Environmental Toxicology and Pharmacology 80 (2020) 103504

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Toxicological assessment and developmental abnormalities induced by butylparaben and ethylparaben exposure in zebrafish early-life stages

C. Merola [a], O. Lai [b], A. Conte [c], G. Crescenzo [b], T. Torelli [a], M. Alloro [a], M. Perugini [a,*]

[a] Faculty of Bioscience and Agro-Food and Environmental Technology, University of Teramo, 64100, Teramo, Italy
[b] Department of Veterinary Medicine, University of Bari, S.P. per Casamassima, Km 3, 70010, Valenzano (Bari), Italy
[c] Istituto Zooprofilattico Sperimentale "G. Caporale", via Campo Boario, 64100, Teramo, Italy

ARTICLE INFO

Keywords:
Acute toxicity test
Parabens
Benchmark dose
Zebrafish larvae
Developmental alterations

ABSTRACT

Toxicological effects of butylparaben (BuP) and ethylparaben (EtP) on zebrafish (*Danio rerio*) early-life stages are not well established. The present study evaluated, using zebrafish embryos and larvae, the toxicity of BuP and EtP through benchmark dose (BMD) approach. BuP was more toxic than EtP to zebrafish larvae. In fact, Lethal Concentration 50 (LC50) values at 96 h post-fertilization (hpf) for BuP and EtP were 2.34 mg/L and 20.86 mg/L, respectively. Indeed, BMD confidence interval (lower bound (BMDL) - upper bound (BMDU) was 0.91–1.92 mg/L for BuP and 10.8–17.4 mg/L for EtP. Zebrafish embryos exposed to 1 mg/L, 2.5 mg/L of BuP and 5 mg/L, 10 mg/L, 20 mg/L, 30 mg/L of EtP showed several developmental abnormalities and teratological effects compared to negative control. Exposed zebrafish developed reduced heartbeat, reduction in blood circulation, blood stasis, pericardial edema, deformed notochord and misshaped yolk sac. Embryos exposed to the highest concentrations of the chemicals (2.5 mg/L of BuP, 10 mg/L, 20 mg/L and 30 mg/L of EtP) showed the developmental abnormalities at 48 hpf while those treated with 1 mg/L of BuP and 10 mg/L of EtP reported behavioral changes at 72 hpf, including trembling of head, pectoral fins and spinal cord. This research identified the lethal and sublethal effects of BuP and EtP in zebrafish early-life stages and could be helpful to elucidate the developmental pathways of toxicity of parabens.

1. Introduction

Parabens are a group of synthetic chemicals used in cosmetics, food, and pharmaceuticals due to their broad-spectrum antimicrobial and antifungal properties (Nowak et al., 2018). In terms of chemical structure, they are esters of *p*-hydroxybenzoic acid with alkyl substituents ranging from methyl to butyl groups (Darbre et al., 2004). With the increase in the length of alkyl chain the value of octanol water-partition coefficient rises, resulting in decrease of their water solubility and in an increase of their lipophilicity (Artacho-Cordón et al., 2018). Antimicrobial activity of parabens is also directly proportional to the chain length of ester group increasing from methyl to n-butyl (Błędzka et al., 2014). *In vivo* studies showed as parabens are rapidly absorbed from the gastrointestinal tract and from blood, hydrolyzed to *p*-hydroxybenzoic acid, conjugated and then excreted in the urine. Parabens can also be absorbed quickly through intact skin and hydrolyzed by skin and subcutaneous fatty tissues carboxylesterases (Boberg et al., 2010). They are classified as endocrine disrupting chemicals (EDCs) due to their ability to bind to several nuclear receptors (Nowak et al., 2018). Indeed, their influence on adipose tissue and neurodevelopment is still less clear. The zebrafish is increasingly used as a model in human toxicological research and is highly suitable to assess developmental and teratogenic effects of EDCs (Staal et al., 2018). The early life stages of development are highly conserved between species and the observation of developmental abnormalities in zebrafish model is considered relevant to understand new modes of action and potential target tissues of EDCs in mammals (Zezza et al., 2019). Specific advantages of the zebrafish model are the relatively transparent body during the early life stages and, according to the new EU Directive 2010/63/EU, the fact that the earliest life-stages do not fall into the regulatory frameworks dealing with animal experimentation (Staal et al., 2018). Recently has been demonstrated that the sublethal exposure to propylparaben (PrP) interferes with lipid utilization in zebrafish larvae, in terms of decrease in neutral lipid mobilization from yolk and alteration of phospholipids metabolism. Moreover, PrP and methylparaben (MeP) exposure in zebrafish early life-stages led to acute toxicity and developmental

* Corresponding author.
E-mail address: mperugini@unite.it (M. Perugini).

https://doi.org/10.1016/j.etap.2020.103504
Received 25 February 2020; Received in revised form 17 September 2020; Accepted 21 September 2020
Available online 24 September 2020

abnormalities due to alterations in gene expression involved in cellular stress response, cell cycle and DNA damage, inflammation and fatty acid metabolism (Dambal et al., 2017; Bereketoglu and Pradhan, 2019). Indeed, available data on the effects of ethylparaben and butylparaben on zebrafish early life stages are still lacking. BuP is recognized to be the most potent paraben in terms of estrogenic activity and was found to be able to interfere with male reproductive functions (Routledge et al., 1998; Oishi, 2001). EtP is admitted as additive in food and it showed genotoxic activity related to telomere shortening *in vitro* studies (EFSA, 2004; Finot et al., 2017).

Data analysis methods for the assessment of zebrafish developmental toxicity end-points are under active evolution and include EC50/LC50 (half maximal effective concentration/median lethal concentration), LOAEL (lowest-observed-adverse-effect level) and NOAEL (no-observed-adverse-effect level) strategies (Hsieh et al., 2018). However, LOAEL and NOAEL approaches have some shortcomings including lack of consideration of concentration-response trend, limiting LOAE/NOAEL options to tested concentration and inability to provide uncertainty factors for reported potency (Davis et al., 2011). For these reasons EFSA Scientific Committee recommends using the BMD (Benchmark Dose) approach instead of NOAEL strategy, since it makes a more extended use of dose-response data and it allows for a quantification of the uncertainties in the dose-response data (EFSA, 2017). Moreover, the Scientific Committee suggests to always report the BMD confidence interval (lower bound, BMDL-upper bound, BMDU) rather than the value of the BMD (EFSA, 2017). The present study focuses on the evaluation of the acute toxicity and developmental abnormalities of butylparaben and ethylparaben on zebrafish early-life stages using the BMD approach as toxicological risk assessment.

