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Effect of cooking on the presence of high production volume chemicals in fish

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ABSTRACT

Contaminants such as 2-(methylthio)-benzothiazole (MeSBT) and tris(2-chloroisopropyl)-phosphate (TCPP) have been identified in fish samples for consumption. Since most fish is cooked before being consumed, it is important to assess how cooking affects these contaminants. This study focuses on the impact of common cooking methods (grilling and steaming) on TCPP and MeSBT in sea bass (*Dicentrarchus labrax*) and tuna (*Thunnus thynnus*), two widely consumed species in Spain. The results revealed that cooking has minimal impact on these contaminants, with one notable exception: a significant 28% loss of MeSBT in grilled sea bass. Other reductions ranged between 3% and 9%, possibly due to the dispersion caused by the cooking method themselves, such as differences in heat distribution, moisture loss, or the handling of samples during the process. These findings suggest that cooking slightly lowers contaminants levels, resulting in reduced exposure to TCPP and MeSBT in cooked fish compared to raw samples, indicating that cooking methods, particularly grilling, may help decrease exposure to these contaminants.

1. Introduction

Fish is an important source of protein and nutrients that are essential for a healthy diet (Maulvault et al., 2012). However, contaminants in fish can pose risks to the health of human consumers. The most widely studied contaminants are heavy metals such as mercury and lead, as well as persistent organic pollutants such as polychlorinated biphenyls (PCBs) and dioxins (Bhavsar et al., 2014; Soerensen et al., 2023). However, several studies have also shown the presence in fish of high production volume chemicals (HPVC) (Borrull et al., 2024; Castro et al., 2020, 2023a; Pantelaki & Voutsa, 2020; Trabalón et al., 2015)such as organophosphate esters (OPEs), benzothiazoles (BTs) and phthalates (PAEs), which are included in the list of HPVC reported by the Organisation for Economic Co-operation and Development (OECD) (OECD, 2004). The above list includes compounds that are produced or imported in quantities above 1000 tonnes per year in at least one OECD member country.

The high production of HPVC and their tendency to accumulate in fish adipose tissues are the main reason for their presence in these types of samples (Bekele et al., 2021; Borrull et al. 2024; Castro et al., 2023a, 2023b; Chen et al., 2020; Sala et al., 2022). In the study by Liu et al., (2019) twelve OPEs were determined in three fish species (mud carp, tilapia and plecostomus) with concentrations ranging from 2.5 to 30 ng g^{-1} (wet weight, w.w.). In the above study, tris (2-chloroisopropyl)-phosphate (TCPP) was one of the four most frequently detected compound, accounting for up to 90% of the total concentration of OPEs determined. In the study by Qin et al. (2024), which determined OPEs, the concentrations of TCPP ranged from undetected (n.d.) to 487 ng g^{-1} (dry weight, d.w.) in crucian carp and silver carp. With regard to other, less studied, families of HPVC, benzothiazoles have also been found in fish. In a previous study by our group, which determined BTs in ten species of fish, including tuna, sea bream and sea bass, MeSBT stood out with concentrations ranging from not detected to 468.7 ng g^{-1} (d.w.) (Borrull et al., 2024). It has been shown that a high presence of HPVC in consumer foods can have adverse effects on human health, including neurological, reproductive and developmental issues (Castro et al., 2020; Cheng et al., 2013; van der Veen & de Boer, 2012).

The concentrations present in raw fish may undergo alterations during the cooking process, with effects depending on the contaminant, the fish species and the cooking method (Alves et al., 2017; Barbosa et al., 2018; Trabalón et al., 2018). Since fish are typically consumed cooked, it is important to evaluate the effects of cooking. However, little is known about these effects. The most common cooking methods for

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evaluation are steaming (Alves et al., 2017; Barbosa et al., 2018; Trabalón et al., 2018), grilling (Perelló et al., 2009; Trabalón et al., 2018), frying (Bhavsar et al., 2014; Perelló et al., 2009) and boiling (Bayen et al., 2005; Perelló et al., 2009). Although it is difficult to generalise on account of the scarcity of studies, most research conducted so far has observed a significant drop in contaminants during the cooking process. For example, when Castro et al. (2024) analysed the effect of steaming and grilling on the concentration of two OPEs and one BT in tuna and hake, they found that organophosphate tributylphosphate (TBP) had the greatest loss (roughly 50%) in both cooking processes and both matrices. The concentration of benzothiazole (BT) also decreased irrespective of the cooking process and matrix, but this decrease was in the 12–20% range. On the other hand, tris (2-chloroethyl)phosphate (TCEP) showed a remarkable disparity in the decrease in concentration (10%–50%) depending on the cooking process and matrix.

