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Storage-driven migration of plastic additives from packaging to fish: influencing factors and human exposure assessment

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ABSTRACT

Plasticizers are widely used in food packaging materials, but their potential migration into food remains largely unknown and of growing concern for human health. This study aimed to evaluate the migration of four classes of compounds, phthalate esters acids (PAEs), non-phthalate plasticizers (NPPs), organophosphate esters (OPEs), and bisphenols (BPs), from common packaging materials into fresh fish (salmon, tuna and hake) under realistic household storage conditions (2 °C for 48 h, and -18 °C for 30 days). After examining the packaging materials, three representative types were selected for migration experiments. Plastic additives were detected in fish after storage, demonstrating that migration occurs under both refrigerated and frozen conditions. Migration rates varied widely (<0.01–100%) and were strongly influenced by storage temperature and time, fish lipid and water content, packaging materials, and physico-chemical properties of the compounds. Lipid content played a key role in the migration of hydrophobic compounds such as di(2-ethylhexyl) adipate (DEHA), while water content influenced the migration of bisphenols. Diethyl phthalate (DHEHP) showed significant migration rates in frozen samples compared to refrigerated ones. The maximum concentrations detected in each class were 243 ng/g wet weight (ww) for PAEs, 358 ng/g ww for NPPs, and 54.0 ng/g ww for BPs, while OPEs were generally below the LOQ. Estimated daily intake (EDI) and hazard index (HI) calculations for adults, children, and infants indicated that packaged fish may contribute to dietary exposure to plastic additives, with worst-case HI values reaching 3.17×10^2 , mainly due to the presence of bisphenol A (BPA). These findings demonstrate how common storage practices can promote migration of plastic additives, potentially increasing human health risks through dietary exposure.

1. Introduction

Over the past decades, plastic has become indispensable in modern food supply chains, and with packaging being one of its major applications globally (Plastics Europe, 2024). Plastic packaging plays a crucial role in maintaining the quality and safety of food products by extending their shelf life, preventing contamination, and facilitating distribution (Gupta et al., 2024; Yenidoğan et al., 2023). However, increasing attention has been paid to the unintended migration of chemical additives, such as plasticizers, from packaging materials into food, raising concerns for consumer health. Historically used as plasticizers, plastic additives including phthalate esters (PAEs), organophosphate esters (OPEs), and bisphenols (BPs), are now known to be harmful, particularly

with regard to human dietary exposure (Catenza et al., 2021; Miao et al., 2024; Rodríguez-Ramos et al., 2024).

PAEs have traditionally dominated the market, but their endocrine and reproductive effects have led to restrictions in several jurisdictions. Several studies have shown that PAEs have toxicological effects, with potential for endocrine disruption, and carcinogenicity (de Lima et al., 2025). To replace PAEs, a wide range of non-phthalate plasticizers (NPPs), including adipates, citrates, and cyclohexane dicarboxylic acids, have been introduced (Qadeer et al., 2022). However, because their health profiles are not yet fully understood (Bui et al., 2016), it is unclear whether they may have similar adverse effects to PAEs. For example, acetyl tributyl citrate (ATBC), di(2-ethylhexyl) adipate (DEHA), and 1,2-cyclohexanedicarboxylic acid diisononyl ester (DINCH) have been

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increasingly linked to emerging health concerns, including metabolic alterations, effects on neurological development, and reduced fertility, as suggested by recent experimental and epidemiological studies (Yun et al., 2024; Zhang et al., 2023).

At the same time, BPs, such as bisphenol A (BPA) and its analogues, and OPEs are also widely used in plastic formulations, both for packaging and other consumer products. BPA is known to interfere with the action of estrogenic and androgenic hormones, thus becoming an endocrine disruptor (Catenza et al., 2021; Miao et al., 2024; Manzoor et al., 2022). Consequently, the use of BPA has been restricted or even banned altogether in many countries, increasing the demand for alternatives, including bisphenol S (BPS) and bisphenol AF (BPAF), whose potential harm to human organs and health concerns remain unclear (Maćczak et al., 2017). Similarly, OPEs have been used in a wide range of plastic materials before being linked to developmental, neurological, and endocrine problems (Ai et al., 2024).

Given their hazardous properties, migration of these substances into foods has become an important focus in food safety assessment. Regulatory bodies such as the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have introduced standards to limit the transfer of hazardous compounds from packaging to food (Lambré et al., 2023; EU, 2011b; EU, 2024a; EU, 2004). These regulations not only establish migration limits for chemicals, such as diisodecyl phthalate (DiDP), di(2-ethylhexyl) phthalate (DEHP), and BPA, but also specific standards to produce plastic products intended for food contact.

However, migration tests often use food simulants under controlled laboratory conditions, which cannot accurately represent the complexity of real foods (EFSA, 2016). Food type and intrinsic properties, e.g., pH, lipid and water content, strongly affect migration, with lipid-rich products such as oils, dairy, and fish favoring the migration of lipophilic plasticizers (Seref and Cufaoglu, 2025). Moreover, the chemical composition of packaging materials and external conditions, including temperature, time, and shelf-life, are also determining factors (Seref and Cufaoglu, 2025; Alp and Yerlikaya, 2020). Fish represent a particularly relevant case due to its high nutritional value, which includes proteins, minerals, and polyunsaturated fatty acids. Dietary standards recommend consuming one to two meals per week (about 130 g each) of fatty fish, such as salmon or herring, or even more for lean fish, such as hake, to support cardiovascular and developmental health (EFSA, 2005). However, due to their potential for bioaccumulation in muscle tissue, fish can also be a significant source of exposure to PAEs (Squillante et al., 2023), BPs (Barboza et al., 2020), OPEs (Sala et al., 2022), and NPPs (Zhang et al., 2022).

Food consumption is considered a major route of human exposure to plasticizers (Miao et al., 2024). Nevertheless, given the widespread use of plastic packaging, no comprehensive dietary study has simultaneously examined PAEs, NPPs, OPEs, and BPs in fish under practical storage conditions. This lack of information highlights the need for further studies to improve our understanding of plastic related chemical exposure through dietary intake, especially regarding dietary exposure studies of newer-generation NPPs, whose replacement with controlled substances makes risk assessment even more difficult. Furthermore, the potential health effects are particularly important for vulnerable populations such as infants and young children, for whom research on estimated daily intakes (EDIs) is still relatively scarce.

Therefore, this study aims to gain a comprehensive understanding of the potential migration of four classes of plasticizers, namely PAEs, NPPs, BPs, and OPEs, from common packaging materials into fresh fish. First, a screening approach was applied to determine the presence and concentration of plasticizers in packaging. Next, a study design was developed to quantify the potential contaminants, previously identified in packaging materials. This work investigates migration under realistic exposure scenarios, using commercially packaged fresh fish stored under real storage conditions. Finally, the EDI and hazard index (HI) of these chemicals based on daily intake were assessed for different age groups, integrating migration data with fish consumption patterns

representative of the Spanish diet. This approach will provide new insights into overall exposure to plasticizers through the consumption of packaged fresh seafood, due to realistic storage-induced migration, and will help inform risk assessment and prevention strategies for food safety.

2. Materials and methods

2.1. Materials and reagents

In this study, a total of 49 plastic additives were investigated, comprising 13 PAEs, 12 NPPs, 20 OPEs, and 4 BPs, along with 23 internal labelled standards (ISs). Details of the targeted compounds and ISs used in this study are reported in Table S1. Quantification of compound concentration in samples was based on the isotope dilution method. HPLC-grade methanol was purchased from J.T. Baker, while acetone, n-hexane, and dichloromethane were purchased from Merck (Darmstadt, Germany). Chromatographic reagents (ammonium acetate, methanol, formic acid, and water) were supplied by Merck (Darmstadt, Germany). Glass wool was purchased from Panreac AppliChem (Barcelona, Spain). Oasis weak anion-exchange (WAX) cartridges (150 mg, 6 cc) were obtained from Waters Corporation (Milford, USA).

2.2. Sample collection

Polymer-based packaging, such as polypropylene (PP), polyethylene (PE), and polystyrene (PS), is widely used to preserve seafood due to its optimal mechanical and thermal properties (Laorenza et al., 2022). For this work, a total of twelve different packaging materials, three fresh fish species, and one canned seafood product, were randomly purchased from local grocery stores in Barcelona (Spain) in February 2025. For privacy concerns, the stores and brands are not reported. Packaging materials were selected based on their prevalence in local markets and household food storage practices. These included trays (n= 4), bags, (n= 5), cling films (n= 2), and can (n= 1) (Table S2, Fig. S1). The chemical composition of each packaging was verified using an attenuated total reflectance (ATR) FTIR spectrometer (Agilent Technologies), equipped with a Ge crystal. Spectra were acquired from 4000 to 400 cm^{-1} , with a resolution of 8 cm^{-1} , by analyzing the surface supposed to be in direct contact with food material. Polymer identification was carried out by comparing the sample spectra with the available published ATR spectra of the polymer standards. Results are shown in Table S2.

