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RESEARCH ARTICLE

Assessment of individual and mixed toxicity of bromoform, tribromoacetic-acid and 2,4,6 tribromophenol, on the embryo-larval development of *Paracentrotus lividus* sea urchin

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Abstract

Water chlorination is the most widely used technique to avoid microbial contamination and biofouling. Adding chlorine to bromide-rich waters leads to the rapid oxidation of bromide ions and leads to the formation of brominated disinfection by-products (bromo-DBPs) that exert adverse effects on various biological models. Bromo-DBPs are regularly encountered within industrialized embayments, potentially impacting marine organisms. Of these, bromoform, tribromoacetic acid and tribromophenol are among the most prevalent. In the present study, we tested the potential toxicity and genotoxicity of these disinfection by-products, using sea urchin, *Paracentrotus lividus*, embryos. We highlighted that tribromophenol showed higher toxicity compared to bromoform and tribromoacetic acid. Furthermore, a synergistic effect was detected when tested in combination. Pluteus cells exposed for 1 h to mixtures of DBPs at several concentrations demonstrated significant DNA damage. Finally, when compared to a non-exposed population, sea urchins living in a bromo-DPB-polluted area produced more resistant progenies, as if they were locally adapted. This hypothesis remains to be tested in order to better understand the obvious impact of complex bromo-DBPs environments on marine wildlife.

Keywords Bromoform · Tribromoacetic acid · Tribromophenol · Disinfection by-products · Sea urchin · *Paracentrotus lividus* · Ecotoxicology · Genotoxicity

Highlights
- Tribromophenol is 10 to 30 times more toxic than bromoform and tribromoacetic acid
- Bromo-DBPs are genotoxic
- Effective concentrations are several orders of magnitude higher than environmental ones
- Progenies of locally exposed adults are resistant to bromoform
- Potential local adaptation to bromo-DBPs (bromoform)

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Introduction

Chlorination has been used worldwide, for decades, and is one of the most effective treatments for water disinfection in treatment plants for the production of tap water. Chlorination is also employed in the management and upkeep of industrial installations, where water is often used for cooling or heating purposes, as well as to prevent and control biofouling and corrosion of pipelines. Hence, in some industrialized embayment areas, huge volumes of coastal seawater are continuously being pumped and chlorinated before being re-released into the original embayment (Allonier et al. 1999; Boudjellaba et al. 2016; Manasfi et al. 2018). As chlorine is very reactive in the presence of natural organic matter, seawater chlorination generates a complex set of brominated and chlorobrominated disinfection by-products (DBPs). Finally, benthic organisms, resident in the embayment, are exposed to these DBPs (Singer 1999; Westerhoff et al. 2004; Halpern et al. 2008). Although the diversity and occurrence of brominated DBPs in contaminated coastal seawater is relatively undocumented (Richardson et al. 2007; Manasfi et al. 2017), bromoform (halomethane), tribromoacetic acid (haloacetic acid) and tribromophenol (halophenol) are among the most prevalent molecules, often measured at relatively high concentrations (i.e. μg/L) (Manasfi et al. 2018).

Most of the DBPs so far tested have been found to be toxic and genotoxic in diverse model systems, such as bacteria, mammalian cells or zebrafish embryos. Furthermore, brominated DBPs have generally been shown to be more toxic than their chlorinated analogues (Richardson et al. 2007, 2010; Yang and Zhang 2013; Hanigan et al. 2017). It is worth noting that at the single molecule level, visible effects have always been observed following exposure to high concentrations of DBPs, suggesting that toxic and genotoxic risks are limited (Teixidó et al. 2015). Nevertheless, recent epidemiological research has suggested that lifetime exposure to DBPs, mainly through ingestion, would significantly increase the risk of bladder cancer in humans (2004; Villanueva et al. 2004, 2006). Hence, there are great concerns about the level of human exposure, causing health and sanitary surveillance agencies to enact maximum concentration limits (MCL). For instance, the US EPA recommends MCLs for total halomethane and haloacetic acid of 80 μM and 60 μM, respectively, in safe drinking water. It is notable that these values are relatively consistent with the maximum concentrations measured in the effluents of water treatment plants from the USA, Canada or France (CAREX Canada 2009; Mouly et al. 2009).

There are currently no MCLs for environmental waters that harbor functional ecosystems, and which are potentially chronically exposed to DBPs. Studies that assess the toxicity of DBPs in aquatic animals remain scarce and to our knowledge, only two have tested brominated DBPs in recent years, using a zebrafish embryo model (Teixidó et al. 2015; Hanigan et al. 2017). These two studies tested a total of 11 brominated DBPs, including bromoform (BMF) and tribromoacetic acid (TBAA). Most of the brominated DBPs that were compared, proved toxic for larval development, though only at high concentrations (i.e. > 100 μM). Moreover, short-term exposure to chlorinated wastewater did not have any significant toxic effect. Finally, a genotoxic effect was only detected with chlorodibromomethane and sodium borate at their EC_{50} levels, 160 μM and 0.049 μM, respectively.