A similar trend was reported by Sungur et al., (2021), who concluded that their three cooking methods (baking, boiling and frying) were effective in reducing the concentrations of polyfluoroalkyl substances (PFAS) in their tested species (bluefish, mullet, whitefish, pandora, mackerel, perch and smelt). Significant reductions in the concentrations of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were observed after cooking. Particularly notable were the drops in concentration after frying: 70%–86% for PFOA and 82%–89% for PFOS. In another study, Del Gobbo et al., (2008) obtained reductions of 54–100% in PFAS concentrations in 18 species of fish (including catfish, mullet and monkfish) after baking, boiling and frying. Perelló et al., (2009) reported losses in PBDE concentrations ranging from 25 to 44% in sardines, hake and tuna as a result of cooking (frying, microwaving, boiling and baking).

Other studies have reported differences in results depending on the contaminants. For example, Barbosa et al., (2018) reported that steaming decreased the concentrations of perfluorinated compounds by 53–68% but increased the concentrations of polycyclic aromatic hydrocarbons by 73–100% in several species (including sea bream, sole, octopus, mackerel, hake, cod, tuna and salmon). A study by Bhavsar et al., (2014) reported an increase (<20%) in PFOS concentrations after cooking (frying, baking and broiling) in all fish species analysed (carp, salmon, trout and walleye) except boiled and fried carp, which showed no significant variations.

This study aims to evaluated, for the first time, the impact of two common cooking methods in Spain, steaming and grilling, on the concentrations of two HPVC (2-(methylthio)-benzothiazole and tris(2-chloroisopropyl)-phosphate), in two widely consumed fish species in Spain, sea bass (representative species of low-lipid content) and tuna (representative species of high-lipid content). Additionally, the study seeks to provide more accurate data on dietary exposure to these contaminants from cooked fish, focusing on the population of Catalonia (Spain).

2. Materials and methods

2.1. Reagents, standards and materials

For this study we selected 2-(methylthio)-benzothiazole (MeSBT) and tris(2-cloroisopropil)-phosphate (TCPP) as the target compounds and benzothiazole deuterate (BT-d_4) and tributylphosphate deuterate (TBP-d_{27}) as the corresponding internal standards. All standards were analytical grade with purity >98% and were supplied by Sigma Aldrich (St. Louis, USA). Two individual working solutions were prepared: one at 100 mg L^{-1} for the cooking study and another at 1 mg L^{-1} for the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction step. Both solutions were prepared in ethyl acetate (>99.9%) sourced from J.T. Baker (Deventer, The Netherlands).

We used GC-quality acetonitrile (>99.9%) also sourced from J.T. Baker. The Synergy purification system from Millipore (Massachusetts, USA) was used to obtain ultrapure water. The extraction procedure was conducted using the QuEChERS extraction kit (Original Sachets) from Scharlab (Barcelona, Spain), while LipiFiltr® filters were obtained from Carlo Erba (Barcelona, Spain). Helium gas with a purity of 99.999% was used as carrier gas for the gas chromatograph and was obtained from Linde Gas España (Tarragona, Spain). Nitrogen for the collision cell of the triple quadrupole mass spectrometer, was from Carburos Metálicos (Barcelona, Spain).

2.2. Sampling and cooking

One specimen of each fish, which were not alive at the time of purchase, were obtained from the central market in the city of Tarragona (Catalonia) in February 2023. The species selected were sea bass (*Dicentrarchus labrax*) and tuna (*Thunnus thynnus*), which are of Mediterranean origin. These species are among the most consumed by the Spanish population, according to a study by the Ministry of Agriculture, Fisheries and Food (Spanish Government, 2023). In addition, sea bass and tuna are representative of species with a low lipid content (<2%) and a high lipid content (>5%), respectively. The edible part of the fish samples was separated, wrapped in aluminium foil, and frozen at −24 °C until cooking treatment and analysis.

