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The bisphenol microplastics issue in marine bivalves

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Abstract

Chemical additives are considered to be one of the main contributors to the toxicity of plastics, especially when they fragment into microplastics (MPs) in the environment. Bisphenols (BPs), as plasticizers, are a group of dozens of organic compounds that have been used as building blocks in the manufacture of polycarbonate plastics, epoxy resins and other products. This review provides an overview of the most commonly produced BPs (BPA, BPB, BPF, BPS, BPAF, BPAP) detected in the marine environment, the methods available for their detection and quantification, particularly in bivalves, and the potential risks of human exposure to BPs as endocrine disrupting chemicals and emerging contaminants. This work shows that the presence of BPA in bivalve molluscs has been investigated worldwide, with most studies conducted on the Asian coast, while the main analogues acting as estrogenic, progesteronic and anti-androgenic compounds have not been studied. The estimated daily intake (EDI) for BPA, BPB, BPF, BPS, BPP, BPAF, BPAP and for the sum of these BPs (ΣBPs) found in bivalves on the South African and Asian coasts at both median and maximum exposure exceeded the tolerable daily intake (TDI), suggesting that the EDI of bivalves with BPs pose a human health hazard. There is a need to conduct and implement studies on the distribution of BPs in the environment and the risk of consumption of bivalves as a potentially significant source of their intake.

Key words: *bisphenols; bivalves; emerging contaminants; occurrence; estimated daily intake*

Introduction

In the Global Chemical Outlook (GCO-II), eleven chemicals or groups of chemicals were identified which new findings have indicated to pose a risk to human health and the environment. These chemicals include the plasticizer bisphenol A (BPA), which is found in the marine environment and could leach from plastic waste and have negative effects on organisms and ecosystems (Bridson et al., 2021; Di Giacinto et al., 2023). There are several thousand different ad-

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ditives for plastic polymers. As the additives are usually not bound to the polymer matrix, have a low molecular weight and can be present in large quantities, they are often responsible for the leaching and emission of chemical substances from plastics. For this reason, chemical additives are considered one of the main contributors to the toxicity of plastics, especially when plastics are fragmented into microplastics (MPs) in the environment (Gallo et al., 2018; Issac and Kandasubramanian, 2021).

MPs are very small plastic particles, usually smaller than 5 mm, that can be created unintentionally through the wear and tear of larger plastic parts, including synthetic textiles, or manufactured and intentionally added to products for a specific purpose, such as exfoliating beads in facial or body scrubs. Once released into the environment, they accumulate in fish and shellfish and enter the food chain (Bogdanović et al., 2022a, b).

Plastic additives can be divided into four main categories: including functional additives, colorants, fillers and reinforcing agents. Among the additives, flame retardants, plasticizers and antioxidants raise significant food safety concerns. Bisphenols (BPs) as plasticizers are a group of dozens of organic compounds that have been used as building blocks in the manufacture of polycarbonate plastics, epoxy resins and other products since the 1960s (Pelch et al., 2019). Among them, BPA has attracted the most attention; the GCO-II identified BPA as a compound of concern, with emerging evidence pointing to risks to human health and the environment with high reproductive toxicity and (potential) endocrine disruption.

The European Food Safety Authority (EFSA) has lowered the Tolerable Daily Intake (TDI) of BPA three times, from originally 50 μg/kg body weight/day to 4 μg/kg body weight/day, and recently to a TDI of 0.2 ng/kg body weight/day (EFSA CEF Panel, 2015, 2023; Di Giacinto et al., 2023). When a harmful chemical is withdrawn from the market, it is usually replaced by a "chemical cousin" with a similar structure and potential for harm. This is the case with BPs analogues (Fantke et al., 2015; Weber et al., 2018). BPs consist of two hydroxylated benzene rings connected by a carbon bridge (Uzzaman et al., 2021). They have a wide range of industrial and consumer applications and different physicochemical properties depending on the different substituents located on the aromatic rings or on the carbon bridge.