Fresh fish species, i.e., salmon, tuna, and hake, were selected based on their lipid content (high, medium, and low, respectively) and typical fresh fish consumption patterns in Spain (Ministerio de Agricultura, 2024). Upon purchase, fresh fish was immediately wrapped in aluminium foil to minimize external contamination and transferred to the laboratory for processing, ensuring the cold chain. Canned tuna was included in the study and stored at room temperature until analysis. All samples were freeze-dried prior to analysis. Additional information on fish samples, such as species, origin, lipid and water content, are included in Table S3.

2.3. Study design

The packaging was initially analyzed for different levels of plasticizer. The results were then analyzed to select four packaging types for a targeted food contact materials experiment, designed to simulate real-life conditions under which fresh fish may come into direct contact with food packaging, and be sold from supermarkets to consumers for home storage. These included a polystyrene tray (V1), a compostable cellulose-based tray (V2), as an example of an alternative derived from renewable sources, a polyethylene cling film (F1), and a polyethylene zip lock bag (B1). A study design was set up to evaluate the potential migration of plasticizers from the packaging to the fish, simulating real-life conditions. The USDA recommends consuming fresh within 1–2 days

if refrigerated, and within 2–3 months and 6–8 months if frozen, for lean fish (i.e., hake), and fatty fish (i.e., tuna, salmon), respectively. For this reason, two storage conditions were applied to simulate home food preservation practices: each fish species was wrapped and stored in the refrigerator at +4 °C for 48 hours, and frozen at –18 °C for 30 days. Immediately after purchase, the fresh fish meat was placed on the tray (V1 and V2) and covered with cling film (F1) to create a sealed surface, while for the bag (B1), the fish meat was placed directly inside, and then zip-locked according to the manufacturer's instructions (Fig. S2). As a control, a sample of each fish species was wrapped in aluminium foil and stored at +4 °C for 48 hours to assess background contamination levels. A total of 21 samples were analyzed (Table 1). Additionally, canned tuna was analyzed by sampling it directly from the can, after carefully decanting the oily medium. Three replicates were performed for each sample and each blank.

2.4. Sample extraction and analysis

2.4.1. Plastic materials

The extraction procedure of PAEs, NPPs and OPEs from plastic materials was adapted from Fernández-Arribas et al. (Fernández-Arribas et al., 2024) and Cioni et al. (Cioni et al., 2025). Approximately 0.1 g of each plastic material was weighed and spiked with 15 ng of ISs. Pieces from each package were cut with a metal blade, selecting the areas intended for direct contact with food. For canned products, both the inner wall of the can and the inner lining of the lid were collected and analyzed. Samples were equilibrated for at least 2 hours, then extracted twice with 40 mL of hexane:acetone (1:1 v/v) using ultrasound-assisted extraction for 15 minutes. The extracts were then filtered through glass wool to remove particulate matter. Solvents were evaporated under nitrogen stream at 20 °C, and residues reconstituted in 500 µL of methanol. A final filtration was performed using PTFE syringe filters (0.2 µm).

Based on Liao & Kannan (Liao and Kannan, 2013), further solid-phase extraction (SPE) treatment was required for BPs analysis. Approximately 0.5 g of each sample was spiked with 20 ng of IS, equilibrated for 2 hours, and extracted twice with 15 mL of hexane:acetone (1:1 v/v) using ultrasound-assisted extraction for 15 minutes. After centrifugation at 4000 r.c.f. for 5 minutes, the solvents were exchanged to 2 mL dichloromethane:hexane (10:90 v/v) via

Table 1

Study design showing the parameters used for the migration experiment (3 packaging x 2 storage conditions x 3 fish species + 3 controls), and a canned fish sample.

Packaging	Storage condition		Fish	Sample ID
	Temperature (°C)	Time (d)		
V1+F1	+4	2	Salmon	S-FR-V1
			Tuna	T-FR-V1
			Hake	H-FR-V1
	–18	30	Salmon	S-FZ-V1
			Tuna	T-FZ-V1
			Hake	H-FZ-V1
V2+F1	+4	2	Salmon	S-FR-V2
			Tuna	T-FR-V2
			Hake	H-FR-V2
	–18	30	Salmon	S-FZ-V2
			Tuna	T-FZ-V2
			Hake	H-FZ-V2
B1	+4	2	Salmon	S-FR-B
			Tuna	T-FR-B
			Hake	H-FR-B
	–18	30	Salmon	S-FZ-B
			Tuna	T-FZ-B
			Hake	H-FZ-B
Aluminium foil	+4	2	Salmon	S-control
			Tuna	T-control
			Hake	H-control
Can	-	-	Tuna	T-can

evaporation under nitrogen. SPE was conducted using Oasis WAX cartridges, preconditioned with methanol:acetone (4:1 v/v) and hexane. Target analytes were eluted with 10 mL methanol:acetone (4:1 v/v), evaporated under nitrogen at 20 °C, and reconstituted in 500 µL of methanol.

2.4.2. Fish

The extraction for NPPs, PAEs, and OPEs followed the methodology described by Fernández-Arribas et al. (Fernández-Arribas et al., 2024). Approximately 1 g dry weight (dw) of freeze-dried fish was extracted twice with hexane:acetone (1:1 v/v) using ultrasound-assisted extraction for 15 minutes. Freeze-drying of the samples is necessary to ensure reliable quantification of the target compounds. After centrifugation at 4000 r.c.f. for 5 minutes, solvent exchange was performed to 2 mL hexane:methanol (1:3 v/v) via nitrogen evaporation. A subsequent centrifugation at 4000 r.c.f. for 5 minutes was performed before 200 µL of the final extract was taken and spiked with 10 ng of IS.

For the analysis of BPs, the followed procedure was the same as that used for plastic materials, extracting 1 g dw of freeze-dried fish (see Section 2.4.1.).

2.5. Instrumental analysis

All samples were analyzed using the same analytical method developed and validated by Fernández-Arribas et al. (Blanco-Zubiaguirre et al., 2021), based on TurboFlow™ TFC-LC-MS/MS (Thermo Fisher Scientific, Waltham, MA, USA). The MS/MS analyses were conducted using a Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer, coupled with a heated-electrospray ionization source (H-ESI +/-) (Thermo Scientific, CA, USA). A ten-point calibration curve in methanol was prepared for all the analytes, ranging from 0.1 to 2000 ng/mL. The ISs (50 ng/mL) was used for the quantitative analysis of the targeted compounds. OPEs, PAEs, and NPPs were injected in positive ionization mode, whereas BPs were injected in negative ionization mode. Instrumental conditions, including chromatographic and MS/MS parameters, are reported in Fernández-Arribas et al. (Fernández-Arribas et al., 2024).

2.6. QA/QC

During the analytical process, plastic items were avoided to reduce the possibility of contamination. Only ultrapure water, ethanol and acetone were used to clean the glassware, metal tweezers, and scissors. All glass equipment was baked at 390 °C for approximately 4 hours and rinsed with the appropriate solvent just before use. Analytical blanks were included in each batch of samples, and processed with the same treatment as for real samples. Blank levels were subtracted from the corresponding samples. During the analysis, a pure solvent (200 µL of methanol) was added every five samples, to control possible carryover phenomena and instrumental background contamination. Analytical parameters such as recoveries, relative standard deviations (RSDs), limits of detection (LODs) and limits of quantification (LOQs) are summarized in Tables S4 and S5. The LOD and LOQ were determined as 3 times and 10 times the ratio of the standard deviation to the slope of the calibration curve, respectively.

2.7. Migration rate

To date, models to calculate the migration of plasticizers from packaging to food products are still based on the use of simulants (Alp and Yerlikaya, 2020). However, real food samples were used in this study. For this reason, an equation to calculate the migration rate (%) of target compounds from packaging to fish was adapted from Blanco-Zubiaguirre et al. (Blanco-Zubiaguirre et al., 2021) and Yuan et al. (Yuan et al., 2019) leading to equation (1)

$$\text{Migration \%} = \frac{(C_{\text{fish sample}} - C_{\text{fish control}}) / (C_{\text{fish sample}} - C_{\text{fish control}} + C_{\text{plastic}}) * 100}{1} \tag{1}$$

with $C_{\text{fish sample}}$ representing the concentration of the detected compound in the fish after being in contact with the plastic material, $C_{\text{fish control}}$ representing the concentration of the detected compound in the fish control, and C_{plastic} representing the concentration of the detected compound in the plastic material, all expressed in ng/g dw. All detected (i.e., >LOD) were taken into account using a conservative approach, in an effort to avoid underestimating potential migration phenomena. In a preliminary assessment, migration rate was also calculated by normalizing the concentration for the total weight (g, dw) of the fish meat and the total weight (g, dw) of the packaging material. However, the differences between the two mass-based approaches were negligible (i.e., < 3%), confirming that expressing the normalized rate per g dw extracted provides consistent and comparable results across samples.