Before cooking, the samples were defrosted and divided into three 200 g fractions: the first was kept raw, the second was grilled, and the third was steamed. Also, before cooking, each fraction was divided into six 30 g portions (three for blank control analysis and three for fortified samples). To fortify the portions, we used a needle connected to a syringe containing a suitable concentration of MeSBT or TCPP. These samples were fortified at 5 μg g^{-1} (w.w.). The whole process was conducted in triplicate for each compound and fish species. All fortified samples were kept in the refrigerator for 24 h to ensure that the compound came into contact with the fish. Fig. S1 schematically summarises the portions and procedure followed until chromatographic analysis.

Grilling was conducted using a preheated pan at 100 °C with each portion of fish kept on either side for 12 min. An oven preheated at 100 °C was used for steaming and each portion of fish was cooked for 30 min while wrapped in aluminium foil. The cooked and raw portions were then freeze-dried using a Genevac miVac system (Ipswich, UK), ground to a fine powder, and stored in a dry room until analysis. Water loss was calculated by weighing the fish portions before and after freeze-drying.

2.3. Analytical method

The procedure used to determine the target contaminants in the samples was based on a method developed by Castro et al. (2023a). First, 0.1 g of freeze-dried fish sample was placed in a 50 mL centrifuge tube, to which 10 mL of ultrapure water and 10 mL of acetonitrile were added and mixed for 1 min. QuEChERS salts (original method) containing 4 g magnesium sulphate and 1 g sodium chloride were then added and vortexed for 3 min. The tube was centrifuged at 4000 rpm for 5 min using a Hettich Universal 32R (Tuttlingen, Germany). The supernatant was removed using a pipette and transferred to a syringe connected to a LipiFiltr® cartridge. The extract was collected in a 20 mL vial and evaporated under a stream of nitrogen to approximately one drop. Then, 100 μL of 1 mg L^{-1} internal standard solution was added to provide a final concentration of 50 μg L^{-1} internal standard. This was then transferred to a flask, filtered through a 0.22 μm PTFE filter (Sharlab), and made up to 2 mL with ethyl acetate.

The analysis was performed using the gas chromatography (GC Agilent 7890A system) coupled to a triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). A ZB-5 capillary column (30 m × 0.25 mm and 0.25 μm film thickness) obtained from Phenomenex (Torrance, CA, USA) was used with a carrier gas flow rate of 1.2 mL/min. The large-volume injector (Agilent multimode inlet, MMI) in solvent vent mode was used to inject 25 μL of the extract. The injector

temperature was gradually increased from 75 °C to 325 °C at a rate of 600 °C/min for 5 min. The oven temperature was set at 75 °C for 3 min, increased to 300 °C at a rate of 30 °C/min, and held for 5 min at 300 °C. The total chromatographic analysis time was 15.5 min.

The triple quadrupole mass spectrometer (QqQ) was operated in multiple reaction monitoring (MRM) mode with an electron ionisation source set at 70 eV and nitrogen gas in the collision cell. The ionisation source, quadrupole one, and quadrupole two were set at temperatures of 280 °C, 150 °C, and 150 °C, respectively. The presence of contaminants was confirmed by matching the retention time, the qualifying ion transitions, and the quantifier/qualifying transition ratio to those of a standard solution. All optimised GC-QqQ parameters are shown in Table S1.

2.4. Dietary exposure

Assessing dietary exposure (E_t) to chemical *t* in the general population involves examining fish consumption and contaminant concentrations for each species, especially after cooking. The results of the survey conducted by the Ministry of Agriculture, Fisheries and Food (Spanish Government, 2023) on fish consumption in the Spanish population in 2023 was used to determine fish consumption.