The analogues of BPA have shown low to moderate acute toxicity and estrogenic activity (Yang et al., 2014). However, bisphenol C (BPC), bisphenol AF (BPAF), bisphenol Z (BPZ), bisphenol P (BPP), bisphenol B (BPB) and bisphenol AP (BPAP) have been reported to have moderate estrogenic activity exceeding that of BPA (Ng et al., 2015). BPS and BPF have also been shown to be toxic and have shown weak estrogenic activity in many studies (Liao et al., 2013). BPs are widely used in various manufacturing processes, mainly as raw materials, so their presence has been detected in various environmental compartments such as water, sediments, air and biomass. BPs can be released into the environment through various pathways, such as atmospheric deposition, urban sewage, and wastewater discharges during the production and treatment of BP materials (Liu et al., 2021).

Bivalve shellfish have been identified as one of the groups most affected by MPs and consequently by MPs leaching additives with BP presence due to their particular properties, such as wide distribution, easy access, stationary lifespan and high tolerance to different environmental conditions (Ward et al., 2019; Baralla et al., 2021). Bivalve shellfish are not only used as indicator organisms for environmental pollution, but are also important species for water quality and safety monitoring (Qu et al., 2018). Bivalve shellfish are the least mobile of the four major aquatic products (fish, shrimp, crab and shellfish); as filter feeders, they are among the species most likely to take up MPs and accumulate BPs (Wright et al., 2013; Germanov et al., 2018). They can be good bioindicators for monitoring BPs and risk assessment of human exposure to MPs ingested by shellfish (Ward et al., 2019).

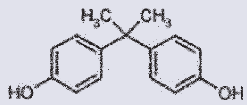
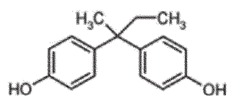
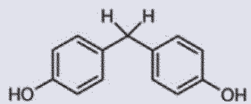
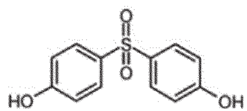
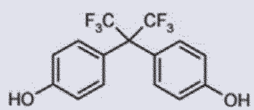
The aim of this review paper was to assess the occurrence of BPs commonly found in MPs in marine bivalves worldwide and to highlight their potential for bioaccumulation. This review provides an overview of the main BPs detected in the marine environment, the methods available to detect and quantify these substances, particularly in bivalves, and the potential risks of human exposure to BPs as endocrine disrupting chemicals and emerging contaminants.

Occurrence of bisphenols in marine aquatic environments

Several organic pollutants have been found in the sea as plastic waste, as well as in sewage sludge and reclaimed wastewater, where they eventually accumulate in aquatic systems (Fotopoulou and Karapanagioti, 2019). This plastic waste accounts for 60 to 95% of all marine litter, from surface waters to deep-sea sediments (Lechthaler et al., 2020) and into food chains (Huerta Lwanga et al., 2017), meaning that it is crucial for the protection of marine health and endocrine systems. The issue of marine plastic litter and MPs is part of three global chemical and waste conventions, the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and their Disposal, the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade, and the Stockholm Convention on Persistent Organic Pollutants (BRS Conventions), as well as a regional seas convention, the Barcelona Convention for the Protection of the Marine and Coastal Environment of the Mediterranean Sea.

However, the Stockholm Convention, which covers additives classified as hazardous to humans, the environment and organisms, cannot cover the vast majority of the growing list of additives not yet covered by existing regulations. The environmentally sound management of plastic waste and potentially hazardous components of plastics in the aquatic environment has been the focus of scientific interest for decades. Considering the wide range of pollution caused by MPs, the almost invisible but equally important toxic chemical components used in plastic production, especially BPs, are of great importance. Currently, at least 148 different substances have the "bisphenol" component (ECHA, 2021). This group includes 17 bisphenols with the general "bisphenol" structure and "bisphenol derivatives", which have components with structural features common to bisphenols (ECHA, 2021). BPs differ both by the chemical group between the two hydroxyphenyls and by the presence of other chemical groups, such as brominated and chlorinated compounds. The chemical structure and physicochemical properties of the most commonly used bisphenols worldwide are shown in Table 1.

