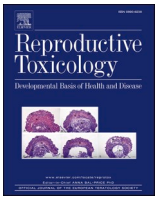
Reproductive Toxicology 128 (2024) 108614

Contents lists available at ScienceDirect

Reproductive Toxicology

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



Effects of combined exposure to two bisphenol plasticizers (BPA and BPB) on *Xenopus laevis* development

M. Battistoni [a,1,2], F. Metruccio [b,1,3], F. Di Renzo [a,*,4], A. Moretto [c,5], R. Bacchetta [a,6], E. Menegola [a,7]

[a] *Università degli Studi di Milano, Department of Environmental Science and Policy, via Celoria, Milan 26-20133, Italy*
[b] *ICPS, ASST Fatebenefratelli Sacco, via GB Grassi, Milan 74-20159, Italy*
[c] *Università degli Studi di Padova, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, via Giustiniani, Padua 2-35128, Italy*

ARTICLE INFO

Handling Editor: Dr. Bal-Price Anna

Keywords:
Endocrine disruptors
Data modelling
Dose-addition
Embryotoxicity
Development
Mixture

ABSTRACT

Due to its endocrine disruptive activity, the plastic additive Bisphenol A (BPA) is classified as substance of very high concern (EU ECHA 2017). A correlation between environmental exposure to BPA and congenital defects has been described in humans and in experimental species including the amphibian *Xenopus laevis*, where severe branchial defects were associated to lethality. The exposure of *X. laevis* embryos to the BPA analogue bisphenol B (BPB) was recently linked to similar teratogenic effects, with BPB having relative potency about 3 times higher than BPA. The combined BPA-BPB exposure is realistic as both BPA and BPB are detected in human samples and environment. Limited experimental data are available on the combined developmental toxicity of BPA and BPB. The aim of the present work is to evaluate the effects of BPA and BPB mixture in the *X. laevis* development model, using R-FETAX procedure. The exposure was limited to the first day of development (corresponding to the phylotypic developmental period, common to all vertebrates). Samples were monitored for lethal effects during the full six-day test period and the external morphology was evaluated at the end of the test. Mixture effects were described by modelling, using the PROAST software package. Overall data modelling showed that dose-addiction could not be rejected, suggesting a health concern for co-exposure.

1. Introduction

Bisphenol A (BPA) is a plastic additive used in the production of polycarbonate plastics, epoxy resins used to line metal cans, and many plastic consumer products including toys, water pipes, drinking containers, eyeglass lenses, sports safety equipment, dental monomers, medical equipment, consumer electronics and, as a colour developer, in thermal paper [1]. Humans are directly or indirectly exposed to BPA through ingestion, inhalation and dermal contact; vertical maternal-to-embryofoetal exposure has also been demonstrated [2].

BPA is currently classified as endocrine-disrupting chemical (EDC) in the European Union (EU) [3], because it shows oestrogen receptor binding and anti-androgenic activity, although with low affinity compared to natural ligands [4]. This classification has prompted regulatory restrictions in production and use in several countries [5,6]. Consequently, one of the urgent issues is finding a safe replacement for BPA and the safety of BPA analogues is under debate.

Among BPA analogues, bisphenol B (BPB) is used as BPA alternative in plastic production in different non-EU countries, including the USA. A direct or indirect release of BPA and BPB into the environment has been demonstrated at any level of the plastic product life cycle (production, consumption, disposal) [2,7]. Even if BPB is not manufactured or used as

* Correspondence to: Università degli Studi di Milano, via Celoria 26, Milan 20133, Italy.
E-mail address: francesca.direnzo@unimi.it (F. Di Renzo).
[1] These authors equally contributed to the work
[2] 0000-0001-5405-458X
[3] 0000-0001-8330-6352
[4] 0000-0003-0200-4673
[5] 0000-0003-4386-5736
[6] 0000-0002-3328-0139
[7] 0000-0001-5558-3459

https://doi.org/10.1016/j.reprotox.2024.108614
Received 20 March 2024; Received in revised form 20 May 2024; Accepted 21 May 2024
Available online 10 June 2024
0890-6238/© 2024 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

a chemical in European Union, it is detectable in the European market in several food products, such as canned foods and commercial milk samples or drinking water [8–11]. Notably, BPB has become one of most frequently detected bisphenols, with reported increasing concentrations in environment matrices, food and beverages [12,13] In 2021 it has been included by ECHA in the list of substances of very high concern [14]. Detectable levels of BPB were found in plasma or urine in both non-EU [15–17] and EU population [18,19]. In a small Italian cohort (Naples), BPA and BPB were found alone or in mixture in blood sera of patients with endometriosis (with maximum concentrations of BPA at 0.03 μM and BPB at 0.05 μM) while they were not detectable in healthy women [18]. BPA concentrations of similar magnitude (0.01 μM) were reported in pregnant women in China [20].

An association between blood/urine levels of bisphenols and adverse pregnancy outcomes, including congenital defects, has been described in humans [21–24]. In different vertebrate developmental models (including mouse, chick, zebrafish and *Xenopus laevis*), lethality, developmental delays, oedema, multiple malformations and neural, cardiac and craniofacial malformations were described [25–36].

Recently, our research group characterized teratogenic and neurobehavioural effects of BPA and BPB in the amphibian *Xenopus laevis* developmental model (using R-FETAX methodology) [37]. In this work, a windowed exposure protocol was applied: when exposure covered the embryogenetic period, common to all vertebrates from both morphological and molecular point of view (phylotypic period) [38–40], both BPA and BPB were teratogenic. Cranio-facial and branchial abnormalities were reported and severe branchial defects were related to time-specific lethality. A 3.42 times higher relative potency factor (RPF) of BPB versus BPA was derived by modelling [37].

Recent papers on mixtures of BPA with some analogues indicate a possible combined effect on estrogenic activity [41,42] and on pregnancy outcomes [43]. In a zebrafish developmental toxicity model, additive lethal and cardiotoxic effects of binary mixture of BPA and its analogues BPF, BPAF and BPB were reported [44], suggesting the need for further evaluation of the environmental impact of bisphenol combined exposure.

The aim of the present work is to evaluate the effects of BPA and BPB mixtures in the amphibian *X. laevis* developmental model, using R-FETAX and data modelling procedures.

2. Materials and methods

2.1. R-FETAX

The R-FETAX methodology is a refined method with marked differences from standard FETAX. R-FETAX was applied according to Battistoni et al., [45]. Briefly, adult amphibians *X. laevis* (Nasco, USA) were maintained under controlled conditions in an automatic breeding system (TecnoPlus, Techniplast, Italy), designed to hold *X. laevis* in a constantly controlled environment, following the Guidance on the housing and care of the African clawed frog *X. laevis* [46]. During recirculation, water was cleaned by both mechanical and active carbon filters, sterilized by UV lamp and automatically re-equilibrated with salt mixes (Instant Ocean ®, Aquarium System, Italy) and sodium bicarbonate (Sigma). Water parameters are set at T=20.5+1, pH= 7.5+1, conductivity= 1150+250 μS. Adults (3 different couples were used) were naturally mated overnight; the following morning, embryos were collected and cleaned by gentle swirling in a 2.25 % L-cysteine solution with an arranged pH of 8.0 and rinsed several times in FETAX solution (625 mg/L NaCl, 96 mg/L $NaHCO_3$, 30 mg/L KCl, 15 mg/L $CaCl_2$, 60 mg/L $CaSO_4 \cdot 2\ H_2O$, and 70 mg/L $MgSO_4$). Normally cleaved embryos at the mid-blastula stage (stage 8, according to Nieuwkoop and Faber [NF] stadiation [47]) were selected and maintained at 23°C during the entire testing period (6 days, corresponding in our laboratory conditions to final NF stage 46, reached in historical unexposed tadpoles). Unlike the classical FETAX protocol described in the guidelines [48], natural mating used in R-FETAX implies later egg deposition, consequently stage 10 (referred by Nieuwkoop and Faber to occur 9 hours post fertilization) is reached in our laboratory in the mid/late-afternoon (day 0.5); furthermore, fine developmental staging in our laboratory conditions indicated that stages up to NF 43 develop at a similar rate compared to Nieuwkoop and Faber staging (1956), while more time is needed to reach stage 46 [49]. The absence of codified *X. laevis* strains, at Nieuwkoop and Faber age until now, does not allow for a strict definition of a match between NF stages and developing hours/days for *X. laevis.* Therefore, we referred to NF stages rather than ASTM codified timing. Exposure (5 embryos/ replicate; at least 3 replicates/group) (Fig. 1) covered the phylotypic developmental window (teratogenicity window, NF stages 10–26), corresponding to the developmental stages common to all vertebrate embryos (gastrula-early morphogenesis, representing the window for species-agnostic teratogenesis purposes). Stage identification was performed referring to Nieuwkoop and Faber and Zhan staging tables (www.xenbase.com).