The difference in migration rates between the levels of the different categories (lipid content, temperature, and packaging), were analyzed using a non-parametric Aligned Rank Transform (ART) ANOVA using R (version 4.5.2, R Core Team) (Table S6). Statistical significance was set

at $\alpha = 0.05$. To assess the direction of the relationship between migration rates and each category, Spearman correlations coefficients were calculated, setting significant correlation strength as $\rho > 0.5$.

2.8. Exposure assessment

To assess exposure to plasticizers through fish consumption, estimated daily intake (EDI) was calculated by multiplying the average Spanish per capita consumption (g/person/day) of fresh fish, by the concentration of each detected compound (i.e., >LOD, ng/g ww), and dividing the result by body weight (kg). In an attempt to identify possible risk categories, EDIs were calculated for three population groups, infants aged 6–12 months, children aged 1–3 years, and adults above 18 years of age. Data on fresh fish consumption in the Spanish population were obtained from Ministerio de Agricultura (Ministerio de Agricultura, 2024) for adults, and from López-Sobaler et al. (López-Sobaler et al., 2019) for infants and toddlers. Consumption values were reported separately for fresh salmon, tuna, hake, and canned tuna (Table S7). Since infants do not appear to consume fresh salmon, fresh tuna, or canned tuna, the EDIs for these categories were not considered

Table 2
Concentrations (ng/g dw) of individual compounds analyzed in packaging samples. The colored grids correspond to the packaging for which migration experiments were performed and the corresponding fish (including canned tuna) was also analyzed.

Class	Analytes	Packaging											
		V1	V2	Vcap1	Vcap2	B1	B2	B3	Bmix	Bvac	F1	F2	Can
PAEs	DMP	nd	6.23	nd	17.3	nd	nd	1.70	nd	nd	nd	nd	nd
	DEP	nd	14.0	nd	39.0	nd	nd	3.84	nd	nd	nd	nd	572
	DiBP	51400	405	nd	nd	nd	nd	nd	nd	nd	nd	nd	95.9
	DnBP	nd	nd	nd	nd	nd	nd	7.66	nd	nd	nd	nd	nd
	DCHP	nd	nd	nd	nd	nd	0.64	nd	237	nd	nd	nd	nd
	DHexP	7.60	1.71	nd	nd	0.91	nd	nd	6.00	nd	nd	nd	nd
	DEHP	648	nd	nd	nd	321	nd	nd	nd	nd	nd	nd	158
	DnOP	nd	nd	nd	317	911	1268	936	2979	411	nd	807	59.3
	DiNP	nd	10774	6713	nd	18863	504	563	1841	484	nd	nd	1499
	DiDP	nd	nd	nd	nd	nd	0.84	nd	nd	nd	1.04	nd	189
Σ PAEs	52055	11201	6713	373	20097	1774	1512	5064	895	1.04	807	2574	
NPPs	DMA	1.23	1.15	nd	0.28	0.55	nd	nd	nd	nd	nd	nd	nd
	TEC	1.58	nd	nd	nd	nd	nd	6.60	nd	nd	nd	nd	nd
	DIPA	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.81	nd	54.8
	TBC	nd	nd	nd	nd	nd	10.3	nd	nd	nd	nd	nd	nd
	DBA	nd	nd	nd	nd	nd	nd	nd	nd	nd	5169	nd	185
	ATBC	26.2	nd	nd	nd	95.7	29622	nd	nd	nd	nd	nd	80.9
	DEHA	18.8	10.6	200	7.44	14.6	5.1	nd	nd	nd	nd	nd	134
	DINA	nd	nd	nd	nd	nd	nd	nd	nd	nd	618	nd	524
	DINCH	15.9	nd	nd	nd	nd	nd	nd	nd	nd	79.9	nd	478
	TOTM	nd	4.30	0.50	0.51	0.72	0.96	3.80	nd	nd	32298	nd	49.1
Σ NPPs	63.7	16.0	200	8.23	112	29639	10.4	nd	nd	38168	nd	1505	
OPEs	TCEP	14.5	31.5	15.6	10.5	nd	8.51	12.3	12.3	29.9	nd	30.0	nd
	TPPO	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.68	nd
	TCIPP	nd	nd	nd	nd	nd	nd	nd	nd	140	320	nd	60.1
	TPHP	nd	nd	nd	nd	5.98	2996	21387	5.48	nd	nd	nd	1.12
	TNBP	nd	nd	nd	nd	nd	128	nd	nd	nd	nd	28.9	25.9
	2IPPDPP	nd	0.25	nd	nd	24.0	nd	nd	nd	nd	nd	nd	nd
	4IPPDPP	nd	nd	nd	nd	4.97	nd	nd	nd	nd	nd	nd	nd
	TCP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	EHDPP	2.70	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	6.58
	B4IPPPP	nd	0.85	nd	nd	34.5	nd	nd	nd	nd	nd	nd	nd
	IDPP	nd	nd	nd	nd	nd	nd	3.45	nd	5.00	nd	nd	nd
	T2IPPP	3.38	0.11	nd	nd	21.1	nd	nd	nd	nd	nd	nd	nd
	TEHP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Σ OPEs	20.5	32.7	15.6	10.5	90.5	3132	21402	17.8	175	320	60.6	94.4	
BPs	BPS	nd	nd	nd	nd	nd	nd	304	nd	nd	157	nd	nd
	BPA	107	33.2	5.75	1002	1.69	3.74	24.8	5.32	0.48	141	2.05	120
	BPB	nd	320	nd	nd	0.28	nd	nd	0.10	nd	nd	0.06	nd
	Σ BPs	106	354	5.75	1002	1.97	3.74	328	5.42	0.48	297	2.11	120
Σ of all classes	52246	11604	6935	1394	20308	34549	23260	5087	1071	38786	869	4293	

for this group. On the other hand, because of its tender texture, hake is often used in processed seafood products intended for children, even those under one year of age (Feketea et al., 2022). In accordance with EFSA guidelines, body weights of 5 kg, 12 kg, and 70 kg were assumed for infants, toddlers, and adults, respectively (Qasim et al., 2020).

Hazard quotients (HQ) were determined by comparing the calculated EDIs with the reference oral doses (RfD) or tolerable daily intakes (TDI), when available, established for certain plasticizers (Table S8). In the case of multiple values, the lowest value was considered to adopt a conservative approach. The HQ was calculated using the following equation (2):

$$HQ = EDI / RfD \text{ or } TDI \quad (2)$$

Finally, the hazard index (HI) was calculated for each sample, as the sum of the individual HQs. An HI greater than 1 was interpreted as an indication of potential health risk associated with dietary exposure.

3. Results and discussion

3.1. Occurrence of plasticizers in packaging

Concentrations of target compounds across twelve packaging types, including trays, films, bags, and a can, are shown in Table 2. This screening aimed to characterize the contamination profiles of materials commonly used for food storage in the domestic environment and to identify potential differences related to polymer composition. All packaging types contained detectable levels of plasticizers, and a total of thirty-four out of forty-nine compounds, representing 69% of the total, were quantified (Table S9). The overall mean concentration of total plasticizers was 1603 ng/g dw, ranging from <LOD to 52246 ng/g dw (in V1 sample, in which DiBP value was 51400 ng/g dw). The average detection frequencies were calculated, i.e. the average of the individual detection frequencies across all packages analyzed (n=11) for each class (n=4), yielding the highest value for PAEs (31%), followed by NPPs and OPEs (both 26%), and BPs (17%) (Fig. 1).

Among the PAEs, DnOP and DiNP were the most prevalent compounds, each with a detection frequency of 67%. DiBP reached the highest individual concentration in sample V1 (51400 ng/g dw) and,

together with DnOP, accounted for most of the total PAEs burden in the packaging. Trays showed the highest total PAE concentrations (52055 ng/g dw in sample V1 to 373 ng/g dw in sample Vcap2), followed by bags (from 20097 ng/g dw in sample B1 to 895 ng/g dw in sample Bvac), the can (2573 ng/g dw), and films (from 807 ng/g dw in sample F2 to 1.04 ng/g dw in sample F1). According to Regulation (EU) No. 10/2011, only five PAEs are authorized for plasticizers and technical support agents in FCMs, namely BBzP, DEHP, DnBP, DiDP, and DiNP. Except for BBzP, which was not detected, traces of the other PAEs were found in at least one sample. The samples also contained compounds not explicitly authorized under Regulation (EU) No 10/2011, as amended by 2023/1442, including DMP, DEP, and DHEXP, some of which showed very high concentrations, indicating their potential widespread presence in food contact materials (EU, 2011b; EU, 2023).

