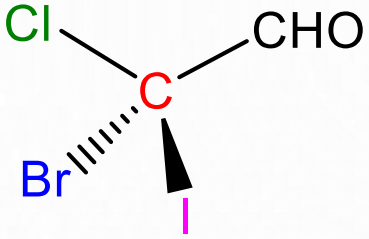
48 different halogenated (Cl, Br, I) THMs, HAAs and haloacetaldehydes are possible.

Assuming mono-, di- and tri-halogenated HAAs and haloacetaldehydes can form.

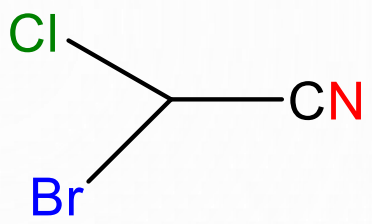
TYPES OF DISINFECTION BYPRODUCTS

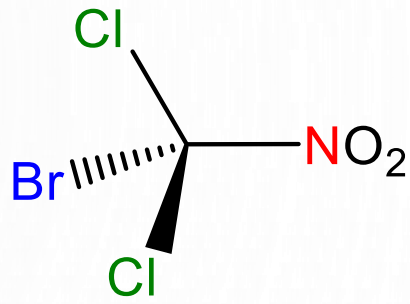


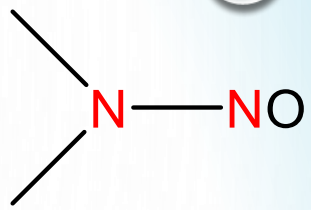




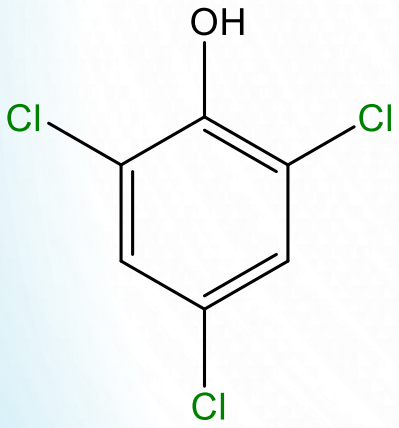
Halogenated aliphatic byproducts

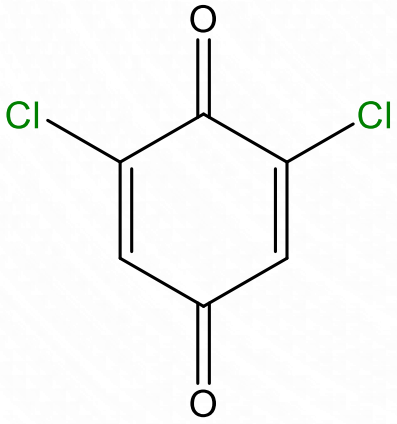


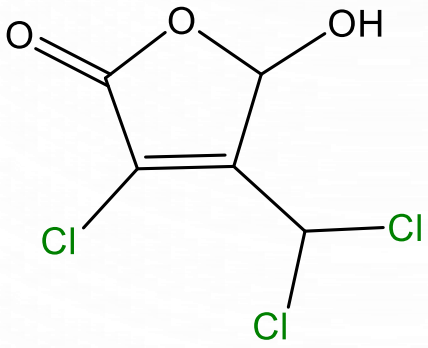




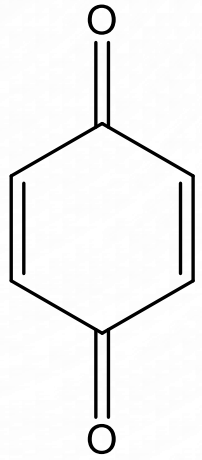
Nitrogenous byproducts

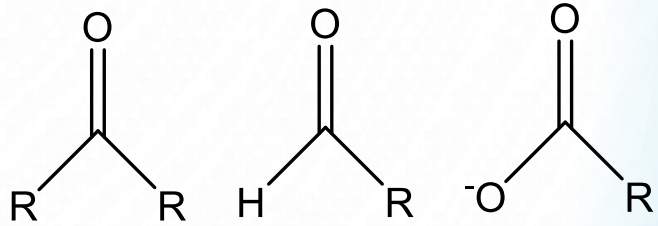






Halogenated cyclic byproducts





Non-halogenated byproducts

700+ disinfection byproducts identified, but this represents ~60% of the halogenated fraction

TRIHALOMETHANES ARE SURROGATES FOR TOTAL DBP FORMATION

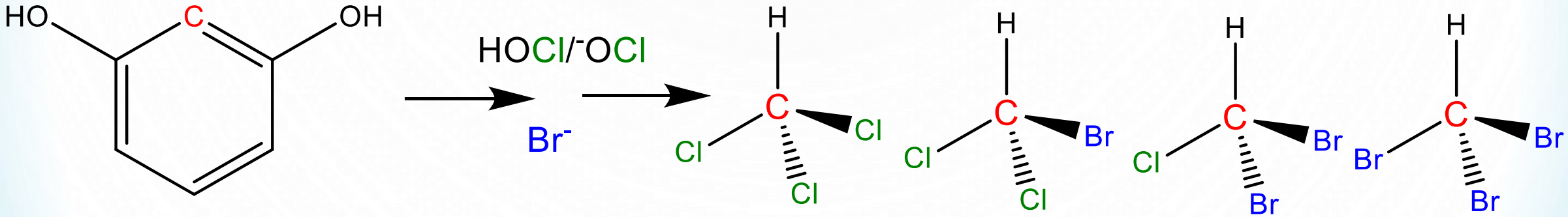
Research

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP4495.