Test chemicals (Sigma, Italy) were dissolved in DMSO (Sigma, Italy) and diluted to obtain stock solutions

stored at −20°C. Final dilutions in DMSO were prepared fresh each time before treatment and added to the FETAX medium (4 μL/mL, as used in our previous paper on BPA and BPB [37] were no effects related to solvent exposure were observed) to reach the final concentrations of BPA (0–12.5–25–30–35 μM), BPB (0–3.25–6.5–8–9.5 μM) or mixtures (MIX). Dose 0 was exposed to DMSO alone (4 μL/mL). An additional group, maintained in pure FETAX solution, was used as an intra-laboratory control. BPA and BPB concentrations were selected based on literature on *X.laevis* BPA and BPB teratogenicity [25,30,37]. BPA concentrations were 10 times higher than BPA concentrations detected in surface water [50–52]. Mixture concentrations were selected on the basis of modelling of previous published data [37] (Fig. 2) from which a RPF of 3.42 was calculated for BPB.

Specifically, BPA and BPB concentrations close to the values derived as BenchMarkDoses for BenchMarkResponse 25 % (BMD_{25}) were selected for the mixture (BPA 25 μM and BPB 6.5 μM). BPA 12.5 μM and BPB 3.25 μM were used as low-dose levels. The groups are listed in Table 1, where the corresponding concentrations in BPA equivalents are also shown.

To assess lethal effects, samples (at list triplicates of 5 embryos/ group) were monitored throughout the entire six-day test period using a cold-light stereomicroscope (Zeiss). At the end of the test (day 6), the functional deglutition test was applied according to Battistoni et al., 2022b. Larvae were maintained for 2 h at 23 ± 0.5 °C in FETAX solution containing 25 μg/mL red polystyrene microparticles (1 μm diameter, Sigma).Tadpoles were anesthetized with MS-222 (Sigma, Italy; 0.01 % in FETAX solution) and evaluated for gross morphology and the presence or absence of red staining in the intestine (deglutition test positive/ negative) under a camera-equipped cold-light illuminated dissecting microscope (Leica). At the end of the evaluation, samples were photographed, euthanized by anaesthetic overdose (MS-222 0.1 % in FETAX

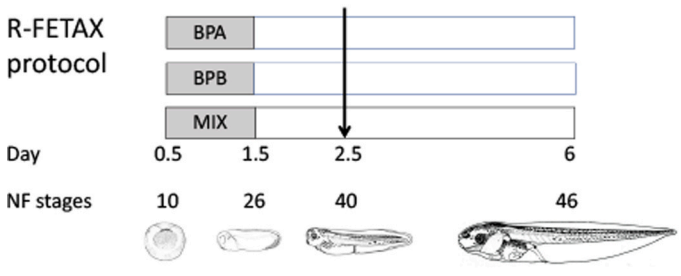


Fig. 1. R-FETAX protocol: coloured boxes represent BPA, BPB or mixture (MIX) exposure window (phylotypic stages); white boxes indicate the maintenance in FETAX solution. Arrow indicates the timing of previously observed BPA- and BPB-related lethality, according to Metruccio et al., (2024).

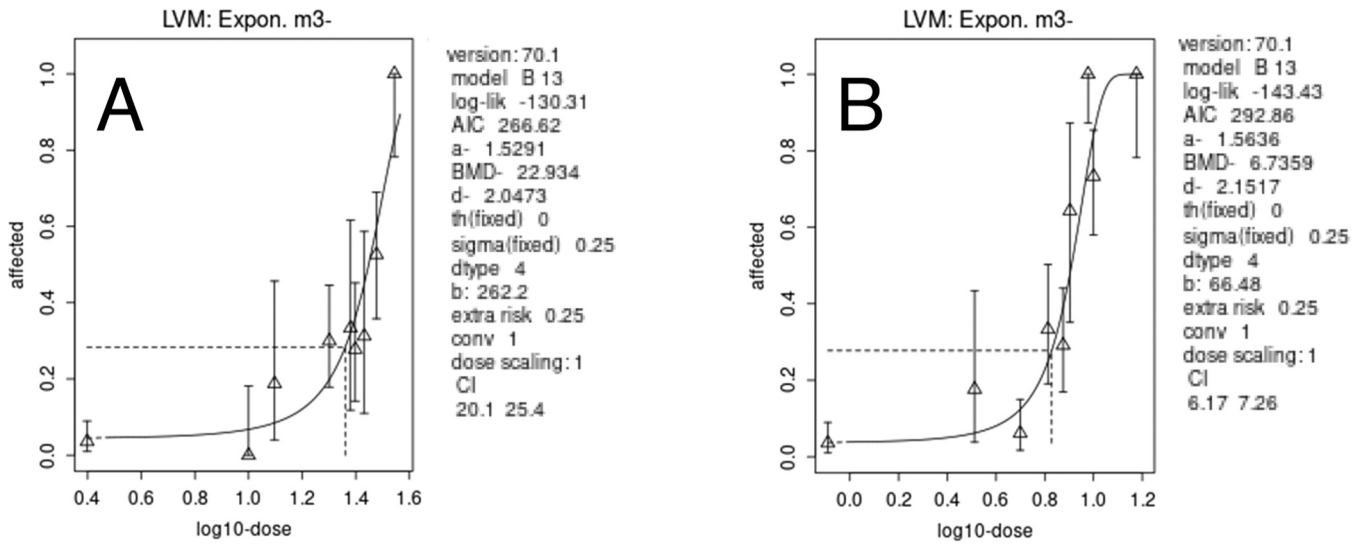


Fig. 2. Dose-response characterization by modelling BPA and BPB previously published datasets (Metruccio et al., 2024) to derive: A) BPA BMD_{25} (22.9 μM; CI 20.1–25.4 μM) and B) BPB BMD_{25}(6.7 μM, CI 6.2–7.3 μM). The obtained values were used to set concentration levels in mixtures. BMD_{25}= BenchMarkDose for BenchMarkResponse 25 %. CI= Confidence Intervals.

Table 1
Experimental groups. Concentrations in BPA equivalents were calculated on the basis of the relative potency factor (RPF= 3.42) derived by modelling in the previous published work (Metruccio et al., 2024). In grey, the mixture groups.

