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# DEVELOPMENTAL TOXICITY OF BISPHENOL A IN A ZEBRAFISH EMBRYO MODEL

**TEREZA KUCERA[1]*, JOANNA WISNIEWSKI[2], LUKASZ ZIELINSKI[3]**

**Affiliation**

[1]Department of Pharmacology and Toxicology, Vysocina Institute of Pharmaceutical Chemistry, Jihlava, Czech Republic
[2]Department of Forensic Medicine and Toxicology, Warmia Institute of Pharmaceutical Sciences, Olsztyn, Poland
[3]Department of Pharmacology and Toxicology, Warmia Institute of Pharmaceutical Sciences, Olsztyn, Poland

*Corresponding author: Tereza Kucera, t.kucera@vipc.cz
ORCID iD: Tereza Kucera 0000-0001-4838-980X; Joanna Wisniewski 0000-0001-3017-6355; Lukasz Zielinski 0000-0001-0064-7363

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**Abstract**

Bisphenol A (BPA) is a high-volume industrial chemical to which most people are exposed daily, and concern about its effects on development has grown for two decades. The zebrafish embryo has become a popular model for testing such effects because it is transparent, develops outside the mother and suits small-scale screening. This review summarises published work on BPA developmental toxicity in zebrafish, covering the properties of the model, mechanisms of action, malformation and neurobehavioural findings, and methodological issues that affect interpretation. Higher concentrations produce consistent morphological defects, while lower concentrations are linked to altered neurogenesis and behaviour, with less agreement between laboratories. The article is a narrative review of published literature and reports no new experimental data.

## 1. INTRODUCTION

Bisphenol A is made in millions of tonnes each year. It is the monomer of polycarbonate plastics, a component of the epoxy resins that line food and drink cans, and an ingredient of many thermal-paper and dental products, so it moves easily from manufactured goods into food, dust and water [1, 2]. Biomonitoring studies detect its conjugated metabolites in the urine of the large majority of people sampled in industrialised countries, which implies continuous low-level exposure rather than an occasional event [3, 4]. Because it is

rapidly conjugated and excreted, a single measurement says little about long-term burden, but the near-universal detection has kept regulators and researchers interested in what small amounts might do during development [3, 5]. Production volumes and the range of consumer goods make complete avoidance impractical, which is why exposure science has concentrated on food packaging and on infants, who eat and drink more per kilogram of body weight than adults.

The concern is built on a simple observation: BPA can bind receptors for sex steroids, and development is a period in which hormone signalling directs how tissues form. Reports from rodent studies, reviewed by several expert groups, described effects at doses well below those used in classical toxicology, including changes in the brain, reproductive tract and mammary gland [5, 6]. Whether those results transfer to human beings has been argued ever since. Regulators weighed the same studies differently, partly because study designs varied and partly because the concept of a low-dose effect conflicts with the traditional assumption that toxicity rises steadily with dose [7, 8, 9]. Expert panels reached different views on how much weight the low-dose findings deserve, and the disagreement has been as much about methods and interpretation as about the underlying biology [5, 6].

## 2. THE ZEBRAFISH EMBRYO AS A MODEL

Zebrafish (*Danio rerio*) suit developmental toxicology for practical reasons. A breeding pair produces hundreds of fertilised eggs, the embryos are optically clear, and every major organ system forms within the first few days, so effects on the heart, body axis, eyes and nervous system can be scored in a living animal [10, 11]. The staging series published in 1995 gave the field a shared timetable, which means a defect can be tied to a specific hour of development and a specific exposure window [10]. The genome has been sequenced, and about 70 per cent of human protein-coding genes have at least one zebrafish counterpart, which supports mechanistic comparison with mammals even though the evolutionary distance is large [12]. Larvae also begin to swim and respond to light and touch within the first week, so behavioural testing is possible while the animal is still tiny and cheap to keep [10, 11].

