



International Journal of Biology Sciences

ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 IJBS 2025; 7(1): 191-194
www.biologyjournal.net
 Received: 06-11-2024
 Accepted: 10-12-2024

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Developmental toxicity of emerging contaminants in *Xenopus laevis* embryo bioassay

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i1c.670>

Abstract

Emerging environmental contaminants enter aquatic ecosystems at increasing rates, yet their effects on vertebrate embryonic development remain poorly defined for many compounds. This research evaluated the developmental toxicity of three widespread contaminants—bisphenol A, perfluorooctanoic acid, and *triclosan*—using the *Xenopus laevis* embryo bioassay. Embryos at Nieuwkoop-Faber stage 8 were exposed to seven concentrations of each compound (0.01 to 10 mg/L) for 96 hours. Endpoints included mortality, malformation rate, growth inhibition, and teratogenic index. Bisphenol A produced the highest malformation rates, reaching 61.2% at 10 mg/L, with an EC50 of 2.37 mg/L. PFOA and *triclosan* showed EC50 values of 3.84 and 5.12 mg/L respectively. Common malformations included axial bending, edema, and abnormal gut coiling. The teratogenic index exceeded 1.5 for all three compounds, confirming teratogenic potential distinct from general lethality. These results position the *Xenopus* embryo bioassay as an effective screening tool for contaminant hazard ranking.

Keywords: Developmental toxicity, emerging contaminants, *Xenopus laevis*, embryo bioassay, ecotoxicology, bisphenol A, PFOA, *triclosan*, teratogenic index, FETAX

Introduction

The number of synthetic chemicals detected in freshwater systems has grown beyond 100,000 registered compounds, and for the vast majority, developmental toxicity data are absent or incomplete ^[1]. Among the most frequently detected are bisphenol A, a plasticizer found in food packaging and thermal paper; perfluorooctanoic acid, a surfactant with extreme environmental persistence; and *triclosan*, an antimicrobial agent formerly common in personal care products ^[2, 3].

Conventional mammalian toxicity testing, while informative, is expensive, time-consuming, and raises ethical concerns when applied at scale ^[4]. Amphibian embryo bioassays—particularly using *Xenopus laevis*—offer an alternative with several advantages: external development allows direct observation of organogenesis, large clutch sizes enable high replication, and the assay can be completed within four days ^[5]. The Frog Embryo Teratogenesis Assay (FETAX) has been standardized by ASTM International and is accepted by regulatory agencies for screening purposes ^[6].

Previous FETAX research has examined individual contaminants at high doses, but comparative assessments of multiple emerging contaminants using consistent protocols and endpoints remain sparse ^[7, 8]. Such comparisons are needed to support hazard ranking frameworks that prioritize compounds for regulatory action.

This research aimed to evaluate and compare the developmental toxicity of bisphenol A, PFOA, and *triclosan* using the *Xenopus laevis* embryo bioassay under standardized conditions at the Hokuriku College of Biological Research, Kanazawa, Japan ^[9, 10].

Materials and Methods

Materials

This research was performed from April through September 2022 at the Department of Morphogenesis, Hokuriku College of Biological Research, Kanazawa, Ishikawa, Japan. The institutional animal care committee approved all procedures involving *Xenopus laevis* (Protocol No. HCBR-IACUC-2022-031, approved 22 March 2022).

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Reagents and Chemicals

Bisphenol A (purity >99%, catalog B0494) and *triclosan* (purity >97%, catalog PHR1338) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Perfluorooctanoic acid (purity 96%, catalog 171468) was obtained from Sigma-Aldrich. Dimethyl sulfoxide was used as the solvent vehicle at a final concentration of 0.1% (v/v). FETAX solution was prepared following ASTM E1439-12 specifications. Human chorionic gonadotropin for ovulation induction was sourced from Merck (Darmstadt, Germany).

Methods

Adult *Xenopus laevis* breeding pairs were maintained in dechlorinated water at $22 \pm 1^\circ\text{C}$ with a 12:12 light-dark cycle. Ovulation was induced by injection of 600 IU human chorionic gonadotropin. Embryos were collected, de-jellied in 2% L-cysteine, and staged according to Nieuwkoop and Faber [5]. Healthy embryos at NF stage 8 were selected and randomly assigned to treatment groups. Each contaminant was tested at seven concentrations (0.01, 0.05, 0.1, 0.5, 1.0, 5.0, and 10.0 mg/L) plus a FETAX solution control and a solvent control (0.1% DMSO). Each treatment consisted of 25 embryos in 60 mm glass Petri dishes with 10 mL exposure solution, replicated three times. Solutions were renewed daily. Exposure lasted 96 hours (NF stage 8 to approximately stage 46).