In this study, we were interested in broadening the understanding of the toxicological impact of DBPs on coastal marine organisms, using the sea urchin, *Paracentrotus lividus*, as a proxy for the echinoderm class. *Paracentrotus lividus* lives in the first few meters of coastal areas. The species is widely distributed along the north-eastern Atlantic coast and on all Mediterranean coasts. Furthermore, they can be encountered in numerous contrasted habitats that include differences in temperature, salinity and chemical contamination (Bellas et al. 2008). While adults are benthic and relatively sedentary, the larvae produced at each reproductive event are pelagic for up to 4 weeks, thus ensuring species dispersion. The sea urchin is a relevant model organism to assess the effect of potentially harmful molecules. It is more closely related to humans than the mussel (Tu et al. 2006), with two species having been fully sequenced. Reproduction is easily performed in vitro, resulting in high numbers of translucid larvae. Sea urchin larvae have been extensively used to assess the embryo toxicity and embryo genotoxicity of molecules (Hose 1985; McGibbon and Moldan 1986; Morroni et al. 2016; Gharred et al. 2016; Trifuoggi et al. 2017; Neves et al. 2018; Messinetti et al. 2018; Pereira et al. 2018; Dorey et al. 2018).

In this study, our objective was to generate additional data in a model marine organism, of the toxic influence of three major brominated contaminants found in coastal seawater that is subjected to massive anthropogenic disturbance. With this aim in mind, we used sea urchin embryos to assess the toxicity and genotoxic potential of bromoform (halomethane), tribromoacetic acid (haloacetic acid) and tribromophenol (halophenol), tested either alone or in combination, on two populations of *P. lividus*, either chronically exposed or unexposed.

Material and methods

Sea urchins

Adult *Paracentrotus lividus* sea urchins were harvested by scuba diving, on the morning of each experiment, at a non-polluted site (NPS) near the Toulon (Var, France) embayment (Garonne Bay: 43.098503–6.018430) and at a polluted site (PS) where seawater is chronically chlorinated (Manasfi

et al. 2018). *P. lividus* were induced to spawn by gentle shaking.

Sperm and eggs were individually collected, respectively dried with a micropipette and kept on ice and in 100 mL of filtered seawater (FSW). Eggs were microscopically observed to verify maturity before adding dry sperm.

Ten independent (i.e. unrelated) larval populations were produced by arbitrarily mixing one sperm with one egg suspension, in 50 mL of FSW at 20 °C at a concentration of 500 eggs/mL, under agitation for 45 min, then the fertilization rates were assessed.

Observed fertilization rates were 100% in every experiment.

Chemicals

Bromoform (CAS 75-25-2), tribromoacetic acid (CAS 75-96-7) and tribromophenol (CAS 118-79-16) were purchased from Sigma-Aldrich. Stock solutions of bromoform and tribromoacetic acid were prepared by direct dissolution in filtered sea water (FSW). Tribromophenol was first dissolved in dimethylsulfoxide solvent (DMSO) to compensate for its very low water solubility, then it was dissolved in FSW, with a final DMSO concentration of 0.1%.

Experimental design

Toxicological assessments

Ten unrelated larval population were used and tested in triplicate, as follows: in 24-well microplates, suspensions of 500 fertilized eggs in 2 mL FSW were exposed for 48 h to 7 concentrations of each chemical (Table 1). At the end of the exposure time, larvae were fixed by adding ethanol at 15% final concentration and kept at 4 °C until microscopic observations were carried out. Normal and abnormal (i.e. delayed growth, developmental anomalies) Pluteus larvae were recorded.

Percentages of normal Pluteus larvae were plotted against chemical concentrations. We then determined the dose-response curves using R software and deduced the half maximal effective concentrations, EC_{50}, here considered as the concentration of chemical at which we could observe only 50% of normal and viable larvae among the pool. The no and lowest observed effective concentrations (respectively NOEC and LOEC) were deduced from the chemical concentrations used in the experiments.

We then assessed the toxicity of the chemicals combined in ten other independent experiments. Specifically, we tested three mixtures named mix NOECs, mix LOECS and mix EC_{50}, that is each chemical within a mixture is at its specific endpoints (NOEC, LOEC or EC50). Depending on the chemical tested, controls were either exposed to FSW alone or FSW containing 0.1% DMSO.

Table 1 Concentrations (μM) of tribromophenol, bromoform and tribromoacetic acid tested for toxicological assessment

Tribromophenol	Bromoform	Tribromoacetic acid
1.51E+00	3.96E+01	1.01E+02
3.02E+00	1.19E+02	1.69E+02
9.07E+00	1. 98E+02	3.37E+02
1.51E+01	3.96E+02	6.74E+02
2.42E+01	7.91E+02	1.35E+03
3.02E+01	1.19E+03	1.69E+03
3.63E+01	1.98E+03	2.36E+03

Genotoxicity test

The genotoxicity of the mix NOECs, mix LOECs and twice mix LOECs was assessed using the comet assays, based on the Tice et al. (2000) procedure.

Five thousand, 48-h, Pluteus were exposed as described above for 1 h to the mixtures. After exposure, larvae were collected by centrifugation at 1500*g* for 10 min at 4 °C. The pellet was resuspended in 1 mL of FSW, then gently mixed with 1 mL of glycine 1 M and incubated on ice for 5 min to allow for complete dissociation of the cells. Fifty thousand cells were then collected and placed in 1 mL of phosphate buffer saline (PBS) 1× and centrifuged at 3000*g* for 5 min at 4 °C. Pelleted cells were then collected and gently mixed with 50 μL of low melting point agarose at 37 °C, then plated onto a pre-coated laboratory microscope slide (Tice et al. 2000).