Table 1. The most produced bisphenols, their chemical characteristics and main application

Bisphenol compound and IUPAC[1] name	Structure	Molecular weight (g/mol)	Log K_{ow}[2]	BF[3]	Main application
Bisphenol A (BPA) 4-[2-(4-hydroxyphenyl) propan-2-yl]phenol		228.29	3.32	71.85	used in epoxy resins, coatings and linings, for pipes and tanks, food, containers (baby bottles, sippy cups and baby food packaging)
Bisphenol B (BPB) 4-[2-(4-hydroxyphenyl) butan-2-yl]phenol		242.31	4.13	304.3	in certain polymers whose unique structure gives them desirable properties; tuna, meat and bear cans
Bisphenol F (BPF) 4-[(4-hydroxyphenyl) methyl]phenol		200.23	2.91	34.73	used in epoxy resins, coatings and linings, for pipes and tanks, food, containers as an alternative to BPA-based products
Bisphenol S (BPS) 4-(4-hydroxyphenyl) sulfonylphenol		250.27	1.65	240.5	heat resistant or less colourful applications, for thermal paper, plastics, epoxy resins, food contact materials, food packaging
Bisphenol AF (BPAF) 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl) propan-2-yl]phenol		336.23	4.47	556.3	applications of fluoropolymer resins, coatings for aerospace applications

IUPAC[1], International Union of Pure and Applied Chemistry; Log Kow[2], the octanol-water partition coefficient; BF[3], bioconcentration factor (source of data: CHEMspider).

Structurally, these BPs have a common structure with two hydroxyphenyl functions. The octanol-water partition coefficient (log Kow) of most BPs produced is between 1.65 (BPS) and 4.47 (BPAF). BPs with a log $K_{ow} \leq 4$, such as BPA, BPF and BPS, are mainly detected in the water phase, while other BPs such as BPAF and BPB (log $K_{ow} \geq 4$) have the potential to adsorb to sediments and accumulate in the tissues of organisms (Liu et al., 2021). BPs can enter the environment via wastewater, refineries, petrochemical plants and other industrial discharges (Dueñas-Moreno et al., 2022). BPs can contaminate the surrounding surface water and eventually enter marine habitats if this discharge is not properly managed. BPs can also migrate through the atmosphere and enter water bodies via rain or other precipitation (Liu et al., 2020; Zhao et al., 2021). They can enter the environment through the manufacture or disposal of plastic items. When it rains or snows, these compounds can be transported by wind currents and deposited in adjacent marine waters (Zainuddin et al., 2024).

The most studied bisphenol in the group of BPs is BPA. It has become one of the most produced chemicals worldwide, reaching a global production of about 8 million tonnes per year. Many countries have banned the use of BPA due to its toxic effects, which has led to the invention of other bisphenol analogues such as BPF, BPB, BPP and BPAF. Marine waters are affected by anthropogenic pollution, as this natural habitat is the final repository for all wastewater. As a result, chemical pollutants, including plastic additives, have been detected in marine waters worldwide (Hermabessiere et al., 2017). BPA is widely distributed and the best-studied analogues of BPS, BPF, BPAF, BPB, 4,4′-dihydroxybenzophenone (DHBP), BPP and bisphenol fluorene (BPFL) have been monitored. The European Union (EU) has set a PNEC (Predicted No-Effect Concentration) value of 150 ng/L for BPA in seawater (EC, 2008). BPA concentrations varied worldwide from a few ng/L to 2470 ng/L (Singapore), and BP analogues varied worldwide from not detected to 1470 ng/L (Tokyo Bay) at the sites studied, including water in the East China Sea and South China Sea (Fabrello and Matozzo, 2022).

Analyses of seawater samples clearly indicate increasing pollution not only with BPA but also with BPS. According to Xie et al. (2022), BPS was the third most important BPA analogue in Chinese marine waters. A similar BPS content to BPA was found in the Baltic Sea (Caban et al., 2022.).

Bisphenols in bivalves

Aquatic organisms can take up a cocktail of chemicals that can accumulate in tissues (Rochman et al., 2015). The constant increase in plastic production and the resulting plastic pollution in the world's oceans (Jambeck et al., 2015; Geyer et al., 2017; Lambert et al., 2020) is therefore likely to increase the concentration of plastic additives such as BPs in the marine environment, through their direct deposition and/or their leaching from the large quantities of plastic waste that enter the oceans every day.

