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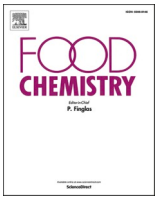

In vitro bioaccessibility of cyclodi-BADGE present in canned seafood: A new approach for the estimation of dietary exposure of the Spanish population

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ABSTRACT

Human dietary exposure to chemical compounds is a priority issue for public health authorities since it constitutes a key step in risk assessment, and food packaging could be an important source of contamination. In this study, the bioaccessibility of cyclodi-BADGE was evaluated in canned seafood samples using a standardized protocol of *in vitro* gastrointestinal digestion and an analytical method based on liquid chromatography coupled to tandem mass spectrometry. The impact of enzymes, different gastric pHs, and food-covering liquids on the bioaccessibility of cyclodi-BADGE was studied. The results highlighted that cyclodi-BADGE was available to be absorbed at the intestinal level (90.9–112.3%), and its bioaccessibility increased substantially in fat food samples. Finally, the estimated dietary exposure to cyclodi-BADGE in the Spanish adult population reached values of 14.26 μg/kg bw/day for tuna in tomato, exceeding the tolerable daily intake (1.5 μg/kg bw/day) recommended for chemicals with high toxicological risk.

1. Introduction

Polymeric coatings are applied on the inner surfaces of cans intended to come into contact with food to protect them from the environment. Typically, these coating materials are complex formulations that may incorporate various components, including intentionally added starting substances (IAS), such as monomers, prepolymers, and additives, and may also include non-intentionally added substances (NIAS), such as products of reactions or degradation that occur during the manufacturing process, which could also migrate into food (Geueke, 2016; Oldring & Nehring, 2007; Paseiro-Cerrato, DeVries, & Begley, 2017). Currently, there is no EU-specific and harmonized legislation for polymeric coatings, and there are only specific regulations on certain substances, such as Regulation (EC) 1895/2005 that restrict the utilization of certain epoxy derivatives (European Commission, 2005).

To ensure food safety, research on the migration of chemical compounds from food contact materials into foodstuffs and the estimation of human exposure to these compounds through the diet has become priority issues for public health authorities, constituting a key step in risk assessment (European Food Safety Authority (EFSA), 2011). Typically, the estimation of dietary exposure to chemicals from packaging materials is primarily based on a combination of data from studies on migration from packaging to food or food simulants and data on the dietary intake of populations (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), et al., 2019; Lestido Cardama et al., 2019; Lestido-Cardama et al., 2021). However, this approach presents some limitations because the levels of specific contaminants detected in food samples do not reflect the amount released from food during gastrointestinal (GI) digestion and its possible absorption in the intestinal epithelium. Therefore, assessing the bioavailable fraction of contaminants that can reach the systemic circulation through food is essential for a more accurate assessment of dietary exposure and its possible effects on consumer health.

Epoxy resins are obtained by the condensation of epichlorohydrin and bisphenol A (BPA), which yields bisphenol A diglycidyl ether (BADGE) (Poças & Hogg, 2007). This study is focused on one of the main compounds detected in epoxy-based coatings, cyclodi BADGE (CdB) (Biedermann, Zurfluh, Grob, Vedani, & Brüschweiler, 2013; Bradley, Driffield, Harmer, Oldring, & Castle, 2008; Bustos et al., 2023; Lestido Cardama et al., 2019; Lestido-Cardama et al., 2021; Lestido-Cardama, Vázquez Loureiro, et al., 2021; Schaefer & Simat, 2004). CdB is not incorporated into the polymer matrix and consequently could migrate

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into food and beverages (Bustos et al., 2023; Lestido Cardama et al., 2019; Lestido-Cardama, Sendón, et al., 2021; Lestido-Cardama, Vázquez Loureiro, et al., 2021). To date, international organizations have not yet established limits on the presence of CdB in food, there are no toxicological data available, and studies related to this chemical compound in food samples are scarce because of the recent accessibility of the analytical standard. However, this compound is classified as type III (high toxicity) according to Cramer's rules due to its chemical structure (Bustos et al., 2023).

The main objective of the work is to present a new approach for the estimation of human dietary exposure to contaminants from packaging materials based on the bioaccessibility of the chemical compound and consumption data. For this purpose, CdB was selected as a model substance and the hypothesis was raised that it is a bioaccessible compound.

The study assesses the chemical behavior of CdB in the different phases of GI digestion and its bioaccessibility using a standardized protocol of *in vitro* GI digestion to estimate more closely the human dietary exposure to this chemical compound. At the same time, the influence of enzymes, gastric pHs (1–4), and different food matrices and covering liquids of canned food were studied. To date, this study is the first approach to evaluate the bioaccessibility and estimate the human dietary exposure to CdB after consumption of several canned food samples.

2. Materials and methods

2.1. Reagents and standards

Solvents: Acetonitrile (ACN) liquid chromatography–mass spectrometry (LC–MS) grade, tetrahydrofuran (THF) high-performance liquid chromatography (HPLC) grade and heptane HPLC grade were provided by Merck (Darmstadt, Germany). Ultrapure type I water was obtained from an Autwomatic Plus purification system Wasserlab (Navarra, Spain).

Analytical standard: cyclodi-BADGE (CdB) 99.5% (CAS 20583–87-3) was obtained from Chiron AS (Trondheim, Norway). The chemical structure and physicochemical properties of CdB are listed in Table S1. The internal standard, BPA-d_{16} ($BPAd_{16}$) 98 atom % D (CAS 96210–87-6), was obtained from Sigma-Aldrich (St. Louis, MO, USA).

A stock solution of CdB was prepared by dissolving the analytical standard in a mixture of ACN:THF (60:40, *v*/v) to obtain a concentration of 200 mg/L. For the internal standard ($BPAd_{16}$), a stock solution of 1000 mg/L was prepared in ACN and, from this, an intermediate solution of 50 mg/L. The CdB calibration curve prepared in 90% ACN:H_2O (90:10, v/v) was from 2.5 to 5000 μg/L, including the internal standard ($BPAd_{16}$) at a concentration of 5 mg/L.

Sample preparation reagents: potassium hexacyanoferrate(II) trihydrate ≥99.5% ($C_6FeK_4N_6$, Carrez I, CAS: 14459–95-1) was purchased from Sigma-Aldrich, and zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$, Carrez II, CAS: 7446–20-0) was obtained from Merck. QuEChERS Extract Pouch was supplied by Agilent Technologies (Sta. Clara, CA, USA).

GI digestion reagents: calcium chloride dihydrate ≥99% ($CaCl_2(H_2O)_2$, CAS: 10035–04-8), potassium chloride ≥99% (KCl, CAS: 7447-40-7), magnesium chloride hexahydrate ≥99% ($MgCl_2(H_2O)_6$, CAS: 7791-18-6), ammonium carbonate ≥30% NH_3 basis ($(NH_4)_2CO_3$, CAS: 506–87-6) were purchased from Sigma-Aldrich; potassium dihydrogen phosphate 98%–102% (KH_2PO_4, CAS: 7778-77-0) from Panreac (Barcelona, Spain); sodium bicarbonate >99.7% ($NaHCO_3$, CAS: 144–55-8) from Probus (Badalona, Spain); sodium chloride ≥99.5% (NaCl, CAS: 7647-14-5), hydrochloric acid 37% (HCl, CAS: 7647-01-0) and sodium hydroxide ≥99% (NaOH, CAS: 1310-73-2) were obtained from Merck (Darmstadt, Germany).