Trihalomethanes in Drinking Water and Bladder Cancer Burden in the European Union

Iro Evlampidou,[1,2,3] Laia Font-Ribera,[1,2,3,4] David Rojas-Rueda,[1,2,3] Esther Gracia-Lavedan,[1,2,3] Nathalie Costet,[5] Neil Pearce,[6] Paolo Vineis,[7] Jouni J.K. Jaakkola,[8] Francis Delloye,[9] Konstantinos C. Makris,[10] Euripides G. Stephanou,[11,12] Sophia Kargaki,[11] Frantisek Kozisek,[13] Torben Sigsgaard,[14] Birgitte Hansen,[15] Jörg Schullehner,[15,16] Ramon Nahkur,[17] Catherine Galey,[18] Christian Zwiener,[19] Marta Vargha,[20] Elena Righi,[21] Gabriella Aggazzotti,[21] Gunda Kalnina,[22] Regina Grazuleviciene,[23] Kinga Polanska,[24] Dasa Gubkova,[25] Katarina Bitenc,[26] Emma H. Goslan,[27] Manolis Kogevinas,[1,2,3,4] and Cristina M. Villanueva[1,2,3,4]

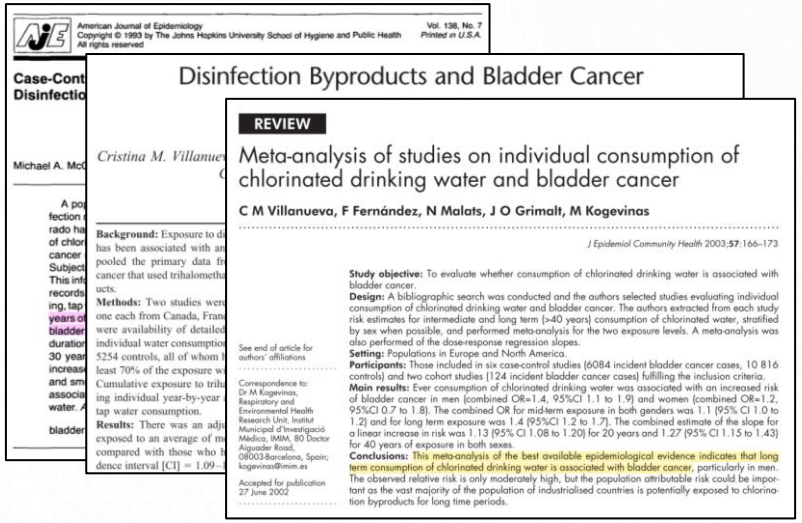
"Byproducts, if consumed in excess of EPA's standard over many years, may increase health risks" (US EPA)



"none of the chlorination DBPs so far identified in drinking water are plausible bladder carcinogens" Hrudey, 2009

MISMATCH BETWEEN EPIDEMIOLOGY AND TOXICOLOGY

Epidemiological studies



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Case-Cont
Disinfectio

Michael A. Mc

Disinfection Byproducts and Bladder Cancer

Cristina M. Villanue

REVIEW

Meta-analysis of studies on individual consumption of chlorinated drinking water and bladder cancer

C M Villanueva, F Fernández, N Malats, J O Grimalt, M Kogevinas

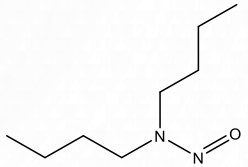
J Epidemiol Community Health 2003;57:166–173

Risk of bladder cancer attributed to DBPs

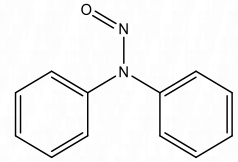
DBPs responsible are unknown

Toxicological studies

Only 2 bladder carcinogens among DBPs



NDBA



NDPhA

Concentrations in water too low to account for risk of bladder cancer observed

Hypothesis: causal agents (bladder carcinogens) yet to be identified.