Once prepared, slides were immersed in a lysis buffer (2.5 M NaCl, 100 mM $Na_2EDTA,2H_2O$, 10 mM Tris pH 10, 10 g N-Lauroylsarcosine, 10% DMSO and 1% Triton X100) for 90 min at 4 °C. Slides were then placed in an electrophoresis tank containing an alkaline solution (200 mM Na_2EDTA $2H_2O$, 10 N NaOH) for 20 min at room temperature, to allow DNA to denature. This was followed by electrophoresis which was conducted for 20 min at 25 V and 350 mA. Thereafter, slides were plunged in a neutralization buffer (0.4 M Tris-HCl pH 7.5) for 20 min. Finally, slides were fixed with 100% methanol and dried at room temperature overnight.

DNA was stained with a solution of SyberGreen (Sigma-Aldrich) deposited onto each slide and left to stand for 20 min in the dark. Slides were then read using an epifluorescence microscope equipped with a digital camera and dedicated software. Whole cells and comets observed within several randomly selected microscope fields were captured, irrespective of the length and the shape of the comet (Gyori et al. 2014). Pictures were then analysed using Open Comet v1.3.1 (cometbio.org) implemented in the ImageJ (SciJava) program (Gyori et al. 2014).

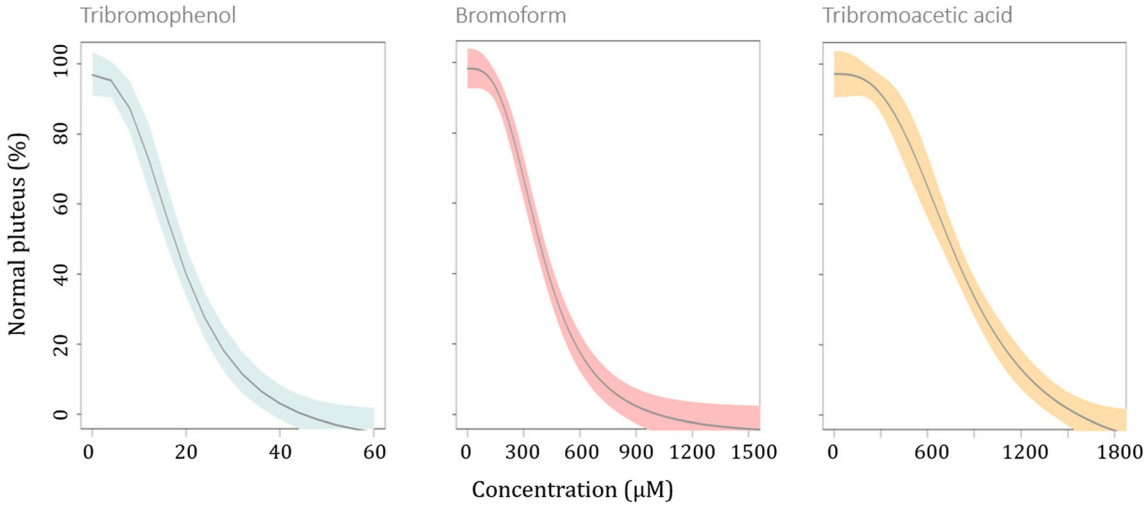

Fig. 1 Effect of tribromophenol, bromoform and tribromoacetic acid alone on the percentage of normal Pluteus growth after 48-h exposure

DNA damage was assessed via the olive tail moment (OTM), which takes into consideration both the length and the distribution of DNA within the comet's tail (Olive et al. 1991).

The positive control experiment consisted of larval suspensions exposed to FSW containing 500 µM of H_20_2. This concentration was chosen after a preliminary experiment in which we tested the genotoxicity of H_20_2 for a range of concentrations ($R = 0.9983$), as previously performed (Nahon et al. 2008).

Statistical analysis

Statistics were performed with Rstudio (version 1.1.442) and XLSTAT (Addinsoft, 2017). Dose-response curves were produced using regression models. Effective concentrations (i.e. EC_{10}, EC_{50} and EC_{90}) values were generated with the "estimating effective doses" (ED.drc) from the R package. Prior to the Kruskal-Wallis test, ecotoxicological and comet assay data were tested for normality. The Mann-Whitney *U* test was proceeded to compare data from non-polluted site (NPS) to polluted site (PS). Differences between each condition were considered significant when p value < 0.05, data were expressed as mean ± S.E.M (standard error of the mean).

Results and discussion

Assessment of BMF, TBAA and TBP toxicity on early embryo development

In this study, embryos were exposed to a range of bromoform (BMF), tribromoacetic acid (TBAA) and tribromophenol (TBP) concentrations for 48 h (Table 1), from fertilization to the Pluteus stage. The control with 0.1% DMSO was not toxic for larvae, and we found typical dose-response curves, from which EC_{50} and EC_{90} were extrapolated (Fig. 1 and Table 2). TBP (halophenol) was by far the more toxic molecule compared to BMF (halomethane) and TBAA (haloacetic acid) (Table 2). BMF was found to be more toxic than TBAA. This order of toxicity has regularly been reported in several aquatic organisms living in fresh or marine water (Yoshioka et al. 1985; Yang and Zhang 2013; Liu and Zhang 2014; Teixidó et al. 2015; Hanigan et al. 2017).