Digestive enzymes: α-amylase from *Bacillus sp.* (CAS: 9000-90-2, 50 units (U) mg^{-1} solid), pepsin from porcine gastric mucosa (CAS: 9001–75–6, ≥250 U mg^{-1} solid), pancreatin from porcine pancreas (CAS: 8049-47-6, 8 USP; Lipase Activity: ≥8 U mg^{-1}; Amylase Activity: ≥100 U mg^{-1}; Protease Activity: ≥100 U/ mg^{-1}), and bovine bile (CAS: 8008-63-7) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Samples

A sample of olive oil (OO; composed of virgin olive oil and refined olive oil, pH 4.84) to study the chemical behavior of CdB in the different phases of GI digestion, and 17 canned seafood samples to determine the presence of the contaminant CdB and further sample selection for the evaluation human dietary exposure, were purchased in a local supermarket of Santiago de Compostela (Spain). The selection of the samples was based on canned food that presented higher levels of CdB according to previous works carried out by Lestido-Cardama, Sendón, et al. (2021) and Bustos et al. (2023), including fish (tuna, sardines) and shellfish (clams, mussels, cockle, variegated scallop). The differences between different covering liquids were also used as a sample's selection criterium to be investigated. Detailed information about the samples included in this study is presented in Table 1. Table 1 also includes the contact surface/volume (S/*V*) of the food ratio for each can sample, which can influence the concentration of CdB. Samples were stored at 20–25 °C until the analysis. Determination of the sample pH was performed in triplicate using a pH meter (Mettler Toledo, SevenCompact). As can be seen in Table 1, all samples showed a slightly acidic character (pH 4.45–6.56). Cans for food and beverages can be constructed using either two or three pieces of metal. The latter usually has a protective seam that was also characterized.

2.3. Coating extraction

To assess possible coating migrants, the cans were opened, emptied, and rinsed using warm water. Then, the entire interior of the can was extracted with ACN by filling (S/*V* ratio shown in Table 1) for 24 h in an oven at 70 °C following the method developed and used in previous works by Lestido-Cardama et al., 2019 and Lestido-Cardama et al., 2021b. The cans were covered with aluminum foil to avoid possible evaporation losses. Finally, 1 mL aliquot of the extract was filtered through a 0.22-μm polytetrafluoroethylene (PTFE) membrane filter, and the internal standard ($BPAd_{16}$) was added at a concentration of 5 mg/L to be analyzed using liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS).

2.4. Foodstuff extraction

The solid and the covering liquid were homogenized using an ultraturrax (IKA T25 digital, Staufen, Germany). The extraction method applied in this study was based on the procedure described in previous work by Sendón García, Paseiro Losada, and Pérez Lamela (2003) with some modifications. Briefly, 5 g of each food sample were taken in triplication. Heptane (5 mL) was added and the mixture was stirred for 1 min using a vibrax mixer (IKA® Vibrax VXR basic, Staufen, Germany) for fat removal. Then, 10 mL of 90% ACN:H_2O (*v*/v) were used for extraction and the sample was stirred in a vortex (VELP Scientifica vortex mixer, Milan, Italy) and placed in an ultrasonic bath (J. P. Selecta, Barcelona, Spain) for 10 min. Then, the sample was stirred for 10 min in the vibrax mixer and centrifuged at 2245 ×*g* for 10 min at 4 °C (Hettich Zentrifugen Universal 320R, Tuttlingen, Germany). Finally, 1 mL aliquot of the ACN/aqueous phase was filtered through a 0.22-μm PTFE membrane filter, and the internal standard ($BPAd_{16}$) was added at a concentration of 5 mg/L to be analyzed using LC–MS/MS.

Recovery was evaluated in triplicate for all samples, spiking them with CdB at three different concentrations (1, 2, and 4 μg/g). The standard solution was allowed to infuse for 15 min into the sample before extraction, following the procedure described above for food samples.

Table 1
Detailed information about the samples included in the study and mean concentrations of CdB in the polymeric can coatings and canned food samples (mean ± SD).

Code	Type of food sample	Covering liquid	Fat content*	Protein content*	Salt content*	pH	S/V ratio (dm^2/mL)	Polymeric can coating (μg/dm^2)	Foodstuff (μg/kg)	Type of can material	
										Internal	External
TS	Tuna	Sunflower oil	35.5 g/100 g (Satur.: 3.7 g)	13.7 g/100 g	0.80 g/100 g	5.85	11.1	0.088 ± 0.004	989 ± 0.07	Lid: PP Lateral: epoxy resin Base: epoxy resin Seam: PET	Lid: acrylic Lateral: acrylic Base: epoxy resin
TN	Tuna	Natural	0.9 g/100 g (Satur.: 0.3 g)	23.0 g/100 g	1.20 g/100 g **	5.91	11.1	0.25 ± 0.07	240 ± 0.09	Lid: PP Lateral: epoxy resin Base: epoxy resin Seam: PET	Lid: PEPU Lateral: PS Base: epoxy resin
TM	Tuna	Marinade	14.3 g/100 g (Satur.: 1.8 g)	19.8 g/100 g	0.90 g/100 g	5.07	11.1	0.07 ± 0.01	1154 ± 0.05	Lid: epoxy base Lateral: epoxy resin Base: epoxy resin Seam: PET	Lid: epoxy resin Lateral: PS Base: epoxy resin
TO	Tuna	Olive oil	25.0 g/100 g (Satur.: 3.9 g)	18.0 g/100 g	0.90 g/100 g	5.83	11.1	0.18 ± 0.02	1091 ± 0.12	Lid: PP Lateral: epoxy resin Base: epoxy resin Seam: PET	Lid: PEPU Lateral: epoxy resin Base: epoxy resin
TT	Tuna	Tomato	12.0 g/100 g (Satur.: 1.5 g)	14.8 g/100 g	1.20 g/100 g	5.47	11.1	0.23 ± 0.01	1508 ± 0.17	Lid: epoxy base Lateral: epoxy resin Base: epoxy base Seam: PET	Lid: epoxy resin Lateral: PS Base: epoxy resin
SL	Sardines	Lemon	27.0 g/100 g (Satur.: 3.4 g)	21.0 g/100 g	0.90 g/100 g	5.64	21.3	0.062 ± 0.07	2400 ± 0.09	Lid: epoxy base Lateral: epoxy base Base: epoxy base Seam: -	Lid: epoxy resin Lateral: epoxy resin Base: epoxy resin
SS	Sardines	Spicy sunflower	30.0 g/100 g (Satur.: 4.7 g)	17.0 g/100 g	1.0 g/100 g	6.10	21.3	<LOD	10 ± 0.01	Lid: PS (PET) Lateral: PS Base: PS Seam: -	Lid: PS Lateral: PS Base: PS
ST	Sardines	Tomato	11.9 g/100 g (Satur.: 2.2 g)	19.0 g/100 g	1.10 g/100 g	5.77	16.6	0.050 ± 0.001	60 ± 0.02	Lid: acrylic Lateral: PS Base: PS Seam: -	Lid: epoxy resin Lateral: epoxy base Base: epoxy base
SO	Sardines	Olive oil	25.0 g/100 g (Satur.: 4.0 g)	16.0 g/100 g	0.90 g/100 g	6.10	21.3	0.026 ± 0.001	3071 ± 0.08	Lid: epoxy base Lateral: epoxy base Base: epoxy base Seam: -	Lid: epoxy resin Lateral: epoxy resin Base: epoxy resin
SM	Sardines	Marinade	16.0 g/100 g (Satur.: 3.0 g)	17.0 g/100 g	0.90 g/100 g	5.02	21.3	0.13 ± 0.04	3260 ± 0.42	Lid: epoxy base Lateral: epoxy base Base: epoxy base Seam: -	Lid: epoxy resin Lateral: epoxy resin Base: epoxy resin
MN	Mussels	Natural	3.2 g/100 g (Satur.: 0.7 g)	15.9 g/100 g	1.52 g/100 g **	6.23	11.1	0.17 ± 0.01	150 ± 0.01	Lid: epoxy base Lateral: acrylic Base: acrylic Seam: -	Lid: epoxy resin Lateral: epoxy base Base: epoxy base