Shellfish are marine organisms that are particularly susceptible to bioaccumulation of pollutants as they are filter feeders (Álvarez-Ruiz et al., 2021; Juhel et al., 2017; Liao and Kannan, 2019). For many years, they have been considered a nutritious and balanced food components in the human diet. Anthropogenic chemicals such as BPA can enter the aquatic environment where they can affect a range of physiological processes in aquatic organisms, including reproduction, growth, development, immune response and endocrine regulation (Aarab et al., 2006; Balbi et al., 2016; Juhel et al., 2017; Tang et al., 2020). The substitution of BPA in several production processes with its analogues did not lead to a reversal of the toxic effects, and their occurrence has been documented in different environmental compartments (Chen et al., 2016).

Baralla et al. (2021) described the occurrence of BPs in bivalves from around the world. In general, with the exception of BPA, reports of BPs analogues in marine invertebrates are still limited. Bivalve species studied worldwide include *Neverita didyma*, *Rapana venosa*, *Mya arenaria*, *Cyclina sinensis*, *Chlamys farreri*, *Scapharca subcrenata*, *Meretrix meretrix*, *Mytilus edulis*, *Crassostrea talienwhanensis*, *Amusium Mactra veneriformis* from the Chinese Bohai Sea (Northeast China) (Liao and Kannan, 2019); bivalve samples from the Pearl River Estuary (South China) (Zhao et al., 2019), and brown mussel (*Perna perna*) samples (Algoa Bay, South Africa (Castro et al., 2022; Nielsen, 2023). The studies investigated the species-specific accumulation and temporal trends of target compound concentrations in molluscs. Based on the daily consumption rates and the measured concentrations, the daily intake of target compounds via the consumption of molluscs was investigated. In the most comprehensive study to date by Liao and Kannana (2019), the mollusc species *Meretrix meretrix* was found to accumulate high concentrations of BPs ranging from undetected to 458 ng/g, while the species *Amusium* had the lowest ΣBPs concentrations ranging from undetected to 58.1 ng/g, which were significant and 6.5 times lower. In the same study, eight BP analogues, including BPA, BPF, BPB, BPS, bisphenol Z (BPZ; 4,4′ cyclohexylidene bisphenol), bisphenol AP (BPAP; 4,4′ (1 phenylethylidene) bisphenol), bisphenol P (BPP; 4,4′ (1,4 phenylenediisopropylidene) bisphenol) and bisphenol M were examined, with BPF showing the widest range (from not detected to 457 ng/g), followed by BPA, BPB, BPP, BPS, BPM, BPZ and BPAP.

The most investigated BPA has been detected in bivalve molluscs harvested around the world ranging from a few to hundreds ng/g of tissue. The lower BPA concentrations were found in the southern area of the Po River Delta in the Adriatic sea (in the clam *Ruditapes philippinarum*, <3.3–9.5 ng/g) (Casatta et al., 2015), Atlantic coasts of Portugal - Tagus estuary (in *Mytilus spp.*, ranged from <MQL up to 12.5 ng/g dw) (Álvarez-Muñoz et al., 2015), Venice Lagoon in the Adriatic sea (*Mytilus galloprovincialis,* 11 ng/g dw) (Pojana et al., 2007), Spanish Atlantic coast and Bay of Biscay (*Mytilus galloprovincialis*, <3.3–714 ng/g dw) (Salgueiro-González et al., 2016), as well as in the southern Asia (in *Perna viridis* ranged from 1–13.4 ng/g dw and *Mytilus galloprovincialis* ranged from 1.1–13.7 ng/g dw) (Isobe et al., 2007).

