

## Butyltin Compounds in Portuguese Wines

MANUEL AZENHA\* AND MARIA TERESA VASCONCELOS

LAQUIPAI, Chemistry Department, Science Faculty, University of Oporto,  
 R. Campo Alegre 687, 4169-007 Porto, Portugal

Butyltin compounds are widespread contaminants that have also been found in some wines. The purpose of the present work was to make a survey of butyltin compounds in Portuguese wines. Forty-three table wines and 14 Port wines were analyzed for butyltin contents by using solid-phase microextraction gas chromatography mass spectrometry (SPME-GC-MS). In 14% of the analyzed wine samples, measurable dibutyltin (DBT) was found at concentrations ranging between 0.05 and 0.15  $\mu\text{g/L}$  as Sn. Monobutyltin (MBT) was also observed (0.05  $\mu\text{g/L}$  as Sn) in just a single wine. A search for the possible sources of DBT residues found in the wines was carried out. Therefore, some plastics and oak wood used in the process of wine-making, which have been directly in contact with the musts or the wines, were studied to check their possible release of butyltins. The eventual presence of DBT was also tested directly along the vinification process, from the must to the finished product. The results suggest that high-density polyethylene containers used in the transfer of wine in an early stage of the vinification process may be the main sources of these contaminants. Therefore, it is recommendable that plastic materials to be used in wineries be previously tested for the release of butyltin compounds.

**KEYWORDS:** Wine; butyltin; plastics; vinification

### INTRODUCTION

Butyltin compounds, monobutyltin (MBT), dibutyltin (DBT), and tributyltin (TBT), are widespread in the environment due to their large-scale use for the production of biocides and polymer stabilizers (1). MBT and DBT are mainly used as heat and light stabilizers for PVC materials. DBT is also increasingly used in water-based varnishes, in which it serves as a binder (2). The main application of TBT is as a biocide in marine antifouling paints.

The negative impact of butyltins on the aquatic environment is well documented and has led to regulations on the use of TBT-based antifouling paints in many countries (3). In contrast, contamination by MBT and DBT released from their use in commercial and household articles has received less attention. Particularly, the extent of exposure of humans to butyltins is relatively unknown.

A few cases of fatal poisoning are reported in the literature (1). Symptoms of dialkyl- and trialkyltin poisoning are vomiting, headache, visual defects, and electroencephalographic abnormalities. Irritation of the skin and the respiratory tract is caused even by a short contact with these compounds (1). Studies dealing with the accumulation of organotins in human tissues are scarcely available. The occurrence of butyltin compounds in human blood and liver (4, 5) has been reported.

In vitro studies have shown that butyltins disrupt the immune response in humans (6, 7) and rats (8, 9). One effect is on the natural killer cells (NK cells) that play a major role in the body's

immune defense against infections and cancer. Also the survival, proliferation, and differentiation of normal human B lymphocytes are compromised. A study (4) involving blood analysis of 38 volunteers from Michigan showed a concentration of butyltin ranging from below the detection limit and to 155  $\mu\text{g/L}$ . Some blood samples had butyltin concentrations comparable to those at which reduced human NK cell activity and B lymphocyte toxicity were recorded.

Two main routes of human exposure are suggested: direct ingestion of contaminated food and drink and indirect exposure from household items containing butyltin compounds. Seafood collected from coastal areas and lakes contain varying amounts of butyltin compounds. Studies have shown that organotins leach from poly(vinyl chloride) (PVC) and related materials, resulting in contamination of foodstuff and beverages (1). Several household commodities made of polyurethane, plastic polymers and silicones contain several organotin species on the order of up to micrograms per gram (5). Another possibility of human contact with organotins is clothes moistened with the vapor or liquid of these chemicals for textile impregnation (1).

Butyltin compounds were found in wines on the Canadian market (10), mainly DBT with concentrations up to 138  $\mu\text{g/L}$ , apparently associated with the use of PVC for wine transportation. In a subsequent study, also with wines on the Canadian market (11), lower values were obtained for DBT concentrations (up to 1.44  $\mu\text{g/L}$ ). The purpose of the present work was to make a survey of butyltin compounds in Portuguese wines.