TOTM and DEHA were the most frequently detected NPPs, with a detection frequency of 67% and 58%, respectively. The highest individual concentration was recorded for TOTM in plastic film (sample F1, 32298 ng/g dw), which also showed the highest total sum of NPPs (38168 ng/g dw). High concentrations were also observed for ATBC in sample B2 (29622 ng/g dw) and DBA in sample F1 (5169 ng/g dw). The sample Vcap2 contained the lowest NPP levels (8.23 ng/g dw). The predominance of TOTM, DEHA, and ATBC is consistent with their widespread use as phthalate substitutes in the formulation of flexible polymers, such as PET and PE (Qadeer et al., 2022). Although DEHA and TOTM are permitted under EU Regulation No. 10/2011, the detection of other added compounds such as DBA, DINCH, and DINA in some of packaging, further supports the growing adoption of high-molecular-weight, less migratory alternatives aimed to comply with European restrictions on phthalates (Qadeer et al., 2022), and indicates potential for migration into fatty foods.

OPEs showed a lowest detection frequency (26%), consistent with reduced use following regulatory attention (EU, 2011b). However, relatively high concentrations of TPHP were detected in compostable bag samples B2 and B3, 2996 and 21387 ng/g dw, respectively, while TCEP showed the highest detection frequency (75%). Plastic bags again exhibited the highest concentrations, from 21402 ng/g dw in compostable bag B3 to 17.8 ng/g dw in sample Bmix, followed by cling films, the can, and the trays (Table 2). These results indicate that flexible polymeric materials may still contain flame-retardant plasticizers, such as TPHP and TCEP, which are now increasingly scrutinized for potential toxicity (Wu et al., 2024).

Among BPs, BPA was ubiquitous, with the highest concentration in sample Vcap2, 1002 ng/g dw, and sample F1, 141 ng/g dw. The can also contained a notable BPA concentration (120 ng/g dw). The compostable tray V2 showed detectable levels of BPB (320 ng/g dw). This finding suggests that BPA analogues can be present in alternative materials, consistent with reports of their growing use in cellulose-based packaging (Qasim et al., 2020). Despite existing bans on BPA in specific applications (EU, 2011a; EU, 2024b), its presence in packaging can be explained by the continued use of pre-ban stock or non-retroactive regulatory enforcement. Moreover, BPA analogues (e.g., BPB, BPAF) are now used as replacements, but their safety profiles remain under evaluation (Souza et al., 2024).

Comparing the concentrations found in packaging samples with other literature works is challenging, as only a few specific studies on food packaging have been conducted (Lestido-Cardama et al., 2022; Fierens et al., 2012). When comparing different polymers, such as PE, PS, PET, and compostable materials, the observed differences in plasticizer patterns are probably due to the different compositions for the different intended applications of each packaging (Fierens et al., 2012). In addition, materials labelled as reusable, such as samples B1 and F2, may present higher exposure risks during repeated use, as plasticizers could be progressively released, particularly when in contact with fatty foods. Also, compostable materials such as samples B2, B3 and V2 contain a significant number of plastic additives, leading to potential migration into food matrices, especially under conditions of heat or

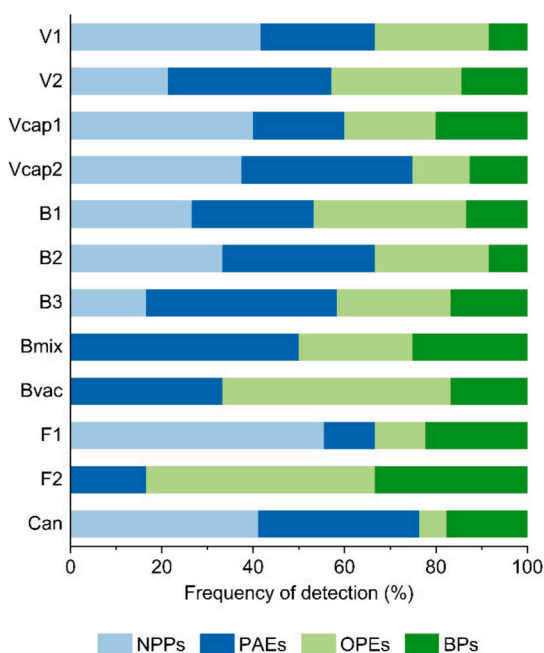


Fig. 1. Frequency of detection (%) of each class of plasticizers for each packaging sample.

prolonged contact. Although marketed as environmentally friendly, their ability to degrade could, unexpectedly, increase the release of these substances, calling into question the assumption that compostable materials are safer in terms of chemical migration. Recent studies have shown that compostable plastics may pose higher levels of chemical risks than conventional ones, raising concerns about their safety profile (Wang et al., 2023). All these distinct contamination profiles are expected to influence migratory behavior during contact with fish.

3.2. Occurrence of plasticizers in fish samples

After verifying the inhomogeneity in the concentration of target compounds across the different packaging materials, three representative packaging types, V1+F1, V2+F1, and B1, were selected for the migration experiments. These selections aim to capture a broader spectrum of chemical migration profiles, reflecting the diversity in polymer composition, additive content, and intended use (e.g., compostable, reusable, conventional), thereby enabling a more comprehensive assessment of potential exposure scenarios. Plasticizers were quantified in twenty-two fish samples, including salmon, tuna, and hake, following contact with the selected packaging materials under two storage conditions, +4 °C for 2 days, and -18 °C for 30 days. These setups were designed to simulate real-life storage conditions for fresh fish under domestic refrigeration. Aluminum-wrapped controls were included for each fish to establish background contamination levels, while canned tuna was analyzed separately. Table 3 reports the total concentrations expressed in wet weight (ww) of the four investigated plasticizers classes in each sample, whereas concentrations for all the forty-nine individual compounds are presented in Table S10. For comparison, the concentrations for all the forty-nine individual compounds are presented in Table S11 expressed in lipid weight (lw) and in Table S12 expressed in dry weight (dw). Overall, 23 out of 49 analyzed plasticizers were detected in fish samples, encompassing 9 PAEs (DMP, DEP, DiBP, DnBP, DHexP, DEHP, DnOP, DiNP, DiDP), 8 NPPs (DIPA, TBC, DBA, ATBC, DEHA, DINA, DINCH, TOTM), 2 OPEs (TCEP, TCIPP), and 4 BPs (BPS, BPA, BPB, BPAF).

BPs and NPPs were the predominant class in fish samples, representing 53% and 28%, respectively, reflecting the contamination pattern in the packaging. Conversely, OPEs were the least frequently detected class (1.4%), reflecting regulatory bans.

Similar linear relationships between additive levels in packaging and

migration into foods have been reported by Fasano et al. (Fasano et al., 2012), confirming that the migration potential of a material largely reflects its additive content. Among individual compounds, the highest detection frequency was found for BpS (86%), DEHA (82%), and BPB (73%). Plasticizers were detected in all experimental fish samples, with a mean concentration of 12.8 ng/g ww, ranging from 0.02 ng/g lw for TOTM in sample T-V1-FR to 358 ng/g ww for DEHA in sample S-V1-FZ, confirming migration occurred from packaging into fish meat. In contrast, control samples showed markedly lower levels, with a mean of 0.08 ng/g ww, indicating that packaging was the primary source of contamination under both refrigerated and frozen conditions.