The highest BPA concentration (53.3 ng/g) was found in mussels sampled along the Italian coast in the study by Bogdanović et al. (2021), which investigated the overall status of BPA and MPs contamination in mussels (*Mytilus galloprovincialis*) collected from aquaculture farms and a natural bed (Croatia) along the Italian and Croatian coasts of the Adriatic Sea. Based on liquid chromatography-tandem mass spectrometry (LC MS/MS) data, a significant spatial distribution trend was found. The group of sites on the Italian Adriatic coast tended to have higher BPA levels. The higher BPA concentrations were found on the Chinese coasts, mostly near highly urbanized and industrial areas (in *Mytilus galloprovincialis* in the range of 170.3–437.2 ng/g dw) (Chiu et al., 2018; Liao and Kannan, 2019). High BPA levels were also found in mussels harvested off the Greek coast (in *Mytilus galloprovincialis* in the range of 342.8–611.9 ng/g dw, in *Venus gallina* in the range of <LOD – 626.3 ng/g dw, in *Modiola barbatus L.* ranged from 209–515.2 ng/g dw) (Gatidou et al., 2010), southern Baltic Sea (in *Mytilus trossulus* ranged from not detected to 273.6 ng/g dw) (Staniszewska et al., 2017), in the Ebro Delta (in *C. edule* 4277.40 ng/g dw BPA but associated with a mortality event) (Álvarez-Muñoz et al., 2019), and in the Persian Gulf (in *Saccostrea* sp., 340.16 ng/g) (Jahromi et al., 2020).

Approaches for the analysis of bisphenols in marine bivalves

In order to determine the presence of xenobiotics in complex matrices, the development and application of a validated method in accordance with the relevant legislation is essential. The validated method fulfils the specific performance criteria of applicability, limit of detection and quantification, precision, recovery and specificity (Commission Regulation (EU) No 333/2007; Commission Implementing Regulation (EU) No 808/2021). BPs as contaminants are present in trace amounts in complex environmental samples and therefore require sophisticated sample isolation and identification steps.

Liquid chromatography (LC) and gas chromatography (GC) coupled with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) are the most widely used analytical techniques in the analysis of BPs in food (Lucarini et al., 2023). Compared to LC methods, GC methods are more laborious due to the required derivatization step, which explains the dominance of LC methods in mussel analyses. Confirmatory methods should be able to unambiguously detect an analyte based on its chemical structure. The criteria for the applicability of the method are laid down in Commission Implementing Regulation (EU) No 808/2021, with the number of identification points as an important selection criterion. Qualification criteria for the analyte are relative ion intensities and retention times. The main characteristics of the methods used for the quantification of BPs in bivalve molluscs are summarised in Table 2.

The procedures for isolating BPs are sophisticated and typically involve the use of matrix solid phase dispersion (MSPD) and the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction procedure in the development and validation of simple, rapid and sensitive methods that capture a mixture of impurities along with BPs. Álvarez-Muñoz et al. (2019) developed the QuEChERS method for several different groups of contaminants and BPA, Cãnadas et al. (2021) applied the MPSD multi-residue method for several plastic additives (BPA, BPF, BPS; phthalate esters and alkylphenols), while Rios-Fuster et al. (2023) quantified BPA, BPF and BPs together with phthalates. Most purification procedures of marine bivalve samples include solid phase extraction and more recently even the step of enzymatic hydrolysis with β-glucuronidase/sulphate lyase, which hydrolyzes bound BPs in the organism and thereby converts them to the free state, resulting in increased concentrations, as observed in the studies by Chen et al. (2024). The results of the comparative analysis of enzymatically and non-enzymatically dissolved BPs showed statistically significant differences ($p < 0.05$) with an increase in mean BPs concentrations by a factor of 1.97 to 26.25 compared to pre-treatment levels in three aquatic product categories: fish, crustaceans and bivalves. Lucarini et al. (2023) extended the QuEChERS method approach to 16 BPs.

Matrix effects, the percentages of signal reduction or enhancement on the analysis of the target compounds, that could appear in LC-MS analyses of shellfish were checked and presented in Table 2. To minimize matrix interferences and avoid under- or overestimation in quantification, the quantification of the target analytes was performed based on the internal standard method and with a corresponding matrix-adjusted calibration (Álvarez-Muñoz et al., 2019; Castro et al., 2022). The analytical methods for BPs with MS-MS detection ensure full recovery of the broadest range of BPs compounds with an LOQ of 0.007 to 10 ng/g (Álvarez-Muñoz et al., 2019; Castro et al., 2022; Lucarini et al., 2023; Nielsen et al., 2023; Chen et al., 2024). Other authors determined BPs in bivalves by LC-fluorescence detection (HPLC/FLD) and diode array detection (LC-DAD) (Staniszewska et al. 2017; Cãnadas et al., 2021) with good recoveries (> 78.5%) and LOQ in the range of 0.16 to 1.12 μg/kg. The LOQ from the table were calculated after converting all reported BP values from dry weight to wet weight using an average moisture content of 80%.