2. Materials and methods

2.1. Chemicals

Ethylparaben (CAS number 120−47-8, Pharmaceutical Secondary Standard; Certified Reference Material) and Butylparaben (CAS number 94−26-8, Pharmaceutical Secondary Standard; Certified Reference Material) were purchased from Sigma Aldrich, Milan, Italy. Dimethyl sulfoxide (DMSO) (> 99.9 % purity) and 3,4-dichloroaniline (> 98 % purity) were obtained from Sigma-Aldrich (Co. St. Louis, MO). Dilution water (DW) was prepared according to OECD TG 203, Annex 2 (OECD, 1992).

2.2. Zebrafish maintenance and eggs collection

Adults zebrafish (wild type AB strain) were bred in University of Teramo facility (code 041TE294). Adults were kept in 3.5 L ZebTec tanks (Tecniplast S.p.a., Buguggiate, Italy) in a recirculating aquatic system. The temperature was maintained at 28 °C, the pH at 7 ± 0.2, the conductivity at 500 ± 100 μS/cm and the dissolved O_2 at 6.1 mg/L. The photoperiod was 14 h light and 10 h dark and chemical parameters were kept as follows: ammonia 0.02 mg/L, nitrite 0.02 mg/L, nitrate 21.3 mg/L. Animals were fed twice a day with live food (*Artemia salina*) and supplemented with 300 μm granules of dry feed (GEMMA Micro 300). The afternoon before spawning, several groups of females and males (1:1) were introduced into 1.7 L breeding tanks (beach style design, Tecniplast S.p.a., Buguggiate, Italy). Immediately after spawning, which was initiated by morning light, fertilized eggs were collected with a sieve and rinsed thoroughly with deionized water and DW. Eggs were transferred to Petri dishes and eggs no fertilized or embryos with injuries were eliminated.

2.3. Fish embryo acute toxicity tests

Fish Embryo Acute Toxicity Tests (FET tests) were performed according to OECD n. 236 (OECD, 2013) and following the published method reported in Perugini et al. (2019).

Ethylparaben was tested at 1 mg/L, 5 mg/L, 10 mg/L, 20 mg/L and 30 mg/L while butylparaben at 0.25 mg/L, 0.5 mg/L, 1 mg/L, 2.5 mg/L and 5 mg/L. Concentrations were chosen based on available literature on other parabens or aquatic model organisms (Yamamoto et al., 2011; Perugini et al., 2019. EtP and BuP were dissolved in DMSO, reaching the final concentration of 0.1 % of DMSO in all experimental groups. Selected embryos were placed individually with 2 mL of solution in each well of 24-well plates. Twenty embryos per concentration were exposed to the chemicals and the working solutions were renewed every 24 h. Experiments were repeated three times in different weeks. Negative control (DW), solvent control (0.1 % DMSO) and positive control (4% 3, 4 dichloroaniline) were also tested. Embryos were exposed for 96 hpf in the incubator at 26 ± 1 °C and photoperiod (14 h light:10 dark) conditions. Embryos were daily observed up to 96 h with the inverted optical microscope (CKX 41, Olympus, Japan) recording the four apical observations as indicators of lethality: coagulation of fertilized eggs, lack of somite formation, lack of detachment of the tail-bud from the yolk sac, and absence of heartbeat. During the exposure period, developmental alterations, teratological parameters, and percentage of hatching were also recorded according to Nagel (2002).

2.4. Statistical analysis

Statistical analysis of FET tests results was performed using a Bayesian approach. Beta distribution was calculated to evaluate differences in the percentage of anomalies and hatching rate in different groups (concentrations and time of exposure), comparing the 95 % confidence intervals. Significant level was set at $p < 0.05$. In addition, LC50 was calculated using ToxRat software version 3.3 (ToxRat Solutions GmbH, Germany). The EFSA web-tool for BMD analysis, which uses the R-package PROAST, version 67.0 was used to obtain BMC confidence interval. The benchmark concentrations were calculated from the mortality dose-response curves, with a predefined value of extra risk of 10 %. The data were fitted using the Model Averaging approach.

3. Results

3.1. Fish embryo acute toxicity tests

FET test acceptance criteria were achieved, by following OECD n.236 since the mortality of negative control at 96 hpf was 0 %, and the mortality in the positive control group was 60 %. LC50 values at 96 hpf for BuP and EtP were 2.34 mg/L and 20.86 mg/L, respectively. BMD confidence intervals were 0.91–1.92 mg/L for BuP and 10.8–17.4 mg/L for EtP (Fig. 1). Coagulation of embryos and no heartbeat were the most common lethal endpoints recorded at 96 hpf. Lack of detachment of the tail-bud from the yolk sac was also reported. The highest EtP concentration (30 mg/L) led to 85 % of mortality at 96 hpf, while all the embryos exposed to 5 mg/L of BuP died at 48 hpf.

3.2. Hatching rate

Significant ($p < 0.05$) delay (dose-dependent) in hatching rate at 72 hpf among control group and embryos exposed to EtP was observed (Fig. 1).

For BuP at 0.25 mg/L the percentage of hatched embryos increased compared to the control ($p < 0.05$) (Fig. 1). Indeed, embryos exposed to the highest concentrations (2.5 and 5 mg/L) of BuP showed a significant decrease in hatching rate ($p < 0.05$).

3.3. Developmental abnormalities and teratological effects

At different time of exposure embryos treated with EtP and BuP showed a similar distribution pattern for the developmental