The concentrations detected in the fish control samples of this study were comparable, or in some cases even lower, than those reported for wild fish of the same species in previous work. For instance, Dettoto et al. (Dettoto et al., 2024) reported concentrations of $0.26 \pm 0.63 - 0.26 \pm 0.63$ ng/g ww for NPPs in hake in Italian coastal areas, values consistent with 1.77 ng/g ww obtained for H-control. However, the same authors found considerably higher PAE levels, ranging from 85.2 ± 106 to 95.7 ± 94.4 ng/g ww, compared to 1.13 ng/g ww detected in this study. Similarly, Sala et al. (Sala et al., 2022) reported a mean concentration of 2.48 ng/g ww for OPEs in hake, collected from the Western Mediterranean Sea, while only TCEP showed detectable concentration in the H-control sample. For salmon, comparable concentrations of OPEs were observed by He et al. (He et al., 2018), who reported levels ranging from 0.05 and 0.63 ng/g ww in Australian market samples, like 0.05 ng/g ww detected in S-control. In contrast, Di Giacinto et al. (Di Giacinto et al., 2023) found 13 ng/g of BPA in Adriatic Sea bluefin tunas, while no BPA was detected in the T-control sample. The lower concentrations found in this study probably reflect differences in the geographical origin of fish and their life stage, as wild species may be more prone to bioaccumulation processes (Qin et al., 2024). Different results were observed for canned tuna. Fattore et al. (Fattore et al., 2015), reported concentrations ranging from not detected to 147.5 ± 4.7 ng/g for BPA and not detected to 145.9 ± 5.3 for BPB in canned tuna in oil from various regions, suggesting that the presence of oil may enhance BPA migration from can linings. In comparison, much lower levels of BPA and BPB, 6.41 and 0.12 ng/g ww respectively, were found in T-can samples. Furthermore, Tian et al. (Tian et al., 2025) did not detect traces of DEHA in canned tuna in oil from Canada and South Africa, nor for DEHP and DINCH in canned tuna from South African markets, in agreement with the results obtained in this study.

Table 3

Total mean concentrations of plasticizers (in ng/g ww) and corresponding standard deviation (SD, n=3) detected in fresh fish after contact with different types of packaging under different storage conditions. The concentration detected in the control is also reported. FR: refrigerator; FZ: freezer.

Fish species	Packaging	Storage mode	Class of compounds Σ PAEs	Σ NPPs	Σ OPEs	Σ BPs	Σ Total	
Salmon	Control	-	3.19	5.54 ± 2.19	0.05	4.04 ± 1.01	12.8 ± 2.31	
	V1+F1	FR	3.52 ± 1.66	145 ± 64.3	nd	0.38	150 ± 72.3	
		FZ	2.61	359 ± 179	nd	4.09 ± 1.53	366 ± 178	
		FZ	246 ± 140	318 ± 142	nd	1.12 ± 0.54	565 ± 165	
	V2+F1	FR	nd	167 ± 82.7	nd	8.26 ± 3.23	175 ± 82.2	
		FZ	2.57 ± 1.56	2.17 ± 0.60	nd	0.33	5.07 ± 1.29	
	B	FR	0.36	1.40 ± 0.41	0.03	0.99	2.78 ± 0.62	
		FZ	nd	nd	nd	0.31	0.31	
Tuna	Control	-	nd	nd	nd	0.31	0.31	
	V1+F1	FR	2.23 ± 1.34	23.5 ± 10.1	nd	15.9 ± 10.6	41.7 ± 11.2	
		FZ	1.35 ± 0.60	12.4 ± 4.72	0.02	1.53 ± 0.55	15.3 ± 5.75	
		FZ	7.16 ± 2.50	32.6 ± 13.7	nd	0.79 ± 0.38	40.6 ± 15.3	
	V2+F1	FR	5.75	7.76 ± 3.07	0.03	64.5 ± 30.7	78.1 ± 30.2	
		FZ	0.09	1.52 ± 0.56	nd	0.51	2.12 ± 0.70	
	B	FR	0.88	0.25 ± 0.06	nd	8.43 ± 2.19	9.56 ± 4.05	
		FZ	1.13 ± 0.28	1.77 ± 0.53	0.02	1.27 ± 0.61	4.19 ± 0.74	
	Hake	Control	-	nd	22.31 ± 12.4	nd	1.33 ± 0.76	23.6 ± 10.9
		V1+F1	FR	nd	5.49 ± 3.83	nd	14.5 ± 2.88	164 ± 68.8
FZ			144 ± 75.3	9.03	0.01	11.22 ± 5.11	20.2 ± 5.91	
FZ			22.5 ± 5.64	2.85 ± 1.55	nd	43.7 ± 17.8	69.0 ± 20.2	
V2+F1		FR	nd	nd	nd	11.4 ± 5.52	11.4 ± 5.71	
		FZ	3.53 ± 2.32	1.94 ± 1.30	nd	5.08 ± 0.90	10.9 ± 2.14	
B		FR	4.00 ± 1.05	2.31 ± 0.98	nd	6.53 ± 4.45	13.3 ± 2.72	
		FZ	nd	nd	nd	nd	nd	
Canned tuna	Can	-	4.00 ± 1.05	2.31 ± 0.98	nd	6.53 ± 4.45	13.3 ± 2.72	

V1 (2.9%), but not in salmon. However, this trend suggests that other factors, such as lipid or water content, may have a preponderant role in the migration of these compounds. Comparable results were reported by Đurić et al. (Đurić et al., 2024), who detected higher concentrations of PAEs residues in smoked fish meat stored in plastic at -18°C for six months compared to shorter storage times.

In contrast, NPPs exhibited higher release values under refrigeration than freezing, as also demonstrated by the migration rate. DIPA migrated at a higher rate in refrigerated salmon, accounting for 7.3% in S-FR-V1 and 3.9% in S-FR-V2, respectively, while DEHA showed higher migration rate in refrigerated tuna, T-FR-V1, T-FR-V2, and T-FR-B (81.1%, 91.0%, and 59.6%, respectively), and hake, 83.2% and 77.7%, in H-FR-V1, and H-FR-V2, respectively. These findings indicate that the migration behavior can be also influenced by physicochemical properties of plasticizers. In the case of NPPs, higher temperature, rather than prolonged contact time, can be a key factor in promoting the migration of these plasticizers into food. In fact, NPPs such as DBA and DEHA generally exhibit lower molecular weights (258.3 and 370.6 g/mol, respectively) and higher volatility ($2.34 \cdot 10^{-1}$ and $4.27 \cdot 10^{-4}$ Pa for vapor pressure) compared to PAEs like DEHP (390.6 g/mol and $2.52 \cdot 10^{-4}$ Pa) (Table S1). Although DEHA and DEHP show similar molecular weights, the combination of higher volatility and lower lipophilicity, likely contributes to greater migration proneness of NPPs at

elevated temperatures, while PAEs, being less volatile and more lipophilic, tend to migrate under prolonged contact time, especially in fatty matrices.

A consistent pattern can be seen for DHexP in all analyzed samples (Fig. 2). Detectable migration occurred only in the frozen samples while for controls and refrigerated samples no significant migration occurred, confirmed by a significantly strong correlation between DHexP migration rate and temperature ($\rho = -0.85$, p-value= 0.001). This can probably be related to the strong influence of storage duration and temperature on the behavior of this compound, concluding that low temperature and long contact time can increase the probability of DHexP migration in fish. A similar trend was observed for BPA (Fig. 2) and BPS (df=1, F= 5.78, p-value= 0.03), which showed higher release percentages under frozen conditions, particularly in tuna and hake packaged with all materials.

Overall, plasticizers migration from packaging to fish was strongly influenced by both storage temperature and contact duration, although to varying degrees. Previous studies have highlighted that prolonged or high-temperature exposure during cooking and storage can enhance additive migration (Fernández-Arribas et al., 2025), underlining the need to control heat treatment to limit the transfer of contaminants. Although migration is generally reduced at lower temperatures, detection of plasticizers even under frozen storage conditions demonstrates