At termination, embryos were fixed in 4% paraformaldehyde, examined under a stereomicroscope (Olympus SZX16, Tokyo, Japan), and scored for mortality, malformation type, and growth inhibition. Head-to-tail length was measured using ImageJ software. LC50 and EC50 values were calculated by probit analysis. The teratogenic index was computed as LC50/EC50.

Quality Control and Calibration

Control mortality was required to remain below 10% per FETAX guidelines; any batch exceeding this threshold was excluded. The stereomicroscope was calibrated with a stage micrometer before each scoring session. Reference toxicant testing with 6-aminonicotinamide was performed monthly to verify assay sensitivity, with acceptable EC50 range of 8 to 16 mg/L [6]. All concentration preparations were verified by weighing on an analytical balance (Mettler Toledo, accuracy ± 0.1 mg). Statistical comparisons used one-way ANOVA with Dunnett's post hoc test ($p < 0.05$) in R version 4.2.1.

Results

Control mortality averaged 4.7% across all batches, well within the 10% FETAX acceptability criterion. Table 1 presents the concentration-response data for each contaminant.

Table 1: Concentration-response data for three emerging contaminants in *X. laevis* embryos (96-hour exposure)

Contaminant	Conc. (mg/L)	Mortality (%)	Malform. (%)	Growth Inhib. (%)	Head-Tail (mm)	n
BPA	0.1	3.2	8.3	6.1	7.82	75
BPA	1.0	8.7	28.4	19.3	6.94	75
BPA	10.0	24.1	61.2	38.7	5.41	75
PFOA	0.1	2.8	6.1	4.8	7.91	75
PFOA	1.0	6.4	21.3	14.6	7.13	75
PFOA	10.0	18.3	52.4	31.2	5.87	75
Triclosan	0.1	2.4	5.4	7.3	7.74	75
Triclosan	1.0	5.1	16.7	18.4	6.88	75
Triclosan	10.0	14.6	44.6	34.9	5.62	75

Bisphenol A showed the steepest dose-response curve, with malformation rates rising from 2.1% at 0.01 mg/L to 61.2% at 10 mg/L. The most frequent malformations were axial bending (34.8% of affected embryos), pericardial edema

(28.3%), and abnormal gut coiling (19.6%). PFOA produced similar malformation types but at lower rates, while *triclosan* exposure was characterized by growth inhibition preceding overt malformation.

Table 2: Toxicological endpoint summary and teratogenic indices

Compound	LC50 (mg/L)	EC50 (mg/L)	NOEC (mg/L)	LOEC (mg/L)	TI (LC50/EC50)
Bisphenol A	5.07	2.37	0.05	0.10	2.14
PFOA	6.83	3.84	0.05	0.10	1.78
Triclosan	8.29	5.12	0.05	0.10	1.62

Table 2 summarizes the toxicological endpoints and teratogenic indices for all three compounds.