(*continued on next page*)

Table 1 (*continued*)

Code	Type of food sample	Covering liquid	Fat content*	Protein content*	Salt content*	pH	S/V ratio (dm^2/mL)	Polymeric can coating (μg/dm^2)	Foodstuff (μg/kg)	Type of can material	
										Internal	External
MSp	Mussels	Spicy marinade	11.0 g/100 g (Satur.: 1.4 g)	20.0 g/100 g	1.30 g/100 g **	4.69	11.1	<LOD	10 ± 0.003	Lid: PS Lateral: PS Base: PS Seam: -	Lid: PS Lateral: PS Base: PS
MSs	Mussels	Scallop Sauce	5.0 g/100 g (Satur.: 0.0 g)	12.8 g/100 g	1.29 g/100 g	5.55	11.1	0.055 ± 0.0003	10 ± 0.001	Lid: PS Base + Lateral: PS Seam: -	Lid: epoxy resin Lateral: epoxy base Base: epoxy base
MM	Mussels	Marinade	11.0 g/100 g (Satur.: 1.4 g)	20.0 g/100 g	1.30 g/100 g	4.45	11.1	0.054 ± 0.01	2410 ± 0.67	Lid: epoxy base Lateral: epoxy resin Base: epoxy base Seam: PET	Lid: epoxy resin Lateral: epoxy resin Base: epoxy resin
CN	Clams	Natural	2.9 g/100 g (Satur.: 0.8 g)	17.0 g/100 g	0.29 g/100 g	6.56	11.1	0.28 ± 0.03	200 ± 0.02	Lid: epoxy base Lateral: epoxy base Base: epoxy base Seam: -	Lid: epoxy resin Lateral: epoxy base Base: epoxy base
CoN	Cockle	Natural	0.5 g/100 g (Satur.: 0.1 g)	10.4 g/100 g	1.50 g/100 g	6.42	16.7	<LOD	<LOD	Lid: PS (PET) Lateral: PS Base: PS Seam: -	Lid: PS Lateral: PS Base: PS
VN	Variegated scallop	Scallop Sauce	7.3 g/100 g (Satur.: 1.0 g)	15.0 g/100 g	1.0 g/100 g	5.55	11.1	<LOD	<LOD	Lid: PS Lateral: PS Base: PS Seam: -	Lid: PS Lateral: PS Base: PS

S: Surface; V: volume; PS: polyester; PET: polyethylene terephthalate; PP: Polypropylene; PEPU: polyester polyurethane; Satur.: saturated; LOD: limit of detection. *Content described in food label; **: drained weight.

2.5. Static in vitro simulation of human GI digestion (INFOGEST protocol)

2.5.1. Preparation of the fluids of each digestion phase and enzymes

The fluids of each digestion phase (oral, gastric, and intestinal) and enzymes were prepared following the methodology described by Minekus et al. (2014), recently improved by Brodkorb et al. (2019). Briefly, each digestion fluid was prepared with its specific electrolyte composition in ultrapure type I water. The pH was adjusted to 7 in the simulated salivary fluid, pH 1, 2, 3, and 4 in the simulated gastric fluid, and pH 7 in the simulated intestinal fluid using 1 M HCl or NaOH. To carry out the digestion assay, enzymes and bile salts were prepared in the respective fluid to reach the following final concentrations: 75 U/mL for α-amylase, 2000 U/mL for pepsin, 100 U/mL for pancreatin, and 10 mM for the bile salts. The fluids from each phase of digestion and the enzyme solutions were prepared the same day as the digestion to avoid variations in pH over time and the potential growth of microorganisms. In addition, to avoid activity losses, enzyme and bile salts were kept in a container with ice.

2.5.2. Static in vitro simulation of GI food digestion

The standardized static *in vitro* GI digestion simulation protocol conducted in this study was based on an international consensus developed by INFOGEST (Brodkorb et al., 2019), where the parameters used (volume of electrolytes, enzymes, bile, pH, and time of digestion) are based on standard physiological data.

On one hand, the chemical behavior of CdB in the different phases of GI digestion was studied in olive oil as a representative sample due to its lipophilic character. For this, the test tubes were prepared, in triplicate, with 5 g of olive oil spiked with CdB at a concentration of 4 μg/g for each digestion phase (oral, gastric, and intestinal) and each gastric pH and their corresponding tubes with intestinal fluid (pH 1, 2, 3, and 4). Briefly, the oral digestion was performed at pH 7 for 2 min with amylase, the gastric phases at pH 1, 2, 3, and 4 for 2 h with pepsin, and finally, the intestinal phase was carried out at pH 7 for 2 h using pancreatin and bile salts. The digestion process was performed in a water bath preheated to 37 °C with rotary shaking (GFL 1083). A blank control test tube without CdB was prepared. A non-enzyme assay was also performed to observe the effect of enzymes, substituting the volume of enzymes with ultrapure type I water. In this study, samples were collected after each phase of digestion and treated for subsequent analysis by LC–MS/MS. In enzyme assays, at the end of each digestion step, the samples were placed in a container with ice to stop/minimize their enzyme activity.

Conversely, the protocol was applied to the 10 canned food samples with CdB values >200 μg/kg (see Table 1) to study the bioaccessibility of CdB in several food matrices with different covering liquids to estimate human dietary exposure to CdB. In this case, the test tubes were prepared, in triplicate, with 5 g of the food sample, and subjected to the complete digestion process previously described. A blank control test tube, without a food matrix, was included in each batch of samples. The samples were collected at the end of the intestinal phase of digestion and treated for subsequent analysis by LC–MS/MS. Once the complete digestion process was finished, the tubes were centrifuged at 2245 ×*g* for 10 min at 4 °C (Hettich Centrifuge Universal 320 R, Tuttlingen, Germany) to separate the supernatant and the pellet. This allows verification of the bioaccessibility of the CdB, to check whether it is soluble in the medium and therefore, could contribute to human dietary exposure to CdB or, conversely, it precipitates and remains in the pellet. In the case of the supernatant, its pH was reduced to 5.4–5.6 to inactivate the enzymes, and a 5 mL aliquot was transferred to another tube and subjected to extraction in the same way as the pellet. Both were extracted using the same procedure previously described for foodstuff (Section

2.3), with an additional concentration step by evaporating 5 mL of the ACN/aqueous phase to dryness using a stream of nitrogen at a temperature of 40 °C (RapidVap Vertex Evaporator, Labconco, Kansas City, MO, USA). Finally, the residue was resuspended in 0.5 mL of 90% ACN: H_2O (*v*/v) by thoroughly shaking in the vortex, and filtered through a 0.2-μm PTFE filter. The internal standard ($BPAd_{16}$) was added at a concentration of 5 mg/L for subsequent analysis by LC–MS/MS.

2.6. Instrumental equipment

2.6.1. Fourier transform infrared spectroscopy

Fourier-transformed infrared spectrometry equipped with an attenuated total reflectance (FTIR-ATR, ATR-PRO ONE, FTIR 4700, Jasco, Tokyo, Japan) was used to verify the type of polymeric coating of the can samples following the method described by Lestido-Cardama et al. (2022). The analysis was done on the internal and external sides of the lid, the lateral, the base, and the seam (when it is present) of the cans by covering the ~~entire~~ complete surface of the diamond optical crystal surface. The FTIR-ATR spectrometer was controlled by the Spectra Manager™ (version 2) software. ATR spectra were measured in the range of 4000–650 cm^{-1}. Spectra identification was done using KnowItAll 17.4.135.B software, comparing sample spectra with polymer IR libraries (Bio-Rad Laboratories, Inc. Philadelphia, PA, USA). These libraries use algorithms to make decisions about the identity of the material, and the matching hits are reported as a hit quality index (HQI), a value that ranges from 0 to 100. The best hits were considered in each comparison.