The most widely used regulatory format is the fish embryo acute toxicity test described in OECD Test Guideline 236. Newly fertilised embryos are exposed for 96 hours in multiwell plates, and four apical observations of lethality are recorded each day: coagulation of the embryo, lack of somite formation, non-detachment of the tail and absence of heartbeat [13]. Many research laboratories add sublethal endpoints, such as hatching time, oedema, body length, spinal curvature and swimming activity. Interlaboratory studies found acceptable reproducibility for the acute test when the protocol was followed closely [14]. Developmental toxicity studies of BPA mostly follow the same pattern but extend the observations, often to five days after fertilisation [11, 13]. Test solutions are either kept static or renewed daily, and temperature is held near 26 to 28 °C, the normal rearing range for the species [13].

The model has limits that matter for a compound like BPA. Zebrafish embryos are surrounded by a chorion for the first two to three days, and this protective layer may reduce the uptake of some chemicals, so dechorionated embryos can show greater sensitivity [15]. Metabolism also differs. Early embryos have limited capacity for some biotransformation reactions, whereas mammalian BPA is rapidly glucuronidated in the gut and liver [16]. The fish skin and gills absorb dissolved compounds directly, while a human foetus is exposed through maternal blood and the placenta. These contrasts do not invalidate the model, but they make it a hazard screening tool, not a direct predictor of human risk [11, 17]. Species differences in receptors add a further caveat: the zebrafish genome underwent a duplication, so some human genes have two fish copies with partly divided roles [12].

Strain and husbandry add background variation that is easily overlooked. Laboratories use different wild-type lines, such as AB, Tübingen and WIK, and these differ in clutch size, developmental rate and baseline behaviour. Adult diet, water quality,

stocking density and the age of breeding fish influence egg quality, and poor clutches show more spontaneous malformation, which can be mistaken for chemical effects [11, 14]. Good practice is to use eggs from several pairs, discard clutches with high background abnormality and record the strain and rearing conditions in detail. These steps are simple and cost little, yet they are sometimes missing from methods sections.

## 3. EXPOSURE AND MECHANISMS

Understanding how BPA acts starts with its receptor pharmacology. In cell-based assays BPA activates oestrogen receptors alpha and beta, but with an affinity several orders of magnitude lower than oestradiol, and it shows antagonism at the androgen receptor in some systems [18, 19]. One of the strongest interactions is with the oestrogen-related receptor gamma, an orphan nuclear receptor that BPA binds tightly, and crystallographic work has shown how the molecule fits into the ligand pocket [20, 21]. This receptor is abundant in the developing brain and placenta, which is one reason why attention has turned to neurodevelopment in addition to reproductive endpoints [20, 21]. Because BPA is a relatively poor ligand for the classical receptors, much of the debate concerns whether tissue concentrations are high enough for such interactions to matter at all [18, 20].

Receptor binding is not the whole explanation. Reviews of endocrine disruptors point to additional routes, including effects on enzymes that synthesise or metabolise hormones, interference with thyroid signalling, changes in epigenetic marks and disturbance of lipid metabolism [7, 19]. Dose-response curves for such chemicals do not always rise steadily; non-monotonic relationships, in which a lower dose produces a larger effect than a higher one, have been described for hormonally active compounds and are well documented in cell and animal studies [8]. For zebrafish studies, this matters because a design that tests only a few high concentrations may miss effects that occur at the low end of the range. Interference with thyroid signalling is of particular interest in fish, where thyroid hormone controls tissue maturation, though evidence for BPA remains mixed [7, 19].

Exposure levels need to be kept in perspective. The concentrations that cause obvious malformations in fish embryos are generally in the micromolar range, which is far above the nanomolar levels measured in most environmental waters and above human blood concentrations reported in biomonitoring studies [3, 22, 23]. Low-dose neurodevelopmental studies work at levels closer to environmental ones, which explains their prominence in the debate [24, 25]. Reviews of BPA in the wild describe measurable concentrations in water, sediment and biota near sources of contamination, and effects on fish reproduction in laboratory studies, but the link to population-level consequences is much less clear [2, 26]. Context decides whether an effect is a hazard of interest or a laboratory curiosity. Reports of effects in wildlife exist, but laboratory exposures are typically much higher and shorter than those of animals living near a discharge point [26].