Exposure levels were calculated using Equation (1) (Castro et al., 2023a), which multiplies average fish consumption (C_f) for each species *f* (g kg bw^{-1} day^{-1}) by the contaminant concentration ($X_{t,f}$) for each species *f* (ng g^{-1}). In line with World Health Organization recommendations (WHO, 2009), values below the limit of quantification were considered to be half the limit of quantification while non-detected values were considered to be half the limit of detection.

$$E_t = C_f X_{t,f} \quad \text{Eq. 1}$$

Two exposure scenarios were simulated: a low-bound (LB) scenario and a high-bound (HB) scenario. The LB scenario was calculated with the geometric mean of the concentrations and the HB scenario was calculated with the 95% percentile of the concentrations.

3. Results and discussion

3.1. Method quality parameters

The analytical method was based on that of a previous study (Castro et al., 2023a). In the present study quality parameters were determined for both species analysed. To check the analytical performance of the method, instrumental linear ranges, instrumental limits of detection (ILODs), instrumental limits of quantification (ILOQs), apparent recoveries (R_{app}), method detection limits (MDLs), method quantification limits (MQLs) and repeatability and reproducibility between days were established. Since the results were comparable for both raw species, only those for sea bass are shown. Procedural blanks and standard solutions were analysed in each bath of samples to confirm the correct performance of the analytical method.

First, instrumental linear ranges were established by injecting standard solutions in ethyl acetate and building the corresponding internal standard calibration curves. Due to the wide range of concentrations used in this study and to enable better quantification, two ranges were obtained for each compound, i.e. from 0.01 to 50 and from 50 to 250 μg L^{-1}, with excellent linearity ($R^2 > 0.999$). Second, the LODs were determined as the concentration that provided a signal-to-background noise ratio of 3, with a value of 0.005 μg L^{-1} for both compounds. LOQs were set at the lowest concentration that could be quantified with the instrumental calibration curves, with a value of 0.01 μg L^{-1} for MeSBT and TCPP. R_{app} (n = 5) for raw and cooked fish were determined by fortifying 0.1 g of freeze-dried fish sample at 2 μg g^{-1} (d.w.). For raw fish results of 80% for MeSBT and 82% for TCPP, were obtained. For the cooked fish, R_{app} (n = 5) ranged from 63% to 82% and were very similar for all species, compounds and cooking methods. The different types of cooking methods therefore did not significantly affect recovery of the extraction process. MDLs and MQLs were estimated for each compound based on their LODs, LOQs and apparent recoveries. MDL for both contaminants was 0.05 ng g^{-1} (d.w.) while MQL was 0.11 ng g^{-1} (d.w.). Repeatability (%RSD, n = 5) and reproducibility between days (%RSD, n = 5) were determined by spiking 0.1 g of the sample at 2 μg g^{-1} (d.w.). Repeatability was 10% for MeSBT and 14% for TCPP, while reproducibility was 20% for both analytes.

3.2. Presence of contaminants in raw samples

Raw samples from both species were analysed. MeSBT was found at concentrations ranging from 2.8 to 3.9 ng g^{-1} (d.w.) in the sea bass samples and from 3.2 to 12.8 ng g^{-1} (d.w.) in the tuna samples. TCPP was not detected in either sea bass or tuna samples.

Wet weight concentrations were also calculated by taking into account loss of water as described in section 2.2. These concentrations were needed to calculate dietary exposure. The water content of the samples was similar in both fish species. Sea bass had a moisture content of 56.2–75.2% while tuna had a moisture content of 73.7–75.1%. The MeSBT concentrations were therefore 1.0–1.6 ng g^{-1} (w.w.) in sea bass and 0.8–3.7 ng g^{-1} (w.w.) in tuna.

MeSBT has been determined in the study by Trabalón et al., (2017) where concentrations of 11–24 ng g^{-1} (d.w.) were reported in seafood samples (cod, salmon, sole, mackerel, mussel, hake, sardine, tuna, shrimp and squid). In a previous study, we found MeSBT concentrations of 3.0 ng g^{-1} (d.w.) in sea bass, while in tuna MeSBT was not detected (Borrull et al., 2024). Other studies found TCPP in species such as mackerel, cod, salmon and hake from the Mediterranean Sea with concentrations ranging from 0.45 to 0.89 ng g^{-1} (d.w.) (Castro et al., 2020) and in carp, tilapia and Plecostomus from South China with concentrations ranging from 0.63 to 8.9 ng g^{-1} (w.w.) (Liu et al., 2019). We also determined TCPP in a previous study at concentrations of 1.1–1862.2 ng g^{-1} (d.w.) in species such as hake, blue whiting, swordfish, squid, mackerel and sardine (Borrull et al., 2024).