Health risk assessment

Humans, who are at the uppermost trophic level of the sea, are potentially exposed to BPs due to possible biomagnification (Akhbarizadeh et al., 2020). Trophic biomagnification of chemicals may be closely correlated with the log K_{OW} and metabolism of chemicals (Gu et al., 2016; Wang et al., 2017). Exposure of marine organisms to BPs poses a potential risk to human health (Ismail et al., 2018). BPs are classified as pollutants with potential endocrine disrupting capabilities that may have adverse effects such as reproductive toxicity. Therefore, it is important to assess the risk that these compounds may have on human health through dietary intake, especially when the main route of the chemical is through food and in countries with high consumption. One of the methods to estimate the extent of exposure is to calculate the Estimated Daily Intake (EDI) using the following equation:

$$\mathbf{EDI = (C_{BP}\ X\ DC)/(bw),}$$

where C_{BP} is the target contaminant concentration in the sample, DC is the estimated daily food consumption and bw is the mean body weight of the consumption.

Estimates of dietary exposure to substances can provide information on: (i) risks to human health by comparing estimated exposure with acceptable or tolerable levels; (ii) the likely relative contribution of different foods to total dietary exposure; and (iii) the impact of risk management measures such as dietary exposure limits (Garrido Gamarro and Costanzo, 2022; Bogdanović et al., 2023). It is currently possible to use estimates of dietary exposure to BP for purposes 1 and 2, i.e., to determine which foods are likely to make the greatest contribution to total dietary exposure to BP and to compare exposure estimates with tolerable levels. Consumption of shellfish, especially mussels, can be determined via FAOSTAT (FAO/WHO, 2022) or national consumption studies. To determine the potential dietary exposure to BPs for shellfish, different amounts of usual consumption were used in the studies. As a rule, median and maximum concentrations are determined in order

Table 2. Overview of analytical methods for the detection of bisphenols in marine bivalves

Bisphenol compound/bivalve specie	Isolation procedure specificity	Analytical method	Method performance criteria			References
			Linear range	Precision (%)/ME presence (%)	LOD-LOQ	
BPA / *Mytilus trossulus*	lyophilized samples Methanol/ammonium acetate extraction and SPE	HPLC-FLD	10–100 ng/mL	91.2% / ME not reported	0.8 ng/g dw	Staniszewska et al., 2017
BPA/mussel *(Mytilus galloprovincialis)*, oyster *(Crassostrea gigas)*, cockle *(Cerastoderma edule)* and razor shell *(Solen marginatus)*.	Freeze dried samples / QuEChERS and SPE	UPLC-HMRS	0.01–50 ng/g	68.4–82.9% (accuracy)/ ME (91.99–14.08)	0.5–10 ng/g dw (mLOD); 1.0–10 ng/g dw (mLOQ)	Álvarez-Muñoz et al., 2019
BPA, BPF, BPS/ *Mytilus galloprovincialis* from aquaculture	. MSPD 5 g Florisil, 0.5 g Na_2SO_4	HPLC-DAD	0.32–6.0 µg/kg	78.5–87%//ME NR	0.07–0.29 µg/kg (LOD); 0.25–1.12 µg/kg	Cãnadas et al., 2021
BPA, BPAF, BPAP, BPF, BPS/ wild brown mussels *(Perna perna)*	Freeze dried samples, β-glucuronidase treatment; LSE extraction12 mL ethyl acetate and 2 mL H_2O	UPLC-MS/MS	0.1–20.0 ng/mL	42–164% (R) / MEs < 50%	0.02–0.5 ng/ml (iLOD); 0.20–52.1 ng/g dw (mLOQ)	Castro et al., 2022
BPA, BPAF, BPAP, BPB, BPF, BPM, BPP, BPS, BPZ/ wild brown mussels *(Perna perna)*, S. lalandi (fish)	freeze-dried mussel or fish; 44 units of β –glucuronidase, LLE ethyl acetate and water	UPLC-MS/MS	0.2–50 ng/mL	NR / NR	2–10 ng/g (mLOD); 6–30 ng/g (mLOQ).	Nielsen et al., 2023
BPA, BPF, BPS/ *Mytilus galloprovincialis* from acquaculture	MSPD	HPLC-ESI-MS	NR	NR	NR	Rios-Fuster et al., 2021
BPA, BPAF, BPAP, BPB, BPBP, BPC, BPE, BPF, BPG, BPM, BPP, BPPH, BPS, BPZ, BP-TMC, tetramethyl BPF/canned food (canned tuna)	10 mL acetonitrile 4 g magnesium sulfate anhydrous ($MgSO_4$) and 1 g (NaCl) Derivatization of BPs with (BSTFA)	QuEChERS GC-MS (SIM); QuEChERS LC-MS/MS	0.1–50 µg/mL	Canned tuna: 68–123% with the exception of BPG, BPAP, BPM, BPP, BPBP, BPPH in the ranges of 25–51% / ME NR	0.03–1.66 µg/L (LOD); 0.1–5.55 µg/L (LOQ)	Lucarini et al., 2023
BPA, BPAF, BPAP, BPB, BPF, BPP, BPS, BPZ/3 aquatic product categories:1 bivalve: *Paphia undulata*, 6 fish species, 1 crustacean	β-glucuronidase treatment, acetonitrile extraction, SPE extraction	HPLC-MS/MS	0.1–50 µg/L	84.8–111% (recovery) / ME NR	0.002–0.090 ng/g (LOD); 0.007–0.03 ng/g (LOQ)	Chen et al., 2024