BPA (μM)	**BPB** (μM)	**Concentration in BPA equivalents** (μM)
0	0	0
0	3.25	11.1
0	6.5	22.2
0	8	27.4
0	9.5	32.5
12.5	0	12.5
25	0	25
30	0	30
35	0	35
12.5	3.25	23.6
12.5	6.5	34.7
25	3.25	36.1
25	6.5	47.2

solution at 4°C), fixed in ethanol 50 % (Sigma, Italy) and conserved in ethanol 70 %.

2.2. Mathematical modelling (PROAST)

The software package PROAST (70.3 version) developed by the Dutch National Institute for Public Health and the Environment (RIVM) (www. proast.nl) for the statistical analysis of dose-response toxicological data, was used for modelling. In order to derive exposure concentrations used in the present work, the benchmark dose (BMD) approach was applied on BPA and BPB previously published datasets [37]: BPA and BPB single dose-response curves were characterized, setting BMD at 25 % benchmark response (BMD_{25}) to derive the Relative Potency Factor (RPF).

Original data obtained in the present work were modelled using the dose-addition option in PROAST. The dose-addition hypothesis was verified and overall BMDs at 10–25–50 % benchmark responses (BMD_{10} BMD_{25} BMD_{50}) were obtained.

2.3. Statistical analysis

Quantal data were analysed using the Chi- square for trend. The level of significance was set at $p < 0.05$.

3. Results

Dose-related lethal and teratogenic effects were evident in groups exposed to BPA, BPB or mixtures (Table 2). In contrast, the vehicle group (dose 0, exposed to DMSO alone) developed similarly to the experimental controls maintained in pure FETAX solutions. Both the vehicle and unexposed controls consisted of seven replicates, which yielded comparable results. Data obtained in controls without solvent were not included in Table 2 because they can't be included in modelling.

Teratogenic effects detected in living tadpoles were classified as anterior defects (shortened craniofacial region, small gill basket), only in few cases associated to ventral oedema and/or bent tail (Table 2; Fig. 3 B-D'). Specifically, the shortened anterior region was correlated with abnormal flexion of the encephalic region (Fig. 3 D). The anterior region, therefore, resulted the main common target for BPA, BPB and their mixture. Deglutition test was positive for all tadpoles, showing no differences among groups and indicating functionally normal facial articulation. As previously reported, exposure-related lethality typically occurred two days after the end of exposure (approximatively NF 42–44, the last tailbud stages), consistent with previously reported data linking lethality to severe branchial defects [37].

B-D') BPA 30 μM (B), BPB 6.5 μM (C) and MIX (BPA 12.5+ BPB 6.5 μM) (D-D')-exposed tadpoles showing abnormal phenotypes characterized by flexed encephalic region (D: yellow dotted line) leading to shortening craniofacial structures (B, C, D': black lines) and small branchial basket (B, C, D': black dotted line). Normal sized and coiling intestine (X) and positive deglutition test (red microplastics in the intestine) are visible in any group. Magnification: B, C, D' 20X; D 32X.

3.1. Data modelling

To test the dose-addiction hypothesis, data were analysed using dose addiction exponential models. As the plotted responses of each group scattered around the fitted curve, the hypothesis that the mixture effect is additive could not be rejected (Fig. 4). Overall BMD values for BenchMarkResponse 10 %, 25 % and 50 % (BMD_{10} BMD_{25} BMD_{50}) were derived, resulting (in BPA equivalents): BMD_{10} [17.6–20.8]; BMD25 [22.2–25.5]; BMD_{50} [26.4–29.8]; RPF BPB vs BPA was 3.7, with Confidence Interval [3.4–3.9] which confirmed the results of the previous experiment (Fig. 4).

Table 2
Main test: dead, abnormal and total affected (dead + abnormal) (%), observed in groups exposed to BPA, BPB or mixtures. Statistics (Chi-square for trend, calculated on frequencies) are shown.

COMPOUND	DOSE BPA (μM)	DOSE BPB (μM)	DOSE (BPA equivalents) (μM)	N	Total affected (%)	Dead (%)	Total abnormal (%)	Head defects (%)	Gill basket defects (%)	Bent tail (%)	Oedema (%)
BPA	0	0	**0**	32	12.5	9.4	3.4	3.4	0.0	0.0	0.0
BPA	12.5	0	**12.5**	24	29.2	25.0	5.6	5.6	0.0	0.0	0.0
BPA	25	0	**25**	23	43.5	21.7	27.8	27.8	0.0	0.0	0.0
BPA	30	0	**30**	25	72.0	52.0	41.7	33.3	16.7	0.0	0.0
BPA	35	0	**35**	15	100.0	100.0					
				p value	**<0.0000001**	**0.000000179**	**0.001001**	**0.003065**	0.051260		
BPB	0	0	**0**	32	12.5	9.4	3.4	3.4	0.0	0.0	0.0
BPB	0	3.25	**11.1**	20	30.0	15.0	17.6	11.8	5.9	5.9	5.9
BPB	0	6.5	**22.2**	39	33.3	17.9	18.8	15.6	0.0	3.1	0.0
BPB	0	8	**27.4**	14	64.3	42.9	37.5	37.5	12.5	0.0	0.0
BPB	0	9.5	**32.5**	30	96.7	96.7					
				p value	**<0.0000001**	**<0.0000001**	**0.01740**	**0.02965**	0.46360	0.7019	0.7271
MIX	0	0	**0**	32	6.4	3.0	3.4	3.4	0.0	0.0	0.0
MIX	12.5	3.25	**23.6**	22	27.3	18.2	11.1	11.1	0.0	0.0	0.0
MIX	12.5	6.5	**34.7**	22	100.0	90.9	100.0	50.0	100.0	50.0	0.0
MIX	25	3.25	**36.1**	22	100.0	95.5	100.0	100.0	100.0	0.0	100.0
MIX	25	6.5	**47.2**	12	100.0	100.0					
				p value	**<0.0000001**	**<0.0000001**	**0.00274**	**0.01818**	**0.0007068**	0.06399	**0.04974**

4. Discussion

Due to regulations on endocrine active substances and public concerns regarding the potential estrogenic and anti-androgenic activity of BPA and its effects on human health [4,53–56], the use of BPA has been banned or restricted in the EU [5,6]. Consequently, alternative bisphenols, such as BPB, have been introduced in plastic production processes in several non-EU countries. Despite EU restrictions, BPB is detected in products marketed in the EU. The widespread use of BPA and alternative bisphenols has resulted in their simultaneous detection in the environment [13], human urine/blood samples [15,17,57–60] and aquatic organisms [61–65]. BPA and BPB (Fig. 5) share a strong structural similarity, as they belong to the group of chemicals with two hydroxyphenyl functional groups. Consistent with their structural similarity, BPB shares the endocrine activity with BPA, albeit with stronger estrogenic and anti-androgenic potency than BPA itself, albeit still orders of magnitude lower than natural ligands [66]. The effects of combined exposure to BPA and BPB need to be considered for both humans and aquatic wild species.

An association between bisphenols levels in blood/urine during pregnancy and congenital birth defects (including neural abnormalities) has been described in humans [21–24]. Relatively high doses of bisphenols in different experimental vertebrate models, including zebrafish and *X. laevis*, have been shown to caused similar defects, particularly head defects [25,26,28,35].

In human pregnancy, the simultaneous detection of different bisphenols in urine has been associated with reduced birth weight, suggesting combined effects [43]. In a zebrafish developmental toxicity model, lethal and cardiotoxic effects were reported in embryos exposed to a binary mixture of BPA and its analogues BPF, BPAF or BPB [44].