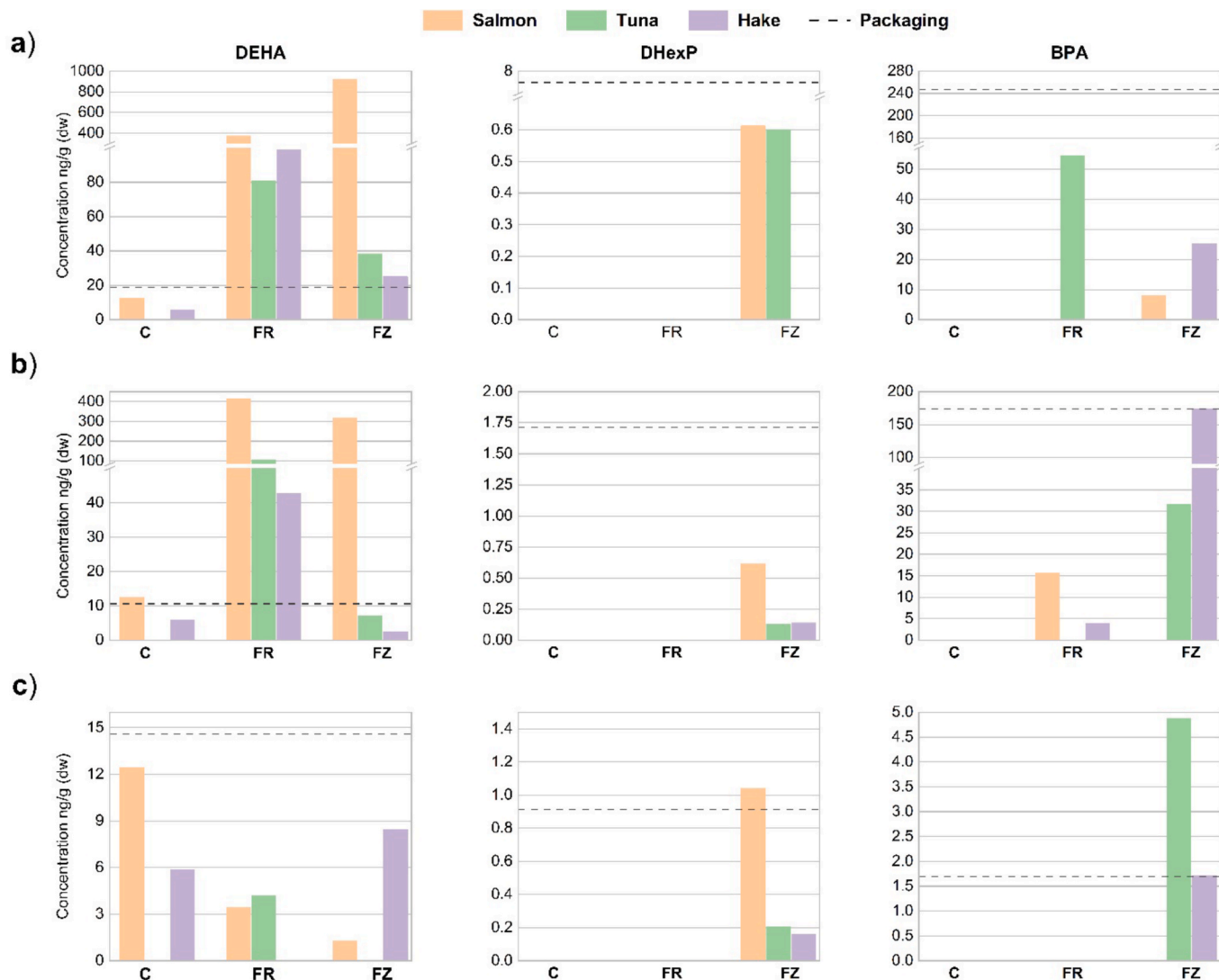


Fig. 2. Concentrations (ng/g dw) of DEHA, DHexP, and BPA in fish control and packaged fish for salmon, tuna, and hake in (a) V1+F1, (b) V2+F1, (c) B1. FR: $+4^{\circ}$, for 2 days; FZ: -18° for 30 days. The dotted line corresponds to the concentration value of the plasticizers in the packaging.

that migration can still occur under these conditions, posing a potential risk to human health.

3.3.2. Effect of lipid content

The nature of the food matrix, such as lipid and moisture content, is a key factor in influencing migration (Đurić et al., 2024). For this reason, the lipid content of the fresh fish was determined, which reached 43.9% in salmon, 10.0% in tuna, and 1.63% in hake (Table S3).

Differences among fish species were observed for almost all classes of analyzed plasticizers. Although salmon, due to its high lipid content, was expected to accumulate the highest total concentrations, hake instead showed the highest overall values (Table S11), mainly driven by high contributions of PAEs, followed by BPs. Tuna showed intermediate values, consistent with its moderate fat content. However, some sort of correlation between lipid content and migration rates can be identified. For example, within NPPs, DIPA showed higher migration rates in salmon, ranging 1.06%–7.26%, followed by tuna, 5.06% in T-FZ-V2, and showed no release in hake. Indeed, a positive correlation with lipid content was found for this compound ($\rho = 0.58$, $p\text{-value} = 0.05$). Similarly, TOTM showed significant rates only in salmon and tuna, although at low levels.

The correlation between lipid content and migration rates was most evident for DEHA in all packaging types (Fig. 2). Salmon exhibited migration values close to 100% in both trays. Lower but still detectable values were found in tuna (46.4–91.0%) and hake (34.7–98.6%). This is in line with physicochemical properties of DEHA, which has a log Kow of 8.1 (Table S1), indicating a high affinity for lipid-rich matrices compared to lean fish such as tuna and hake.

For many plasticizers, including PAEs and OPEs, the migration rate is typically higher in fatty foods compared to those with low-fat content (Miao et al., 2024; Fierens et al., 2012; Đurić et al., 2024). As a result, DEHP was expected to show increased migration into fat-rich fish such as salmon. However, an inverse correlation was observed, with no detectable release in salmon, similar to what reported by Alp & Yerlikaya (Alp and Yerlikaya, 2020). This apparent discrepancy may reflect the combined influence of compound-specific physicochemical properties (for DEHP MW of 390.6 g/mol, and LogKow 7.72), and matrix-related factors affecting partitioning behavior.

Interestingly, BPs also showed an opposite trend, with migration rates following the order hake > tuna > salmon (Fig. 2). The physicochemical properties of the compounds likely drive this behavior. BPS, BPA, and BPB have lower log Kow values (1.65, 3.43, and 4.13 respectively), and higher water solubility (3518.0, 120.0, and 29.2 mg/L, respectively), compared to the other plasticizer classes (Table S1). Specifically, BPB showed significant correlation with lipid content ($\rho = -0.5$, $p\text{-value} = 0.05$). Given that hake has the highest water content, 78.5%, compared to salmon, 61.9%, and tuna, 71.4%, it is plausible that the migration rates of BPs in lean fish are higher, thus offering a new perspective on the behavior of BPs migration from packaging to food.

The susceptibility of foods to migration is highly dependent on the intrinsic physicochemical properties of migrants, such as lipophilicity and solubility, and should not be underestimated when assessing the risk associated with the migration of plasticizers from packaging materials to food.

3.3.3. Influence of packaging composition

The chemical structure, density and permeability properties of packaging material are also among the factors that influence migration processes in foods.

In this study, three types of packaging, differing in chemical composition, were used for migration experiments, namely, a polystyrene tray (V1), a compostable tray (V2) and a polyethylene bag (B1). A polyethylene cling film (F1) was also applied to ensure the correct sealing of the trays. The initial concentration of plasticizers in the packaging can represent a critical parameter that can influence migration rates. Considering the total concentration of all compounds in the

packaging materials, the following trend was observed: $V1+F1 > V2+F1 > B$, with total values indicated of 91032 ng/g dw, 50389 ng/g dw and 20308 ng/g dw, respectively. However, the calculated migration rates showed a reverse trend, following $B > V2+F1 > V1+F1$, with average migration values across all analyzed compounds of 46.8%, 21.0% and 19.8%, respectively. This inverse relationship suggests that factors beyond the initial concentration play a significant role in determining the migration behavior of chemicals from packaging to food. Among these, the contact surface between food and packaging is particularly relevant. Direct contact between food and packaging has been shown to facilitate faster migration of compounds, while indirect contact, in which a gaseous layer separates the surfaces and acts as a diffusion medium, results in slower migration rates (Alamri et al., 2021). In the trays, the fish meat was placed on the base and covered with transparent film, following the classic packaging procedures for this type of packaging, while in the plastic bag, the fish was enclosed and sealed using the zip-lock system, resulting in close contact with the material over almost the entire surface of the meat (Fig. S2). This likely resulted in a larger contact surface between the fish and the plastic bag, facilitating a higher migration rate compared to the trays, as also found by Đurić et al. (Đurić et al., 2024) for vacuum bags. The presence of airspace limited direct contact and may have reduced the transfer of compounds into the fish, despite their potential volatilization.

This phenomenon could partly explain the behavior of DHexP, which showed a marked increase in concentration in all fish samples packaged in the plastic bag. The same trend was observed for BPs. In tuna, migration rates in the plastic bag ranged from 86.5% to 91.0%, while in hake they reached values between 81.2% and 100%. Particularly high values, such as 100% migration rate for BPB in H-FZ-B ($F = 5.25$, $p\text{-value} = 0.02$), likely reflect matrix effects, variations in extraction efficiency between packaging and fish, and the heterogeneous distribution of additives within packaging materials (Han et al., 2021), although such conditions are plausible due to the methodological complexities inherent in real food packaging systems.

Considering the vapor pressure (Pa) of the individual compounds, substances with relatively high vapor pressures, such as TCEP (8.17 Pa), TCIPP (7.52×10^{-3} Pa), DBA (2.34×10^{-1} Pa), DINCH (8.4×10^{-4} Pa) and ATBC (6.07×10^{-4} Pa), showed higher migration rates in V1 and V2 in all fish species. In contrast, compounds with lower vapor pressures, such as BPA (5.3×10^{-6} Pa) and BPS (6.3×10^{-8} Pa), showed higher migration from B. These observations highlight the critical influence of the contact surface on migratory behavior, beyond the intrinsic properties of the packaging materials themselves.