Like other aquatic organisms (Yoshioka et al. 1985; Delacroix et al. 2013; Liu and Zhang 2014; Teixidó et al. 2015; Hanigan et al. 2017), *P. lividus* embryos were sensitive to relatively high concentrations of the tested molecules. The EC_{50} was 14.5 µM for TBP, 526.31 µM for BMF and 923.5 µM for TBAA, and the lowest LOEC was 3 µM with TBP (Table 2). Except for bromoform, *P. lividus* appears to be more sensitive to TBP and TBAA, than the embryos of the marine polychaete, *Platynereis dumerilii* (Yang and Zhang 2013), or the phytoplanktonic green algae, *Tetraselmis marina* (Liu and Zhang 2014). Indeed, according to three previous studies, the EC_{50} for bromoform in *P. lividus* is situated between the EC_{50} in zebrafish (200 µM,(Teixidó et al. 2015)) and the EC_{50} in polychaete embryos (730 µM, Yang and Zhang (2013)). With regard to TBP and TBAA, our model seems 4 to 14 times more sensitive to TBP and 4 to 6 times more sensitive to TBAA, when compared with other, different, biological systems (Yang and Zhang 2013; Liu and Zhang 2014; Teixidó et al. 2015).

Table 2 Different endpoints (µM) for tribromophenol, bromoform and tribromoacetic acid after 48 h of exposure

	Tribromophenol	Bromoform	Tribromoacetic acid
NOEC µM	1.51E+00	3.96E+01	1.69E+02
LOEC µM	3.02E+00	1.98E+02	3.37E+02
EC_{50} µM	1.45E+01	5.26E+02	9.23E+02
EC_{90} µM	3.14E+01	1.05E+03	1.78E+03

The first observable effect (LOEC) of the three chemicals in our model was the abnormal development of a fraction of Plutei. At higher concentrations, we observed both abnormal and delayed development, mostly between the morula and gastrula stages, although in different relative proportion, depending upon the molecule and the concentration. Of the developmental anomalies triggered by all three chemicals, we mainly observed crossed spicules, which are among the most regularly reported malformations in sea urchin larvae (Gharred et al. 2016). Interestingly, TBAA-induced mortality was detected early, since we observed a majority of black-coloured dead eggs (*unpublished data*).

Exposure for 48 h to mix NOECs and mix LOECs critically reduced the proportion of normal Pluteus, to 63% ± 2 and 18% ± 1 respectively, while none survived exposure to mix EC_{50} condition, demonstrating the combined effect of these chemicals (Table 2). The results obtained with a mixture of chemicals were entirely expected. However, in zebrafish embryos, the influence of chlorinated water had no detectable phenotypic impact after 5 days of exposure, suggesting that a complex water environment, containing a wide diversity of DBPs at much lower concentrations than those tested, may not be deleterious for the population (Hanigan et al. 2017). Nevertheless, chronic exposure of adult fish to 0.9 nM of TBP could potentially have a significant effect over a number of generations, on survival, larval development and the malformation rate (Deng et al. 2010).

We further tested the impact of embryo exposure at polluted sites (PS), with embryos generated by *P. lividus* adults chronically exposed to chlorinated sea water. These PS embryos were exposed to TBP, BMF and TBAA at their respective LOEC, EC_{50} and EC_{90}, and to mix LOECs condition. They were found to be slightly less sensitive to TBP and TBAA than NPS embryos, while very resistant to BMF, which is the most prevalent molecule found in sea water where genitors were harvested (Fig. 2) (Manasfi et al. 2018). Moreover, exposure to mix LOECs reduced the proportion of normal Pluteus by 40% instead of 80% for the NPS embryos. This increased PS embryo resistance could be explained by either natural larval selection, with higher fitness for the polluted site, or a parental effect (Ross et al. 2016).

Evaluation of mixture genotoxicity

The alkaline comet assay has been widely used to test potential genotoxic effects of environmental pollutants, in several model organisms such as animal embryos and plant roots, as it is an efficient method for detecting single- and double-strand DNA breaks (Yıldız et al. 2009; Liman et al. 2011). The results obtained from the comet assay are summarized in Fig. 3. Here we tested whether 1 h exposure to mixtures of TBP, BMF and TBAA, at their respective NOEC, LOEC and twice their LOEC concentrations, could induce DNA strand breakages in Pluteus larvae. It is likely that given the short-term exposure to the mixtures, we observed the obvious potential of our mixtures to induce DNA strand breakages, prior to any activation of DNA repair mechanisms that would counteract the induced DNA damage. The level of genotoxicity was assessed in positive and negative controls and exposed groups according to the OTM and the percentage of DNA (%DNA) measured in the comet tail. Furthermore, we only assessed the proportion of cells within each group that exceeded a

