# LEVELS OF ORGANOTIN COMPOUNDS IN DIET SAMPLES FROM PORTUGAL - PRELIMINARY RESULTS FROM A DUPLICATE DIET STUDY

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## Introduction

Organotin compounds (OTs) are ubiquitous contaminants used as PVC stabilizers, antifouling biocides, agricultural pesticides and catalysts for the production of polyurethanes and silicones[1]. Despite broad range of applications, their notoriety is due to the outstanding biocidal properties of triorganotin derivatives, particularly tributyltin (TBT), used for decades as an active ingredient in antifouling paint formulations. Its widespread use led to the global contamination of the marine environment at an incomparable scale and to a wide range of deleterious effects in aquatic ecosystems[1]. Due to its toxicity and persistency, TBT use was banned in September 2008 on a global scale and as a result the levels of this compound in the aquatic environment (including seafood) are declining[1]. However, the TBT story is far from reaching an end; in fact, the discovery of its obesogenic potential placed it again in the forefront of scientific research. Several *in vitro* and *in vivo* studies with gastropods, amphibians, fish[5] and particularly with mice[2- 6], demonstrated that TBT is able to interfere with adipocyte differentiation and/or proliferation. Such results demonstrated for the first time that an environmental contaminant was able to alter the metabolic homeostasis and could therefore play a major role in the development of obesity[7]. TBT was the first obesogenic compound described and is probably the most studied, being considered a model obesogen[8]. Bearing in mind that obesity is a global epidemic, a great deal of effort has been undertaken not only to better describe the role of environmental contaminants in the etiology of this disease but also in their mode of action. Hence, basic knowledge on the average intake of these contaminants is mandatory.

In the present work we quantified the levels of organotin compounds, including the model obesogen TBT, in duplicate diet samples from members of University of Aveiro community, in order to characterize the intake of these compounds.

## Materials and Methods

### Sample collection

In May 2012 a duplicate diet study was launched at the University of Aveiro. Volunteers willing to provide a duplicate sample of their diet for seven consecutive days, while maintaining their regular dietary habits, were recruited. A total of 51 volunteers (including students, researchers and professors) participated in this study by providing samples of all the solid food items consumed at every meal, including small snacks, for one week. Participants kept the food items in polyethylene bags, maintained at their home freezer and by the end of the sampling period the samples were handed to the researchers involved in the project. A daily registry of all the food items consumed was also provided by each participant.

Once in the laboratory, the content of the different bags provided by each participant were pooled together and homogenized. The samples were freeze dried and preserved at -20$^0$C until chemical analysis. To date, nineteen samples were analyzed and the results here presented correspond to those samples.

### Organotin analysis

Organotin compounds were quantified by isotope dilution method according to the protocol described by Sousa et al.[9]. Approximately 1.0 g of freeze dried sample was spiked with deuterated labeled standards ($d_9$-MBT, $d_{18}$-DBT, $d_{27}$-TBT, $d_{10}$-DPT, $d_{15}$-TPT, $d_{17}$-MOT, $d_{34}$-DOT) before extraction. OTs in the samples were extracted by 1N HBr/methanol-ethyl acetate (1:1), transferred into ethylacetate/*n*-hexane (3:2) and concentrated by rotary evaporation. OTs in the extract were then ethylated by adding 1mL of 5% tetraethyl sodium borate. After ethylation, the extract was cleaned up by 2M KOH and SEP-PAK plus florisil cartridge (Waters). OTs were

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eluted by 5% diethylether/hexane and the solutions were concentrated to 1 mL and spiked with 50 ng of deuterated tetrabutyltin (used as a recovery standard). The final solutions were injected into a gas chromatograph-mass spectrometric detector (GC-MSD) (Hewlett-Packard 6870 GC system with 5973 mass selective detector and 7683 series auto sampler) and measured in selected ion monitoring mode (EI-SIM). Average recovery rates ± St Dev (%) for each surrogate compound in the 19 analyzed samples were: $d_9$-MBT: 63.4 ±24.0, $d_{18}$-DBT: 72.6±9.0, $d_{27}$-TBT: 79.7±6.0, $d_{10}$-DPT: 76.8±24.2, $d_{15}$-TPT: 153.5±13.1, $d_{17}$-MOT: 68.0±19.1 and $d_{34}$-DOT: 154.0±22.4. QA/QC assessment was performed analyzing two certified reference materials - fish tissue (NIES CRM No.11) and mussel tissue (ERM-CE 477) by the same method. The obtained results from 3 replicate analyses of both CRMs were within ±10% of the certified values. In addition, a procedural blank was included, with each analytical batch, to check for interfering compounds and to correct sample values, if necessary. Methods gave a tin detection limit (in terms of ng Sn $g^{-1}$ ww) between 0.04-1.3 for MBT; 0.15-1.07 for DBT; 0.04-0.11 for TBT; 0.04-0.19 for DPT; 0.04 for TPT; 0.07-2.3 for MOT and 0.19-0.37 for DOT.

**Results and Discussion**

Organotin compounds were detected in 47% of the diet samples analyzed (Figure 1). Generally, the levels of butyltins and octyltins are low whereas phenyltins were never detected. Amongst butyltins, tributyltin was detected only in one sample (0.19 ng Sn $g^{-1}$ ww); dibutyltin in two samples (0.25 and 0.54 ng Sn $g^{-1}$ ww) and monobutyltin in four samples with levels between 0.18 and 0.69 ng Sn $g^{-1}$ ww. A similar trend with higher detection frequencies for the mono substituted compounds was observed for octyltins with monooctyltin detected in four samples (0.12 - 3.42 ng Sn $g^{-1}$ ww) and dioctyltin in one sample (0.51 ng Sn $g^{-1}$ ww). Organotin distribution pattern is probably associated with the profiles of OTs usage. TBT was used as a biocide in antifouling paints but its global ban lead to a decrease on its levels in the aquatic environment, and therefore, in seafood items. Mono- and di- substituted compounds are mainly used as stabilizers in the plastics industry and as catalysts in the production of polyurethane foams and silicones and, consequently, these compounds can be found in many products that are in contact with food such as silicone molds, plastic containers, kitchen utensils and water pipes, for example[1].