The aim of the present work was to evaluate the teratogenic effects of the BPA-BPB binary mixture in the amphibian *X. laevis* developmental model. The *X. laevis* embryo model is widely used in embryotoxicity research fields [67–70], and has been also selected to test EDCs [71]. The R-FETAX procedure was applied, with the exposure covering NF stage 10–26, considered in *X. laevis* as the phylotypic period, common to all vertebrates at both morphological and molecular levels [38–40]. Our results confirm that both BPA and BPB are teratogenic, BPB being more than 3 times more potent than BPA (RPF= 3.7, CI 3.4–3.9). Moreover, co-exposure to BPA-BPB resulted in dose-additivity, a hypothesis that was not rejected by data modelling, as expected by molecules sharing the same molecular targets [72].

In a small Italian cohort [18] of patients with endometriosis, BPA and BPB human blood serum concentrations were up to 0.03 μM BPA and 0.05 μM BPB (equal to 0.15 μM BPA equivalents, considering BPB RPF=3), resulting in a cumulative BPA_BPB dose of 0.18 μM BPA equivalents. The $BMDL_{10}$ (the CI lower value referred to 10 % extra risk) is used as the point of departure in current risk assessment [73]. The point of departure is intended to be the values from which to derive exposure limits by applying uncertainty factors to account for inter and intraspecies differences. The value of these uncertainty factors is 10 for both differences. Applying the total uncertainty factor of 100 to $BMDL_{10}$ derived from our modelling, the exposure limit for concentration in human embryos of BPA-equivalents would be 0.17 μM (considering $BMDL_{10}$ / 100), falling within the range of theoretical human exposure [18]. The similarity of effects reported in human literature (neural defects) with our results (flexed encephalic region) suggests R-FETAX as an alternative predictive model.

The use of *X.laevis* model has application in ecotoxicological risk assessment of the presence of bisphenols in water. Concentrations of BPA up to 29920 ng/L (0.13 μM) in surface water and 16929 ng/L (0.07 μM) in seawater have been found in Turkey; in China, BPB levels reached 46 ng/L (0.0002 μM) in surface waters [74–76]. In Europe (Poland), BPB concentrations were found to be around 60 ng/L (0.0003 μM) in raw wastewaters and about 30 ng/L (0.0001 μM) [77], confirming that, despite a series of treatment processes, bisphenols can still be detected in the effluent, which could allow them to enter natural waters [50,51]. Concerning wild species exposure, the simultaneous detection of several bisphenols, including BPA and BPB, has been described in several fish samples [52,78], in concentration ranges providing insights into the bioaccumulation of this molecules in animals. Additionally, the possibility of BPA accumulation in fish eggs was experimentally demonstrated and correlates with endocrine deregulation during development [79,80]. Considering all these pieces of evidence, a complex picture emerges, indicating the necessity for a more comprehensive evaluation in the context of assessing the risks of bisphenol co-exposure to wild species as well. Although wild species extinction increased awareness of the need for conservation, there are still many gaps in research on the effects of bisphenols on wild amphibians. Considering that embryos and larvae are the most sensitive [81], our data on bisphenol-induced lethality during development stress the need for severe legislations limiting bisphenol direct or indirect

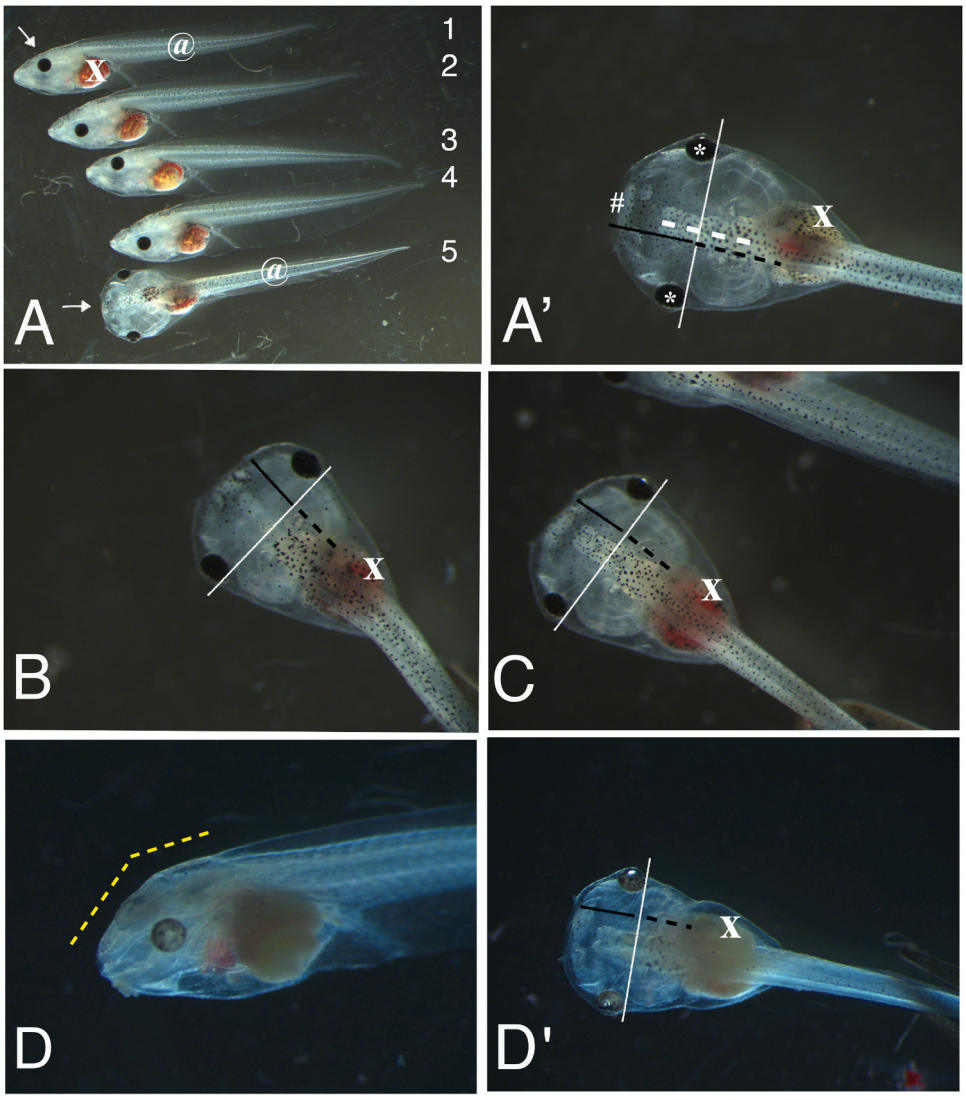


Fig. 3. Phenotypes observed at the end of R-FETAX in groups exposed to BPA, BPB or mixtures. A) Tadpoles with normal phenotype (1–4 lateral view; 5 dorsal view). Note the anterior region (→), the tail (@), the coiled intestine (X, index of NF 46 developmental stage reached). Magnification 8X; A') dorsal view of tadpole normal anterior region. Note the linear encephalon (white dotted line), the eyes (*) representing the limit border (white line) between the anterior craniofacial region (black line) and the branchial region (black dotted line). (#) open mouth; X coiled intestine. Magnification 20X.

release into aquatic ecosystems.

In summary, our findings suggest that BPB is unlikely to be a superior substitute for BPA [82], highlighting the importance of re-evaluating the impacts of bisphenols when co-exposure occurs, both for human and environmental health. Moreover, our study demonstrates that the R-FETAX methodology resulted sensitive in detecting teratogenic mixture effects associated with EDC exposure. We propose R-FETAX as a rapid, cost-effective, and sensitive method of choice for screening mixtures that may be harmful to aquatic and terrestrial ecosystems, as well as human development.