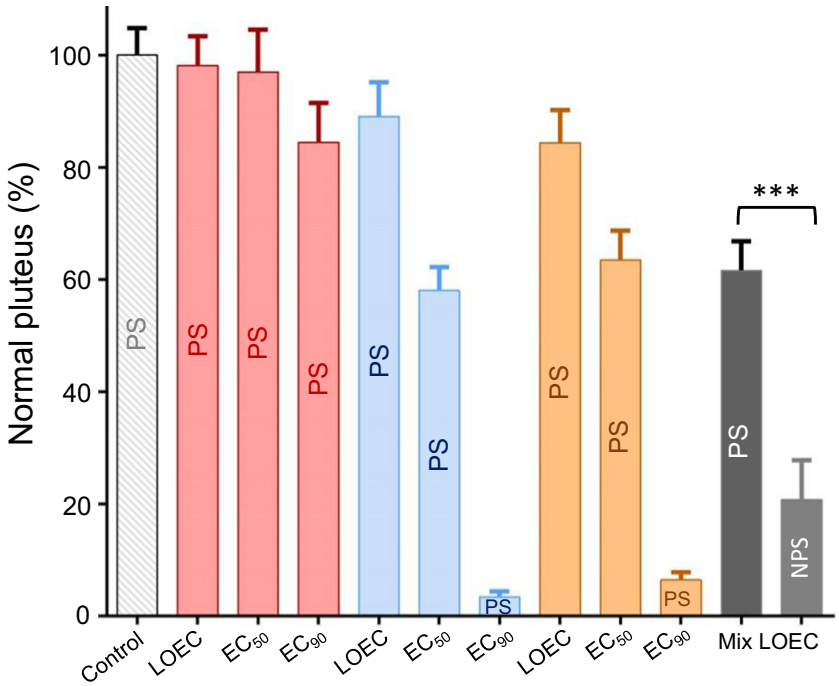

Fig. 2 Effect of bromoform (pink), tribromophenol (blue) and tribromoacetic acid (orange), alone and mix LOECs (grey), on the percentage of normal Pluteus generated by adults harvested from polluted site (PS) or non-polluted site (NPS), after 48-h exposure.*** $p < 0.05$ (Dunnet's test)