2.6.2. LC–MS/MS analysis

CdB analysis was carried out using an LC–MS/MS system from Thermo Fisher Scientific (San José, CA, USA). It consisted of an Accela autosampler, an Accela 1250 pump, and a column thermostated system coupled to a triple-stage quadrupole mass spectrometer TSQ Quantum Access MAX. Data acquisition and processing were performed using the Xcalibur 2.1.0 software.

Chromatographic separation was performed using a Kinetex® EVO C18 100 Å (150 mm * 3.0 mm internal diameter, 5-μm particle size) column thermostat at 30 °C, with a mobile phase composed of ultrapure type I water (A) and ACN (B). The gradients were as follows: 40% B in an isocratic mode for 2 min, followed by a gradient to 50% B for 5 min, another gradient to 70% B for 9 min, and a final gradient to 100% B for 0.5 min. Finally, an isocratic elution with 100% organic phase for 3.5 min and the initial conditions were retaken. The flow rate remained steady at 0.5 mL/min, and the injection volume was 10 μL.

The mass spectrometer was used in positive atmospheric pressure chemical ionization (APCI) mode. The optimized settings of the MS/MS detector were: capillary temperature at 350 °C, vaporizer temperature at 400 °C, nitrogen as the sheath gas at 35 psi and as an auxiliary gas at 10 arbitrary units. Argon was used as collision gas under a pressure of 1.5 mTorr. MS data were acquired in selected reaction monitoring (SRM). The precursor ion selected for CdB was *m/z* 569.0, which was the most sensitive ion in the Q1 mass spectra. Three SRM transitions of *m/z* 569.0 > 134.8 (transition used for quantification purposes), *m/z* 569.0 > 294.9, and *m/z* 569.0 > 106.9 were monitored with a collision gas energy of 29, 37, and 39 *V*, respectively. For the internal standard ($BPAd_{16}$), the SRM transitions of *m/z* 242.0 > 224.0 (transition used for quantification purposes), *m/z* 242.0 > 125.0, and *m/z* 242.0 > 97.0 were monitored with a collision gas energy of 15, 27 and 34 V, respectively. CdB was eluted at 12.6 min and $BPAd_{16}$ at 3.2 min.

2.7. Estimation of human exposure to CdB

Dietary exposure was estimated by considering the obtained concentration of the positive food samples for CdB after the *in vitro* digestion process and the Spanish consumption data for this type of food (Spanish Agency for Food Safety and Nutrition (AESAN), 2021). The average daily consumption of canned seafood by the adult Spanish population is shown in Table S2.

ENALIA 2 is a dietary survey conducted in Spain that includes the adult population between 18 and 74 years of age. It is an individual survey, which allows knowing the category of food and the intake (g/day) of this population and the frequency of food consumption, which is essential for scientific research on exposure studies to chemical substances through food. This survey study took place between the spring of 2014 and the first half of 2015. The methodology followed the EFSA guidance recommendations on the "General principles for the collection of national food consumption data in the view of a pan-European dietary survey." The survey included 933 adults and elderly (623, 18–64 years old and 310, 65–74 years old) (AESAN, 2021). In this study, we focused on the adult population group from 18 to 74 years old because this group represents the largest consumer group of this type of canned food. Pregnant women were also considered since it is a vulnerable group of the population.

The estimated dietary intake (EDI) was calculated taking into account the obtained concentration of CdB in the bioaccessible phase after the *in vitro* digestion process at each pH and the Spanish consumption data for this type of food (Table S2), through the following equation:

$$EDI = C \times W$$

where C is the concentration of CdB in the bioaccessible phase for each sample and each pH (μg/g), W is the estimated consumption rate of each type of food per individual (g/day) and EDI is the estimated dietary intake expressed as μg/kg body weight (bw) per day.

2.8. Statistical analysis

The results are presented as the mean ± standard deviation. Data were compared by one-way ANOVA, and significant differences were assessed by Duncan's *post hoc* test at a 95% confidence level using the IBM SPSS Statistics software (version 28.0; StatSoft, Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Characterization of the type of coating by FTIR-ATR analysis

The exact composition of the polymeric can coatings was unknown prior to testing, so the initial procedure involved determining the type of coating through FTIR-ATR analysis. Only the best matches with the spectral libraries were considered. As can be seen in Table 1, most of the samples (13 of 17) had an epoxy resin on the inside (internal) or outside (external) (sardines in tomato (ST), mussels in scallop sauce (MSs)), except for the SS, mussels in spicy marinade (MSp), natural cockle (CoN), and variegated scallop in scallop sauce (VN) samples that presented only polyester-type coatings. Fig. 1 shows an IR spectrum of the lateral internal side of the sample tuna in tomato (TT) sample, superimposed with the initial entry of the libraries, an epoxy resin with an HQI of 98.23.

The most common epoxy-based coatings result from the condensation reaction between BPA and epichlorohydrin forming epoxy resins based on BADGE. However, because of the uncertainties regarding the potential adverse effects of BPA, public debates, and recent regulatory decisions, food industries and can manufacturers, are now exploring and implementing alternatives such as polyester or acrylic-based coatings. Further, polyesters are obtained by polycondensation reactions between polycarboxylic acids and polyols. Numerous raw materials have been thoroughly assessed for toxicity and meet food packaging regulations (Zhang, Scarsella, & Hartman, 2020). Acrylic-based coatings result from the polymerization of acrylic resins and their derivatives (Piergiovanni & Limbo, 2016). The most used monomer to produce acrylic coatings is ethylacrylate (Geueke, 2016).

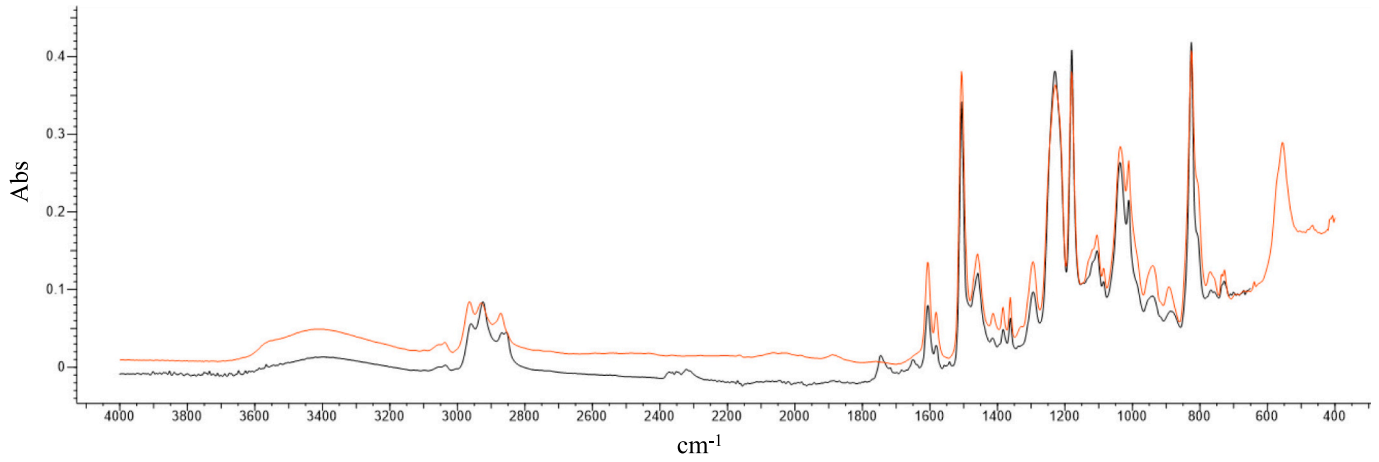


Fig. 1. IR spectrum of sample TT (dark line) compared with the epoxy resin spectrum of the IR spectral libraries (red line). Absorbance *vs.* wavenumber (cm^{-1}). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Can samples consisting of three parts (lid, lateral, and base) were coated internally with a "side seam strip" to safeguard uncovered metal (U. S. Environmental Protection Agency, 1998) that was also analyzed on the FTIR and turned out to be PET. PET is a type of polyester produced in an esterification reaction where ethylene glycol reacts with terephthalic acid (Robertson, 2016). Three samples, including tuna in sunflower oil (TS), natural tuna (TN), and tuna in olive oil (TO), had a simple lid opening mechanism made of polypropylene on the inner side.