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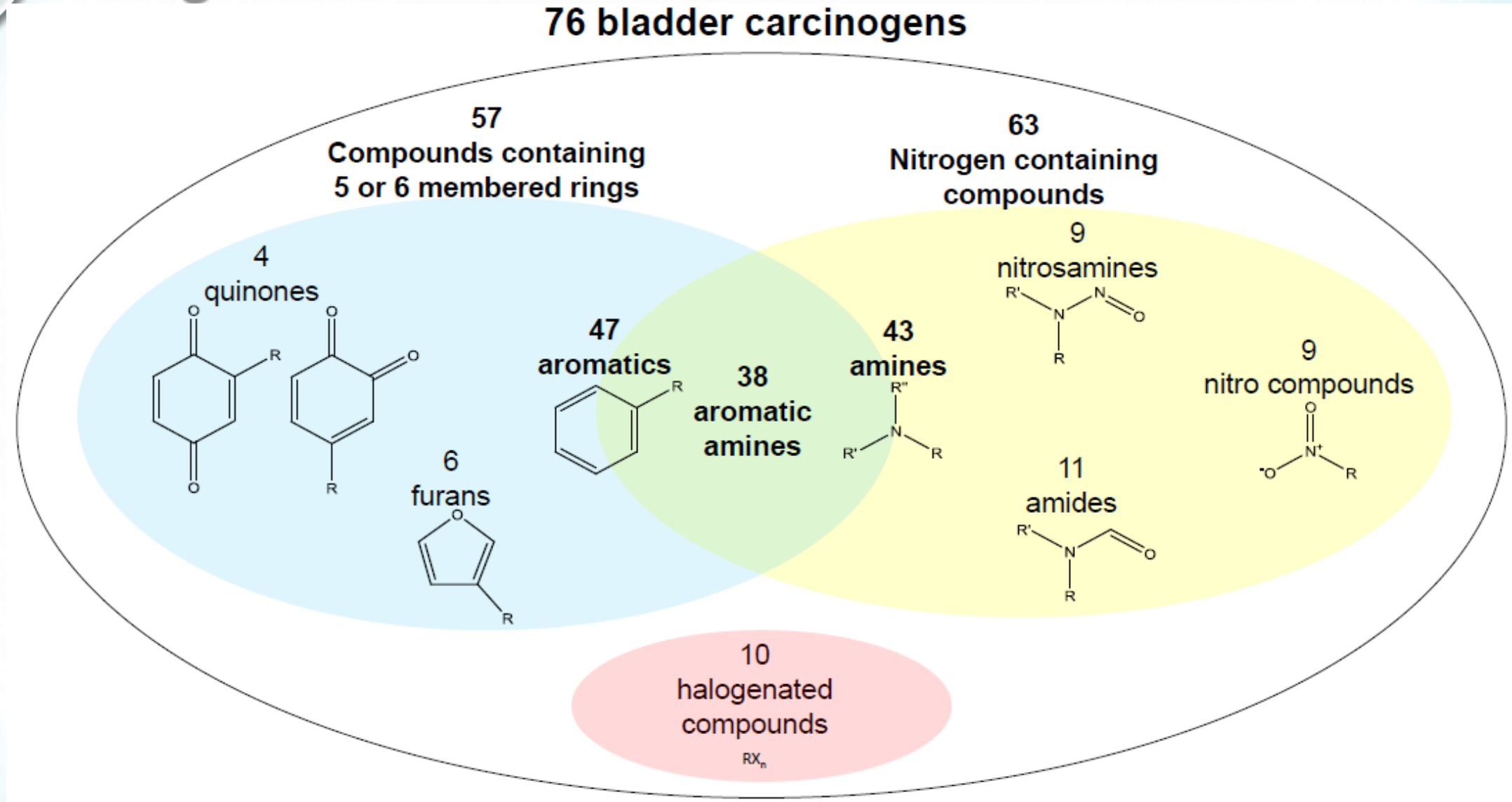
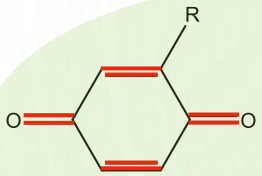
STRUCTURE OF KNOWN BLADDER CARCINOGENS

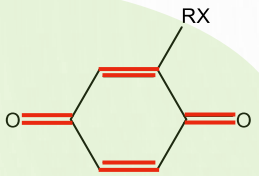


CANDIDATE CHLORINATION BYPRODUCTS AND MODEL PRECURSORS

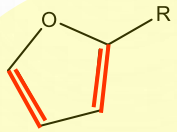
Candidate DBPs:



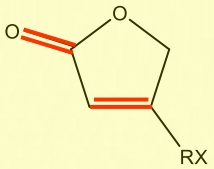
Quinones



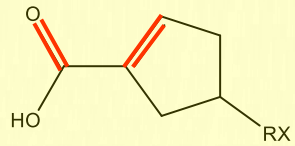
Haloquinones



Furans

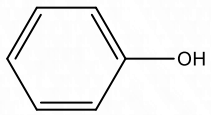


Halofuranones (MX compounds)



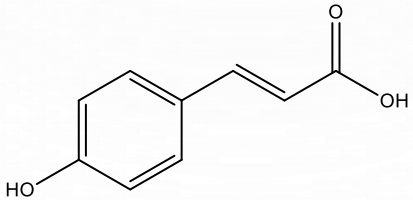
Halocyclopentenoic acids

Precursors selected:

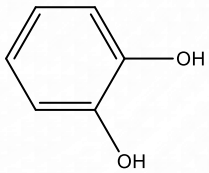


Phenol

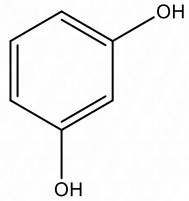
Hydroquinone



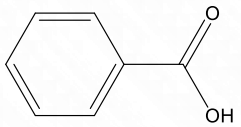
4-hydroxycinnamic acid



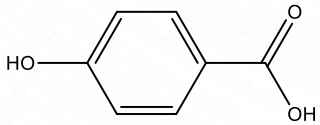
Pyrocatechol



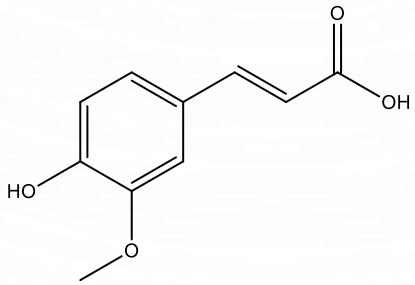
Resorcinol



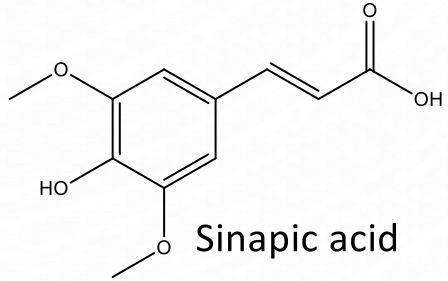
Benzoic acid



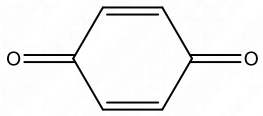
4-hydroxybenzoic acid



Trans-ferulic acid



Sinapic acid



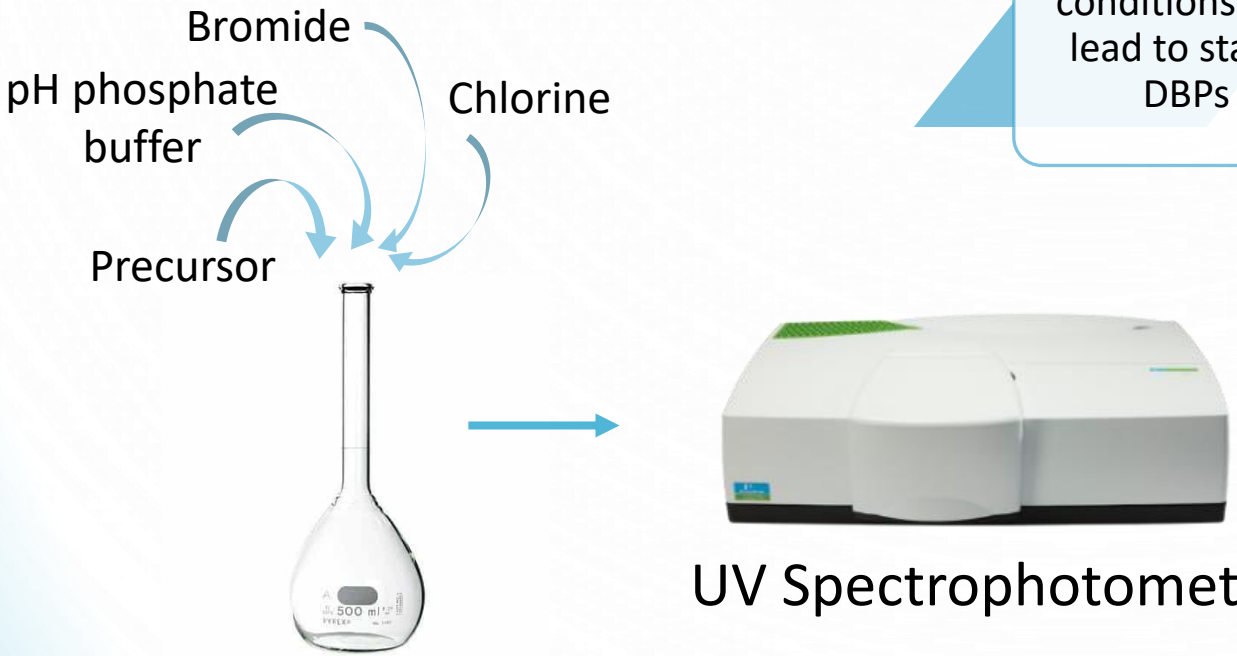
1,4-benzoquinone

UV ABSORBANCE SCREENING EXPERIMENTS

Literature research of potential candidates and their precursors

Chlorination of precursors under different conditions

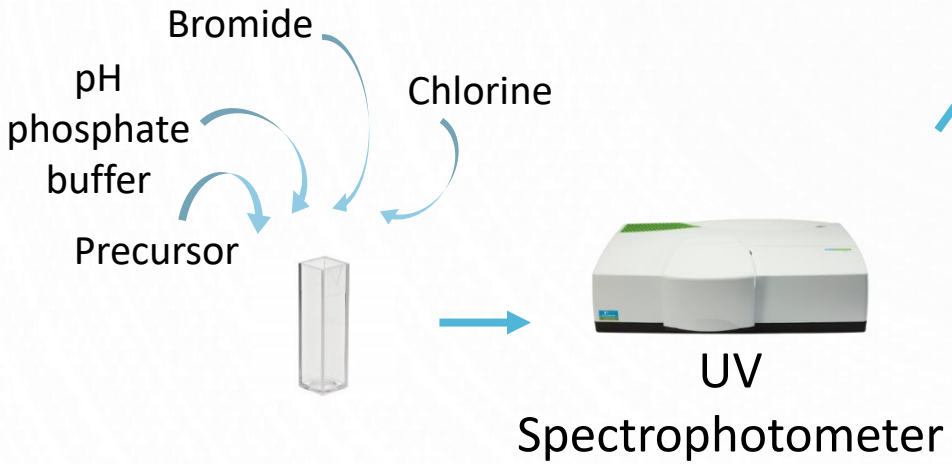
Selection of conditions that lead to stable DBPs