Food safety concerns also arise from the fact that plastic bag B1 was labeled as reusable. The repeated use of packaging materials has been identified as a factor that can significantly influence migration. Tisler & Christensen (Tisler and Christensen, 2022) reported increasing concentrations of DEHA in plastic bottles, indicating continued migration even after repeated flushing cycles. Similarly, Kubwabo et al. (Kubwabo et al., 2009) observed that BPA levels increased with each reuse of baby bottles.

Finally, by comparing chemical composition of the packaging, the compostable cellulose-based tray V2 showed slightly higher average migration rate, compared to the polystyrene tray V1, and some compounds selectively migrate from this material, for example, DiBP, DiNP, and TCIPP, although with low rate. Compostable trays, in particular cellulose-based ones, have emerged as environmentally friendly alternatives from renewable materials and offer compostable options at the end of their life (Qasim et al., 2020). However, these materials may still contain substances that can migrate into foods (Fierens et al., 2012; Selin et al., 2021). In addition, Vinggaard et al. (Vinggaard et al., 2000) found higher concentrations of BPA in kitchen rolls made from recycled paper than in those made from virgin paper. Alternatives from renewable resources, as compostable ones, do not necessarily ensure less chemical transfer, highlighting the importance of assessing both environmental and chemical safety aspects when selecting materials for food contact

applications. Therefore, adapting packaging design and material selection according to the physical characteristics of the food product should be essential to minimize the transfer of contaminants.

4. Human exposure assessment

Data obtained from monitoring plasticizer levels in real food systems were used to assess potential adverse effects to human health from dietary exposure through fresh fish consumption. Concerns have been raised regarding exposure levels for the most vulnerable population groups, such as infants (6–12 months) and toddlers (1–3 years). In addition, the identification of which combinations, among fish species, packaging, conservation conditions and populations, have the highest exposure and risk was assessed.

Fig. 3 gives the total EDI and HI values in the target population groups, while the explicit values for the class of compounds are presented in Table S13. Overall, Σ EDI for adults showed values between 0.01 and 30.5 ng/kg bw/day, while for toddlers they ranged between 0.02 and 277 ng/g bw/day. The risk assessment for infants was limited by the fact that only hake was shown to be consumed in this population group, therefore Σ EDI ranged from 0.0 and 248 ng/kg bw/day for this fish. However, newborns consistently showed the highest exposure per body weight, with mean EDI values more than ten times higher than those of adults. Consequently, even small amounts of consumed fish result in a much higher exposure per kg of body weight. For adults and toddlers, among fish species salmon and hake contributed in higher proportion to EDIs, as consumption of these fish species was higher than tuna for both groups (Table S7). Even though recent reports have shown a decrease in purchases of fresh fish, the Spanish population consumes about 7.31 Kg of fresh fish, it is therefore the type of fishery product that concentrates the highest per capita consumption of the year (Ministerio de Agricultura, 2024).

The contribution made by the studied class of plasticizers to total EDI was different among population groups. For children and infants, a similar trend was observed, following PAEs (50.5, 56.4%) > BPs (25.7, 29.2%) > NPPs (23.7, 14.4%) > OPEs (<0.01%), while for adults NPPs (67.3%) > PAEs (24.8%) > BPs (7.87%) > OPEs (0.03%). PAEs represented the largest group in daily intake for both toddlers and infants, with Σ EDI of 308 and 259 ng/kg bw/day, respectively, while a greater contribution was made by NPPs for intake in adults, showing a Σ EDI of 57.3 ng/g bw/day. OPEs contributed to a lesser extent of 0.01, 0.07, and 0.05 ng/kg bw/day as also found by Fernández-Arribas et al. (Fernández-Arribas et al., 2025) for other food products.

Concerning storage conditions, highest EDIs were observed for frozen preserved fish, in line with migration rate found under these storage conditions (Table 4). For example, Σ EDI for frozen fish represented 62.7, 481, and 370 ng/kg bw/day for adults, toddlers and children, respectively, an order of magnitude higher than the values under refrigerated conditions, 22.0, 120, and 83.9 ng/kg bw/day. Moreover, EDIs in fish-control were lower than in packaged samples, reflecting the concentration pattern found in fish (Table 3), thus suggesting packaging as a major route of exposure to plastic additives through dietary intake. In addition, higher EDIs were found for fish in V1 for almost all population groups (365 and 320 ng/kg bw/day for toddlers and infants, respectively), followed by V2, 207 and 135 ng/g bw/day, and B, 39.1 and 33.9 ng/kg bw/day. Although NPPs are the least studied class of plasticizers, other studies have reported varying estimates of dietary intake, although incomparability can occur depending on the type of food and population group. For example, a study of the Spanish population found EDI as of 244 ng/kg bw/day for adults, 936 ng/kg bw/day for toddlers, and $1.62 \cdot 10^3$ ng/kg bw/day for infants (Fernández-Arribas et al., 2025), whereas observed values of 610 ng/kg bw/day were found for individuals following a vegan diet (Macan Schönleben et al., 2025).

For PAEs, a study on U.S. food found adult EDIs ranging from 4 to 673 ng/kg bw/day (Schechter et al., 2013), while an average of 1737 ng/kg bw/day was found for different food groups in the Belgian adult

population (Fierens et al., 2012). Analyses of food simulants in contact with Chinese takeaway food containers indicated EDI as 0.03–2668 ng/kg bw/day, with the highest median total detected in expanded polystyrene samples (764 ng/kg bw/day), consistent with the higher values observed in this study for fish in polystyrene tray (V1) consumed by toddlers and infants (Han et al., 2021). Considering OPEs, EDIs reached 1128 ng/kg bw/day in different food categories for the Swedish adult population (den Ouden et al., 2025), whereas values between 62.3 and 420 ng/kg bw/day were reported for different population groups in the UK (Gbadamosi et al., 2022).

Dietary intake is considered the main route of exposure to BPA and has also been studied in several food groups (Manzoor et al., 2022). For example, Sakhi et al. (Sakhi et al., 2014) calculated an EDI of 5 ng/kg bw/day for BPA in the Norwegian adult population, considering different food products, while Cao et al. (Cao et al., 2011) found that canned food is the main contributing factor to BPA ingestion, especially fish. In this study, adults showed a Σ EDI of 6.70 ng/g bw/day for BPs, with a higher contribution coming from hake samples (3.36 ng/g bw/day), and the same was evident for toddlers, with an Σ EDI for BPs of 157 ng/g bw/day (with 150 ng/g bw/day from hake) (Fig. 3). BPA represented the highest portion of Σ EDI for BPs, followed by BPS, as also found by Qu et al. (Qu et al., 2024) for the Chinese adult population in several food products.

To better understand the potential non-carcinogenic risks associated with the ingestion of packaged food, hazard index (HI) was also evaluated, as the sum of individual HQs (Table S13). As shown in Fig. 3, some samples exceeded the threshold of 1, indicating potential risk for human health under specific exposure conditions. Across all population groups, HI values ranged from $2.57 \cdot 10^{-7}$ to $3.17 \cdot 10^2$, reflecting wide variability among fish species, packaging materials, and storage conditions. All control samples and those packaged in refrigerated plastic bag (B-FR), showed HI values below the threshold, which suggests a negligible health risk. In contrast, the highest HI estimates were associated with hake samples packaged in compostable trays and stored frozen (H-V2-FZ), with values of 7.12, $3.17 \cdot 10^2$, and $2.84 \cdot 10^2$ for adults, toddlers, and infants, respectively. Such high values for toddlers and infants can be attributed to the body weight normalization for HI, thus highlighting greater vulnerability in these population groups. These results indicate that freezing fish in compostable trays may not be an ideal preservation method. This trend is in line with both concentration data and migration behavior, where hake showed the highest migration rates among the studied species.

The cumulative non-carcinogenic risk was predominantly driven by BPA, detected in almost all samples, which contributed to nearly 100% of the HI. In contrast, the combined contribution of all other contaminants detected, including PAEs, OPEs, and NPPs, was negligible, remaining consistently several orders of magnitude lower than that of BPA, and thus much lower than the critical value of 1. The European Food Safety Authority (EFSA) has paid particular attention to exposure to BPA in foodstuffs. In 2015, EFSA established a temporary Tolerable Daily Intake (TDI) of 4 μ g/kg bw/day, concluding that such exposure was not a health concern for any age group. However, following a comprehensive re-evaluation in 2023, TDI was reduced by a factor of 20000, to 0.2 ng/kg bw/day (Lambré et al., 2023), reflecting growing concern over its endocrine-disrupting potential (Agarwal et al., 2025). Considering this new threshold, the HI > 1 for several hake samples, particularly among infants, indicates a potential concern for chronic exposure to BPA through the consumption of packaged fish, and underscores the central role of this compound in dietary exposure assessments. The disparity in HI contributions reflects both the relatively low toxicological TDI established for BPA and its relatively high migration rates in the sample analyzed.

