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## Disinfection by-products in drinking water: Where do they come from and are they safe to drink?

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**Abstract:** During drinking water disinfection, potentially harmful disinfection by-products are formed due to the reaction of organic matter with chlorine. Therefore, in this research, human intestinal epithelial cells (Caco-2) were exposed to haloacetamides (HAcAm) and haloacetic acids (HAAs). A 24, 48 and 72h exposure was conducted to determine the cytotoxicity of these compounds. The unregulated HAcAms were clearly more cytotoxic than the regulated HAAs. Moreover, changing the halogen from Cl to I increased the toxicity extensively. Hence, a membrane fractionation process is developed to split organic matter into three fractions which should facilitate the identification of both HAA and HAcAm precursors after chlorination.

**Keywords:** Membrane fractionation; Disinfection by-products; Modelling

### Introduction

Disinfection of drinking water is a widely used technique to inactivate pathogens. However, harmful disinfection by-products (DBPs) are formed due to the reaction with natural organic matter (NOM) [1]. Unregulated DBPs, such as haloacetamides (HAcAms), are found in lower concentrations compared to the regulated trihalomethanes and haloacetic acids (HAAs), but it is suggested that their toxicity is much higher [2].

Research with Chinese Hamster Ovary (CHO) cells showed that iodoacetamide has 2 times higher cytotoxicity than iodoacetic acid [3]. However, limited studies conducted on human cell lines (colonic epithelial cells, CCD 841 CoN) showed that iodoacetic acid is 4 times more cytotoxic than iodoacetamide [4].

To identify the precursors that are responsible for the formation of these by-products from the complex NOM mixture, the latter is split into smaller fractions with similar chemical or physical properties. Dead-end ultrafiltration membrane fractionation is the most commonly used technique to split NOM by size, but is unable to produce sharply separated fractions [5].

This research will therefore, (i) seek for a new membrane fractionation method to split NOM into biopolymers (BP), humic substances (HS) and low molecular weight compounds (LMW) to identify DBP precursors after chlorination and (ii) couple this information to the toxicity of HAAs and HAcAms on human intestinal epithelial cells (Caco-2).

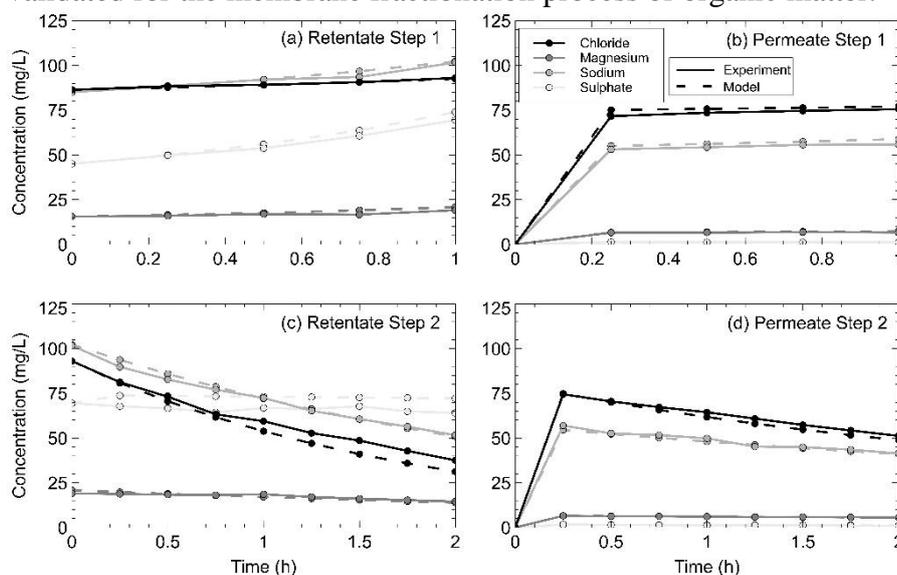
### Material and Methods

During fractionation, a nano- and ultrafiltration membrane in crossflow is used where both a normal filtration step (step 1) and a diafiltration step (step 2) is executed to ensure a good separation between the three fractions. So far, a model has been constructed that predicts the concentration change for a certain compound during these two steps and was validated with a nanofiltration for different ions in a synthetic ion matrix (Dow NF270).