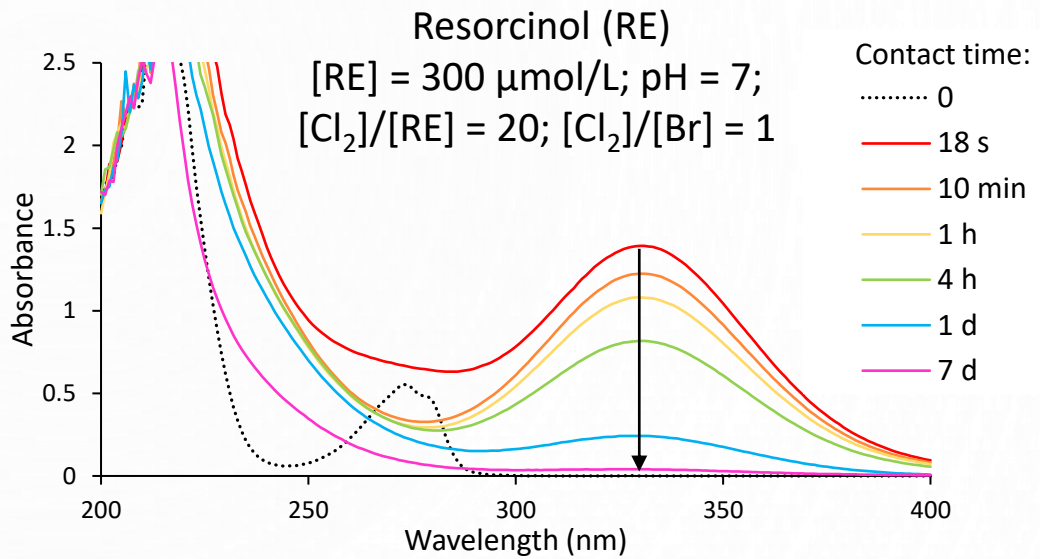


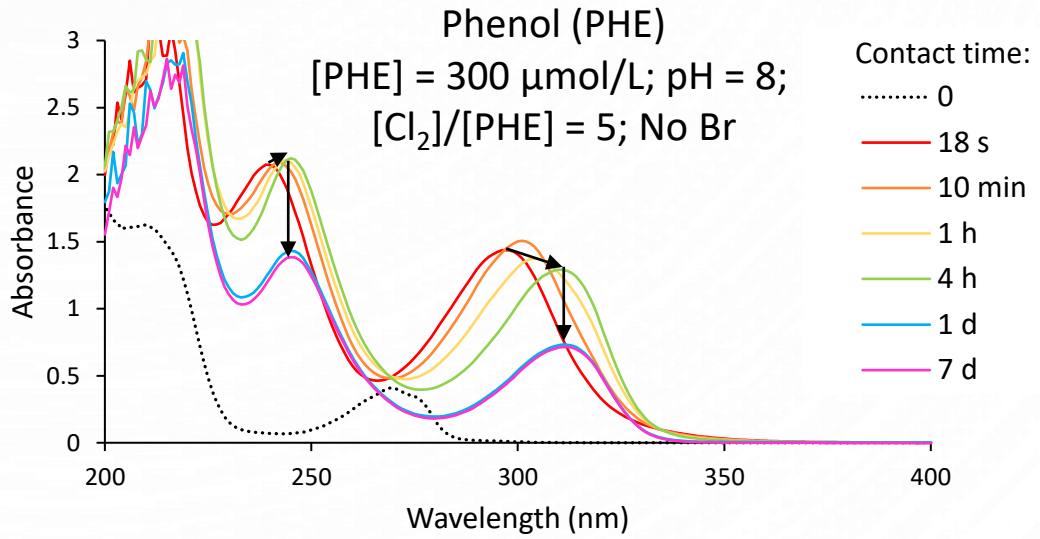
UV Spectrophotometer

Variables	Values selected
pH	6; 7; 8
Cl_2 / precursor molar ratio	5; 10; 20
Cl_2 / Br molar ratio	1; no bromide
Contact time	From 18 s to 7 days

RESULTS OF UV SCREENING EXPERIMENTS







97 different experimental conditions.
10, from six model precursors, selected for subsequent analysis.

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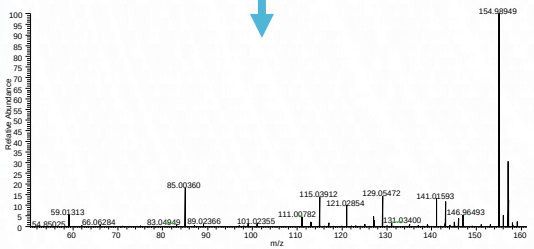
BYPRODUCT IDENTIFICATION USING HIGH RESOLUTION LIQUID CHROMATOGRAPHY- MASS SPECTROMETRY

Analysis HPLC MS/MS (Orbitrap)
Positive + negative mode
C18 column

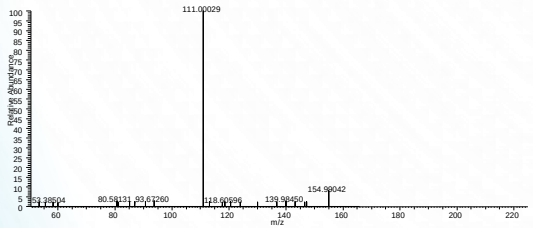


MS spectrum

Compound discoverer software (Thermo)