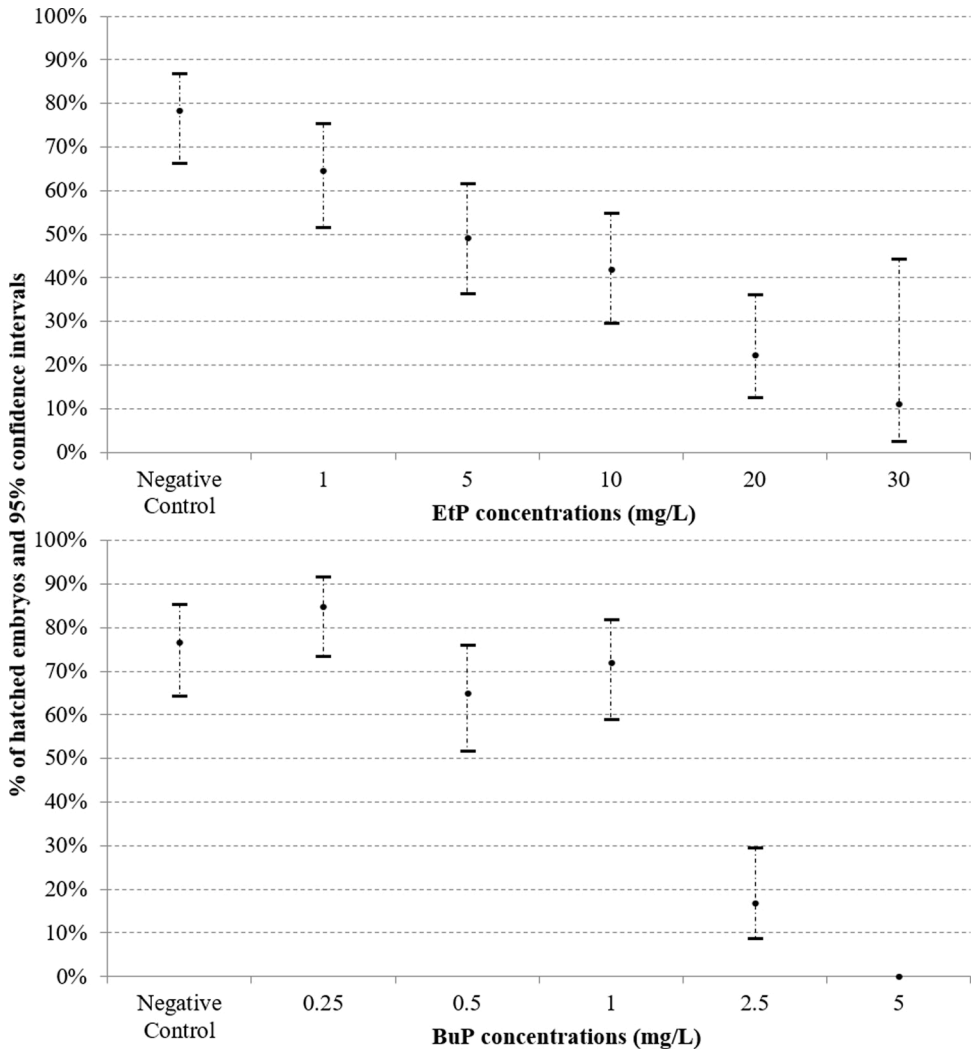

Fig. 1. Percentage and 95 % confidence interval of hatching embryos treated with BuP and EtP for 72 hpf compared to negative control group. Data were reported as sum of hatched embryos in three independent experiments.

abnormalities and teratological effects. The absence of spontaneous movements was registered for all exposed embryos except for the lowest concentrations of both compounds.

Zebrafish early-life stages did not show any significant developmental or teratological abnormality at the concentrations of 0.25 mg/L, 0.5 mg/L of BuP and 1 mg/L of EtP compared to negative control (Figs. 2,3).

Zebrafish larvae exposed to 5 mg/L of EtP showed only misshaped yolk sac. The embryos with misshaped yolk sac exposed to 5 mg/L of EtP showed a significant difference ($p < 0.05$) at 96 hpf (26.8 %) compared to those at 72 hpf (17.5 %).

Reduction in blood circulation (Video 1), blood stasis, pericardial edema, misshaped yolk sac and deformed notochord (Fig. 4) were reported at 72 hpf in zebrafish larvae treated with 10 mg/L of EtP and 1 mg/L of BuP. In the same experimental groups at 96 hpf, the number of zebrafish larvae with misshaped yolk sac and deformed notochord was higher ($p < 0.05$) than those reported at 72 hpf.

In the experimental group of BuP 1 mg/L the number of embryos that exhibited reduction in blood circulation, blood stasis and pericardial edema registered an increase at 96 hpf compared to 72 hpf ($p < 0.05$). Indeed, larvae treated with EtP at 10 mg/L, showed reduced heartbeat that significantly increased from 72 hpf to 96 hpf ($p < 0.05$) (Fig. 4, Video 2). Starting from 72 hpf, larvae exposed to 10 mg/L of EtP and 1 mg/L of BuP showed defects in pectoral fins development and behavioral symptoms including trembling of head, pectoral fins and spinal cord and circling behavior (Video 3) compared to negative control group (Video 4).

Zebrafish embryos exposed to 20 mg/L of EtP and 2.5 mg/L of BuP reported more significant effects ($p < 0.05$) as reduced heartbeat, blood stasis, reduction in blood circulation, misshaped yolk sac and pericardial edema at 48 hpf than at 24 hpf; moreover the number of embryos that reported these sublethal alterations increased at 72 hpf ($p < 0.05$) compared to 48 hpf (Fig. 4). Indeed, deformed notochord was reported only at 72 hpf in zebrafish larvae exposed to 20 mg/L of EtP and 2.5 mg/L of BuP (Fig. 4). The number of zebrafish larvae treated with 20 mg/L of EtP and 2.5 mg/L of BuP that showed deformed notochord, increased at 96 hpf ($p < 0.05$) compared to 72 hpf.

All survived embryos exposed to 30 mg/L of EtP, showed reduced heartbeat, blood stasis, reduction in blood circulation, pericardial edema and misshaped yolk sac at 48 hpf ($p < 0.05$) compared to 24 hpf.

4. Discussion

The present study demonstrated that BuP and EtP exposure in zebrafish early-life stages resulted in acute toxicity leading to developmental abnormalities and teratological effects. According to their physical and chemical properties, LC50 of BuP (2.34 mg/L) was lower than LC50 of EtP (20.86 mg/L). These results agree with data obtained