3.3. Cooking effect

To evaluate the cooking effect, 0.1 g of samples of sea bass (n = 3) and tuna (n = 3) were fortified at 5 μg g^{-1} (w.w.) as described in section 2.3. These samples were fortified at a higher concentration than was found in the raw samples to ensure that the amount of compound in the raw samples did not affect the calculation of the cooking effect. Our results are summarised in Fig. 1, which shows the variations in the mean concentration of the analytes for the cooking treatments of the two fish species.

The MeSBT concentration in grilled sea bass showed a significant reduction of 28%. In contrast, all other results were very low or almost negligible with losses between 3 and 9%, which can be attributed to the precision of the method used, as well as factors such as variations in heat distribution, moisture loss, or sample manipulation during the process. Some authors assert that certain neutral organic compounds tend to associate with fatty tissues, causing them to associate in the form of oil during cooking (Bhavsar et al., 2014). For example, TCPP, which has a logK_{ow} of 2.59, tends to accumulate in fatty substances, which makes its loss less likely. On the other hand, MeSBT, which has a logK_{ow} of 1–2, tends to accumulate less in fatty tissues and is removed more easily. This could explain why MeSBT shows higher average losses in some cooked fish than TCPP, whose losses are almost negligible. However, in our study there seems to be no correlation between loss and lipid content.

Other characteristics should also be taken into account. For example, differences in fish measurements may contribute to differences in results (for example, using small pieces or large portions affects moisture/fat loss). The part of the fish where the portion is extracted should also be taken into account since areas closer to the tail have more lipids than the centre or back of the fish. The study by Elmasry and Wold (2008)

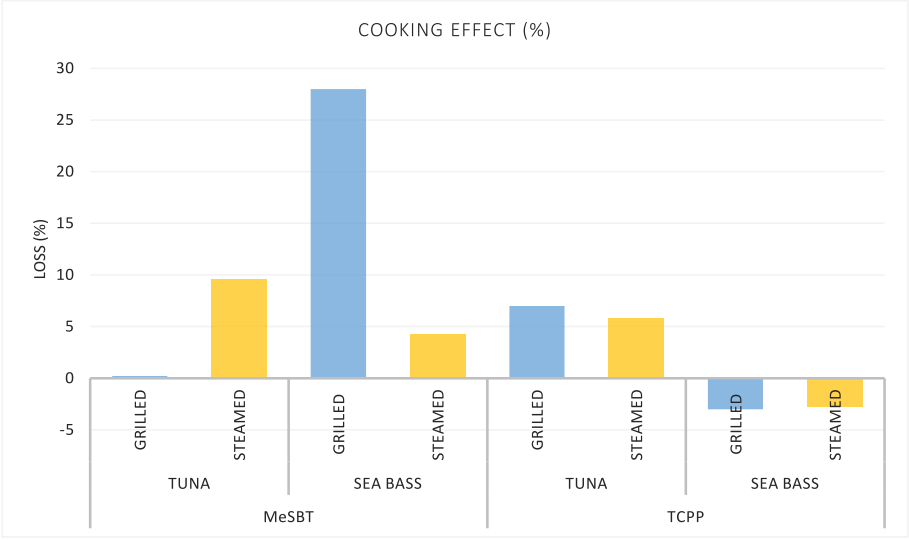

Fig. 1. Effect of the cooking process on analyte concentration, where positive values represent contaminant losses and negative values represent contaminant gains.

illustrated the distribution of fat and water contents in various fish fillets, with more fat content observed at the edges and in the belly of the fillet than in the central part. To minimise this error, in this study we tried to make all portions as identical as possible and to extract them from the same areas of the fish.