\* mazenha@fc.up.pt

Table 1. GC-ITMS Operation Parameters

GC Parameters	
injection mode	splitless
injection port temperature (°C)	250
column flow (mL/min)	1.3 (He)
temperature program	80 °C (1 min) × 1.5 °C/min; 135 °C × 20 °C/min; 250 °C (5 min)
ITMS Parameters	
transfer line temperature (°C)	170
ion trap temperature (°C)	170
ionization mode	electrical ionization/autmode
filament current (μA)	10
ion preparation	selective ion storage ( <i>m/z</i> range 150–300)

## EXPERIMENTAL PROCEDURES

**Apparatus.** The gas chromatography–ion trap mass spectrometry (GC-ITMS) system consists of a gas chromatograph (Varian 3800, Varian, Walnut Creek, CA) equipped with a split/splitless injector port, an SPME 0.8 μm desorption liner, a microseal septum system (Merlin, Half Moon Bay, CA), a DB-5MS 60 m length × 0.250 mm diameter column (J&W Scientific, Folsom, CA), and a Saturn 2000 ion trap mass detector (Varian). The system was computer-controlled using the Varian Saturn workstation software.

**Operation of the GC-ITMS system.** The polydimethylsiloxane (PDMS) fiber with the extracted ethyl-derivatized butyltin compounds was introduced through the Merlin microseal and thermally desorbed during 1.5 min in the liner. Operation parameters of the system are listed in Table 1.

**Reagents.** The carrier gas for the chromatography was helium of high purity, 99.9999%, supplied by Praxair, Madrid, Spain. The organotin compounds, tetramethylammonium hydroxide 25% w/v solution (TMAH), and sodium tetraethylborate (NaBEt<sub>4</sub>) were obtained from Sigma-Aldrich (Milwaukee, WI). Other solvents and common laboratory reagents were of analytical grade. Deionized water with conductivity below 0.1 μS/cm was used throughout the work. Prior to use, all of the glass- and plasticware was immersed overnight in a 20% nitric acid bath. Individual stock solutions of organotin compounds (2 g/L as Sn) were prepared by dissolving the equivalent amounts in ethanol and stored in the freezer. A mixed MBT, DBT, and TBT 50 mg/L as Sn solution was prepared in ethanol by dilution of the individual stock solutions and was also stored in the freezer. A more diluted mixed solution, 0.25 mg/L, was prepared daily in ethanol. NaBEt<sub>4</sub> solution (2.5% w/w) was prepared monthly in THF, under N<sub>2</sub> atmosphere, and stored at 4 °C in the dark.

**Wine Samples and Winery Materials.** The 43 different table wines analyzed were commercially available on the market (price range = 1.5–8 Euros/0.75 L), which included red, white and Verde white wines. Verde designates a type of wine exclusively produced in a demarcated region of northwestern Portugal, where unique varieties, which produce musts moderately rich in sugar but rich in acid, with relatively low pH, result in wines with ethanol percentages ranging between 8.5 and 11%. All of the major Portuguese wine regions were represented: Alentejo (8 samples), Douro (11), Verde (7), Dão (3), Varosa (1), Beiras (3), Terras do Sado (3), Trás-Os-Montes (1), Bairrada (2), Pico-Açores (1), Ribatejo (1), and Extremadura (2). The study was extended to 14 different Port wine samples, all supplied by the Port Wine Institute, Oporto, Portugal.

To investigate the possible release of butyltins into musts or wine, samples collected from different stages of the process of wine production were also analyzed, as well as wine or an acetic acid/acetate buffer (pH 4) solution after it had been in contact with some plastic materials and oak wood for 1 or 2 h. A wine producer from the Douro region provided the authors with all of the samples and materials used in this study. A brief scheme of the vinification procedure used by that producer is presented in Figure 1, where the sampling points are indicated.