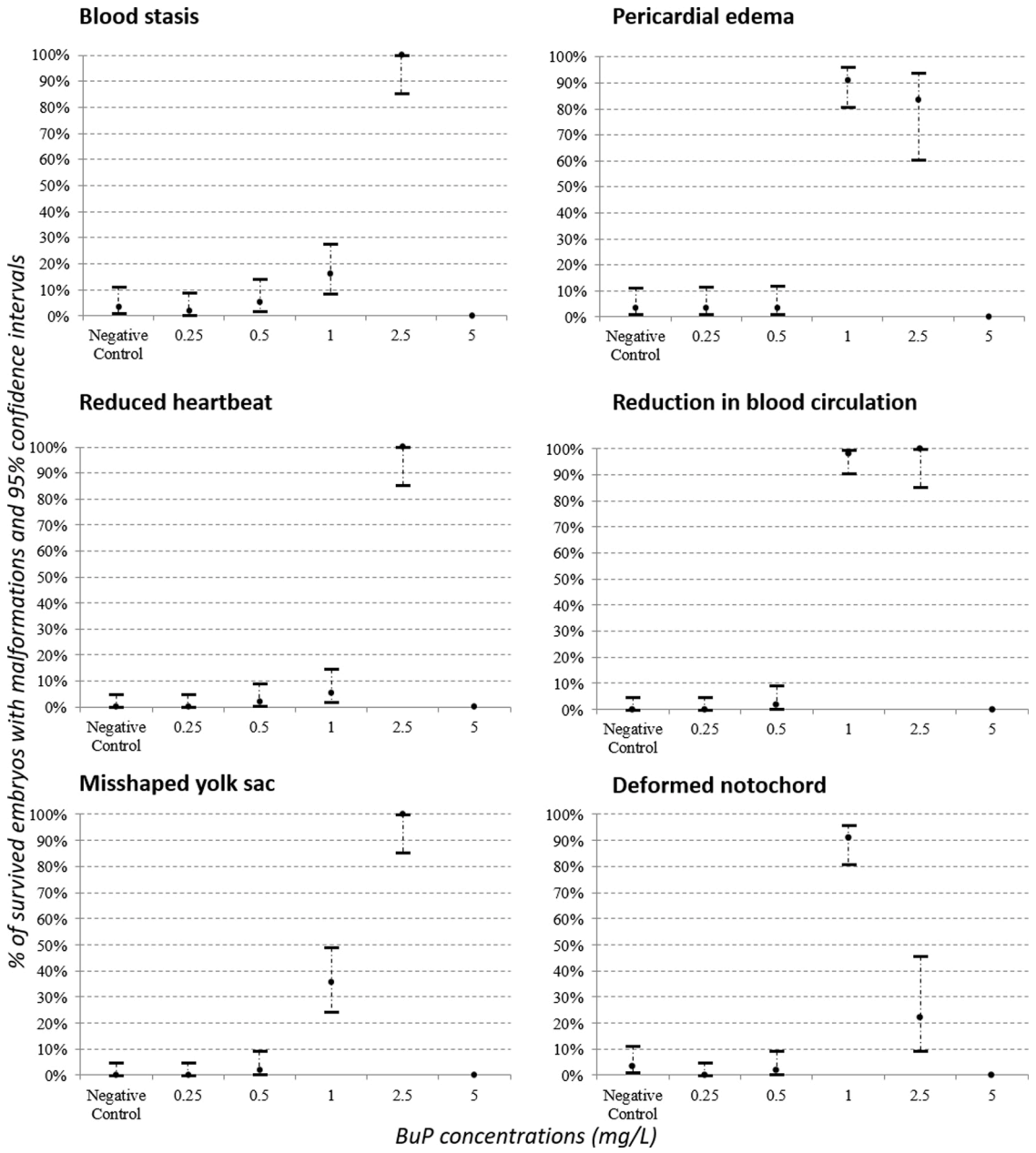

Fig. 2. Percentage and 95 % confidence interval of embryos exposed to BuP concentrations for 96 hpf that developed sublethal alterations compared to negative control group. Data were reported as sum of survived embryos with sublethal alterations in three independent experiments.

from several acute studies on parabens toxicity towards fish as animal models. Medaka (*Oryzias latipes*) early life stages were used to assess the acute toxicity of parabens according to OECD guideline n°203 and LC50 was 2.9 mg/L for BuP and 14 mg/L for EtP (Yamamoto et al., 2007, 2011). On fathead minnow (*Pimephales promelas*), LC50 values were 4.2 mg/L and 34.3 mg/L respectively for BuP and EtP (Dobbins et al., 2009). Fathead minnow growth was adversely affected at 1 mg/L for BuP and 17 mg/L for EtP (Dobbins et al., 2009). EFSA Scientific Committee recommended to use BMDL values as potential reference point for risk assessment and the BMDL values reported in the present study were 0.91 mg/L for BuP and 10.8 mg/L for EtP, respectively. The BMDL value reflects the dose level where the associated effect size was unlikely to be larger than benchmark response (BMR) (EFSA, 2017). In our case, the BMR was defined in terms of an increase in the incidence of the number of dead embryos compared with the background incidence. This increase was expressed as an extra risk and a BMR of 10 % was used, as suggested for quantal data (EFSA, 2017). Together with results of previous studies, these toxicological data may be considered as an important contribution to provide an alternative method for human health risk assessment of BuP and EtP. Despite these two parabens are not the most common parabens used in personal care products and they are less detected in the environment, their potential adverse effects on human health are not negligible. Recently, a positive association between maternal urinary concentrations of BuP and childhood overweight within the first eight years of life was reported (Leppert et al., 2020).

BuP and EtP exposure, led also to the development of several sublethal effects in zebrafish embryos and larvae (Fig. 5).

At the highest concentrations (2.5 mg/L for BuP, 10 mg/L, 20 mg/L and 30 mg/L for EtP) the developmental abnormalities appeared at 48 hpf, while in the others experimental groups, at 72 hpf (BuP 1 mg/L, EtP 5 mg/L). The percentage of zebrafish embryos and larvae exposed to 2.5 mg/L of BuP that developed cardiac alterations (reduced heartbeat and pericardial edema) and defects in the blood circulation (blood stasis