Ethical approval

The study was conducted according to the relevant European (EU Directive 2010/63/EU for animal experiments) and Italian (Legislative Decree No. 26/2014) laws, rules, and regulations. All procedures were examined and approved by the Animal Welfare Organization of the Università degli Studi di Milano. Facility authorization number: 198283; date: 19/12/2019.

Funding

This work was supported by the European Union's Horizon2020 Research and Innovation program [grant agreement 633172 (EuroMix; www.euromixproject.eu)] and by the Università degli Studi di Milano grant Linea2_2018 and Linea2_2019.

CRediT authorship contribution statement

E. Menegola: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **A. Moretto:** Writing – review & editing, Funding acquisition, Conceptualization. **R. Bacchetta:** Writing – review & editing, Methodology, Conceptualization. **F. Metruccio:** Writing – review & editing, Software, Data curation, Conceptualization. **Francesca Di Renzo:** Writing – review & editing, Resources, Methodology, Data curation, Conceptualization. **M. Battistoni:** Writing – review & editing, Methodology, Data curation, Conceptualization.

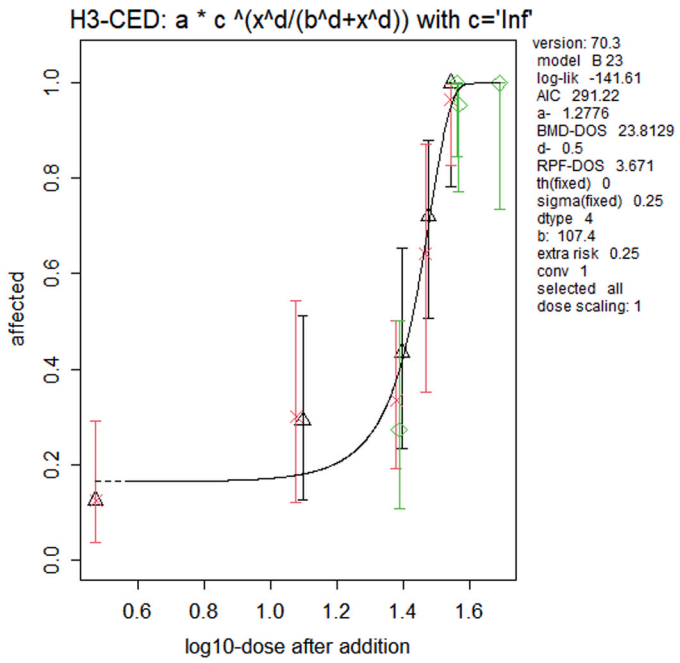


Fig. 4. BPA and BPB single and mixture effects showing the additive hypothesis not rejected, with the plotted responses of each group scattered around the fitted curve. Overall BMD_{10}; BMD_{25} and BMD_{50} Confidence Intervals were derived using dose addiction exponential models and were: BMD_{10} [17.6–20.8]; BMD_{25} [22.2–25.5]; BMD_{50} [26.4–29.8]. Dose after addition= BPA equivalent dose. BPB was confirmed the sensitive subgroup: RPF BPB vs BPA was 3.7, with Confidence Interval resulted [3.4–3.9].

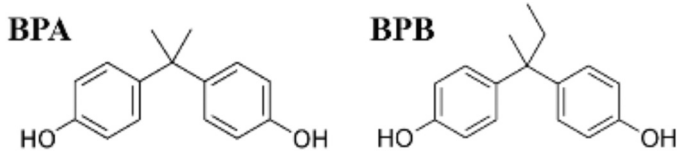


Fig. 5. BPA and BPB chemical structures. Note the extreme similarity between the two molecules.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Elena Menegola reports financial support was provided by University of Milan. Angelo Moretto reports financial support was provided by European Union. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

The Authors thank the staff of Xenopus facility at the Università degli Studi di Milano. The Authors acknowledge the Euromix project.

References

[1] L.N. Vandenberg, R. Hauser, M. Marcus, N. Olea, W.V. Welshons, Human exposure to bisphenol A (BPA), Reprod. Toxicol. 24 (2007) 139–177, https://doi.org/10.1016/j.reprotox.2007.07.010.

[2] S. Almeida, A. Raposo, M. Almeida-González, C. Carrascosa, Bisphenol A: food exposure and impact on human health: Bisphenol A and human health effect…, Compr. Rev. Food Sci. Food Saf. 17 (2018) 1503–1517, https://doi.org/10.1111/1541-4337.12388.

[3] ECHA, ECHA, 2017. One new substance added to the Candidate List, several entries updated [https://echa.europa.eu/-/one-new-substance-added-to-the-candidate-list]., (2017). https://echa.europa.eu/-/one-new-substance-added-to-the-candidate-list.

[4] W.V. Welshons, S.C. Nagel, F.S. vom Saal, Large effects from small exposures. III. Endocrine mechanisms mediating effects of Bisphenol A at levels of human exposure, Endocrinology 147 (2006) s56–s69, https://doi.org/10.1210/en.2005-1159.

[5] European Food Safety Authority CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Aids), Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs, EFSA J. (2005), https://doi.org/10.2903/j.efsa.2015.3978.

[6] EFSA, Bisphenol A.: EFSA draft opinion proposes lowering the tolerable daily intake. [⟨https://www.efsa.europa.eu/en/news/bisphenol-efsa-draft-opinion-proposes-lowering-tolerable-daily-intake⟩], (2021).

[7] J. Liu, L. Zhang, G. Lu, R. Jiang, Z. Yan, Y. Li, Occurrence, toxicity and ecological risk of Bisphenol A analogues in aquatic environment – a review, Ecotoxicol. Environ. Saf. 208 (2021) 111481, https://doi.org/10.1016/j.ecoenv.2020.111481.

[8] L. Grumetto, D. Montesano, S. Seccia, S. Albrizio, F. Barbato, Determination of Bisphenol A and Bisphenol B residues in canned peeled tomatoes by reversed-phase liquid chromatography, J. Agric. Food Chem. 56 (2008) 10633–10637, https://doi.org/10.1021/jf802297z.

[9] L. Grumetto, O. Gennari, D. Montesano, R. Ferracane, A. Ritieni, S. Albrizio, F. Barbato, Determination of five bisphenols in commercial milk samples by liquid chromatography coupled to fluorescence detection, J. Food Prot. 76 (2013) 1590–1596, https://doi.org/10.4315/0362-028X.JFP-13-054.

[10] S.C. Cunha, C. Almeida, E. Mendes, J.O. Fernandes, Simultaneous determination of bisphenol A and bisphenol B in beverages and powdered infant formula by dispersive liquid–liquid micro-extraction and heart-cutting multidimensional gas chromatography-mass spectrometry, Food Addit. Contam. Part A 28 (2011) 513–526, https://doi.org/10.1080/19440049.2010.542551.

[11] M. Fattore, G. Russo, F. Barbato, L. Grumetto, S. Albrizio, Monitoring of bisphenols in canned tuna from Italian markets, Food Chem. Toxicol. 83 (2015) 68–75, https://doi.org/10.1016/j.fct.2015.05.010.

[12] M.T. García-Córcoles, M. Cipa, R. Rodríguez-Gómez, A. Rivas, F. Olea-Serrano, J. L. Vílchez, A. Zafra-Gómez, Determination of bisphenols with estrogenic activity in plastic packaged baby food samples using solid-liquid extraction and clean-up with dispersive sorbents followed by gas chromatography tandem mass spectrometry analysis, Talanta 178 (2018) 441–448, https://doi.org/10.1016/j.talanta.2017.09.067.

[13] C.J. Catenza, A. Farooq, N.S. Shubear, K.K. Donkor, A targeted review on fate, occurrence, risk and health implications of bisphenol analogues, Chemosphere 268 (2021) 129273, https://doi.org/10.1016/j.chemosphere.2020.129273.