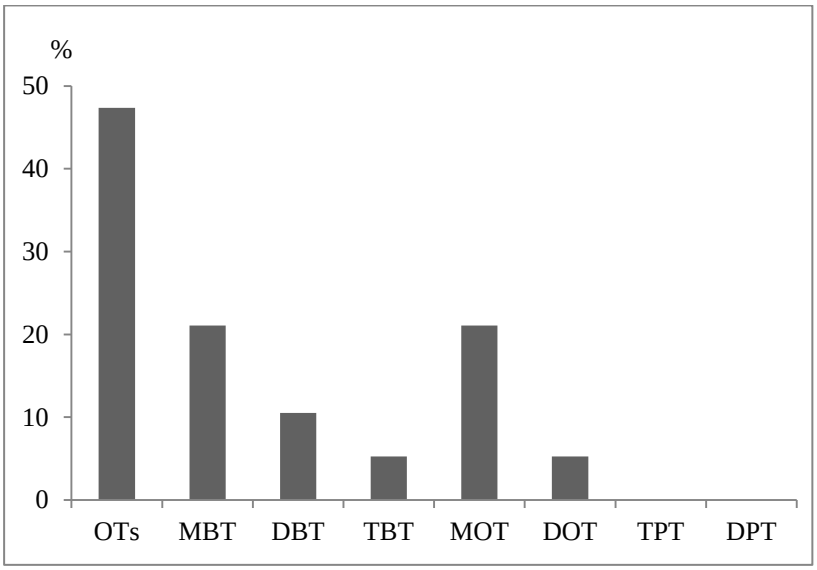


**Figure 1.** Detection frequency of each organotin compound in the nineteen duplicate diet samples analysed.

The overall low levels of organotins can be explained if we consider the intrinsic characteristics of the duplicate diet samples collected. As people tend to eat greater amounts of carbohydrates than proteins, the duplicate samples provided also reflected this tendency. Hence, the amount of bread, pasta, and rice was usually much higher than the amount of meat or fish. Data on the levels of organotins in food items other than seafood are limited but even so, available publications disclose levels below the limit of detection. Results from a survey of butyltins in food items back in the early 1990s revealed that farm products were not contaminated by butyltins

(<3.5 ng $g^{-1}$ wet weight (ww)) whereas fish samples exhibited very high levels (up to 340 ng $g^{-1}$ ww)[10]. More recent data on a market-basket study performed in Finland, exposed the same tendency, with organotins (MBT, DBT, TBT, MPT, DPT, TPT and DOT) always below the limit of detection in most of the food items (e.g. cereals, dairy products, fats) except for seafood and some vegetables and fruits[11]. Hence, the low levels of organotins in duplicate diet samples such as the ones collected in the present survey were expectable.
In order to estimate organotin intakes from diet and considering that most of the OTs in the analyzed samples were below their limits of detection, we followed the recommendation made by Rantakokko et al.[11] and attributed zero to all the samples that exhibited values below the limit of detection. Table 1 depicts the obtained results, taking into consideration only those samples for which organotins were detected.

**Table 1.** Estimated daily intakes (on Sn basis) based on an average adult weight of 70 kg and the average food consumption in Portugal for 2012 (1870 g. $inhabitant^{-1}$. $day^{-1}$). Data provided by the National Institute of Statistics (INE, 2014)[12].

| | Estimated daily intake (ng Sn $kg^{-1}$ body weight. $day^{-1}$) |
|---|---|
| MBT | 4.8-18 |
| DBT | 6.6-14 |
| TBT | 0-5.0 |
| MOT | 3.3-91 |
| DOT | 0-14 |
| ∑OTs | 4.8-95 |
| ∑DBT, TBT, DOT | 5-14 |

The total daily intake for the sums of all the organotins detected varied between 4.8 and 95 ng $kg^{-1}$ body weight per day. If we consider only those organotins, that, according to the European Food Safety Authority (EFSA)[13] have similar mode of action and equivalent potency, i.e., DBT, TBT, TPT and DOT the estimated intakes varied between 5 and 14 ng $kg^{-1}$ body weight per day. The tolerable daily intake set by EFSA for the sum of DBT, TBT, TPT and DOT of 100 ng $kg^{-1}$ body weight per day (on Sn basis) is much higher than our maximum daily intake and therefore risks are negligible. However the critical toxicological endpoint used by EFSA for risk assessment was immunotoxicity. Recent studies suggest that more sensitive endpoints (e.g. endocrine disruption and obesogenic effects) should also be considered[1, 14], and therefore it is expected that this TDI will be revised in the future. In addition, TDIs are not available for other OT compounds such as MBT and MOT, which also should be considered. Nevertheless, our results disclose low levels of organotins in duplicate diet samples that represent in average less than 10% for the recommended TDI; therefore the risk associated with food ingestion is low. Future work on human exposure to organotin compounds should focus on other exposure pathways such as dermal contact, inhalation and especially ingestion of house dust in which considerable high concentrations of organotins were often detected[15,16].

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