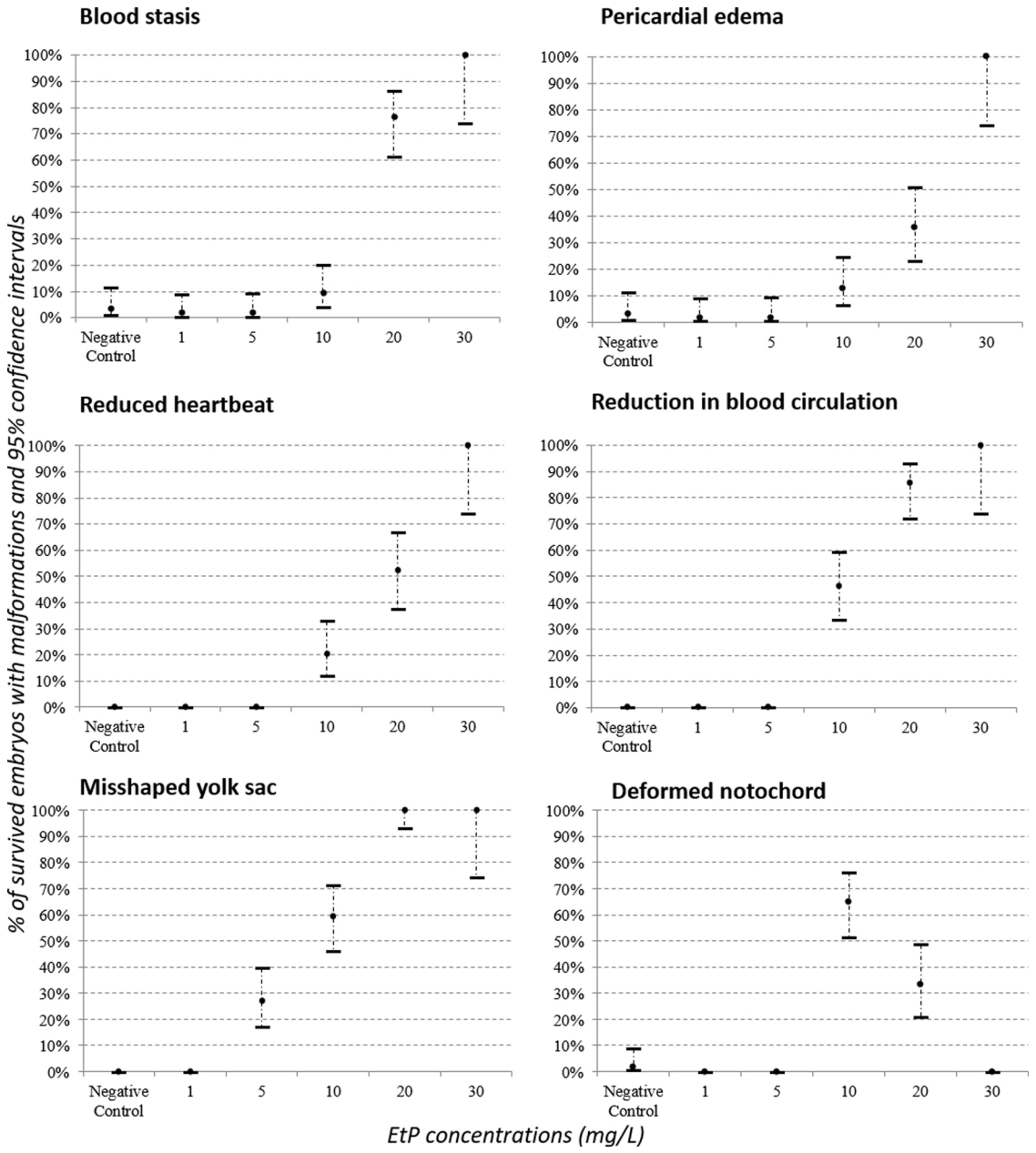

Fig. 3. Percentage and 95 % confidence interval of embryos exposed to EtP concentrations for 96 hpf that developed sublethal alterations compared to negative control group. Data were reported as sum of survived embryos with sublethal alterations in three independent experiments.

and reduction in blood circulation) at 48 hpf was higher compared to the experimental group of EtP 20 mg/L. Moreover, the effects of BuP and EtP exposure on zebrafish hatching were different. In fact, zebrafish embryos exposed to EtP showed a delay in hatching at 72 hpf compared to negative control group (Fig. 1), while embryos exposed to BuP reported differences in hatching rate depending on the tested concentration. Interestingly, zebrafish larvae exposed to the lowest concentrations of BuP and EtP (BuP 0.25 mg/L, BuP 0.5 mg/L and EtP 1 mg/L) did not reported significant sublethal effects compared to negative control (Figs. 2,3). Among teratogenic effects, zebrafish embryos exposed to the highest concentrations of BuP and EtP developed misshaped yolk sac at 48 hpf. Several EDCs showed the ability to interfere with yolk sac mobilization and utilization leading to yolk retention in zebrafish larvae (Sant and Timme-Laragy, 2018). Misshaped yolk and hyperexcitability were recorded in zebrafish larvae exposed to sublethal concentrations of PrP and were associated with increase in the amount of neutral lipid in the yolk, alteration of phospholipids metabolism, shorter body length, reduction in head size and delay in swim bladder inflation (Perugini et al., 2019).

Reduction in blood circulation, blood stasis, pericardial edema, reduced heartbeat, deformed notochord and hatching delay were recently reported in zebrafish early-life stages following to PrP and MeP exposure (Bereketoglu and Pradhan, 2019; Perugini et al., 2019). These sublethal effects were accompanied by impairment in several biochemical pathways involved in cellular stress response, cell cycle and DNA damage, inflammation as well altered genes expression of androgen receptor (ar) and estrogen receptor 2 alpha (esr2a) indicating an antiandrogenic and estrogenic activity of parabens in zebrafish (Bereketoglu and Pradhan, 2019). Brown et al., exposed transgenic insulin GFP zebrafish embryos to BuP at 0.048 mg/L, 0.097 mg/L, 0.194 mg/L and 0.583 mg/L. They found that the lowest concentrations of BuP increased islet area of pancreatic cells and the prevalence of aberrant beta cell architecture in Islets of Langherans in persistent way. Qualitative increase in yolk sac utilization, intestinal effusion,