3.2. Method optimization and validation

To find a suitable CdB extraction method in food samples, different methodologies were tested using olive oil (OO) as a representative fatty food and spiking it with CdB at a concentration of 10 μg/g in duplicate: extraction with ACN and application of Carrez solutions I and II, extraction with ACN and the use of QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), and extraction following the methodology previously reported by Sendón García et al. (2003). The recoveries obtained for the Carrez and QuEChERS methods were < 70%, and for this reason, the methodology described previously by the research group was selected for this study.

The analytical method selected and described above (Section 2.3) was validated in terms of linearity, sensitivity, accuracy, and precision. For this, calibration curves of CdB were prepared daily in 90% ACN:H_2O (90:10, *v*/v) at 12 different concentrations in the range of 2.5–5000 μg/L, and the internal standard $BPAd_{16}$ was added (5 mg/L) for quantitative analysis. Calibration curves were created by plotting concentration of standard solution against the ratio of peak area/internal standard area using the transitions for quantification purposes. For CdB, the most powerful precursor ion was the protonated molecular ion $[M + H]^+$, while $BPAd_{16}$ is transformed into $BPAd_{14}$ in water, and for that reason, we detected *m/z* 242 (Inoue, Kawaguchi, Funakoshi, & Nakazawa, 2003). The quantification of CdB was carried out as the sum of the two isomers (cis- and trans-). As a result, the method showed good linearity with correlation coefficients (r) higher than 0.99. Regarding sensitivity, this was evaluated based on limits of detection (LOD) and quantification (LOQ), estimated as the lowest concentration that provided a signal-to-noise higher than 3 or 10, respectively, obtaining 1 μg/L (2 μg/kg food) and 2.5 μg/L (5 μg/kg food), respectively. The repeatability of the method, expressed as the percentage of relative standard deviation (RSD %), was determined by a repetitive analysis ($n = 10$) of a standard solution at a concentration of 50 μg/L and the value obtained was lower than 15%. The precision and accuracy of the extraction method were determined, in triplicate, by spiking the food samples with a known amount of CdB at three different concentration levels (1, 2, and 4 μg/g). The mean recoveries (%) and the repeatability (RSD%) obtained are shown in Table S3. The recoveries obtained were > 70% except in two cases, while the repeatability was <15% for almost all experiments. The extraction was considered complete after one extraction step since a second extraction step resulted in recovery values of <10% of those obtained in the first extraction. This data indicates that the method is suitable for extracting CdB from canned food samples.

3.3. Analysis of polymeric can coatings and foodstuffs

The developed analytical method was applied to identify and quantify CdB by LC–MS/MS in the ACN extracts of polymeric can coatings and their corresponding foodstuffs after the extraction as described above. The analysis of each can extract was performed in triplicate, and each food sample was extracted in triplicate. In addition, to check for possible background contamination, blanks were injected with each batch of sequences. The quantification was performed using the external calibration curve method. Table 1 presents a summary of the samples with their concentrations expressed as μg of CdB per dm^2 in the case of the cans and as μg of CdB per kg for the foodstuffs, with their corresponding standard deviations.

Regarding the polymeric can coatings, as shown in Table 1, CdB levels were detected in the extracts of all the coatings, reaching values of 28 μg/dm^2 in the natural clams (CN) extract, except in four samples (sardines in spicy sunflower (SS), MSp, CoN, and VN), which presented only polyester-type coatings. Low CdB concentrations were also quantified in two samples with PS-type coatings on the internal side and with epoxy-type coating on the external side (ST and MSs). This migration of CdB to the internal side of the can could be due to the possible set-off phenomena during production and/or storage of these materials in the industry. After coatings application, the sheets are stacked and stored. At the same time, there is a close connection between both the internal and external sides of the material, which may lead to the transfer of compounds and subsequently migrate to the packaged food (Lestido-Cardama et al., 2019). It is worth noting the CdB contamination (10 μg/kg) found in samples SS and MSp for which CdB could not be detected in the can extract (<LOD) and whose internal and external coatings were not identified as epoxy resins. In these cases, a possible explanation could be that either or both the food sample and its covering liquid were previously contaminated. For example, analogs of bisphenols were identified in seafood samples (Akhbarizadeh, Moore, Monteiro, Fernandes, & Cunha, 2020; Barboza, Cunha, Monteiro, Fernandes, & Guilhermino, 2020).

Regarding the food samples analyzed, the CdB levels ranged between <LOD and 3260 μg/kg in the sample of sardines in marinade sauce (SM).

Data in Table 1 show that those samples with the highest fat content and packed in cans with an epoxy-type coating had a higher concentration of CdB. This may be due to the fact that this kind of compound usually has a greater preference for the lipid-soluble surroundings (*e.g.*, samples covered with sunflower or olive oil, such as TS, TO, or sardines in olive oil (SO)). The log P of 7.79 of CdB (data obtained from ChemDraw Professional v. 16.0.1.4) also supports its affinity for a lipid medium. Based on the results found, it could be said that sardine samples are the most contaminated, followed by tuna and mussels. Regarding the food sauces, we observed that samples with marinade sauce, such as SM and mussels in marinade (MM), displayed higher levels of CdB than those canned without sauce (natural ones). For example, tuna with marinade sauce (TM) showed a CdB concentration of 680 μg/kg, while for TN, the measured concentration was 35% lower (240 μg/kg). However, in this case, in addition to having different covering liquids, the polymeric coatings of the internal part of the lid are different (see Table 1). The LC–MS/MS chromatograms of sample SM, after the extraction of the polymeric coating (A) and the food sample (B), are shown in Fig. S1.

Because the analytical standard has only recently become available, there is a lack of data on studies regarding the presence of CdB in food samples. The results are in line with those reported by Biedermann et al. (2013) and more recently confirmed by Lestido-Cardama, Sendón, et al. (2021) and Bustos et al. (2023). CdB was determined in canned food, including canned fish, in two campaigns by the Official Food Control Authority of the Canton of Zurich in 2010 and 2012. In 2012, CdB was detectable in 13 of the 44 samples with an average concentration of 807 μg/kg, and the highest value of 2640 μg/kg in sardines in oil (Biedermann et al., 2013). In the study of Lestido-Cardama et al. (2021a), CdB was one of the most detected compounds in the canned food samples analyzed, and the concentration ranged from 134 μg/kg to 3590 μg/kg in the pickled sauce of mussels. In the study by Bustos et al. (2023), 20 of the 48 samples tested positive for CdB, with levels reaching up to 2623 μg/kg.

Currently, no EU-harmonized specific legislation for polymeric coatings is available, and there are only a few substances with specific legislation. To date, international organizations have not yet established limits on the presence of CdB in food. Comparing these values with the acceptable migration level of 50 μg of CdB/kg of food reported in 2016 by the German Federal Institute of Risk Assessment (BfR Opinion 022/2016, 2016), 70% of the samples analyzed in this study exceeded this recommendation.