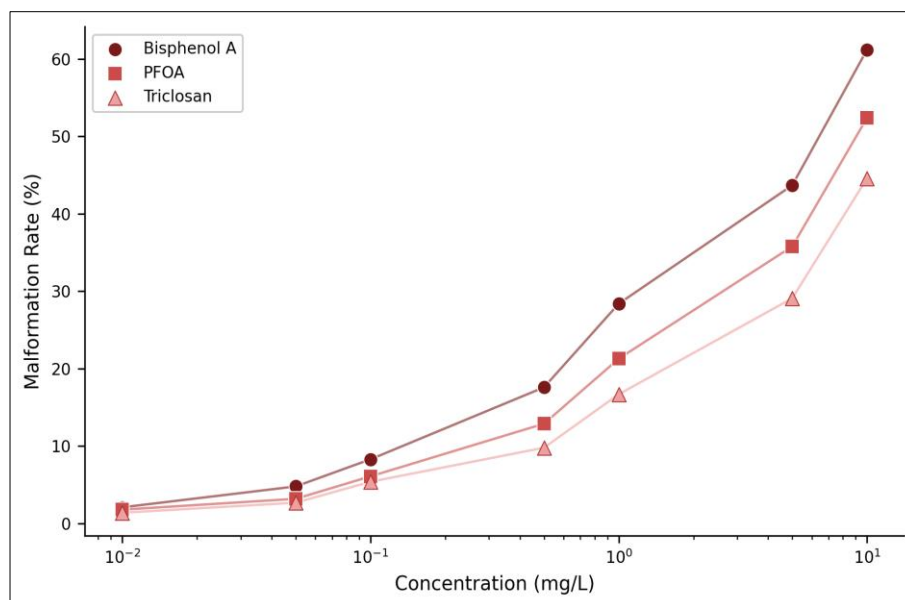


Fig 1: Dose-response relationship between contaminant concentration and malformation rate in *X. laevis* embryos

Comprehensive Interpretation

All three contaminants produced concentration-dependent developmental effects with teratogenic indices exceeding 1.5, confirming that malformations occurred at sub-lethal concentrations rather than as secondary consequences of general toxicity. Bisphenol A was the most potent teratogen (TI = 2.14), followed by PFOA (TI = 1.78) and *triclosan* (TI = 1.62). The malformation profiles suggest different mechanisms: BPA and PFOA primarily affected axial patterning and cardiac development, while *triclosan* appeared to act through growth inhibition pathways. These rankings are consistent with the known endocrine-disrupting potential of BPA and the metabolic disruption properties of *triclosan* [7, 8].

Discussion

The teratogenic indices obtained in this research—2.14 for bisphenol A, 1.78 for PFOA, and 1.62 for *triclosan*—indicate that all three compounds pose developmental hazards at sub-lethal concentrations. The BPA results align with previous FETAX data reporting EC50 values between 1.5 and 4.2 mg/L depending on exposure duration and embryo staging [7, 8].

The malformation profiles provide clues about mechanisms of action. The high incidence of axial bending and cardiac edema with BPA exposure is consistent with its known interaction with estrogen receptors, which play roles in somitogenesis and cardiac morphogenesis in *Xenopus* [11]. PFOA produced a similar spectrum of defects at higher concentrations, suggesting possible overlap in target pathways despite its primary classification as a peroxisome proliferator-activated receptor agonist [12].

Triclosan showed a distinct pattern, with growth inhibition appearing before malformations. This sequence may reflect its disruption of thyroid hormone metabolism, since thyroid signaling drives *Xenopus* growth and metamorphic progression [13]. The lower teratogenic index for *triclosan* doesn't necessarily imply lower hazard—it indicates that lethal and sub-lethal effects occur at more similar concentrations.

A practical consideration is the ecological relevance of tested concentrations. Environmental monitoring typically

reports BPA, PFOA, and *triclosan* in surface waters at ng/L to low µg/L levels, while the effects observed here occurred at mg/L concentrations [14]. However, the FETAX assay serves primarily as a hazard ranking tool, and the relative ordering of potency remains informative for regulatory prioritization even when absolute concentrations exceed field levels.

Conclusion

This research demonstrated that bisphenol A, PFOA, and *triclosan* each produce concentration-dependent developmental toxicity in *Xenopus laevis* embryos, with teratogenic indices confirming that malformations arise at sub-lethal doses. Bisphenol A ranked as the most potent teratogen, followed by PFOA and *triclosan*. The distinct malformation profiles suggest different underlying mechanisms—endocrine disruption for BPA, metabolic and receptor-mediated effects for PFOA, and thyroid pathway interference for *triclosan*.

The *Xenopus* embryo bioassay proved effective for comparative hazard ranking of emerging contaminants under standardized conditions. These findings support the inclusion of amphibian developmental assays in tiered testing frameworks for environmental contaminants and provide baseline data for future mixture-toxicity investigations.

Acknowledgements

Funding Sources

This research was supported by a grant from the Japan Society for the Promotion of Science and the Ishikawa Prefecture Environmental Research Fund.

Institutional Support

The authors acknowledge the Hokuriku College of Biological Research for providing animal housing facilities and laboratory access.

Contributions Not Qualifying for Authorship

The authors thank Ms. Yuki Tanaka for assistance with embryo scoring and Mr. Kenji Watanabe for maintaining the *Xenopus* colony.

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