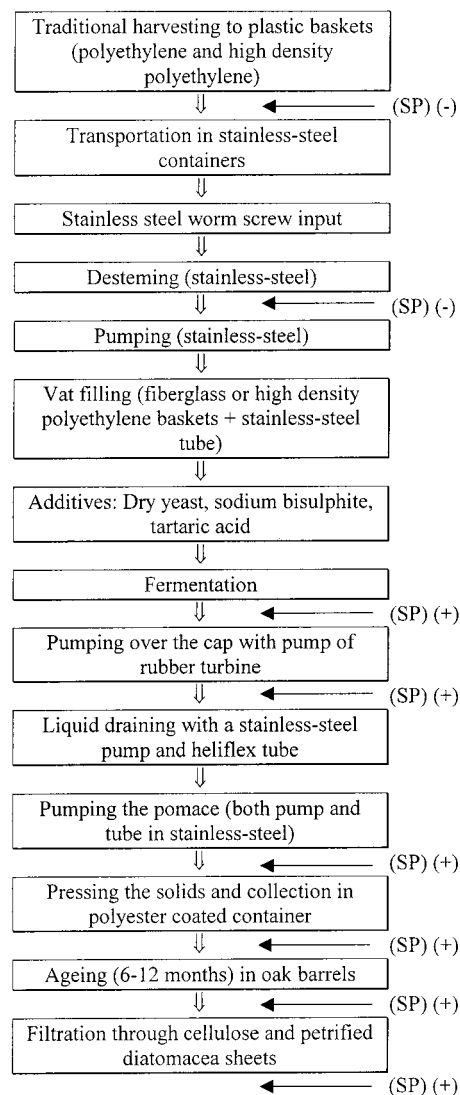


Figure 1. Brief scheme of the vinification procedure. The sampling points (SP) used for collection of samples are indicated, as well as the respective qualitative result for DBT analysis: (+) detected or (–) not detected.

Table 2. Headspace Operation Conditions

TMAH pretreatment (sample/TMAH ratio)	8:2 or 5:5 when analyzing DBT only; 50 min at 40 °C
pH	adjusted to 5 with acetic acid
matrix modification	10% salt (table wines only)
extraction mode	headspace
fiber type	100 μm PDMS
derivatization	in situ, with 5 μL of NaBEt <sub>4</sub> 2.5% w/w in THF
extraction temperature (°C)	40
extraction time (min)	20 or 30

**Procedures. Headspace SPME Conditions.** The conditions set for the analysis were optimized in a previous work (12) and are presented in Table 2. The fibers (Supelco, Bellefonte, PA) were handled with a Supelco manual holder. The extractions were performed inside 22 mL Supelco SPME vials (22 × 75 mm) filled with a volume of 10 or 11 mL. The vial contents were magnetically stirred at the maximum rate allowed by the stirrer (model M21/1, Framo-Gerätetechnik, Eisenbach, Germany) by means of 12 × 4.5 mm magnetic stir bars (Kartell, Noviglio, Italy).

**Analytical Figures of Merit of the Method.** The reproducibility of three independent analyses carried out on three consecutive days was 9% for MBT and 8% for DBT and TBT (relative standard deviation, RSD, *n* = 3).

**Table 3.** Conditions Used for Screening the Dibutyltin Release by Some Materials That Have Been Directly in Contact with the Product in Some Steps of the Vinification Process

material	use in wine-making/mean contact time	experimental conditions used to test the release of dibutyltin
polyethylene	harvest/3–4 h with grapes	2 h with acetate/acetic acid buffer (pH 4) at 20 or 40 °C
glass fiber with undetermined coating	must handling/0.5 h	1 h with acetate/acetic acid buffer (pH 4) at 20 or 40 °C
high-density polyethylene	wine handling/4 h	4 h with Port or red wine, at 20 or 40 °C
flexible PVC	must transfer/2 h wine refilling/30 min	2.5 h with Port or red wine, at 20 °C
oak wood	wine aging/6–12 months	7 days at 40 °C with red wine