It is difficult to find studies similar to the present one since this is the first time that losses of these two compounds during fish cooking processes have been evaluated. An example with similar compounds could be the study by Castro et al. (2024), who examined the losses of TBP, TCEP and BT in grilled and steamed tuna and hake samples. Their study showed greater losses (11%–61%) for TBP, TCEP and BT, with the greatest losses being found in grilled samples. In the study by Fierens

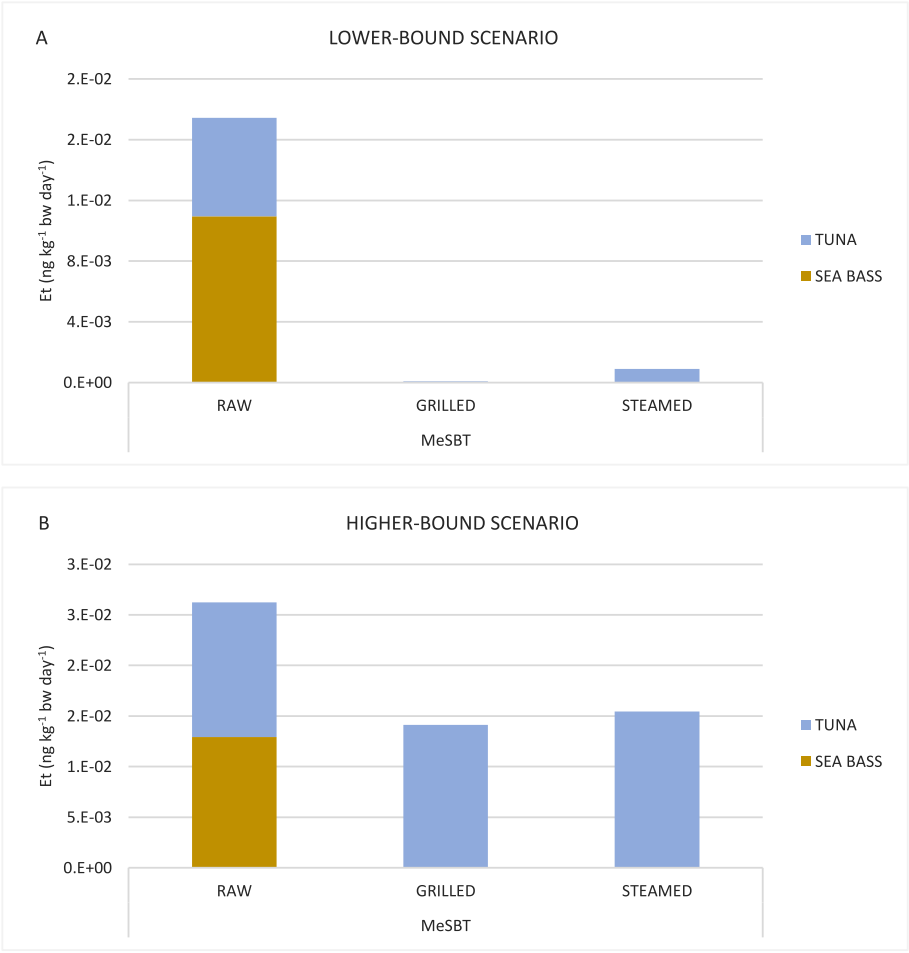

Fig. 2. MeSBT dietary exposure index scenarios (ng kg^{-1} bw day^{-1}): a) LB scenario and b) HB scenario.

et al., (2012), losses of various compounds in fish were analysed using different cooking methods. These authors found differences between steaming with and without aluminium foil. Grilling with aluminium foil increased the content of di-(2-ethylhexyl) phthalate in salmon but a sixfold increase was observed when aluminium foil was not used. Moreover, at least a 27-fold increase was observed when salmon was steamed while wrapped in aluminium foil. Grilled salmon without aluminium foil also contained less dimethyl phthalate, isobutyl phthalate and di-n-butyl phthalate but more benzylbutyl phthalate than steamed salmon. In conclusion, using aluminium foil when cooking salmon may affect contaminants such as phthalates, with some compounds increasing and others decreasing. The study by Barbosa et al., (2018) also reported that different cooking methods have different effects on compounds. Other compounds, such as musk fragrances, showed an increase in compound concentration after steaming: some species whereas others showed a decrease. Galaxolide, for example, showed a 60% increase in sole and a 37% decrease in mackerel, while tonalide showed a 75% increase in sole and a 21% decrease in crab after steaming (Barbosa et al., 2018).