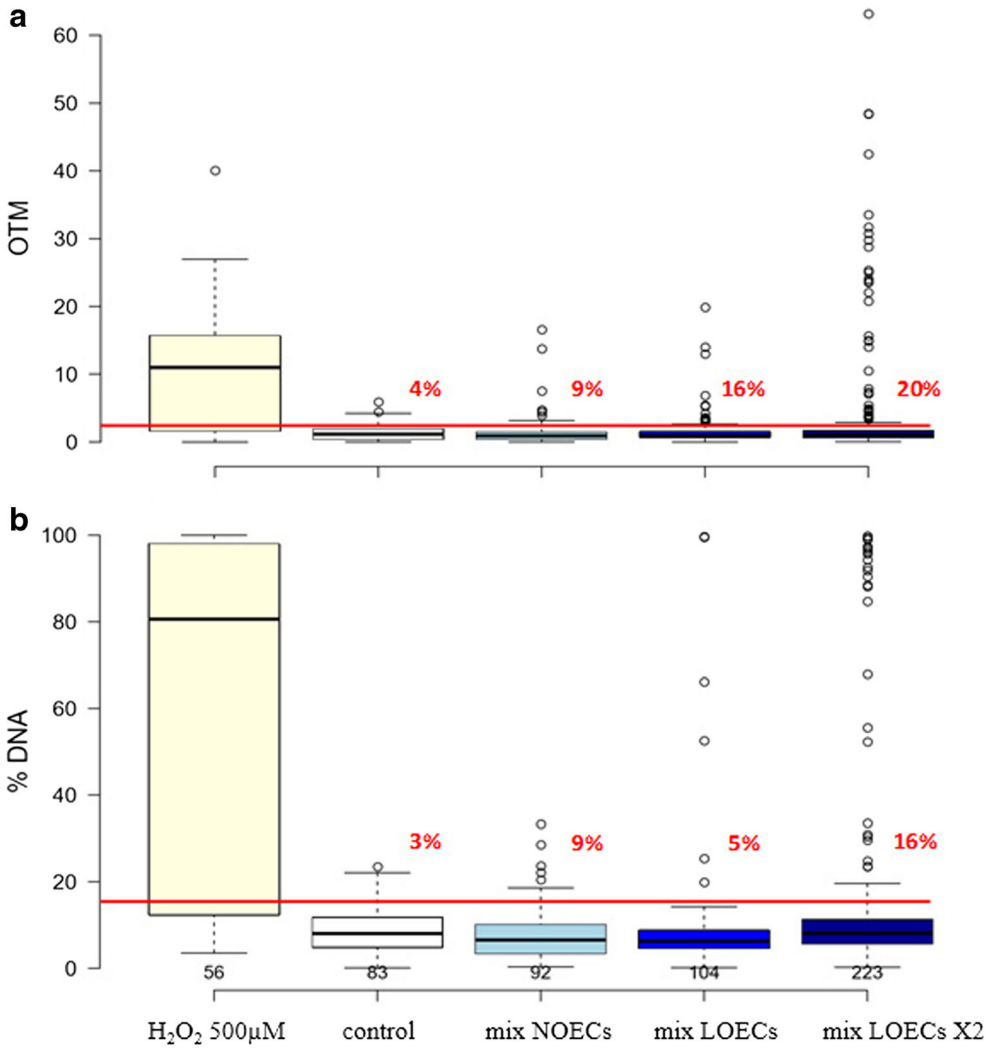

Fig. 3 Distribution of OTM (**a**) and % of DNA within the comet tail (**b**) according to the level of exposure of Pluteus larvae to the chemical mixture. Boxplots encompass the 1st and the 3rd quartiles. The black line within the boxplot represents the median. Tukey's whisker extents are presented (defined as 1.5 times the 1st and 3rd quartiles). The red horizontal line indicates the 95% percentile of the control condition used as a reference, and the numbers in red indicate the proportion of comets presenting a higher value than the reference

threshold value, corresponding to the 95th percentile of the control group for the OTM and %DNA. Under controlled conditions, the 95th percentiles for OTM and %DNA were 2.6% and 16.5%, respectively.

For the two parameters considered, we found normal data distributions within the negative control group (Shapiro-Wilk, $p = 0.1$) but not in the tested groups or in the positive control (Shapiro-Wilk, $p < 0.0001$). The non-parametric test did not reveal any influence of exposure level based on the OTM but demonstrated that the group exposed to the mixture at twice the LOEC concentration (mix LOEC_X2), had significantly more DNA within the comet tail, compared to all other groups (Kruskal-Wallis, $p < 0.033$). This result would suggest that this last condition only had a genotoxic effect. However, the discrepancy in the results between controls and tested groups for normality of data distribution indicates that exposure had an influence on a fraction of the whole cells within each group, as previously reported (Taban et al. 2004). Indeed, the proportion of cells with an OTM higher than 2.6 regularly increases with the concentration and gives higher OTM values than observed in the negative control group (Fig. 3a). This increase in the proportion of cells has also been observed for the %DNA parameter, although it is clearly noticeable at the mix LOEC_X2 condition (Fig. 3b). Hence, overall, our results suggest a dose response when taking into consideration both the effect on DNA and the occurrence of responding cells. Similar results have already been reported in short-term exposure experiments, such as those performed on CHO (Chinese hamster ovary) cells exposed to TBA for 4 h (Plewa et al. 2008) and occurrence (Taban et al. 2004).

It is interesting to note that at the mix LOEC_X2 condition, we found that 7% of the cells were distributed with more than 90% of their DNA within the comet tail. Among these, a few cells also displayed a high OTM value, which relates to the length of the comet. A similar result was also obtained with some cells from the positive control group (Fig. 3b). This could be indicative of ongoing apoptosis (Ríos et al. 2003; Collins 2004). Evidence for this has already been demonstrated in other studies. It has been shown that hydrogen peroxide can induce apoptosis, causing cell death in less than 2 h in

293T cells exposed to more than 400 μM of H_2O_2 (Xiang et al. 2016). A recent study has also suggested that halogenated DBPs might trigger apoptosis at high concentrations (Ríos et al. 2003; Collins 2004). Thus, in our study, the mixtures might have the potential to also trigger apoptosis in a fraction of the larval cells.

This distribution pattern is difficult to explain at this stage, and it would be interesting to assess whether a certain cell type would be more sensitive to mixtures than others, or if the external cells are more impacted than those embedded within the larval body, for instance. This has already been reported in zebrafish exposed for a much longer time (i.e. 72 h) to BMF (Teixidó et al. 2015).

Finally, we have highlighted with our experimental conditions that a significant genotoxic effect, observed with high doses of a cocktail of DBPs, are generally consistent with previous findings (Teixidó et al. 2015; Hanigan et al. 2017).

Conclusion

This study has furthered our knowledge of the impact of bromoform, tribromophenol and tribromoacetic acid, tested alone and in combination, on sea urchin, *Paracentrotus lividus*, development. We have revealed that all three molecules could impair embryo development with the order of toxicity TBP > BMF > TBAA. TBP is 10 to 30 times more toxic compared to the other two molecules. The mixture of the three DBPs also significantly impaired development and were proved weakly genotoxic at low doses (i.e. mix NOECs). Increasing the concentration of the mixture to mix LOECs significantly increases cytotoxicity, as well as increasing the level of DNA damage and the proportion of cells affected.

In this study, we have highlighted a potential genome by environment interaction, particularly regarding BMF. Further experiments will be required, however, in order to assess the relative influence of a potential parental effect, as well as local genetic adaptation in the observed resistance.