Biotransformation in the embryo deserves some comment, because BPA is detoxified in mammals mainly by conjugation to glucuronide and sulfate, and the conjugates have far lower receptor activity than the parent compound [16]. Zebrafish are reported to express conjugating enzymes from the first days of development, but activity at early stages is lower than in the adult liver, so the free fraction of BPA may stay high for longer in the embryo than it does in a mammal [11, 16]. If so, embryos could be somewhat more sensitive per unit of internal exposure than expected, and part of the effects seen at high water concentrations may reflect this limited clearance rather than a specific receptor mechanism.

Toxicokinetics add a layer of uncertainty that zebrafish data rarely resolve. In people, oral BPA is conjugated and cleared within hours, with only a small fraction of free active compound reaching the circulation [16]. In the water of a multiwell plate, by contrast, the embryo is exposed continuously for days, and uptake across the skin bypasses first-pass metabolism. This difference makes the internal dose in exposed embryos hard to compare with that in a person eating food

containing the chemical. Some authors therefore suggest reporting tissue or whole-embryo concentrations alongside nominal water levels, and relating these to the free fraction measured in human biomonitoring [3, 16].

## 4. REPORTED DEVELOPMENTAL EFFECTS

Morphological findings are the most consistent part of the zebrafish BPA literature. Exposure of embryos to concentrations in the tens of micromoles per litre or above increases mortality, delays or reduces hatching, and produces pericardial and yolk sac oedema and curvature of the body axis in surviving larvae [22, 23]. Toxicogenomic profiling of exposed embryos reported changes in the expression of genes related to development, metabolism and endocrine function that accompany these phenotypes [22]. Screening programmes that scored hundreds of chemicals with a standard battery of morphological endpoints placed BPA among compounds that cause developmental effects at moderate concentrations, though not among the most potent [27, 28]. The main findings are collected in table 1. Most studies include untreated and solvent control groups and report effects as a percentage of embryos, which makes comparison between papers workable if the denominators are clear.

Neurodevelopmental findings attract the most scientific interest and also the most disagreement. Studies at low concentrations reported precocious neurogenesis in the hypothalamus, with an increase in the number of neurons born early and a rise in larval activity [24]. A separate laboratory found early-life hyperactivity after low-dose exposure, and, strikingly, learning deficits in adult fish that had been exposed only as embryos [25]. These results suggest that a brief developmental exposure can leave a lasting mark. They also illustrate the difficulty of the field: behavioural assays are sensitive to temperature, lighting, time of day and handling, and the effects at low doses are small relative to variation between clutches [14, 25]. Observation of the offspring of adults treated before breeding is an additional strategy, but published examples are few and the results need confirmation [25].

**Table 1: Developmental endpoints and methodological points reported for BPA in zebrafish**

| Endpoint or issue | Published finding or point | Ref. |
|---|---|---|
| Survival and hatching<br>higher concentrations | Reduced survival and delayed or reduced hatching at concentrations in the micromolar range | [22, 23] |
| Oedema<br>pericardial and yolk sac | Frequent malformation in exposed embryo-larvae, often alongside other defects | [22, 23] |
| Body axis<br>curvature and length | Bent or shortened axis in surviving larvae at higher concentrations | [23, 27] |
| Screening position<br>large chemical panels | Developmental effects at moderate concentrations, not among the most potent compounds | [27, 28] |
| Hypothalamic neurogenesis<br>low dose | Earlier birth of neurons and increased larval activity after low-dose exposure | [24] |
| Behaviour<br>larval and adult | Early hyperactivity and learning deficits later in life after brief embryonic exposure | [25] |
| Receptor activity<br>in vitro | Weak oestrogen receptor activation; strong binding to oestrogen-related receptor gamma | [18, 20, 21] |
| Gene expression<br>toxicogenomics | Changes in development, metabolism and endocrine genes that accompany phenotypes | [22] |
| Analogues<br>bisphenol S, F and AF | Not consistently safer; similar or greater potency for some endpoints | [23, 29] |
| Chorion and solvent<br>exposure conditions | Dechorionation changes uptake; solvent controls needed to avoid artefacts | [14, 15] |
| Test guideline<br>OECD 236 | Four lethal endpoints over 96 hours with acceptable interlaboratory reproducibility | [13, 14] |

