Bioanalytical assessment of toxicity change during ozonation of methyl paraben and its halogenated derivatives

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Abstract

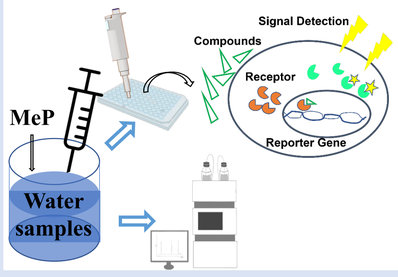
Methylparaben (MeP), a common ingredient in personal care products, is frequently detected in aquatic environments at concentrations ranging from ng/L to µg/L. Its halogenated derivatives, including mono- (Cl-MeP and Br-MeP) and di-halogenated (diCl-MeP, Br,Cl-MeP, diBr-MeP) forms, are also prevalent due to the transformation of MeP during water chlorination. Previous research has indicated that MeP and halo-MePs can undergo oxidative degradation during water treatment processes like ozonation, potentially giving rise to toxic byproducts. However, relevant information on this matter is currently limited. This study aimed to assess the toxicity of transformation products formed during ozonation of MeP and halo-MePs using bioanalytical methods, specifically *in vitro* bioassays covering oxidative stress response (Nrf2) and genotoxicity (p53 alone and p53 with S9 activation). Each paraben, prepared at a concentration of 100 uM, underwent treatment with ozone concentration ranging from 0 from 230 uM in the presence of *tert*-butanol (as OH radical scavenger). The resulting samples were analyzed using both the bioassays and HPLC/UV for residual paraben concentrations. The results showed measurable bioactivities in Nrf2 and p53 with S9 activation for the tested parabens, but negligible activities (below LOQ) in p53 alone. In addition, the bioactivites of ozonated samples were significantly higher than those of MeP and halo-MePs prior to ozonation, with increases of up to ~25-fold for Nrf2 and ~5-fold for p53/S9. These heightened bioactivities persisted even after the complete degradation of parent parabens by ozone, indicating the presence of stable toxic transformation products. Future studies should focus on identification of these toxic byproducts, and the efficacy of post-treatment processes such as biological filtration for their removal should be assessed.

Research objectives

- To determine the reaction stoichiometries of MeP and halo-MePs with ozone
- To assess the bioactivities of MeP and halo-MePs using in vitro bioassays (Cytox, Nrf2, p53)
- To assess the bioactivity changes (Nrf2 and p53±S9) induced by ozone treatment of MeP and halo-MePs

Materials & Methods

Experimental Scheme



Responses of samples' bioactivities were expressed as bioanalytical equivalent concentrations (BEQ) relative to the reference compounds used in each CALUX bioassay.

$$BEQ = \frac{EC_{IR1.5}(ref)}{EC_{IR1.5}(sample)}$$

Table 1. Summary of bioassays, end point, method reference, reference compound, effect concentrations (EC), limit of quantifications (LOQ)

CALUX assay	End point	Method reference	Reference compound	Reference compound EC value	Limit of quantification
Cytox	Cytotoxicity	Van der Linden et al.,2014	Tributyltin (TBT)	7.13×10^{-5} M (EC_{50}) [a]	1.0×10^{-9} M
Nrf2	Oxidative stress	Van der Linden et al.,2014	Curcumin (Cur)	3.0×10^{-6} M ($EC_{IR1.5}$) [b]	1.0×10^{-9} M
p53(±S9)	Genotoxicity	Van der Linden et al.,2014	Actinomycin D (Act)	2.12×10^{-8} M ($EC_{IR1.5}$) [b]	1.0×10^{-11} M