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References

(2004) Some drinking-water disinfectants and contaminants, including arsenic. IARC, Lyon

Allonier A-S, Khalanski M, Camel V, Bermond A (1999) Determination of dihaloacetonitriles and halophenols in chlorinated sea water. Talanta 50:227–236

Bellas J, Fernández N, Lorenzo I, Beiras R (2008) Integrative assessment of coastal pollution in a Ría coastal system (Galicia, NW Spain): correspondence between sediment chemistry and toxicity. Chemosphere 72:826–835. https://doi.org/10.1016/j.chemosphere.2008.02.039

Boudjellaba D, Dron J, Revenko G, Démelas C, Boudenne JL (2016) Chlorination by-product concentration levels in seawater and fish of an industrialised bay (gulf of Fos, France) exposed to multiple chlorinated effluents. Sci Total Environ 541:391–399. https://doi.org/10.1016/j.scitotenv.2015.09.046

CAREX Canada (2009) Les sous-produits de chloration. School of Environmental Health University of British Columbia

Collins AR (2004) The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol 26:249–261. https://doi.org/10.1385/MB:26:3:249

Delacroix S, Vogelsang C, Tobiesen A, Liltved H (2013) Disinfection by-products and ecotoxicity of ballast water after oxidative treatment – results and experiences from seven years of full-scale testing of ballast water management systems. Mar Pollut Bull 73:24–36. https://doi.org/10.1016/j.marpolbul.2013.06.014

Deng J, Liu C, Yu L, Zhou B (2010) Chronic exposure to environmental levels of tribromophenol impairs zebrafish reproduction. Toxicol Appl Pharmacol 243:87–95. https://doi.org/10.1016/j.taap.2009.11.016

Dorey N, Maboloc E, Chan KYK (2018) Development of the sea urchin Heliocidaris crassispina from Hong Kong is robust to ocean acidification and copper contamination. Aquat Toxicol 205:1–10. https://doi.org/10.1016/j.aquatox.2018.09.006

Gharred T, Jebali J, Belgacem M, Mannai R, Achour S (2016) Assessment of the individual and mixture toxicity of cadmium, copper and oxytetracycline, on the embryo-larval development of the sea urchin Paracentrotus lividus. Environ Sci Pollut Res 23:18064–18072. https://doi.org/10.1007/s11356-016-6988-3

Gyori BM, Venkatachalam G, Thiagarajan PS, Hsu D, Clement MV (2014) OpenComet: an automated tool for comet assay image analysis. Redox Biol 2:457–465. https://doi.org/10.1016/j.redox.2013.12.020

Halpern B, Walbridge S, Watson R (2008) A global map of human impact on marine ecosystems. Science 319:948–952. https://doi.org/10.1126/science.1149345

Hanigan D, Truong L, Simonich M, Tanguay R, Westerhoff P (2017) Zebrafish embryo toxicity of 15 chlorinated, brominated, and iodinated disinfection by-products. J Environ Sci 58:302–310. https://doi.org/10.1016/j.jes.2017.05.008

Hose JE (1985) Potential uses of sea urchin embryos for identifying toxic chemicals: description of a bioassay incorporating cytologic, cytogenetic and embryologic endpoints. J Appl Toxicol 5:245–254. https://doi.org/10.1002/jat.2550050406

Liman R, Ciğerci İH, Akyıl D, Eren Y, Konuk M (2011) Determination of genotoxicity of Fenaminosulf by Allium and comet tests. Pestic Biochem Physiol 99:61–64. https://doi.org/10.1016/j.pestbp.2010.10.006

Liu J, Zhang X (2014) Comparative toxicity of new halophenolic DBPs in chlorinated saline wastewater effluents against a marine alga: Halophenolic DBPs are generally more toxic than haloaliphatic ones. Water Res 65:64–72. https://doi.org/10.1016/j.watres.2014.07.024

Manasfi T, De Méo M, Di Giorgio C et al (2017) Assessing the genotoxicity of two commonly occurring byproducts of water disinfection: chloral hydrate and bromal hydrate. Mutat Res Genet Toxicol Environ Mutagen 813:37–44. https://doi.org/10.1016/j.mrgentox.2016.11.009

Manasfi T, Lebaron K, Boudenne J-L (2018) Characterization of chlorination byproducts in marine waters and sediments in a semi-enclosed bay exposed to multiple industrial chlorinated effluents

McGibbon S, Moldan AGS (1986) Routine toxicity testing of toxicants using a sea urchin gamete bioassay. Mar Pollut Bull 17:68–72. https://doi.org/10.1016/0025-326X(86)90294-8

Messinetti S, Mercurio S, Parolini M, Sugni M, Pennati R (2018) Effects of polystyrene microplastics on early stages of two marine invertebrates with different feeding strategies. Environ Pollut 237:1080–1087. https://doi.org/10.1016/j.envpol.2017.11.030

Morroni L, Pinsino A, Pellegrini D, Regoli F, Matranga V (2016) Development of a new integrative toxicity index based on an improvement of the sea urchin embryo toxicity test. Ecotoxicol Environ Saf 123:2–7. https://doi.org/10.1016/j.ecoenv.2015.09.026

Mouly D, Joulin E, Rosin C et al (2009) Les sous-produits de chloration dans l'eau destinée à la consommation humaine en France 76

Nahon S, Charles F, Pruski AM (2008) Improved comet assay for the assessment of UV genotoxicity in Mediterranean sea urchin eggs. Environ Mol Mutagen 49:351–359. https://doi.org/10.1002/em.20391

Neves RAF, Contins M, Nascimento SM (2018) Effects of the toxic benthic dinoflagellate Ostreopsis cf. ovata on fertilization and early development of the sea urchin Lytechinus variegatus. Mar Environ Res 135:11–17. https://doi.org/10.1016/j.marenvres.2018.01.014