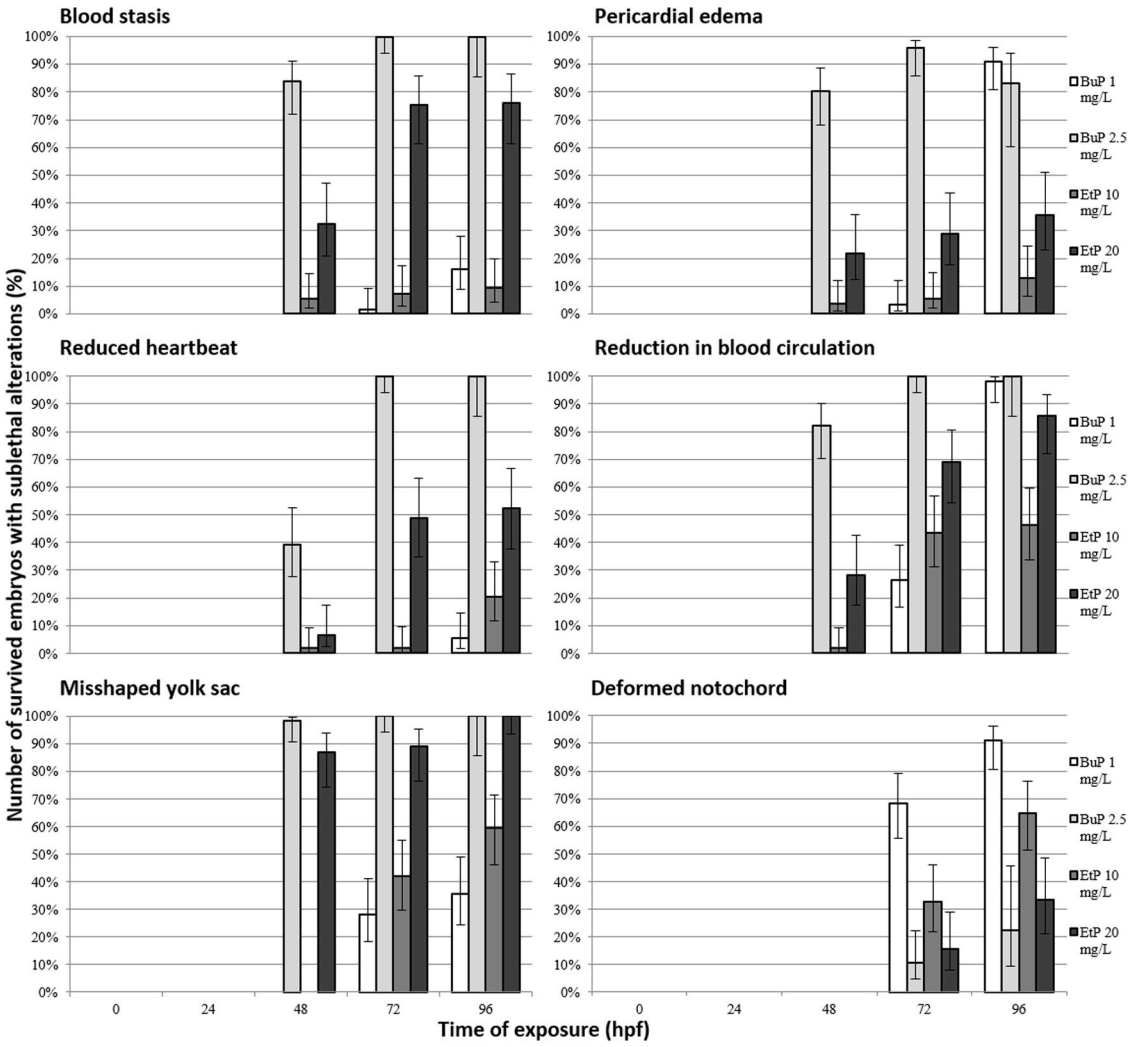

Fig. 4. Percentage and 95 % confidence interval of zebrafish embryos with sublethal alterations at 24, 48, 72 and 96 hpf, following the exposure to 1 mg/L, 2.5 mg/L of BuP and 10 mg/L, 20 mg/L of EtP. Data were reported as sum of survived embryos that developed sublethal alterations in three independent experiments.

pericardial edema, craniofacial malformations, and spinal malformations was also reported in dose-dependent manner (Brown et al., 2018). However, yolk sac area and embryonic body length were not significantly affected by BuP exposure at any concentration, as well no clear relationship was observed between the occurrence of developmental deformities and islet architecture, suggesting that these are independent events (Brown et al., 2018). Behavioral changes in zebrafish larvae exposed to 1 mg/L of BuP and 10 mg/L of EtP were due to involuntary, rapid movements of body with corkscrew (spiral) swimming, head shake movements, pectoral fins tremor, and jittery locomotion. This behavioral phenotype was similar to tremor behavior evoked by selected neurotoxic/convulsant drugs, such as domoic acid (DA) or pentylenetetrazole (PTZ) acting on glutaminergic system and GABA-A receptors, respectively (Kalueff et al., 2013; Tiedeken and Ramsdell, 2007). Neurobehavioral changes are neurotoxic endpoints frequently investigated and addressed in zebrafish exposed to chemicals (d'Amora and Giordani, 2018). In particular, larvae exposed to several pesticides, including chlorpyrifos, diazinon, and parathion showed reduced acetylcholinesterase activity and larval motility (d'Amora and Giordani, 2018). Indeed, zebrafish larvae treated with different pyrethroids presented neurotoxicity characterized by increased motility (De Micco et al., 2010). The neurotoxic effects of parabens on zebrafish early-life stages are not well established, however, MeP exposure led to anxiety-like behaviour, increased cortisol levels, and caused significant inhibition of the acetylcholinesterase activity in exposed larvae (Raja et al., 2019). Therefore, BuP and EtP effects on GABA and glutamatergic system, as well as their ability to led to anxiety-like behaviors in zebrafish early-life stages deserve to be investigated. Indeed, anxiety was evaluated as comorbid symptom of autism spectrum disorder both in zebrafish model and humans (Chen et al., 2018; Zaboski and Storch, 2018). Prenatal exposure to BuP induced neuro-developmental disorders like some of the neurodevelopmental disorders observed in the valproic model of autism in offspring male rats (Ali and Elgoly, 2013). Recently, the correlation between elevated levels of prenatal estrogens and autism likelihood was demonstrated in humans (sleBaron-Cohen et al., 2019). Therefore, the estrogenic properties of BuP and EtP should be investigated on target genes that play an essential role in early brain development and which are also used as indicators of estrogenic chemicals.

5. Conclusion

The data of the present study showed that BuP and EtP exposure was able to induce acute toxicity in zebrafish early-life stages and that BuP was more toxic than EtP. LC50 values enriched data on toxicological properties of BuP and EtP in aquatic organism as there was deficient information available on acute toxicity of these parabens on zebrafish early-life stages. BMD confidence interval could be used as a starting point for risk assessment. Moreover, BuP and EtP exposure led to