- Compound formula
- Relative mass error Δm

➢ Several possible isomers



MS/MS spectrum

Metfrag software

- Comparison with predicted fragmentation spectra

➢ Reduction of number of possible isomers

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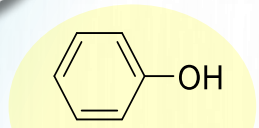
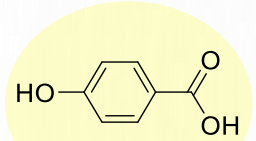
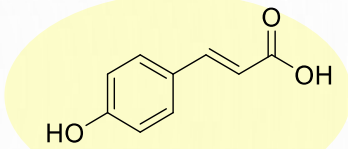
TENTATIVE IDENTIFICATION OF STABLE CHLORINATION BYPRODUCTS



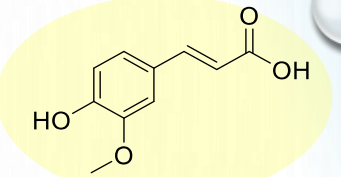
Phenol



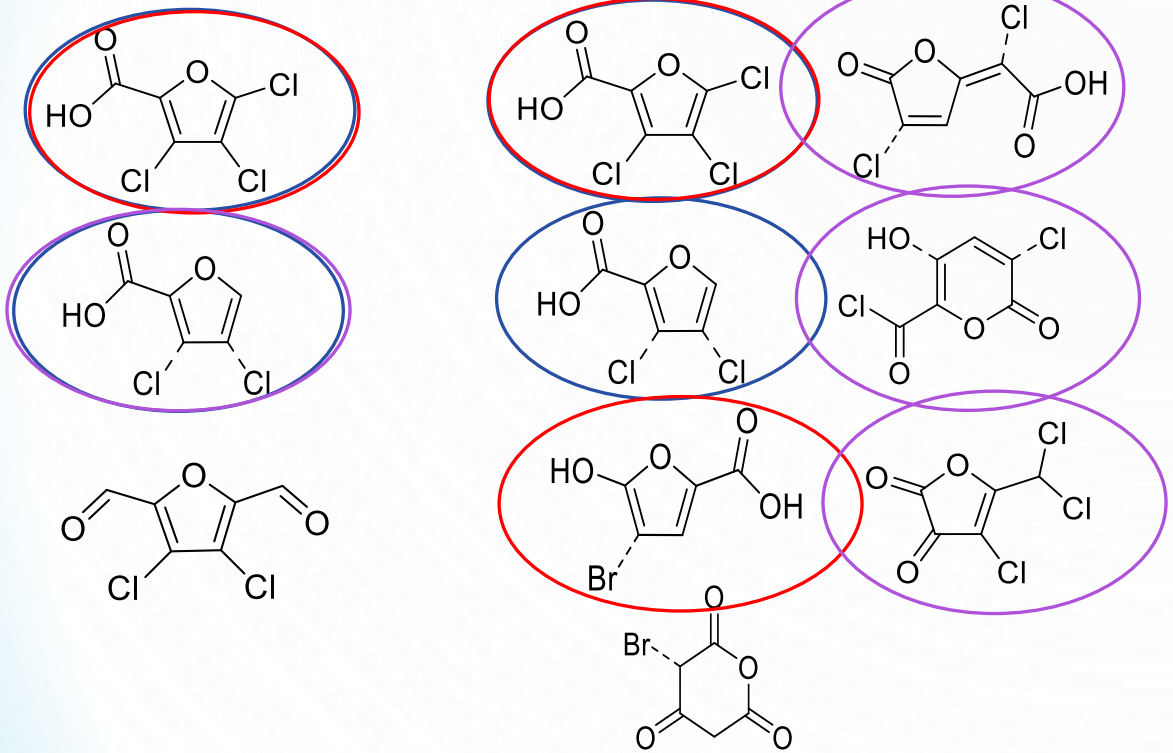
4-hydroxybenzoic acid

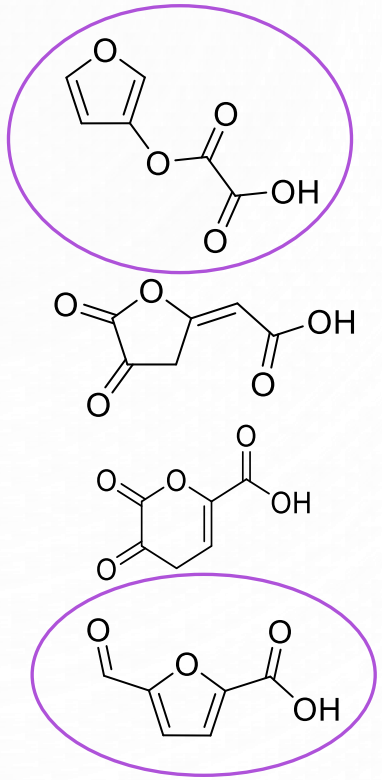


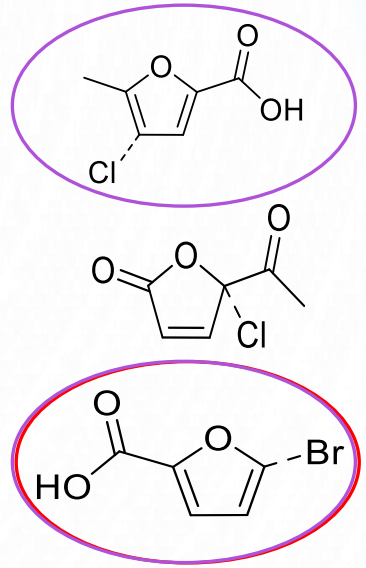
4-hydroxycinnamic acid



Trans-ferulic acid



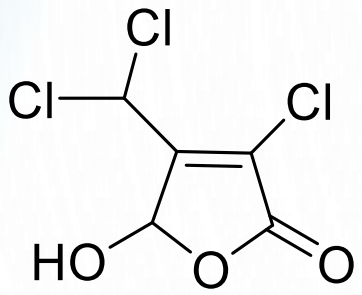




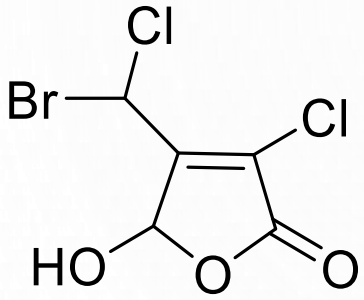
Chemical formulas for 30 stable DBPs elucidated, including 12 furan-like compounds

Eight predicted mutagens and three predicted bladder carcinogens.

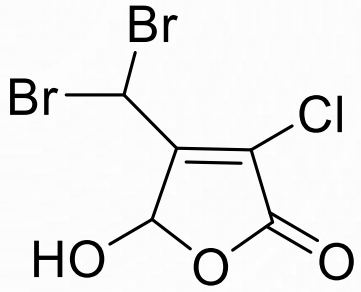
FURANS AS CHLORINATION BYPRODUCTS



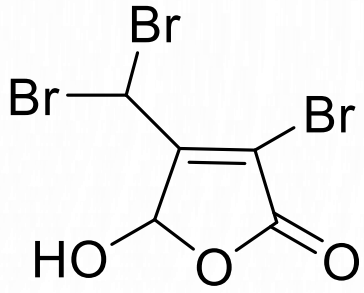
MX



BMX-1

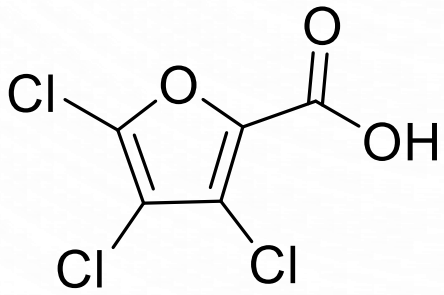


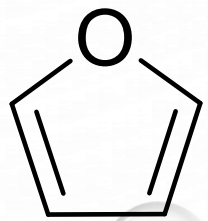
BMX-2



BMX-3

57 furan-type compounds reported as DBPs in literature; 44 predicted mutagens, 10 potential bladder carcinogens.





CONCLUSIONS

- Cyclic byproducts can be stable terminal products of chlorination reactions (not just intermediates).

- 12 furan-type compounds were tentatively identified from model precursors. 11 have never been reported previously as DBPs.