Results & Discussion

Reaction stoichiometries of MeP and halo-MePs with ozone

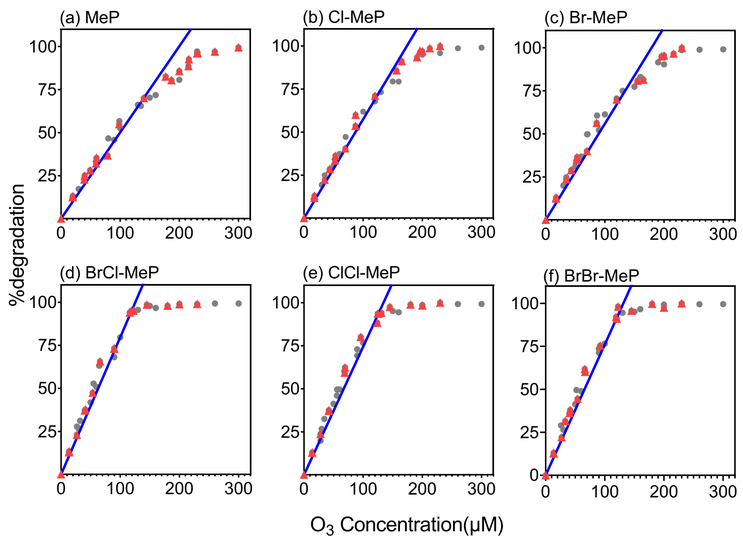


Figure 1. % degradation of parabens as a function of ozone concentration. 100 µM of each paraben was treated by ozone (0 to 230 µM) in the presence of 10 mM *tert*-butanol as OH radical scavenger. Dots indicate the degradation percentage, the triangles represent the sample used for the bioassay. Solid lines are used to guide the data.

- Dihalogenation increases the methylparaben's reactivity with ozone.
- The observation that degradation rates plateau at higher ozone concentrations (>100 µM) suggests formation of transformation products. These products may still react with ozone, leading to further breakdown, but at a slower rate.

Changes of adaptive stress responses (Nrf2) during degradation of MeP and halo-MePs by ozone

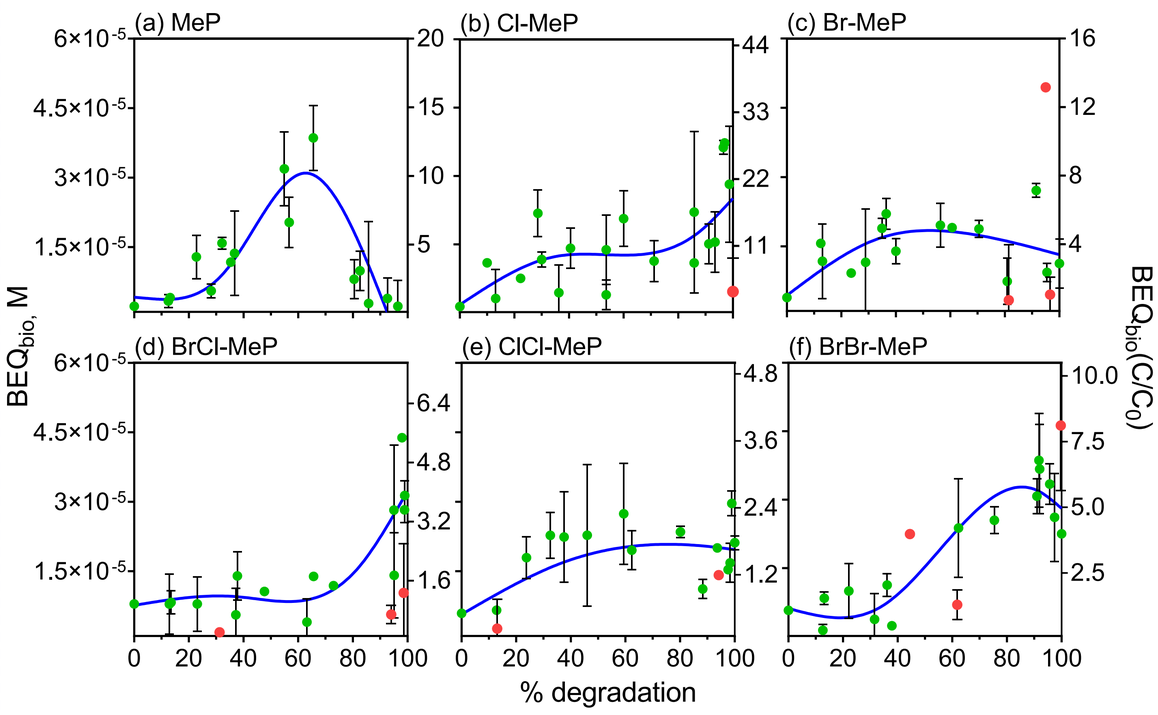


Figure 2. Evolution of adaptive stress responses (expressed as BEQ (left) and relative BEQ (right) as a function of % degradation of parabens by ozone. Dots represent the measured data, and solid lines depict the non-linear regressions to guide the data. Red dots are classified as outliner and not included in the non-linear regressions.