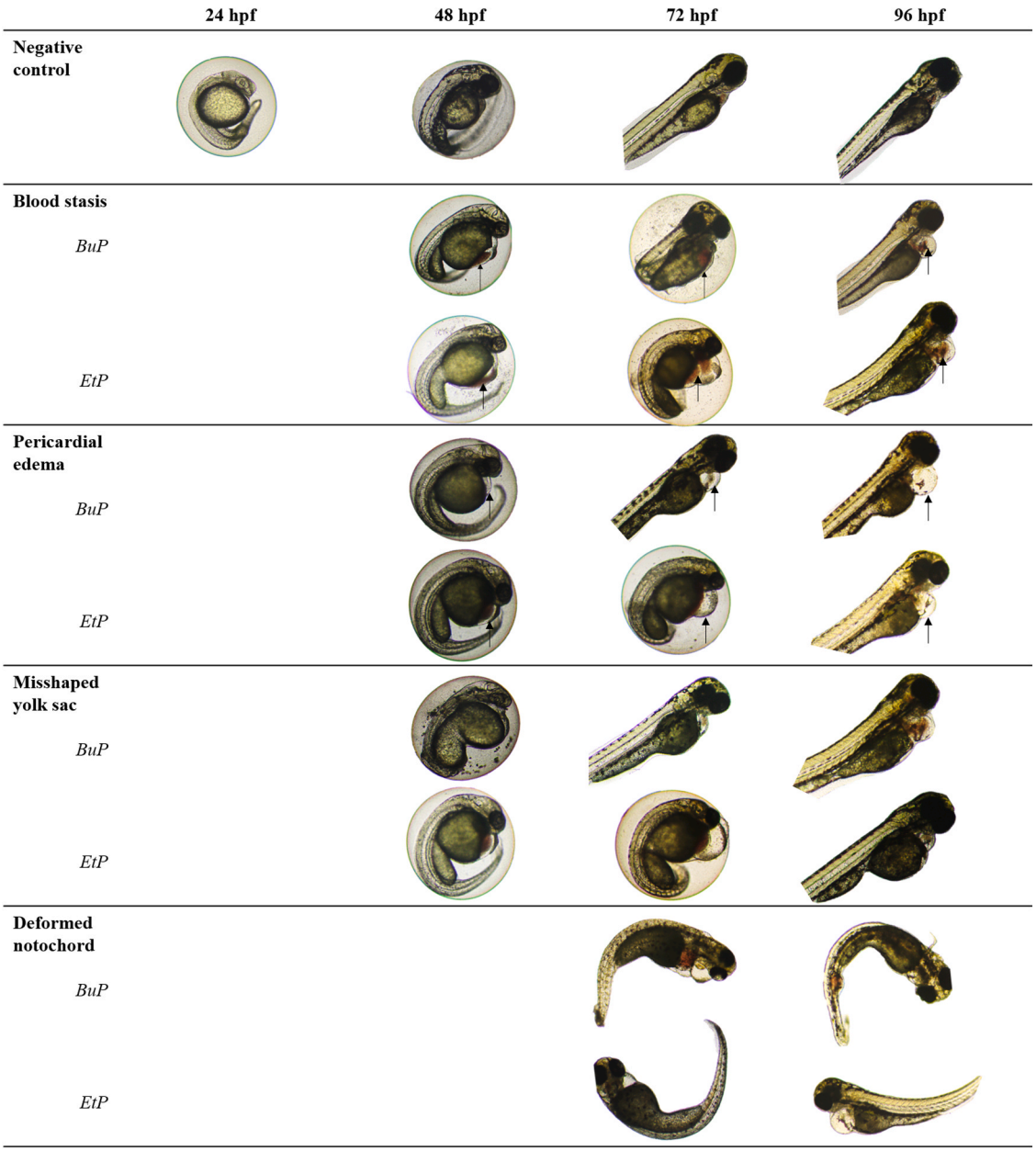

Fig. 5. Sublethal alterations reported at 24, 48, 72 and 96 hpf in zebrafish early-life stages.

developmental abnormalities and teratological effects in zebrafish embryos and larvae. Reduced heartbeat, reduction in blood circulation, blood stasis, deformed notochord, pericardial edema and misshaped yolk sac were also reported in zebrafish early-life stages following PrP and MeP exposure. Behavioral symptoms were also registered and could be related to neurotoxic potential of BuP and EtP. Therefore, further studies are needed to establish new potential modes of action of parabens on central nervous system.

CRediT authorship contribution statement

C. Merola: Methodology, Validation, Investigation. **O. Lai:** Writing - original draft. **A. Conte:** Formal analysis, Software. **G. Crescenzo:** Writing - review & editing. **T. Torelli:** Methodology, Data curation. **M. Alloro:** Writing - original draft. **M. Perugini:** Resources, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.etap.2020.103504.

References

Ali, E.H., Elgoly, A.H.M., 2013. Combined prenatal and postnatal butyl paraben exposure produces autism-like symptoms in offspring: comparison with valproic acid autistic model. Pharmacol. Biochem. Behav. 111, 102–110.

Artacho-Cordón, F., Fernández, M.F., Frederiksen, H., Iribarne-Durán, L.M., Jiménez-Díaz, I., Vela-Soria, F., Andersson, A.M., Martin-Olmedo, P., Peinado, F.M., Olea, N., Arrebola, J.P., 2018. Environmental phenols and parabens in adipose tissue from hospitalized adults in Southern Spain. Environ. Int. 119, 203–211.