Olive PL, Wlodek D, Banáth JP (1991) DNA Double-Strand Breaks Measured in Individual CellsSubjected to Gel Electrophoresis. Cancer Res 51:4671–4676

Pereira TM, Merçon J, Passos LS, Coppo GC, Lopes TOM, Cabral DS, Scherer R, Chippari-Gomes AR (2018) Effects of the water-soluble fraction of diesel oil (WSD) on the fertilization and development of a sea urchin (Echinometra lucunter). Ecotoxicol Environ Saf 162:59–62. https://doi.org/10.1016/j.ecoenv.2018.06.040

Plewa MJ, Wagner ED, Muellner MG, Hsu KM, Richardson SD (2008) Comparative mammalian cell toxicity of N-DBPs and C-DBPs. In: Karanfil T, Krasner SW, Westerhoff P, Xie Y (eds) Disinfection by-products in drinking water. American Chemical Society, Washington, DC, pp 36–50

Richardson S, Plewa M, Wagner E et al (2007) Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. Mutat Res Rev Mutat Res 636:178–242. https://doi.org/10.1016/j.mrrev.2007.09.001

Richardson SD, DeMarini DM, Kogevinas M et al (2010) What's in the Pool? A comprehensive identification of disinfection by-products and assessment of mutagenicity of chlorinated and brominated swimming pool water. Environ Health Perspect 118:1523–1530. https://doi.org/10.1289/ehp.1001965

Ríos JC, Repetto G, Jos A et al (2003) Tribromophenol induces the differentiation of SH-SY5Y human neuroblastoma cells in vitro. Toxicol in Vitro 17:635–641. https://doi.org/10.1016/S0887-2333(03)00110-3

Ross PM, Parker L, Byrne M (2016) Transgenerational responses of molluscs and echinoderms to changing ocean conditions. ICES Journal of Marine Science 73:537–549. https://doi.org/10.1093/icesjms/fsv254

Singer P (1999) Humic substances as precursors for potentially harmful disinfection by-products. Water Sci Technol 40. https://doi.org/10.1016/S0273-1223(99)00636-8

Taban IC, Bechmann RK, Torgrimsen S, Baussant T, Sanni S (2004) Detection of DNA damage in mussels and sea urchins exposed to crude oil using comet assay. Mar Environ Res 58:701–705. https://doi.org/10.1016/j.marenvres.2004.03.018

Teixidó E, Piqué E, Gonzalez-Linares J, Llobet JM, Gómez-Catalán J (2015) Developmental effects and genotoxicity of 10 water disinfection by-products in zebrafish. J Water Health 13:54–66. https://doi.org/10.2166/wh.2014.006

Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 35:206–221. https://doi.org/10.1002/(SICI)1098-2280(2000)35:3<206::AID-EM8>3.0.CO;2-J

Trifuoggi M, Pagano G, Guida M, Palumbo A, Siciliano A, Gravina M, Lyons DM, Burić P, Levak M, Thomas PJ, Giarra A, Oral R (2017) Comparative toxicity of seven rare earth elements in sea urchin early life stages. Environ Sci Pollut Res 24:20803–20810. https://doi.org/10.1007/s11356-017-9658-1

Tu Q, Brown CT, Davidson EH, Oliveri P (2006) Sea urchin Forkhead gene family: phylogeny and embryonic expression. Dev Biol 300:49–62. https://doi.org/10.1016/j.ydbio.2006.09.031

Villanueva CM, Cantor KP, Cordier S, Jaakkola JJK, King WD, Lynch CF, Porru S, Kogevinas M (2004) Disinfection byproducts and bladder cancer: a pooled analysis. Epidemiology 15:357–367

Villanueva CM, Cantor KP, Grimalt JO, Malats N, Silverman D, Tardon A, Garcia-Closas R, Serra C, Carrato A, Castano-Vinyals G, Marcos R, Rothman N, Real FX, Dosemeci M, Kogevinas M (2006) Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering, and swimming in pools. Am J Epidemiol 165:148–156. https://doi.org/10.1093/aje/kwj364

Westerhoff P, Chao P, Mash H (2004) Reactivity of natural organic matter with aqueous chlorine and bromine. Water Res 38:1502–1513. https://doi.org/10.1016/j.watres.2003.12.014

Xiang J, Wan C, Guo R, Guo D (2016) Is hydrogen peroxide a suitable apoptosis inducer for all cell types? Biomed Res Int 2016:1–6. https://doi.org/10.1155/2016/7343965

Yang M, Zhang X (2013) Comparative developmental toxicity of new aromatic halogenated DBPs in a chlorinated saline sewage effluent to the marine Polychaete *Platynereis dumerilii*. Environ Sci Technol 47:10868–10876. https://doi.org/10.1021/es401841t

Yıldız M, Ciğerci İH, Konuk M, Fatih Fidan A, Terzi H (2009) Determination of genotoxic effects of copper sulphate and cobalt chloride in Allium cepa root cells by chromosome aberration and comet assays. Chemosphere 75:934–938. https://doi.org/10.1016/j.chemosphere.2009.01.023

Yoshioka Y, Ose Y, Sato T (1985) Testing for the toxicity of chemicals with Tetrahymena pyriformis. Sci Total Environ 43:149–157. https://doi.org/10.1016/0048-9697(85)90037-3