3.4. Static in vitro simulation of human GI digestion (INFOGEST protocol)

3.4.1. Chemical behavior of CdB in the different phases of GI digestion

For the study of the chemical behavior of CdB through the different phases of digestion, olive oil (OO) was spiked in triplicate with CdB at 1.5 mg/L. Fig. 2 shows the mean recovery of CdB obtained at each digestion phase and different pH tested, with the percentage corresponding to the bioaccessible (soluble in the supernatant) and non-bioaccessible (insoluble in the pellet) fractions (see Supplementary Table S4). This percentage of CdB recovery was determined considering the initial concentration of CdB determined in the sample after spiking. Different gastric pH values, within the range of 1 to 4, were tested to evaluate the possible effect of an acidic environment on the solubility and chemical stability of CdB in the present study. In addition, a non-enzyme test was also carried out to observe the effect of enzymes (Supplementary Table S4).

In the test without enzymes, all the recovered CdB was determined just in the bioaccessible fraction, considering that in this test, no pellet fraction was obtained by centrifugation. Comparing the four pHs tested, lower recoveries were observed in the intestinal phase after the gastric phase at pH 4 (48.3%), which may be due to the lower solubility of CdB under these pH conditions. This effect could be related to the partial emulsion formation at lower pH values that may contribute to the high dispersion/solubility of CdB due to its apolar nature and affinity to oil.

In the second test to evaluate the effect of enzymes, the presence of enzymes leads to the precipitation of CdB into the non-bioaccessible fraction (pellet), significantly reducing its bioaccessibility in the oral ($p < 0.001$) and gastric stages ($p < 0.01$ for pH 1 and 3, and $p < 0.001$ for pH 2) compared with the test without enzymes. This effect could be related to the presence of enzymes, which at optimal pH may create stable interactions with CdB and lead to the precipitation of CdB, as

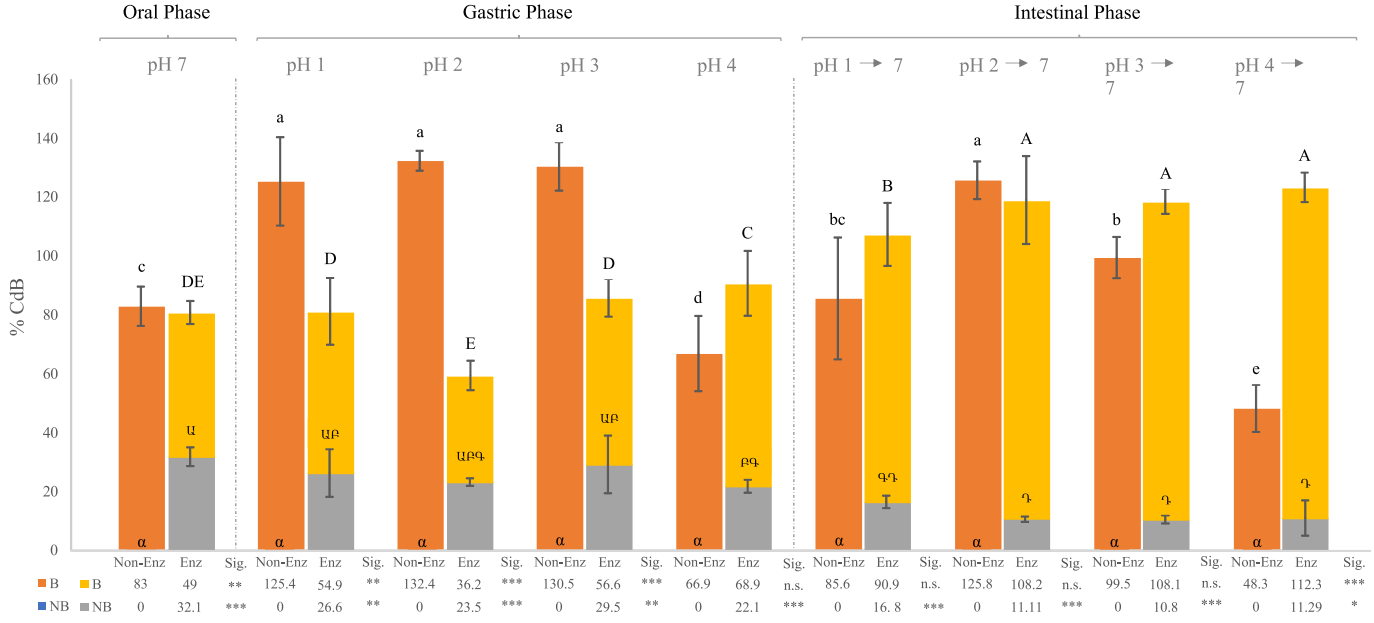


Fig. 2. Effect of the presence (Enz) and absence (Non-Enz) of enzymes on the solubility and bioaccessibility (B) and non-bioaccessibility (NB) of CdB (percentage) during the different stages of GI digestion at different pH values. The lowercase letters indicate a significant difference at $p < 0.05$ among CdB percentages for the NB and B fractions for the assays in the absence of enzymes; whereas different uppercase letters indicate a significant difference at $p < 0.05$ for different digestion phases and pH values tested for the assays with enzymes; Significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s. = not significant. Armenian alphabet letters were used to indicate significant differences among NB fractions and Latin alphabet for B fractions.

observed with the bisphenol S molecule (Lestido-Cardama, Sendón, & Rodríguez Bernaldo de Quirós, 2022; Wang & Zhang, 2014). However, after the gastric phase, an increase in pH in the intestinal phase and the presence of bile salts and pancreatin that contain, among others, lipases, increased the solubility of CdB, and the bioaccessibility was complete, including for pH 4. As shown in Fig. 2, in general, most of the percentage of CdB was found in the bioaccessible fraction. The CdB bioaccessibility values ranged between 90.9 (after pH 1 in the gastric phase) and 112.3% (for pH 4) at the intestinal level, meaning CdB is available for absorption. A similar effect of the increment of the solubility of the bisphenol A in the intestinal phase after the gastric phase at pH 2 was previously described by Cunha, Alves, Fernandes, Casal, & Marques, 2017. The authors also correlated this behavior to the surfactant-like effect of bile that stimulates the formation of micelles and increases the BPA bioaccessibility by up to 81% (Cunha et al., 2017).

3.4.2. *Bioaccessibility of CdB in different food samples*

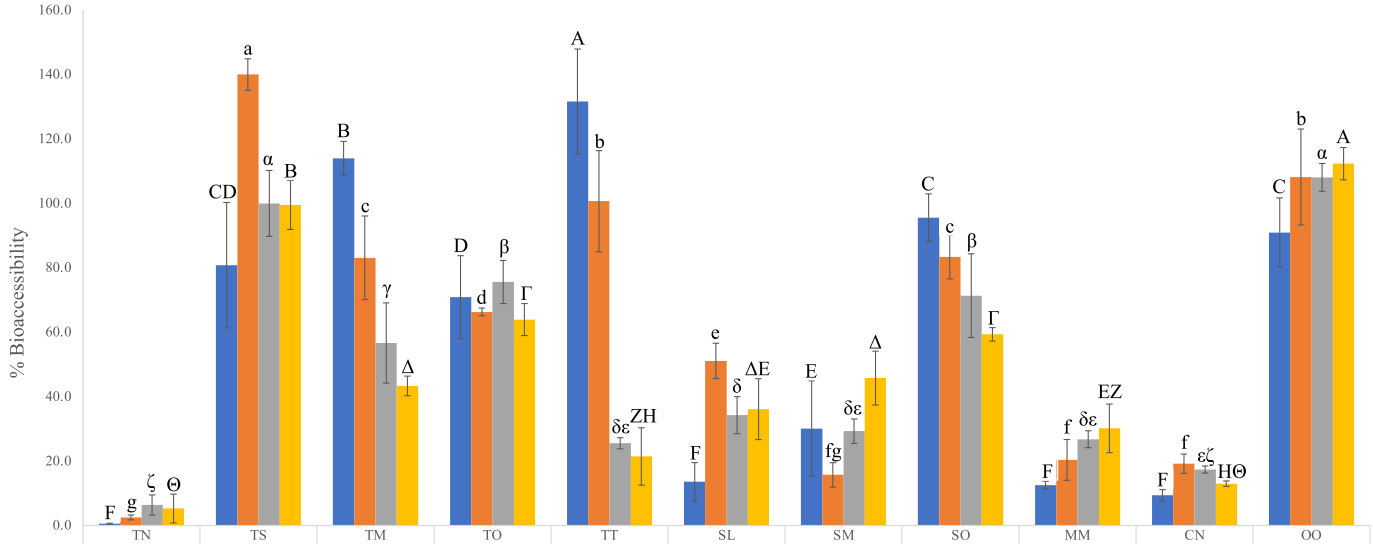
The simulated human GI digestion was applied to the canned food samples with concentrations of CdB equal to or higher than 200 μg/kg (see Table 1). In addition to the several seafood matrices selected, the nature of different commercially available covering liquids was also considered in the study of oral CdB bioaccessibility. In this case, the study focused on the complete food digestion process, and the resulting samples from the intestinal phase were analyzed in triplicate. Due to human gastric pH variability (Scott, Weeks, Melchers, & Sachs, 1998), different gastric pH values within the range of 1–4 were tested to evaluate the bioaccessibility of CdB, and the results obtained are reported in Fig. 3 (see also Supplementary Table S5). There is evidence of age-related changes in the stomach. The basal pH of the stomach in humans is approximately 1.5. However, children and the elderly have less acidic stomachs (pH > 4) (Beasley, Koltz, Lambert, Fierer, & Dunn, 2015).