[14] ECHA, Agreement of the member state committee on the identification of 4,4'-(1-methylpropylidene)bisphenol (Bisphenol B) as a substance of very high concern [https://echa.europa.eu/documents/10162/b8baaee8-e3ae-64f6–371a-dd188c8d4268], (2021). https://echa.europa.eu/documents/10162/b8baaee8-e3ae-64f6–371a-dd188c8d4268..

[15] A.G. Asimakopoulos, J. Xue, B.P. De Carvalho, A. Iyer, K.O. Abualnaja, S. S. Yaghmoor, T.A. Kumosani, K. Kannan, Urinary biomarkers of exposure to 57 xenobiotics and its association with oxidative stress in a population in Jeddah, Saudi Arabia, Environ. Res. 150 (2016) 573–581, https://doi.org/10.1016/j.envres.2015.11.029.

[16] C. Gao, H. He, W. Qiu, Y. Zheng, Y. Chen, S. Hu, X. Zhao, Oxidative stress, endocrine disturbance, and immune interference in humans showed relationships to serum bisphenol concentrations in a dense industrial area, Environ. Sci. Technol. 55 (2021) 1953–1963, https://doi.org/10.1021/acs.est.0c07587.

[17] Hao Wang, R. Gao, W. Liang, S. Wei, Y. Zhou, Z. Wang, L. Lan, J. Chen, F. Zeng, Large-scale biomonitoring of bisphenol analogues and their metabolites in human urine from Guangzhou, China: Implications for health risk assessment, Chemosphere 338 (2023) 139601, https://doi.org/10.1016/j.chemosphere.2023.139601.

[18] L. Cobellis, N. Colacurci, E. Trabucco, C. Carpentiero, L. Grumetto, Measurement of bisphenol A and bisphenol B levels in human blood sera from healthy and endometriotic women, Biomed. Chromatogr. 23 (2009) 1186–1190, https://doi.org/10.1002/bmc.1241.

[19] S.C. Cunha, J.O. Fernandes, Quantification of free and total bisphenol A and bisphenol B in human urine by dispersive liquid–liquid microextraction (DLLME) and heart-cutting multidimensional gas chromatography–mass spectrometry (MD–GC/MS), Talanta 83 (2010) 117–125, https://doi.org/10.1016/j.talanta.2010.08.048.

[20] A. Li, T. Zhuang, W. Shi, Y. Liang, C. Liao, M. Song, G. Jiang, Serum concentration of bisphenol analogues in pregnant women in China, Sci. Total Environ. 707 (2020) 136100, https://doi.org/10.1016/j.scitotenv.2019.136100.

[21] M. Guida, J. Troisi, C. Ciccone, G. Granozio, C. Cosimato, A.D.S. Sardo, C. Ferrara, M. Guida, C. Nappi, F. Zullo, C. Di Carlo, Bisphenol A and congenital developmental defects in humans, Mutat. Res. /Fundam. Mol. Mech. Mutagen. 774 (2015) 33–39, https://doi.org/10.1016/j.mrfmmm.2015.02.007.

[22] V. Pergialiotis, P. Kotrogianni, E. Christopoulos-Timogiannakis, D. Koutaki, G. Daskalakis, N. Papantoniou, Bisphenol A and adverse pregnancy outcomes: a systematic review of the literature, J. Matern. Fetal Neonatal Med. 31 (2018) 3320–3327, https://doi.org/10.1080/14767058.2017.1368076.