Bereketoglu, C., Pradhan, A., 2019. Comparative transcriptional analysis of methylparaben and propylparaben in zebrafish. Sci. Total Environ. 671, 129–139.
Błędzka, D., Gromadzińska, J., Wąsowicz, W., 2014. Parabens. From environmental studies to human health. Environ. Int. 67, 27–42.
Boberg, J., Taxvig, C., Christiansen, S., Hass, U., 2010. Possible endocrine disrupting effects of parabens and their metabolites. Reprod. Toxicol. 30 (2), 301–312.
Brown, S.E., Sant, K.E., Fleischman, S.M., Venezia, O., Roy, M.A., Zhao, L., TimmeLaragy, A.R., 2018. Pancreatic beta cells are a sensitive target of embryonic exposure to butylparaben in zebrafish (*Danio rerio*). Birth Defects Res. 110 (11), 933–948.
Chen, J., Lei, L., Tian, L., Hou, F., Roper, C., Ge, X., Zhao, Y., Chen, Y., Dong, Q., Tanquay, R.L., Huang, C., 2018. Developmental and behavioral alterations in zebrafish embryonically exposed to valproic acid (VPA): an aquatic model for autism. Neurotoxicol. Teratol. 66, 8–16.
d'Amora, M., Giordani, S., 2018. The utility of zebrafish as a model for screening developmental neurotoxicity. Front. Neurosci-Switz. 12, 976.
Dambal, V.Y., Selvan, K.P., Lite, C., Barathi, S., Santosh, W., 2017. Developmental toxicity and induction of vitellogenin in embryo-larval stages of zebrafish (Danio rerio) exposed to methyl Paraben. Ecotox. Environ. Safe. 141, 113–118.
Darbre, P.D., Aljarrah, A., Miller, W.R., Coldham, N.G., Sauer, M.J., Pope, G.S., 2004. Concentrations of parabens in human breast tumours. J. Appl. Toxicol. 24 (1), 5–13.
Davis, J.A., Gift, J.S., Zhao, Q.J., 2011. Introduction to benchmark dose methods and US EPA's benchmark dose software (BMDS) version 2.1. 1. Toxicol. Appl. Pharmacol. 254 (2), 181–191.
De Micco, A., Cooper, K.R., Richardson, J.R., White, L.A., 2010. Developmental neurotoxicity of pyrethroid insecticides in zebrafish embryos. Toxicol. Sci. 113, 177–186.
Dobbins, L.L., Usenko, S., Brain, R.A., Brooks, B.W., 2009. Probabilistic ecological hazard assessment of parabens using Daphnia magna and Pimephales promelas. Environ. Toxicol. Chem. 28 (12), 2744–2753.
EFSA, 2004. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to para hydroxybenzoates (E 214–219). EFSA J. 83, 1–26.
EFSA Scientific Committee, 2017. Update: use of the benchmark dose approach in risk assessment. EFSA J. 15 (1), e04658.
Finot, F., Kaddour, A., Morat, L., Mouche, I., Zaguia, N., Cuceu, C., Souverville, D., Négrault, S., Cariou, O., Essahli, A., Prigent, N., et al., 2017. Genotoxic risk of ethyl-paraben could be related to telomere shortening. J. Appl. Toxicol. 37 (6), 758–771.
Hsieh, J.H., Ryan, K., Sedykh, A., Lin, J.A., Shapiro, A.J., Parham, F., Behl, M., 2018. Application of benchmark concentration (BMC) analysis on zebrafish data: a new perspective for quantifying toxicity in alternative animal models. Toxicol. Sci. 167 (1), 92–104.
Kalueff, A.V., Gebhardt, M., Stewart, A.M., Cachat, J.M., Brimmer, M., Chawla, J.S., Gaikwad, S., et al., 2013. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. Zebrafish 10 (1), 70–86.
Leppert, B., Strunz, S., Seiwert, B., Schlittenbauer, L., Schlichting, R., Pfeiffer, C., et al., 2020. Maternal paraben exposure triggers childhood overweight development. Nature Commun. 11 (1), 1–12.
Nagel, R., 2002. DarT: the embryo test with the Zebrafish Danio rerio–a general model in ecotoxicology and toxicology. Altex 19, 38–48.
Nowak, K., Ratajczak–Wrona, W., Górska, M., Jabłońska, E., 2018. Parabens and their effects on the endocrine system. Mol. Cell. Endocrinol. 474, 238–251.
OECD, 1992. Test No. 203: Fish, Acute Toxicity Test. OECD, Guideline for Testing of Chemicals. OECD pub.
OECD, 2013. Test No. 236: Fish Embryo Acute Toxicity (FET) Test. OECD, Guideline for Testing of Chemicals. OECD pub.
Oishi, S., 2001. Effects of butylparaben on the male reproductive system in rats. Toxicol. Ind. Health 17 (1), 31–39.
Perugini, M., Merola, C., Amorena, M., D'Angelo, M., Cimini, A., Benedetti, E., 2019. Sublethal exposure to propylparaben leads to lipid metabolism impairment in zebrafish early-life stages. J. Appl. Toxicol. https://doi.org/10.1002/jat.3921.
Raja, G.L., Subhashree, K.D., Lite, C., Santosh, W., Barathi, S., 2019. Transient exposure of methylparaben to zebrafish (Danio rerio) embryos altered cortisol level, acetylcholinesterase activity and induced anxiety-like behaviour. Gen. Comp. Endocr. 279, 53–59.
Routledge, E.J., Parker, J., Odum, J., Ashby, J., Sumpter, J.P., 1998. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. Toxicol. Appl. Pharmacol. 153 (1), 12–19.
Sant, K.E., Timme-Laragy, A.R., 2018. Zebrafish as a model for toxicological perturbation of yolk and nutrition in the early embryo. Curr. Environ. Health Rep. 5 (1), 125–133.
sleBaron-Cohen, S., Tsompanidis, A., Auyeung, B., Nørgaard-Pedersen, B., Hougaard, D. M., Abdallah, M., Cohen, A., Pohl, A., 2019. Foetal oestrogens and autism. Mol. Psychiatry 1–9.
Staal, Y.C., Meijer, J., van der Kris, R.J., de Bruijn, A.C., Boersma, A.Y., Gremmer, E.R., Zwart, E.P., Beekhof, P.K., Slob, W.H., Van der Ven, L.T.M., 2018. Head skeleton malformations in zebrafish (*Danio rerio*) to assess adverse effects of mixtures of compounds. Arch. Toxicol. 92 (12), 3549–3564.
Tiedeken, J.A., Ramsdell, J.S., 2007. Embryonic exposure to domoic acid increases the susceptibility of zebrafish larvae to the chemical convulsant pentylenetetrazole. Environ. Health Perspect. 115 (11), 1547–1552.
Yamamoto, H., Watanabe, M., Hirata, Y., Nakamura, Y., Nakamura, Y., Kitani, C., Sekizawa, J., Uchida, M., Nakamura, H., Kagami, Y., Koshio, M., Hirai, N., Tatarazako, N., 2007. Preliminary ecological risk assessment of Butylparaben and benzylparaben ^{2}1. Removal efficiency in wastewater treatment, acute/chronic toxicity for aquatic organisms, and effects on medaka gene expression. Environ. Sci. 14, 073–087.
Yamamoto, H., Tamura, I., Hirata, Y., Kato, J., Kagota, K., Katsuki, S., Yamamoto, A., Kagami, Y., Tatarazako, N., 2011. Aquatic toxicity and ecological risk assessment of seven parabens: individual and additive approach. Sci. Total Environ. 410, 102–111.
Zaboski, B.A., Storch, E.A., 2018. Comorbid autism spectrum disorder and anxiety disorders: a brief review. Future Neurol. 13 (1), 31–37.
Zezza, D., Tait, S., Della Salda, L., Amorena, M., Merola, C., Perugini, M., 2019. Toxicological, gene expression and histopathological evaluations of environmentally realistic concentrations of polybrominated diphenyl ethers PBDE-47, PBDE-99 and PBDE-209 on zebrafish embryos. Ecotoxicol. Environ. Safety 183, 109566.