As shown in Fig. 3, comparing the different food samples analyzed, the TS, TO, and SO presented the highest bioaccessibility at all tested pH values that ranged from 59.3 (pH 4 for SO) up to 100% (pH 3 for TS). In general, the tuna samples showed a higher bioaccessibility, followed by the sardines, mussels, and clams, successively.

Most of the percentage of CdB was found in the bioaccessible fraction, except for those samples without any sauce as covering liquids and lower fat content (TN (0.9%) and CN (2.9%)). For these two samples, the non-bioaccessible fraction was higher than the bioaccessible. The highest values of CdB bioaccessibility were observed for food samples with fat content that ranged from 11% (MM) up to 35% (for TS). Considering the same type of food, *i.e.* tuna, with different fat contents and covering liquids (TS, TN, TM, TO, and TT), samples with a high-fat content (TS-35% and TO-25%) are those with high CdB bioaccessibility values for all gastric pH values studied. Therefore, it can be deduced that the different covering liquids influence the bioaccessibility, and the correlation indicated that more lipophilic molecules had higher bioaccessibility in foods with high-fat content, as shown in other studies (Cunha et al., 2017; Yu et al., 2010). In addition, despite food samples covered with marinade sauce (TM and SM) being more contaminated with CdB, in general, its bioaccessibility was significantly lower ($p < 0.05$) than that observed for canned seafood covered with sunflower oil (TS) and olive oils (TO and SO), especially after the gastric phases at higher pH values (pH 3 and pH 4).

Other parameter that should be considered is the protein content. In general, samples with a protein content higher than 17%, such as TN (23%), TM (19.8%), SL (21%), SM (17%), MM (20%), and CN (17%) are those with low bioaccessibility of CdB. These effects of the different components of the food matrix were described by Faria, Melo, & Ferreira, 2020 for several chemical contaminants. These authors observed that food matrix components affected differently the contaminants bioaccessibility according to their chemical nature (Faria et al., 2020).

Comparing the different gastric pHs tested for some samples such as TT, TM, and SO, the percentage of bioaccessible CdB decreases as the pH increases. In contrast, the opposite behavior occurs in the MM sample. For 60% of the samples, the highest bioaccessibility was found at pH 1 or 2. Therefore, adult people with a more acidic gastric environment will be slightly more exposed to the possible effects of CdB in their organism



	TN	TS	TM	TO	TT	SL	SM	SO	MM	CN	OO
pH1	0.5 ^{b}	80.8 ^{c}	113.9 ^{a}	70.9 ^{a}	131.6 ^{a}	13.5 ^{c}	30.1 ^{b}	95.6 ^{a}	12.5 ^{b}	9.4 ^{c}	91.0 ^{b}
pH2	2.5 ^{ab}	140.0 ^{a}	83.1 ^{b}	66.3 ^{a}	100.7 ^{b}	51.1 ^{a}	15.7 ^{c}	83.3 ^{ab}	20.4 ^{ab}	19.2 ^{a}	108.2 ^{ab}
pH3	6.4 ^{a}	100.0 ^{b}	56.7 ^{c}	75.6 ^{a}	25.5 ^{c}	34.3 ^{b}	29.3 ^{b}	71.4 ^{bc}	26.8 ^{a}	17.4 ^{a}	108.1 ^{ab}
pH4	5.3 ^{ab}	99.5 ^{b}	43.3 ^{c}	63.9 ^{a}	21.4 ^{c}	36.1 ^{b}	45.8 ^{a}	59.3 ^{c}	30.1 ^{a}	13.0 ^{b}	112.3 ^{a}

Fig. 3. Mean recoveries (percentage, $n = 3$) of CdB in the bioaccessible fraction obtained from the intestinal phase coming from each pH and food sample tested. The uppercase Latin alphabet letters indicate a significant difference at $p < 0.05$ among CdB percentages of the different food samples for the B fractions for the assays at pH 1; while different lowercase Latin alphabet letters indicate a significant difference at $p < 0.05$ for the assays at pH 2, different lowercase Greek alphabet letters were used to indicate significant differences at $p < 0.05$ for the assays at pH 3 and different uppercase Greek alphabet letters were used to indicate significant differences at $p < 0.05$ for the assays at pH 4. Lowercase Latin alphabet letters were also used in the table at the bottom of the figure to indicate significant differences at $p < 0.05$ among CdB percentages obtained at different pHs for each food sample.

than children and the elderly.

3.5. Estimation of human exposure to CdB

Consumers' dietary intake of chemical contaminants plays a key role in risk assessment. For this, human dietary exposure is usually estimated based on the concentration of the analyte measured in each food sample type, and this data is combined with the national consumption data for that type of food (European Food Safety Authority (EFSA), 2011). However, the objective of this study was to estimate the human dietary exposure to CdB more accurately, based on the concentration of CdB that is bioaccessible obtained from the positive food samples after performing the *in vitro* digestion process. Dietary exposure was estimated considering the four pHs tested for each sample. Estimated dietary exposure values to CdB in the different canned food samples for the Spanish adult population are shown in Table 2. For the tuna in tomato (TT) sample, since consumption data was not available, it was calculated based on the maximum consumption according to the product category that corresponds to tuna in oil.

Observing the results, it is possible to verify a low dietary exposure to CdB for all population groups and all the pH tested with the consumption of natural tuna (0.000 to 0.014 μg/kg bw per day at pH 3 for the population groups of 18–39 and 40–64 years), marinade tuna (0.012 to 0.329 μg/kg bw per day at pH 1 for the population group of 40–64 years), sardines in lemon (0.012 to 1.378 μg/kg bw per day at pH 2 for the population group of 65–74 years), marinade sardines (0.020 to 1.227 μg/kg bw per day at pH 1 for the population group of 65–74 years), clams and similar products (0.000 to 0.008 μg/kg bw per day at pH 2 for the population group of 40–64 years), and marinade mussels (0.012 to 0.117 μg/kg bw per day at pH 4 for the population group of 40–64 years). However, higher CdB dietary exposure values were found with the consumption of tuna in olive oil (3.144 to 6.003 μg/kg bw per day at pH 3 for the population group of 18–39 years), tuna in tomato (1.437 to 14.255 μg/kg bw per day at pH 1 for the population group of 18–39 years), tuna in sunflower oil (4.110 to 10.105 μg/kg bw per day at pH 2 for the population group of 18–39 years), and sardines in olive oil (0.074 to 3.707 at pH 1 for the population group of 65–74 years).