[23] S. Basak, M.K. Das, A.K. Duttaroy, Plastics derived endocrine-disrupting compounds and their effects on early development, Birth Defects Res. 112 (2020) 1308–1325, https://doi.org/10.1002/bdr2.1741.
[24] J. Liang, C. Yang, T. Liu, H.J.J. Tan, Y. Sheng, L. Wei, P. Tang, H. Huang, X. Zeng, S. Liu, D. Huang, X. Qiu, Prenatal exposure to bisphenols and risk of preterm birth: findings from Guangxi Zhuang birth cohort in China, Ecotoxicol. Environ. Saf. 228 (2021) 112960, https://doi.org/10.1016/j.ecoenv.2021.112960.
[25] S. Iwamuro, M. Sakakibara, M. Terao, A. Ozawa, C. Kurobe, T. Shigeura, M. Kato, S. Kikuyama, Teratogenic and anti-metamorphic effects of bisphenol A on embryonic and larval Xenopus laevis, Gen. Comp. Endocrinol. 133 (2003) 189–198, https://doi.org/10.1016/S0016-6480(03)00188-6.
[26] A.L. Arancio, K.D. Cole, A.R. Dominguez, E.R. Cohenour, J. Kadie, W.C. Maloney, C. Cilliers, S.M. Schuh, Bisphenol A, Bisphenol AF, di-n-butyl phthalate, and 17β-estradiol have shared and unique dose-dependent effects on early embryo cleavage divisions and development in Xenopus laevis, Reprod. Toxicol. 84 (2019) 65–74, https://doi.org/10.1016/j.reprotox.2018.12.005.
[27] E. Atay, A. Ertekin, E. Bozkurt, E. Aslan, Impact of Bisphenol A on neural tube development in 48-hr chicken embryos, Birth Defects Res. 112 (2020) 1386–1396, https://doi.org/10.1002/bdr2.1791.
[28] W. Huang, X. Wang, S. Zheng, R. Wu, C. Liu, K. Wu, Effect of bisphenol A on craniofacial cartilage development in zebrafish (Danio rerio) embryos: a morphological study, Ecotoxicol. Environ. Saf. 212 (2021) 111991, https://doi.org/10.1016/j.ecoenv.2021.111991.
[29] C.F.V. Scopel, C. Sousa, M.R.F. Machado, W.G.D. Santos, BPA toxicity during development of zebrafish embryo, Braz. J. Biol. 81 (2021) 437–447, https://doi.org/10.1590/1519-6984.230562.
[30] Y. Ge, F. Ren, L. Chen, D. Hu, X. Wang, Y. Cui, Y. Suo, H. Zhang, J. He, Z. Yin, H. Ning, Bisphenol A exposure induces apoptosis and impairs early embryonic development in Xenopus laevis, Environ. Pollut. 280 (2021) 116901, https://doi.org/10.1016/j.envpol.2021.116901.
[31] Q. Yang, Z. Zhu, Q. Liu, L. Chen, Adverse effects of bisphenol B exposure on the thyroid and nervous system in early life stages of zebrafish, Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 250 (2021) 109167, https://doi.org/10.1016/j.cbpc.2021.109167.
[32] R. Dasiman, N.M. Nor, Z. Eshak, S.S.M. Mutalip, N.R. Suwandi, H. Bidin, R. Abas, A systematic review of the effects of Bisphenol analogs on embryonic development and cytoskeletal organization of Zebrafish (Danio rerio) embryos, Biointerface Res Appl. Chem. 13 (2022) 75, https://doi.org/10.33263/BRIAC131.075.
[33] K. Mashay Al-Anazi, M. Jabr Aljuaid, M. Abul Farah, A. Hossam Mahmoud, A. I. Algefare, M. Ajmal Ali, F.M. Abou-Tarboush, Maternal and developmental toxicity of Bisphenol-A in SWR/J mice, Saudi J. Biol. Sci. 29 (2022) 1543–1549, https://doi.org/10.1016/j.sjbs.2021.11.014.
[34] H. Chen, K. Zhong, Y. Zhang, L. Xie, P. Chen, Bisphenol A interferes with redox balance and the Nrf2 signaling pathway in Xenopus tropicalis during embryonic development, Animals 12 (2022) 937, https://doi.org/10.3390/ani12070937.
[35] G. Heredia-García, L.M. Gómez-Oliván, G.A. Elizalde-Velázquez, J.D. Cardoso-Vera, J.M. Orozco-Hernández, K.E. Rosales-Pérez, S. García-Medina, H. Islas-Flores, M. Galar-Martínez, O. Dublán-García, Multi-biomarker approach and IBR index to evaluate the effects of bisphenol A on embryonic stages of zebrafish (Danio rerio), Environ. Toxicol. Pharmacol. 94 (2022) 103925, https://doi.org/10.1016/j.etap.2022.103925.
[36] F. Wang, X. Ma, Q. Sun, Y. Zhang, Y. Liu, J. Gu, L. Wang, Bisphenol B induces developmental toxicity in zebrafish via oxidative stress, Environ. Sci. Pollut. Res (2023), https://doi.org/10.1007/s11356-023-31161-9.
[37] F. Metruccio, M. Battistoni, F. Di Renzo, R. Bacchetta, N. Santo, E. Menegola, Teratogenic and neuro-behavioural toxic effects of bisphenol A (BPA) and B (BPB) on Xenopus laevis development, Reprod. Toxicol. 123 (2024) 108496, https://doi.org/10.1016/j.reprotox.2023.108496.
[38] R.P. Elinson, L. Kezmoh, Molecular Haeckel, Dev. Dyn. 239 (2010) 1905–1918, https://doi.org/10.1002/dvdy.22337.
[39] N. Irie, S. Kuratani, Comparative transcriptome analysis reveals vertebrate phylotypic period during organogenesis, Nat. Commun. 2 (2011) 248, https://doi.org/10.1038/ncomms1248.
[40] H.-G. Drost, P. Janitza, I. Grosse, M. Quint, Cross-kingdom comparison of the developmental hourglass, Curr. Opin. Genet. Dev. 45 (2017) 69–75, https://doi.org/10.1016/j.gde.2017.03.003.
[41] D.G. Skledar, L.P. Mašič, In vitro estrogenic activity of binary and multicomponent mixtures with bisphenol A, Sci. Total Environ. 707 (2020) 135211, https://doi.org/10.1016/j.scitotenv.2019.135211.
[42] H. Lee, J. Park, K. Park, Mixture effects of Bisphenol A and its structural analogs on estrogen receptor transcriptional activation, Toxics 11 (2023) 986, https://doi.org/10.3390/toxics11120986.
[43] S. Kim, E. Park, E.-K. Park, S. Lee, J.-A. Kwon, B.-H. Shin, S. Kang, E.-Y. Park, B. Kim, Urinary concentrations of bisphenol mixtures during pregnancy and birth outcomes: the MAKE study, IJERPH 18 (2021) 10098, https://doi.org/10.3390/ijerph181910098.
[44] S. Arrokhman, Y.-H. Luo, P. Lin, Additive cardiotoxicity of a bisphenol mixture in zebrafish embryos: The involvement of calcium channel and pump, Ecotoxicol. Environ. Saf. 263 (2023) 115225, https://doi.org/10.1016/j.ecoenv.2023.115225.
[45] M. Battistoni, R. Bacchetta, F. Di Renzo, F. Metruccio, A. Moretto, E. Menegola, Modified Xenopus laevis approach (R-FETAX) as an alternative test for the evaluation of foetal valproate spectrum disorder, Reprod. Toxicol. 107 (2022) 140–149, https://doi.org/10.1016/j.reprotox.2021.12.005.
[46] B.T. ReedXenopus Laevis Guidance on the Housing and Care of the African Clawed Frog, 2005.
[47] Amsterdam: North Holland Publishing Co , Normal table of Xenopus laevis (Daudin) (Eds.), P.D. Nieuwkoop, J. Faber , in: 1956, P.D. NieuwkoopJ. Faberin: (Eds.).
[48] ASTM, Standard guide for conducting the frog embryo teratogenesis assay-Xenopus (FETAX), Annu. Book ASTM Stand. (1998) 826–836.
[49] E. Menegola, M. Battistoni, R. Bacchetta, F. Metruccio, F. Di Renzo, Evaluation of temperature- and ethanol-related developmental degree variations by a new scoring system (FETAX-score) applicable to Frog Embryo Teratogenicity Assay: Xenopus, Submitt. Reprod. Toxicol. (2024).
[50] M. Česen, K. Lenarčič, V. Mislej, M. Levstek, A. Kovačič, B. Cimrmančič, N. Uranjek, T. Kosjek, D. Heath, M.S. Dolenc, E. Heath, The occurrence and source identification of bisphenol compounds in wastewaters, Sci. Total Environ. 616–617 (2018) 744–752, https://doi.org/10.1016/j.scitotenv.2017.10.252.
[51] M. Caban, P. Stepnowski, The quantification of bisphenols and their analogues in wastewaters and surface water by an improved solid-phase extraction gas chromatography/mass spectrometry method, Environ. Sci. Pollut. Res 27 (2020) 28829–28839, https://doi.org/10.1007/s11356-020-09123-2.
[52] N.A.H. Ismail, S.Y. Wee, A.Z. Aris, Bisphenol A and alkylphenols concentrations in selected mariculture fish species from Pulau Kukup, Johor, Malaysia, Mar. Pollut. Bull. 127 (2018) 536–540, https://doi.org/10.1016/j.marpolbul.2017.12.043.
[53] R. Kuruto-Niwa, R. Nozawa, T. Miyakoshi, T. Shiozawa, Y. Terao, Estrogenic activity of alkylphenols, bisphenol S, and their chlorinated derivatives using a GFP expression system, Environ. Toxicol. Pharmacol. 19 (2005) 121–130, https://doi.org/10.1016/j.etap.2004.05.009.
[54] N. Kunz, E.J. Camm, E. Somm, G. Lodygensky, S. Darbre, M.L. Aubert, P.S. Hüppi, S.V. Sizonenko, R. Gruetter, Developmental and metabolic brain alterations in rats exposed to bisphenol A during gestation and lactation, Int. J. Dev. Neurosci. 29 (2011) 37–43, https://doi.org/10.1016/j.ijdevneu.2010.09.009.
[55] K. Kim, H. Park, W. Yang, J.H. Lee, Urinary concentrations of bisphenol A and triclosan and associations with demographic factors in the Korean population, Environ. Res. 111 (2011) 1280–1285, https://doi.org/10.1016/j.envres.2011.09.003.
[56] S. Ehrlich, A.M. Calafat, O. Humblet, T. Smith, R. Hauser, Handling of thermal receipts as a source of exposure to Bisphenol A, JAMA 311 (2014) 859, https://doi.org/10.1001/jama.2013.283735.
[57] D. Chen, K. Kannan, H. Tan, Z. Zheng, Y.-L. Feng, Y. Wu, M. Widelka, Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity—a review, Environ. Sci. Technol. 50 (2016) 5438–5453, https://doi.org/10.1021/acs.est.5b05387.
[58] H.-C. Chen, J.-W. Chang, Y.-C. Sun, W.-T. Chang, P.-C. Huang, Determination of Parabens, Bisphenol A and Its Analogs, Triclosan, and Benzophenone-3 levels in human urine by isotope-dilution-UPLC-MS/MS method followed by supported liquid extraction, Toxics 10 (2022) 21, https://doi.org/10.3390/toxics10010021.
[59] C. Gys, M. Bastiaensen, L. Bruckers, A. Colles, E. Govarts, L.R. Martin, V. Verheyen, G. Koppen, B. Morrens, E. Den Hond, A. De Decker, G. Schoeters, A. Covaci, Determinants of exposure levels of bisphenols in flemish adolescents, Environ. Res. 193 (2021) 110567, https://doi.org/10.1016/j.envres.2020.110567.
[60] S. Mok, J.-E. Lim, A. Lee, S. Kim, S. Kim, I. Lee, Y. Kho, J. Park, S. Kim, K. Choi, H.-B. Moon, Within- and between-person variability of urinary phthalate metabolites and bisphenol analogues over seven days: considerations of biomonitoring study design, Environ. Res. 209 (2022) 112885, https://doi.org/10.1016/j.envres.2022.112885.
[61] Q. Wang, M. Chen, G. Shan, P. Chen, S. Cui, S. Yi, L. Zhu, Bioaccumulation and biomagnification of emerging bisphenol analogues in aquatic organisms from Taihu Lake, China, Sci. Total Environ. 598 (2017) 814–820, https://doi.org/10.1016/j.scitotenv.2017.04.167.
[62] R. Akhbarizadeh, F. Moore, C. Monteiro, J.O. Fernandes, S.C. Cunha, Occurrence, trophic transfer, and health risk assessment of bisphenol analogues in seafood from the Persian Gulf, Mar. Pollut. Bull. 154 (2020) 111036, https://doi.org/10.1016/j.marpolbul.2020.111036.
[63] L.G.A. Barboza, S.C. Cunha, C. Monteiro, J.O. Fernandes, L. Guilhermino, Bisphenol A and its analogs in muscle and liver of fish from the North East Atlantic Ocean in relation to microplastic contamination. Exposure and risk to human consumers, J. Hazard. Mater. 393 (2020) 122419, https://doi.org/10.1016/j.jhazmat.2020.122419.
[64] N. Zhao, H. Hu, M. Zhao, W. Liu, H. Jin, Occurrence of free-form and conjugated bisphenol analogues in marine organisms, Environ. Sci. Technol. 55 (2021) 4914–4922, https://doi.org/10.1021/acs.est.0c08458.
[65] K. Czarny-Krzymińska, B. Krawczyk, D. Szczukocki, Bisphenol A and its substitutes in the aquatic environment: occurrence and toxicity assessment, Chemosphere 315 (2023) 137763, https://doi.org/10.1016/j.chemosphere.2023.137763.
[66] S. Kitamura, Comparative study of the endocrine-disrupting activity of Bisphenol A and 19 related compounds, Toxicol. Sci. 84 (2005) 249–259, https://doi.org/10.1093/toxsci/kfi074.
[67] R.A. Hoke, G.T. Ankley, Application of frog embryo teratogenesis assay-xenopus to ecological risk assessment, Environ. Toxicol. Chem. 24 (2005) 2677, https://doi.org/10.1897/04-506R.1.
[68] I. Mouche, L. Malésic, O. Gillardeaux, FETAX assay for evaluation of developmental toxicity, Methods Mol. Biol. 1641 (2017) 311–324, https://doi.org/10.1007/978-1-4939-7172-5_17.
[69] D.J. Fort, M. Mathis, Frog embryo teratogenesis assay— *Xenopus* (FETAX): use in alternative preclinical safety assessment, Cold Spring Harb. Protoc. 2018 (2018) pdb.prot098319, https://doi.org/10.1101/pdb.prot098319.
[70] E. Menegola, M. Battistoni, F. Metruccio, F. Di Renzo, Advantages and disadvantages of the use of Xenopus laevis embryos and zebra fish as alternative