- Eight were predicted to be mutagenic and three were predicted to be bladder carcinogens; this group of byproducts may be toxicologically significant for the urinary bladder.

WHAT DOES THIS HAVE TO DO WITH EPIDEMIOLOGICAL EXPOSURE ASSESSMENT?

- Toxicological and (particularly) epidemiological assessments lag behind the identification of new byproducts using increasingly sophisticated analytical chemistry techniques. Insufficient occurrence data for most byproducts to use as exposure metrics.

- THMs are surrogates for total exposure to halogenated byproducts – but are they good surrogates?

- Can widespread water quality parameters (e.g. TOC, SUVA, pH, Br, I) be used as the basis for alternative exposure metrics?

- Profiles of byproducts in pristine and impacted water sources are different – are different exposure metrics appropriate?

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THANKS FOR YOUR ATTENTION

ANY QUESTIONS?

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2. CONDITIONS FORMING STABLE DBPS

10 sets of conditions selected for DBP identification:

	Precursor	pH	Cl_2 dose	Br dose	Contact Time	λ_{max} of new peaks (nm)
1	Phenol	8	5	0	> 1 d	310
2	Benzoic acid	6	20	0	> 1 min	224
3	4-hydroxybenzoic acid	8	10	0	1 min	280
4	4-hydroxybenzoic acid	8	10	0	30 min	310
5	4-hydroxybenzoic acid	8	5	1	> 4 d	270
6	4-hydroxybenzoic acid	8	5	0	> 1 d	310
7	4-hydroxycinnamic acid	6	5	0	> 1 d	255; 300
8	Trans-ferulic acid	8	5	0	> 3 h	255
9	Trans-ferulic acid	6	5	1	> 1 d	300
10	Sinapic acid	6	5	0	> 1 d	265