Comparing these results of the estimation of dietary exposure to CdB (Table 2), which were calculated considering the bioaccessibilities at different pHs, with the typical estimation of the dietary exposure (see Supplementary Table S6), which is based on a combination of the concentrations detected in canned food samples (Table 1) and data on the dietary intake of populations (Table S2), it can be observed how this exposure is overestimated in many of the cases since it does not reflect the amount released from food during GI digestion and its possible absorption in the intestinal epithelium. Therefore, the methodology presented in this study allows a more accurate assessment of dietary exposure and its possible effects on consumer health.

Comparing the results obtained in the estimation of dietary exposure to CdB (Table 2) in the population aged 18–74 years with the results obtained in the previous work of Lestido-Cardama et al. (2021a), it can be observed that, taking into account bioaccessibility, the dietary exposure of CdB turned out to be lower in the samples of natural tuna, clams and marinade mussels for all the pHs tested, while the dietary exposure to CdB turned out to be much higher in the samples of tuna and sardines in olive oil (0.013 μg/kg bw per day *vs* 4.798 μg/kg bw per day at pH 3 in tuna in olive oil, 0.055 μg/kg bw per day *vs* 2.402 μg/kg bw per day at pH 1 in sardines in olive oil). When the results of this study are compared with those obtained in the work of Bustos et al. (2023) for the samples of tuna and sardines in oil (0.5319 μg/kg bw per day in tuna in oil and 0.9797 μg/kg bw per day in sardines in oil), the same conclusion was drawn.

Since there is little toxicological data on CdB and no tolerable daily intake (TDI) established, the threshold of toxicological concern (TTC) concept was applied to determine human exposure levels. The Cramer classification was the most widely recognized method for predicting TTC

Table 2
Dietary exposure to CdB (μg/kg bw/day) in Spanish adult population.

	pH 1				pH 2				pH 3				pH 4			
	18–39 years	40–64 years	65–74 years	18–74 years	Pregnant women	18–39 years	40–64 years	65–74 years	18–74 years	Pregnant women	18–39 years	40–64 years	65–74 years	18–74 years	Pregnant women	18–39 years	40–64 years	65–74 years	18–74 years	Pregnant women
Tuna natural	0.005	0.005	0.000	0.004	0.004	0.008	0.008	0.001	0.007	0.006	0.014	0.014	0.001	0.012	0.011	0.011	0.012	0.001	0.010	0.009
Tuna marinade	0.106	0.329	0.021	0.201	–	0.097	0.301	0.019	0.185	–	0.065	0.200	0.013	0.123	–	0.059	0.184	0.012	0.113	–
Tuna olive oil	5.629	3.805	3.485	4.499	4.670	5.266	3.559	3.260	4.208	4.369	6.003	4.058	3.717	4.798	4.981	5.078	3.432	3.144	4.058	4.213
Tuna sunflower oil	6.638	4.487	4.110	5.305	5.507	10.105	6.830	6.256	8.076	8.384	7.941	5.368	4.917	6.347	6.589	7.235	4.890	4.479	5.782	6.002
Tuna tomato*	14.255	9.635	8.825	11.392	11.826	10.903	7.369	6.750	8.713	9.045	2.765	1.869	1.712	2.210	2.294	2.322	1.569	1.437	1.855	1.926
Sardines lemon	0.096	0.324	0.365	0.237	0.012	0.364	1.223	1.378	0.893	0.044	0.244	0.820	0.923	0.598	0.030	0.257	0.865	0.974	0.631	0.031
Sardines marinade	0.324	1.090	1.227	0.795	0.039	0.166	0.557	0.627	0.406	0.020	0.315	1.060	1.194	0.774	0.038	0.493	1.657	1.866	1.209	0.060
Sardines olive oil	0.979	3.292	3.707	2.402	0.119	0.864	2.906	3.272	2.120	0.105	0.731	2.458	2.768	1.794	0.089	0.609	2.049	2.307	1.495	0.074
Clams and similar products	0.003	0.004	0.001	0.003	0.000	0.006	0.008	0.002	0.006	0.000	0.005	0.007	0.002	0.006	0.000	0.004	0.005	0.001	0.004	0.000
Mussels marinade	0.036	0.048	0.033	0.042	0.012	0.059	0.079	0.054	0.069	0.020	0.078	0.104	0.071	0.091	0.026	0.087	0.117	0.080	0.102	0.029

* As the consumption data was not available, it was calculated based on the maximum consumption according to the product category that corresponds to tuna in oil.

(Cramer, Ford, & Hall, 1976). This approach is recommended by the EFSA (European Food Safety Authority (EFSA), 2012). The Cramer decision tree enables the assessment of a compound's toxicological hazard based on its molecular structure, categorizing it into three classes: low toxicity (class I), intermediate toxicity (class II), or high toxicity (class III). To achieve this goal, Toxtree v3.1.0 (Ideaconsult Ltd., Sofia, Bulgaria) software was used, and CdB was classified as class III. Therefore, the assigned value of 1.5 μg/kg bw/day or 90 μg/person/day (based on a person weighing 60 kg) is suggested to be a useful tool for the evaluation of the exposure. Therefore, comparing this reference value with the estimated dietary exposure values obtained in the analyzed samples, some tuna (in olive oil and sunflower oil) and sardine (in the marinade and olive oil) samples exceeded the limit, reaching values of 14.255 μg/kg bw/day for tuna. People with a gastric pH value of 2 will be more exposed to this chemical compound through the diet.

Vulnerable groups of the population are more exposed to this chemical compound due to the high consumption of canned tuna and sardines for seniors (65–74 years) and tuna in olive oil and sunflower oil for pregnant women.

4. Conclusions

This work is the first approach to evaluate the bioaccessibility of CdB after the intake of several canned foods using an *in vitro* simulated GI digestion model. This methodology allows the estimation of the human dietary exposure to this molecule more accurately and contributes to their risk assessment. Despite the tendency to change the coatings used in food cans, most coatings were composed of epoxy resin, thus a source of CdB contamination. CdB was determined and quantified in the extracts of the cans and their corresponding foodstuffs exceeding the recommended limit of 50 μg/kg reported by the BfR. The results showed that, after complete digestion, CdB was available and might be susceptible to being absorbed at the intestinal level. Moreover, CdB in seafood samples with different covering liquids and fat contents showed different behaviors. Therefore, this highlights the importance of analyzing real samples considering the food matrix and how different factors are relevant. Finally, the dietary exposure to CdB was estimated in the Spanish adult population and it was found that the recommended TDI value for substances classified as Cramer class III was exceeded in some of the samples. Vulnerable groups of the population are more exposed to this chemical compound due to their consumption habits, high consumption of canned tuna and sardines, in the case of seniors (65–74 years), and tuna in olive and sunflower oils for pregnant women. Considering this new methodology, the estimated dietary exposure turned out to be lower than what is being calculated so far. This is a more realistic estimate that should be integrated as a tool to complete dietary exposure studies. Further studies related to the toxicological effects of this chemical compound are advisable to complete the risk assessment, as well as absorption studies in cell models to assess how much CdB would pass through the intestine.

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CRediT authorship contribution statement

Antía Lestido-Cardama: Writing – original draft, Visualization, Methodology, Investigation, Data curation. **Patricia Vázquez-Loureiro:** Methodology, Investigation, Data curation. **Raquel Sendón:** Writing – review & editing, Validation, Conceptualization. **Juana Bustos:** Writing – review & editing, Validation, Conceptualization. **Perfecto Paseiro-Losada:** Writing – review & editing, Validation, Conceptualization. **Ana Rodríguez Bernaldo de Quirós:** Writing – review & editing, Validation, Resources, Conceptualization. **Letricia Barbosa-Pereira:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, 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.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

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