methods to assess teratogens, Curr. Opin. Toxicol. 34 (2023) 100387, https://doi.org/10.1016/j.cotox.2023.100387.
[71] Y. Xu, J. Jang, M.C. Gye, The Xenopus laevis teratogenesis assay for developmental toxicity of phthalate plasticizers and alternatives, Environ. Pollut. 300 (2022) 118985, https://doi.org/10.1016/j.envpol.2022.118985.
[72] E. Ribeiro, C. Ladeira, S. Viegas, EDCs Mixtures: a stealthy hazard for human health? Toxics 5 (2017) 5, https://doi.org/10.3390/toxics5010005.
[73] EFSA Scientific Committee, A. Hardy, D. Benford, T. Halldorsson, M.J. Jeger, K. H. Knutsen, S. More, A. Mortensen, H. Naegeli, H. Noteborn, C. Ockleford, A. Ricci, G. Rychen, V. Silano, R. Solecki, D. Turck, M. Aerts, L. Bodin, A. Davis, L. Edler, U. Gundert-Remy, S. Sand, W. Slob, B. Bottex, J.C. Abrahantes, D.C. Marques, G. Kass, J.R. Schlatter, Update: use of the benchmark dose approach in risk assessment, EFS2 15 (2017), https://doi.org/10.2903/j.efsa.2017.4658.
[74] X.M. Shan, D.H. Shen, B.S. Wang, B.B. Lu, F.Y. Huang, Simultaneous determination of bisphenols and alkylphenols in water by solid phase extraction and ultra performance liquid chromatography-tandem mass spectrometry, Biomed. Environ. Sci. 27 (2014) 471–474, https://doi.org/10.3967/bes2014.076.
[75] Z. Yan, Y. Liu, K. Yan, S. Wu, Z. Han, R. Guo, M. Chen, Q. Yang, S. Zhang, J. Chen, Bisphenol analogues in surface water and sediment from the shallow Chinese freshwater lakes: occurrence, distribution, source apportionment, and ecological and human health risk, Chemosphere 184 (2017) 318–328, https://doi.org/10.1016/j.chemosphere.2017.06.010.
[76] X. Zhao, W. Qiu, Y. Zheng, J. Xiong, C. Gao, S. Hu, Occurrence, distribution, bioaccumulation, and ecological risk of bisphenol analogues, parabens and their metabolites in the Pearl River Estuary, South China, Ecotoxicol. Environ. Saf. 180 (2019) 43–52, https://doi.org/10.1016/j.ecoenv.2019.04.083.
[77] D. Kiejza, U. Kotowska, W. Polińska, J. Karpińska, USAEME-GC/MS method for easy and sensitive determination of nine Bisphenol analogues in water and wastewater, Molecules 27 (2022) 4977, https://doi.org/10.3390/molecules27154977.
[78] Y.-H. Liu, J.-W. Huang, Z. Huang, Y.-X. Mei, J.-L. Zhao, G.-G. Ying, Tissue-specific bioaccumulation and health risks of bisphenols in wild fish from West and North Rivers, South China, J. Environ. Expo. Assess. 2 (2023), https://doi.org/10.20517/jeea.2023.26.
[79] O. Birceanu, M.R. Servos, M.M. Vijayan, Bisphenol A accumulation in eggs disrupts the endocrine regulation of growth in rainbow trout larvae, Aquat. Toxicol. 161 (2015) 51–60, https://doi.org/10.1016/j.aquatox.2015.01.028.
[80] B. Sadoul, O. Birceanu, N. Aluru, J.K. Thomas, M.M. Vijayan, Bisphenol A in eggs causes development-specific liver molecular reprogramming in two generations of rainbow trout, Sci. Rep. 7 (2017) 14131, https://doi.org/10.1038/s41598-017-13301-7.
[81] N.C. Wu, F. Seebacher, Effect of the plastic pollutant bisphenol A on the biology of aquatic organisms: a meta-analysis, Glob. Change Biol. 26 (2020) 3821–3833, https://doi.org/10.1111/gcb.15127.
[82] ECHA, Group assessment of bisphenols identifies need for restriction [https://echa.europa.eu/da/-/group-assessment-of-bisphenols-identifies-need-for-restriction], (2022). https://echa.europa.eu/da/-/group-assessment-of-bisphenols-identifies-need-for-restriction.