Bisphenol A, BPA and bisphenol analogues: BPA, BPAF, BPAP, BPB, BPBP, BPC, BPE, BPF, BPG, BPM, BPP, BPPH, BPS, BPZ, BP-TMC, tetramethyl BPF; BSTFA, bis(trimethylsilyl)trifluoroacetamide derivatization reagent; GC-MS (SIM), gas chromatography-mass spectrometry (single ion monitoring); HPLC-ESI-MS, high-performance liquid chromatography-electrospray ionization-mass spectrometry; HRMS - High-Resolution Mass Spectrometry; LC-MS/MS, liquid chromatography, tandem mass spectrometry; i-LOD (instrument limit of detection); m-LOD (method limit of detection); m-LOQ (method limit of quantification); LC, liquid chromatography; LSE, liquid solid extraction; ME (%) matrix effects; MSPD, matrix-solid phase dispersion; NR, not reported; QuEChERS - (Quick, Easy, Cheap, Effective, Rugged and Safe) R, absolute recoveries (%); SPE, solid phase extraction to evaluate the medium and high intake scenarios. The most extensive study currently available on the occurrence of BPs in shellfish reports an average daily shellfish consumption for adults of 27.5 to 33 g in the marine areas of South Africa and China (Liao and Kannan, 2019; Castro et al., 2022; Nielsen et al., 2023).

The EDI of BPA, BPB, BPF, BPS, BPP, BPAF, BPAP and for the sum of these BPs found in shellfish are listed in Table 3. The values of the highest BPs BPA have concentrations ranging from 0.105 to 16 ng/kg bw/day for the overall mean and median. The maximum EDI values for BPA range from 4.06 to 20.5 ng/kg bw/day. Taking into account the mean BPA concentration reported in the review by Barala et al. (2021) and the average consumption of shellfish in the different countries, the EDI varies between 1.1 ng/kg bw/day in Europe and 7.1 ng/kg bw/day in Asia, for a standard adult of 70 kg.

The results of recent studies presented in Table 3 show that the median BPA levels are between 0.1 and 5.90 ng/kg bw/day. The maximum BPA values are three to twenty times higher. The respective mean, median and maximum ∑BPs values in Africa and Asia were in the ranges of 1.34–13.2 ng/kg bw/day and 17.22–135 ng/kg bw/day, respectively. BPA, BPF, BPAP and BPB contributed to the majority of BPs intake. The TDI limit for BPA set by EFSA was 0.2 ng/kg bw/day. Exceeding these limits can pose a significant health risk to humans (EFSA, 2023). The EDI for both the mean and maximum exposure scenarios for BPA and ΣBPs in shellfish exceed the limit of 0.2 ng/kg bw/day, except for the overall mean value in the study by Liao and Kannan, (2019).