$10^4$  cells/well were seeded in a 96-well plate and incubated with 100  $\mu$ L cell medium containing increasing concentrations of either chloroacetamide (Cl-AcAm), bromoacetamide (Br-AcAm), iodoacetamide (I-AcAm), dichloroacetamide (Cl<sub>2</sub>-AcAm), trichloroacetamide (Cl<sub>3</sub>-AcAm), chloroacetic acid (Cl-AA), bromoacetic acid (Br-AA) or iodoacetic acid (I-AA) for 24h, 48h and 72h. 10  $\mu$ L of 5 mg/mL MTT (3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl tetrazolium bromide) stock solution was added at the end of the stimulation period. The MTT is reduced by metabolic active cells and converted into formazan crystals. These crystals were dissolved with 100  $\mu$ L DMSO and absorbance was measured at 570 nm.

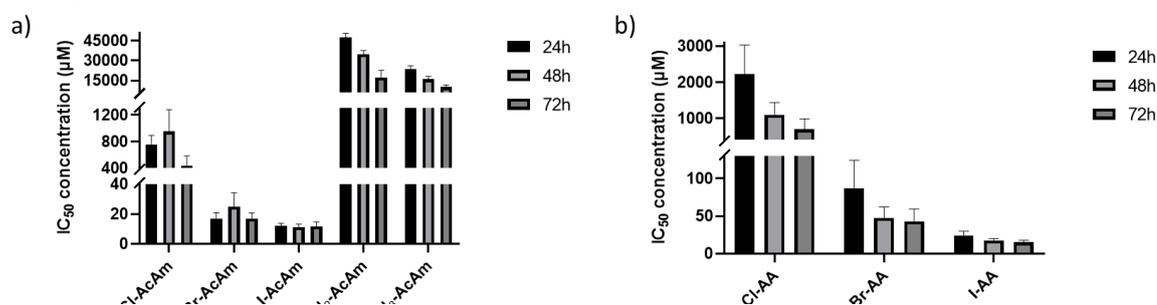
## Results and Conclusions

Figure 1.1. clearly shows that the model predicts the experimental data very well. The model is ready to be validated for the membrane fractionation process of organic matter.



**Figure 1.1.:** Concentration change of chloride, magnesium, sodium and sulphate during normal filtration (step 1 (a,b)) and diafiltration (step 2 (c,d)). Full lines represent experimental data, dashed lines represent predicted data.

Figure 1.2. shows the half maximal inhibitory concentration ( $IC_{50}$ ) deduced from the dose-response curves ( $r^2 > 0.75$ ) for HAcAms and HAAs. HAcAms have overall lower  $IC_{50}$  values compared to the HAAs, similar to the conclusions drawn from CHO cells. However, the  $IC_{50}$  values found in this study were generally higher than the ones reported for CHO [3]. Furthermore, the toxicity within one family increases when changing the halogen from Cl to Br to I, except for Cl<sub>2</sub>-AcAm and Cl<sub>3</sub>-AcAm ( $IC_{50} = 15\ 000 - 40\ 000\ \mu$ M).



**Figure 1.2.:**  $IC_{50}$  values ( $\mu$ M) determined after 24h, 48h and 72h for, (a) chloro- (Cl-AcAm), bromo- (Br-AcAm), iodo- (I-AcAm), dichloro- (Cl<sub>2</sub>-AcAm), trichloroacetamide (Cl<sub>3</sub>-AcAm) and, (b) chloro- (Cl-AA), bromo- (Br-AA) and iodoacetic acid (I-AA). Error bars represent the 95% confidence interval.

To conclude, the unregulated HAcAms appear to be more cytotoxic than the regulated HAAs. Therefore, it is important to identify their precursors through the membrane fractions, and remove them if needed, to control the HAcAm concentrations in drinking water.



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