3.4. Dietary exposure

Dietary exposure values were calculated only for MeSBT because TCPP was not found in the raw samples. Exposure to MeSBT was significantly higher in raw sea bass than in grilled sea bass. No significant differences were observed for all other species and cooking types, though the raw samples displayed slightly higher dietary exposure values. The LB scenario results can be seen in Fig. 2A and the HB scenario results, in Fig. 2B.

Both the LB and the HB scenarios showed lower exposure in the cooked samples than in the raw samples for both fish species, except for tuna in the HB scenario, whose values were similar for both the cooked and the raw samples (approximately $2.5{\cdot}10^{-2}$ ng kg^{-1} bw day^{-1}). In this scenario, the minimal cooking losses were negligible in the exposure calculations. The most significant reductions in exposure values were also found in the HB scenario, where sea bass displayed an exposure value of $1.3{\cdot}10^{-2}$ ng kg^{-1} bw day^{-1} in the raw samples and 10^{-6} ng kg^{-1} bw day^{-1} in the cooked samples. Similarity, in the LB scenario, significant decreases were observed, with values in raw fish reaching $2{\cdot}10^{-2}$ ng kg^{-1} bw day^{-1} after cooking (e.g. steaming).Calculating dietary exposure from these cooking values obtains a more realistic E_t since the vast majority of consumers cook fish before eating it. Exposure values for MeSBT decreased significantly from raw to cooked fish due to loss of the analyte during cooking.

Our results could not be compared with other studies as this is the first time these compounds have been determined in cooked samples. The comparison was therefore made with studies that used raw samples. For example, the following studies compared similar compounds but did not consider the cooking effects: Castro et al. (2023b) showed comparable E_t values, with contaminant BT ranging from $1.0{\cdot}10^{-1}$ to 2.26 ng kg^{-1} bw day^{-1}; Trabalón et al., (2017) identified MeSBT with the highest dietary intake value (22 ng kg^{-1} bw day^{-1}); in a recent study, Borrull et al. (2024) reported slightly higher TCPP values ranging from $1.5{\cdot}10^{-3}$ (LB) to $4.4{\cdot}10^{-1}$ (HB) ng kg^{-1} bw day^{-1} in sea bass; Liu et al., (2019) presented higher TCPP values ranging from $3.0{\cdot}10^{-2}$ to 6.3 ng kg^{-1} bw day^{-1}; and Castro et al., (2020) reported OPEs ranging from 1.2 to 1.9 (LB) and from 2.5 to 4.7 (HB) ng kg^{-1} bw day^{-1}.

4. Conclusions

The cooking effect on MeSBT and TCPP in fish samples has been determined for the first time. The results showed a significant reduction of MeSBT in grilled sea bass, on the other hand the results of steaming MeSBT and grilled and steamed TCPP in sea bass and tuna showed very low reductions which is attributed to the precision of the method.

This study underlines the importance of the cooking method in dietary intake assessments, as it can alter contaminant concentrations to a greater or lesser extent. The loss of contaminant concentrations during cooking results in slightly lower exposure values for cooked samples compared to uncooked samples. The highest exposure was observed in the HB scenario, which showed a significant decrease for cooked sea bass samples, i.e. 10^{-6} ng kg^{-1} bw day^{-1} compared to the raw samples at $1.3{\cdot}10^{-2}$ ng kg^{-1} bw day^{-1}, which represents a substantial reduction.

CRediT authorship contribution statement

Sílvia Borrull: Writing – original draft, Validation, Resources, Investigation, Formal analysis. **Francesc Borrull:** Supervision, Funding acquisition. **Eva Pocurull:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Rosa M. Marcé:** Writing – review & editing, Supervision, Methodology, 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.foodcont.2025.111175.

Data availability

Data will be made available on request.

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