- Parabens with more halogen substitution showed higher initial bioactivity.
- MeP showed an increase in bioactivity up to approximately 60% degradation. This suggests that initial transformation products may be more bioactive than the parent compound. However, further degradation led to a decrease in bioactivity, possibly due to the breakdown of these primary transformation products.
- In the case of Cl-MeP and BrCl-MeP, a steady increase in bioactivity with increasing degradation was observed. This indicates that their transformation products continued to exhibit bioactivity.
- Unlike MeP, halo-MePs did not completely lose their bioactivity even after full degradation. This indicates that the bioactive transformation products of halo-MePs are relatively stable.
- Cytotoxicity in all samples was below LOQ levels.

Changes of genotoxicity (p53 alone-left figure set and p53 with S9 activation-right figure set) during degradation of MeP and halo-MePs by ozone

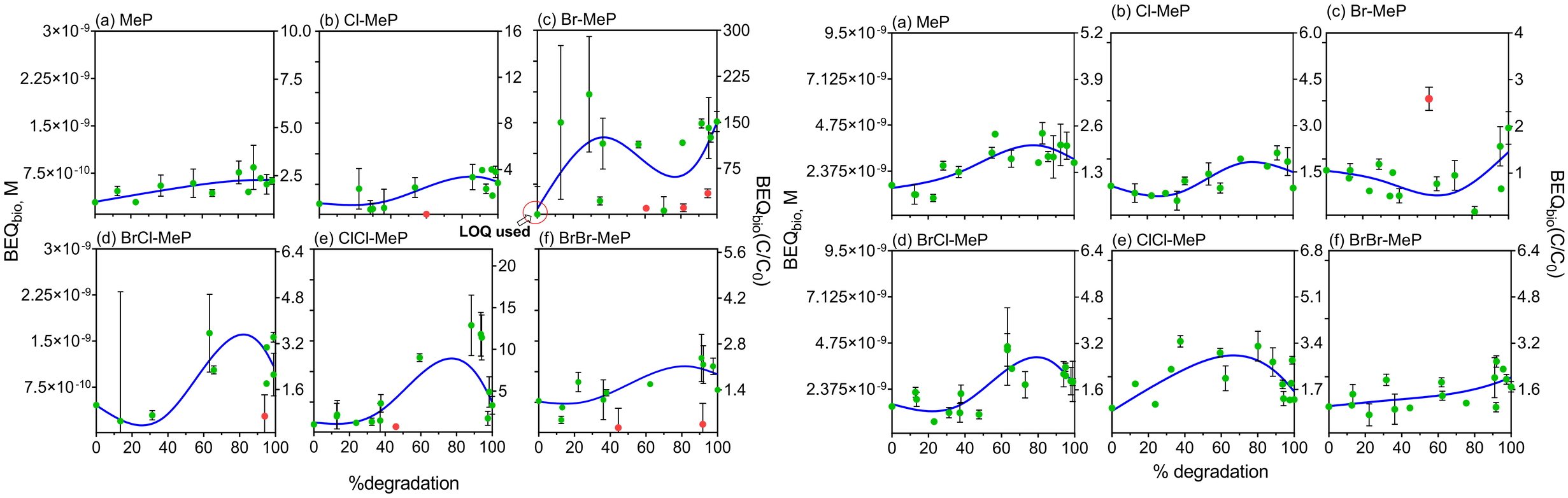


Figure 3. Evolution of genotoxicity measured by p53 alone (left figure set) and p53 with S9 activation (right figure set) as a function of % degradation of parabens by ozone. The bioactivities were expressed as bioanalytical equivalent concentrations (BEQ) of the reference compounds in each bioassay (see Table 1). Dots represent the measured data, and solid lines depict the non-linear regressions to guide the data. Red dots are classified as outliers and not included in the non-linear regressions.

In p53 alone-(left)

- Initial BEQbio values were significantly low.
- Dihalo-MePs exhibited similar patterns, with an initial increase in bioactivity followed by a decrease.
- Among halo-MePs, Cl groups attached show the significant changes due to the small initial values specifically, for ClCl-MeP, significant changes were observed at 90% degradation.

In p53 with S9 activation set -(right)

- BEQ_{bio} values are higher regardless of the presence of ozone treatment than p53 cell alone.
- Dihalo-MePs show increased bioactivity up to 5 fold when using the ozonated samples.

Conclusions

- Bioactivities were observed for Nrf2 and p53 with S9 activation for the tested parabens, but negligible bioactivities (below LOQ) were found for p53 alone.
- The bioactivities of ozonated samples were significantly higher than those of MeP and halo-MePs prior to ozonation, with increases of up to ~25-fold for Nrf2 and ~5-fold for p53/S9.
- The increased bioactivities persisted even after the complete degradation of parent parabens. Structural identification, and options for post-treatment processes for the removal of these toxic transformation products are warranted

Acknowledgement

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