CARCINOGENIC DBPS AND THEIR POTENCY

	DBP	Animal	Target organ	TD_{50} (mg/kg/day)	Concentration in drinking water (µg/L)	TD_{50} / concentration (L/kg/day)	References
THMs	Chloroform	Rat, mouse	Kidney, liver	262 (rat) 111 (mouse)	< 120	925	(Gold, 2011; Lipmann, 2009)
	Bromodichloromethane	Rat	Liver, pituitary gland	72.5	< 8	9,063	(Gold, 2011; Lipmann, 2009)
HAAs	Dichloroacetic acid	Rat, mouse	Liver	161 (rat) 119 (mouse)	< 40	2,975	(Gold, 2011; Lipmann, 2009)
	Bromochloroacetic acid	Rat, mouse	Large intestine, mammary gland, peritoneal mesothelium, liver		< 19		("National Toxicology Program (NTP)," n.d.; Lipmann, 2009)
	Dibromoacetic acid	Rat, mouse	Peritoneal mesothelium, hematopoietic system, liver, lung		< 18		("National Toxicology Program (NTP)," n.d.; Lipmann, 2009)
	Trichloroacetic acid	Rat, mouse	Liver	584 (mouse)	< 80	7,300	(Gold, 2011; Lipmann, 2009)
HANs	Dibromoacetonitrile	Rat, mouse	Oral cavity, stomach		< 3		("National Toxicology Program (NTP)," n.d.; Lipmann, 2009)
Halofuranones	**MX (mutagen X; 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]furanone)**	Rat	Thyroid, liver, adrenal, lungs, pancreas	**0.583**	**< 0.85**	**686**	(Gold, 2011; Richardson, 2011)
Aldehydes	Chloroacetaldehyde	Mouse	Liver	36.1	< 2.4	15,042	(Gold, 2011; Lipmann, 2009)
Nitrosamines	NDMA (N-nitrosodimethylamine)	Rat, mouse	Liver, lung	0.0959 (rat) 0.189 (mouse)	< 10	9.6	(Gold, 2011; Lipmann, 2009)
	NDEA (N-nitrosodiethylamine)	Rat	Liver, oesophagus	**0.0265**	**< 10**	**2.7**	(Gold, 2011; Lipmann, 2009)
	NDMOR (N-nitrosomorpholine)	Rat, hamster	Liver, vasculature, nasal, oral cavity	0.109 (rat) 3.57 (hamster)	< 10	11	(Gold, 2011; Lipmann, 2009)
	NDPYR (N-nitrosopyrrolidine)	Rat, mouse, hamster	Kidney, liver, vasculature	0.199 (rat) 0.679 (mouse) 14.2 (hamster)	< 10	20	(Gold, 2011; Lipmann, 2009)
	NDPIP (N-nitrosopiperidine)	Rat, hamster	Liver, nasal cavity, oral cavity, oesophagus	1.43 (rat) 83.3 (hamster)	< 10	143	(Gold, 2011; Lipmann, 2009)
	NDBA (Nitrosodibutylamine)	Rat, mouse	Liver, lung, stomach, **urinary bladder**, oesophagus	**0.691 (rat)** **1.09 (mouse)**	**< 0.1**	**6,910**	(Gold, 2011; Li et al., 2011; Wang et al., 2011)
	NDPhA (N-nitrosodiphenylamine)	Rat	**Urinary bladder**	**167 (rat)**	**< 0.1**	**1,670,000**	(Gold, 2011; Li et al., 2011)
Oxyhalides	Bromate	Rat, hamster, mouse	Kidney, thyroid, liver, lung, tunica vaginalis, peritoneum	9.82 (rat) 53.8 (mouse) 533 (hamster)	< 25	393	(Gold, 2011; Richardson, 2011)
	Chlorate	Rat	Thyroid		< 190		(Lipmann, 2009; Richardson, 2011)
Other	Chloral hydrate	Mouse	Liver	99.9	< 16	6,243	(Gold, 2011; Krasner et al., 2006)
	2-bromoethanol	Mouse	Stomach, liver, lung	70.1			(Gold, 2011)
	Hydrogen peroxide	Mouse	Small intestine	7540			(Gold, 2011)
	1,4-dioxane	Rat, mouse	Liver, mammary gland, nasal cavity, peritoneum, subcutaneous tissue	267 (rat) 204 (mouse)			(Gold, 2011)

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METHOD HPLC-MS/MS

HPLC-Orbitrap : 1250 Pump + Velos LTQ Orbitrap (Thermo Scientific)

Injection volume : 20 µL
Flow rate: 0.5 mL/min
Column: Zorbax Eclipse XDB-C18 analytical column 4.6 x 150 mm, 5 µm (Agilent)
Elution gradient using acetonitrile (eluent A) and water with 0.1% ammonium acetate (eluent B) as eluents

Ionisation: electrospray source
Polarity: negative mode
Full-scan mode 50 – 450 m/z

Initial precursor concentration: 10 mg/L

Step	Start (min)	Duration of step (sec)	Gradient type	% A	% B
1	0.00	60	Step	5	95
2	1.00	540	Ramp	95	5
3	10.00	180	Ramp	95	5
4	13.00	120	Ramp	95	5
5	15.00	60	Ramp	5	95
6	16.00	60	Step	5	95

RISK OF BLADDER CANCER IN WATER

2 - 17% of bladder cancers attributed to the consumption of drinking water

Strength of association between bladder cancer and drinking water consumption quite low compared to that of other epidemiological studies

Risk of death for drinking 2 L of tap water / day:

9 times less than drinking 1 can of diet soda / day
2000 times less than smoking 10 cigarettes / day

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