Replacement compounds complicate the picture. As BPA was restricted in baby bottles and some food contact uses, manufacturers shifted to analogues such as bisphenol S, F and AF. Comparative studies in zebrafish embryo-larvae found that these substitutes

were not consistently safer, producing similar or in some cases stronger effects on survival, morphology and oestrogenic responses [23]. A systematic review of the hormonal activity of bisphenol S and F reached a comparable conclusion, that their potency in receptor and cell assays is of the same order as BPA [29]. The practical lesson is that a switch of chemical name does not by itself guarantee reduced hazard [23, 24, 29]. Other bisphenols were examined in the same way, and the pattern of effects on hatching, heart and nervous system looked broadly similar [23, 24].

Put simply, the findings fall into groups that are easy to describe even if their relevance to human beings is debated, and the distribution of entries across those groups is shown in fig. 1. Morphological and lethal effects at higher concentrations are reproducible across laboratories and fit with the test guideline literature [13, 22, 23]. Neurodevelopmental and behavioural effects at low concentrations are fewer in number, depend on specialised assays and, in several instances, come from single laboratories [24, 25]. Endocrine activity is supported by binding and reporter work but connects to developmental outcome only indirectly [18, 20, 21]. Each group deserves a different level of confidence when its findings are applied to risk assessment [7, 9]. These distinctions help a reader decide quickly which results are firm enough to rely on and which need independent confirmation [9, 14].

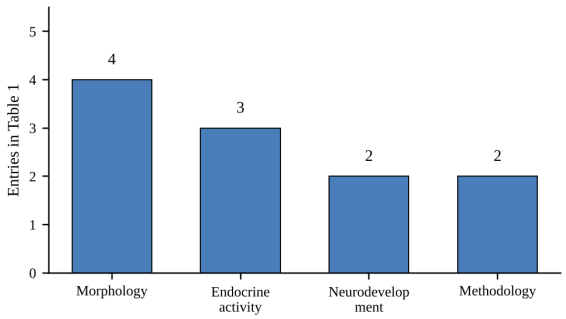



**Fig. 1: Number of entries in table 1 by type of endpoint or issue**

## 5. METHODOLOGICAL ISSUES

Several design choices explain why results from different laboratories disagree. The first is exposure window: embryos exposed from fertilisation to five days after fertilisation, those exposed only during gastrulation, and those exposed from 24 hours on are in different developmental states, and a window that misses a sensitive period will underestimate toxicity [10, 11]. The second is the solvent. Dimethyl sulfoxide or ethanol is often used to dissolve BPA, and although the final concentration is kept low, the solvent itself can influence behaviour and gene expression if it is not matched in the control group [14, 15]. Third, the number of independent clutches used is frequently small, which limits the power to detect modest effects [14]. Temperature and light cycle during exposure are rarely compared across studies, even though both influence developmental speed and therefore the stage reached at the time of scoring [10, 13].

Concentration reporting is a recurrent weakness. Most papers state nominal concentrations, calculated from the amount added, without verifying them by chemical analysis. BPA is moderately hydrophobic and can adsorb to plastic surfaces, while polycarbonate or other plastic labware can itself leach traces of the compound into exposure media, which may contaminate controls [1, 2]. Glass vessels, solvent-matched controls and analytical confirmation of media concentrations by liquid chromatography, measured at the beginning and end of the exposure, answer these concerns. In semi-static designs, where the medium is renewed daily, measurements before and after renewal show how much the concentration falls [11, 14]. Cleaning procedures for exposure vessels, including the use of separate glassware for BPA work, are seldom described but make a practical difference at low concentrations.

Endpoint selection and scoring contribute to variability as well. Subjective grading of oedema or curvature varies between observers unless scoring criteria are defined and ideally blinded, and automated imaging and tracking systems reduce this problem for behavioural studies [27, 28]. Statistical practice also deserves attention: embryos from the same clutch are not independent, and treating each embryo as a separate unit inflates the apparent sample size [14]. Pre-registered protocols, clutch as the experimental unit and sharing of

raw tracking data would make low-dose behavioural findings far easier to evaluate. Such practices are standard in clinical research and are becoming more common in toxicology, although progress is gradual [14, 17]. A shared reporting checklist, listing exposure window, solvent, measured concentration and scoring method, would help readers compare papers at a glance.