Overall, these results highlight the need for careful selection of packaging materials, for frozen or long-stored products, and reinforce the importance of considering updated regulatory limits when assessing cumulative dietary exposure to plasticizers and BPs, as it has been

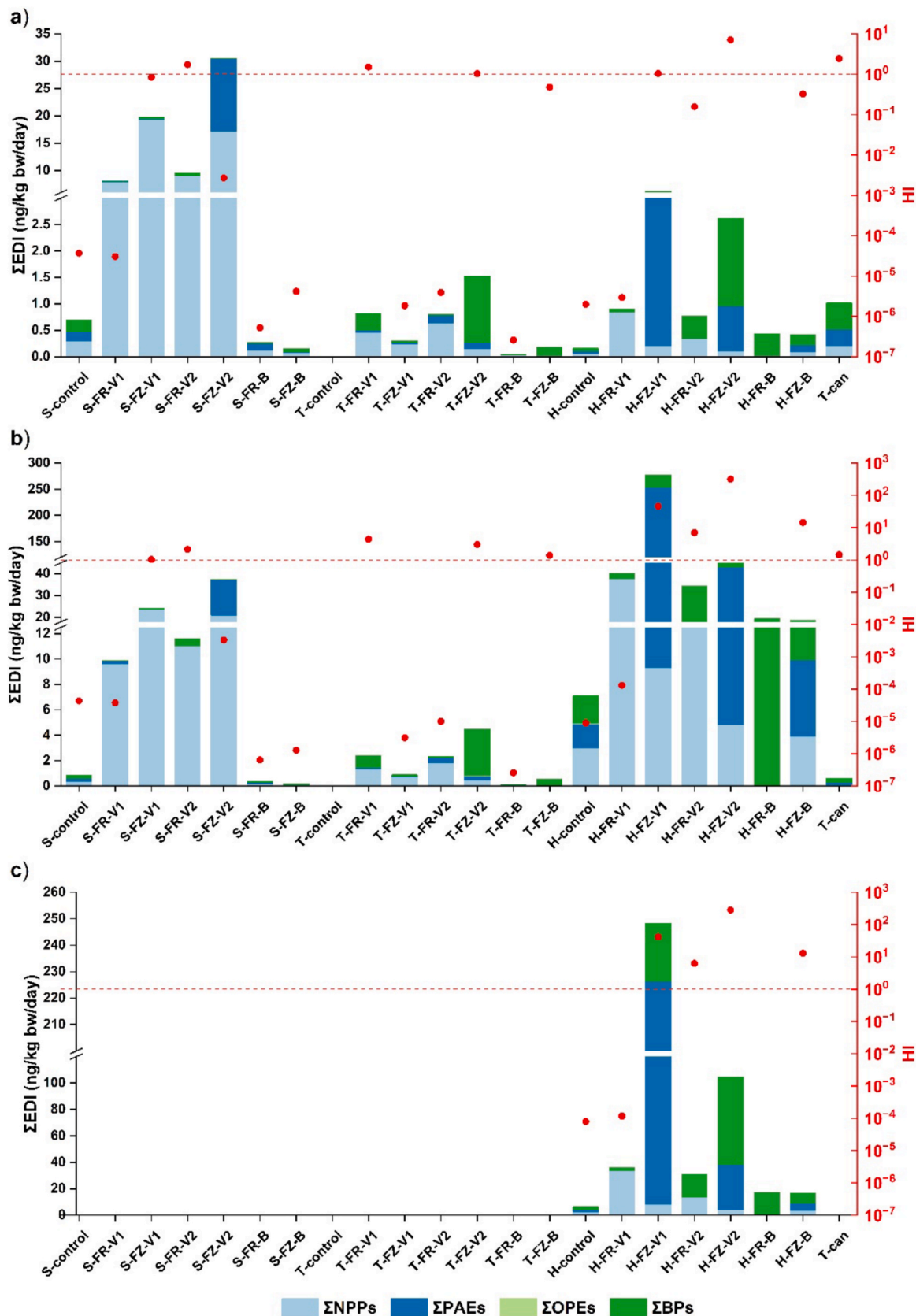


Fig. 3. Total estimated daily intake (ΣEDI) expressed in stacked bar charts, showing the contributions of plasticizer classes, and hazard index (HI) expressed in red dots for each sample, for a) adults, b) toddlers, and c) infants, via fish intake. HI in logarithmic scale (dotted lines correspond to an HI of 1).

shown that HI can approach or exceed the risk threshold under specific conditions.

5. Implications and perspectives

For the first time, this study provides new insight into the migration behavior of plastic additives and BPs from packaging materials to fish under realistic home storage conditions. The study design included three species of fresh fish and three types of packaging (two fossil-based and one compostable), purchased from Spanish retail sales, representing a selection of materials currently available on the market. Migration tests conducted under frozen and refrigerated storage conditions allowed the simultaneous evaluation of 49 compounds, including PAEs, NPPs, OPEs and BPs. Migratory behavior was found to be highly dependent on both food matrix and storage conditions. The results obtained revealed the detection and quantification of both regulated and unregulated contaminants in packaging material and fish following their release and migration, representing both an analytical and a normative challenge. Moreover, a comprehensive chemical screening allowed an assessment of the combined exposure to multiple migrants across different population groups (adults, toddlers and infants) and their potential health implications.

Further considerations may be taken into account following the results of this study:

- Performing migration tests under realistic conditions is of paramount importance, as experiments using food simulants fail to fully capture the complexity of real matrices and their interactions with packaging materials (Rekibi et al., 2025). Moreover, improved analytical strategies for complex food matrices are needed to detect both targeted and unknown contaminants (Rekibi et al., 2025). More importantly, the maximum permissible concentrations for many newer plasticizers, such as NPPs and bisphenol substitutes are still lacking, due to insufficient data on their presence, release potential, and toxicological relevance (Qadeer et al., 2022). Future studies should therefore address these gaps, potentially incorporating biomonitoring to link dietary exposure with human health outcomes.
- Although diet is an important pathway for exposure to BPs and plasticizers, human exposure would increase if more pathways were considered, resulting in a higher risk. It can therefore be assumed that a comprehensive exposure assessment could eventually be carried out either by including multiple relevant pathways and taking into account their contribution to total mixture exposure, or by using a biomonitoring approach and reflecting on the contribution of each pathway to design mitigation strategies. In addition, current regulations mainly concern individual compounds and rarely consider the effects of mixtures, despite growing evidence of additive or synergistic toxic effects (Macan Schönleben et al., 2025).
- It is recommended to extend investigation to other food categories, such as dairy products and meats, to assess migration tests in relation to their specific properties (e.g., lipid content, water content). A larger-scale study would ensure a more balanced representation across the different categories and greater statistical power. Moreover, a preliminary analysis of packaging materials can be useful to understand the compounds more likely to migrate, while analysis of packaging after contact with food can help to assess actual migration levels. The actual composition of packaging materials in terms of plastic additives is not disclosed to consumers, leading to misconceptions about which products are truly safe and appropriate to use. Although plastic remains the dominant packaging for fish, alternatives made of compostable and renewable materials are often perceived as safer (Fernández-Arribas et al., 2025), though our study suggests that the presence of additives may challenge this assumption. The shift to alternatives from renewable sources introduces new functional challenges, while also raising concerns about the risks to humans through additive migration into food (Qasim et al., 2020).

Sustainable materials offer environmental benefits but must still meet safety and performance requirements to ensure food safety and minimize waste. Furthermore, the influence of repeated use on the additive release should not be underestimated.

Overall, this study underlines the urgent need to strengthen risk assessment frameworks for plastic additives and BPs in food contact materials. To mitigate the risks of human exposure associated with food packaging, it is essential to continue efforts towards comprehensive migration modelling, regulatory harmonization and the development of safer material formulations.

CRedit authorship contribution statement

Laura Sforzi: Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis. **Valentina Araya Piqué:** Writing – original draft, Methodology, Investigation, Formal analysis. **Tania Martellini:** Writing – review & editing, Visualization. **Alessandra Cincinelli:** Writing – review & editing, Visualization, Funding acquisition. **Ethel Eljarrat:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Maria Vittoria Barbieri:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2026.110261>.

Data availability

No data was used for the research described in the article.

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