Table 3. Estimated daily intake (EDI, ng/kg bw/day) of bisphenols from the consumption of different shellfish species by adults

Location and reference	Aegean sea, Greece (Gatidou et al., 2010)	North-Eastern China (Liao and Kannan, 2019)		South Africa (Castro et al., 2022)		South Africa (Nielsen, 2023)	
Shellfish Species	3 bivalve species	11 mollusc species		Mussels *(Perna Perna)*		Mussels *(Perna Perna)*	
Bisphenol Compound	General Mean	General Mean	Maximum	Median	Maximum	Median	Maximum
BPA	5.90	0.105	20.5	2.63	9.81	1.12	4.06
BPB	NDr	0.033	6.72	0.82	2.09	ND	ND
BPF	NDr	0.077	46.9	0.63	6.47	ND	ND
BPS	NDr	0.015	0.481	ND	ND	1.23	4.98
BPP	NDr	0.018	3.74	ND	ND	ND	ND
BPAF	NDr	ND	ND	0.09	2.20	0.71	1.97
BPAP	NDr	ND	ND	12.2	121	2.58	16.63
∑BPs	NDr	1.34	47.1	13.2	135	2.87	17.22

Bisphenol A, BPA and bisphenol analogues: BPA, BPAF, BPAP, BPB, BPF, BPS, BPP; NDr, not determined; ND, not detected

This indicates that the consumption of shellfish poses a threat to human health and that exposure to these contaminants is of concern.

Conclusions

The summarized scientific findings on the occurrence of and exposure to bisphenols in shellfish require further investigation. The EDI for BPA and the most produced BPs found in shellfish worldwide exceeded the TDI, suggesting that the EDIs of shellfish containing BPs pose a health risk to humans. The uptake and accumulation of BPs in bivalves is influenced by many biotic and abiotic factors and is species-specific. BP monitoring programmes using bivalves as sentinel organisms should be conducted in all countries to investigate the global distribution of these emerging contaminants and the resulting consequences for animals and human exposure. BPs as contaminants are present in trace amounts in complex environmental samples and therefore require sophisticated sample isolation and identification steps using validated methods that meet the specific performance criteria.

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Bisfenoli – aditivi mikroplastike u morskim školjkašima

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Kemijski su aditivi prepoznati kao jedan od glavnih čimbenika koji doprinose toksičnosti plastike, osobito kada se plastika u okolišu fragmentira u mikroplastiku. Bisfenoli su skupina umjetno proizvedenih kemikalija koje se upotrebljavaju u izradi polikarbonatne plastike i epoksi smola. Ovaj rad donosi prikaz najzastupljenijih bisfenola (BPA, BPB, BPF, BPS, BPAF, BPAP) iz morskog okoliša i dostupnih analitičkih postupaka za njihovo prepoznavanje i kvantifikaciju, s posebnim osvrtom na školjkaše. Kako su bisfenoli kao endokrini disruptori štetne kemikalije procijenjen je rizik izloženosti konzumenata školjaka. U dosadašnjim istraživanjima prisutnosti bisfenola širom svijeta najviše je bio zastupljen BPA, a većina studija su istraživanja duž azijske obale. Utvrđen je nedostatak istraživanja analoga bisfenola, koji djeluju kao estrogenski. progesteronski i anti-androgeni spojevi. Procijenjeni dnevni unosi (EDI) za BPA, BPB, BPF, BPS, BPP, BPAF i BPAP te za zbroj ovih bisfenola ΣBPs utvrđenih u školjkašima duž južnoafričke i azijske obale. U oba scenarija srednje i maksimalne izloženosti prekoračen je podnošljivi dnevni unos (TDI), što ukazuje na to da EDI školjkaša s BP predstavljaju zdravstvenu prijetnju konzumentima. Istražena je potreba za provedbom istraživanja distribucije bisfenola u okolišu i opasnosti konzumacije školjkaša kao mogućnost znatnog izvora njihovog unosa.

Ključne riječi: *bisfenoli, školjkaši, kontaminanti s rizikom u nastajanju, pojavnost, procijenjeni dnevni unosi*