Concentration-response design is another place where care pays off. A range-finding experiment with widely spaced concentrations normally comes first, followed by a definitive series of at least five geometrically spaced concentrations, so that the slope of the curve can be estimated and not just a single no-effect level [13, 14]. Modelling approaches that fit a curve and report a benchmark concentration are more informative than a simple comparison of each group against control, because they use all the data and do not depend on arbitrary group spacing. Low-dose designs also need many more embryos per group than acute tests, since small effects are easily buried in clutch-to-clutch noise [8, 14].

## 6. LIMITATIONS AND FUTURE DIRECTIONS

The strengths and weaknesses of the evidence are now fairly clear. Reproducible malformations at high concentrations show that BPA is bioactive in the embryo, but those concentrations lie far above typical exposures [3, 22, 23]. The low-dose neurodevelopmental findings are more relevant to exposure but are drawn from a small number of laboratories and depend on sensitive behavioural measures [24, 25]. Mechanistic explanations remain incomplete: receptor binding data are strong, yet the path from binding to a specific developmental outcome has been traced for few endpoints [7, 20, 21]. Finally, extrapolation to human foetal development is limited by differences in metabolism, placental transfer and physiology [16, 17]. Species differences in sensitivity should be kept in mind, because other fish tested for BPA effects respond at different concentrations [26].

Progress will probably come from three directions. First, better exposure characterisation, including measured concentrations and internal dose estimates, would let zebrafish results be compared directly with human biomonitoring data [3, 16]. Second, independent replication of the low-dose neurodevelopmental findings, across laboratories and strains, would show how much weight they can bear [14, 24, 25]. Third, mixture studies, combining BPA with its analogues and other endocrine-active chemicals at realistic levels, would address the fact that people are exposed to many compounds at once [19, 29]. The zebrafish is well placed to support all three, provided that methodological discipline matches the attractiveness of the model [11, 27]. Funding bodies and journals could support such work by valuing replication studies, which often attract less recognition than novel findings [14, 17]. Regulators need such evidence before drawing firm conclusions for people, and agencies that rely on weight of evidence will only be moved by results that survive replication [6, 9].

## 7. CONCLUSION

Published zebrafish studies show that BPA reliably disturbs development at concentrations in the micromolar range, producing lethality, delayed hatching, oedema and axis curvature, and that lower concentrations have been linked to altered neurogenesis and behaviour [22, 23, 24, 25]. Mechanistic data point to receptor interactions, especially with oestrogen-related receptor gamma, although the chain from binding to outcome is incomplete [20, 21]. Replacement bisphenols do not appear to be a simple solution [23, 29]. These conclusions are consistent with expert panel reviews of the wider endocrine-disruptor literature [7, 19]. The picture is therefore one of a bioactive compound whose effects are dose-dependent, window-dependent and not yet fully explained at the lowest exposures.

The key message for readers is to weigh each finding according to its group, as table 1 and fig. 1 suggest. Morphological results are robust but far from typical exposures, whereas low-dose behavioural results are closer to real exposures and less firmly replicated. Better chemical verification of exposure, blinded and clutch-aware analysis,

and independent replication would strengthen the evidence most [6, 9, 14]. Until then, zebrafish data are best used to identify hazards and compare compounds, not to set human exposure limits [17, 30]. The choice of compounds to replace BPA should likewise be informed by comparative testing, not assumed safe on the basis of lower regulatory attention [29]. Readers who commission or evaluate toxicity work will find these points more useful than any single headline concentration.

## 8. ABBREVIATIONS

BPA-Bisphenol A, BPS-Bisphenol S, BPF-Bisphenol F, BPAF-Bisphenol AF, ER-Oestrogen receptor, ERRγ-Oestrogen-related receptor gamma, OECD-Organisation for Economic Co-operation and Development, EFSA-European Food Safety Authority, DMSO-Dimethyl sulfoxide, hpf-Hours post fertilisation, FET-Fish embryo acute toxicity test

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## 10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## 11. CONFLICT OF INTERESTS

Declared none

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