**Table 4.** Butyltin Compound Concentrations Found in Portuguese Wine Samples

wine type	origin	[butyltin] ( $\mu\text{g/L}$ as Sn)
	DBT	
red	Douro	0.15 $\pm$ 0.02
red	Douro	0.13 $\pm$ 0.01
red	Alentejo	0.07 $\pm$ 0.01
white	Douro	0.10 $\pm$ 0.01
white	Extremadura	0.08 $\pm$ 0.01
white Verde	Verde wine region	0.15 $\pm$ 0.02
Port	Douro	0.15 $\pm$ 0.03
	MBT	
Port	Douro	0.05 $\pm$ 0.01

The limit of detection (LOD) determined from the data of the linear regression of the standard addition curve (13) was greatly dependent on the butyltin species and on the type of wine (white, red, and Port), as was demonstrated previously (12). The general trend for the LOD values was MBT (0.05–0.2  $\mu\text{g/L}$  as Sn) > DBT (0.02–0.1  $\mu\text{g/L}$  as Sn) > TBT (0.01–0.05  $\mu\text{g/L}$  as Sn). Among the different types of wines, in general, the LOD decreased as follows: white (0.02–0.2  $\mu\text{g/L}$  as Sn) > red (0.02–0.1  $\mu\text{g/L}$  as Sn) > Port (0.01–0.08  $\mu\text{g/L}$  as Sn).

*Release of Butyltins from Winery Plastics and Oak Wood.* Some plastics and oak wood used in the process of wine-making by a collaborate winery, having direct contact with the musts or the wines, were studied to check their possible release of butyltins. **Table 3** summarizes the conditions used for the experiments, which were considered to broadly mimic the real winery conditions. The release from materials having contact with must was studied at typical must pH with an acetic acid/acetate buffer (pH 4) at 20 and 40 °C. The reason for using the acetate buffer is that it favors a high ethylation yield for the reaction of butyltin compounds with  $\text{NaBEt}_4$  (14).

## RESULTS AND DISCUSSION

**Survey of Butyltin Compounds in Portuguese Wines.** Butyltin compounds were assessed in 57 different Portuguese wines from different regions, including 14 Port wine samples. Measurable amounts of DBT were found in seven (12%) of the analyzed wines, and MBT was found in just one sample (details in **Table 4**). The DBT was also detected (but not quantified) in another 6 samples (2 Ports, 3 whites, and 1 red table wine), and MBT was measurable in 1 wine and detected in another 8 wines representing the three types of studied wines (Port, white, and red table). TBT was not detectable in any of the studied wines.

Therefore, DBT was the predominant butyltin compound, and the determined levels were between 0.07 and 0.15  $\mu\text{g/L}$  (as Sn). MBT was quantified in one Port wine at 0.05  $\mu\text{g/L}$  (as Sn). In a previous study on Canadian wines and other wines imported by Canada, Forsyth and co-workers (10, 11) also observed the predominance of DBT among the organotin compounds tested. However, in some wines, those authors found much higher concentrations [up to 138  $\mu\text{g/L}$  in a study carried out in 1992 (10) and up to 1.44  $\mu\text{g/L}$  in another study in 1994 (11)] than

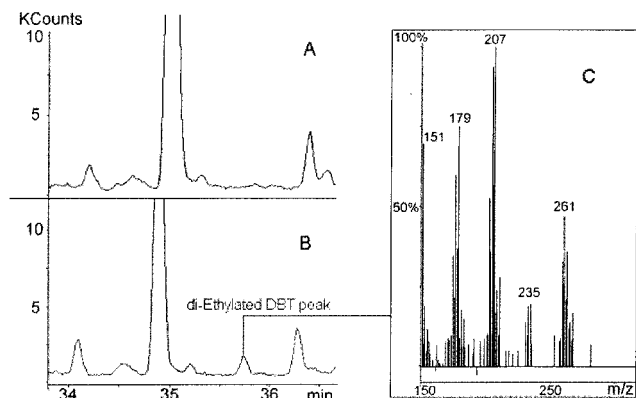
those present in the Portuguese wines studied in the present work. Those high concentrations were found in imported wines (bottled in Canada), which were exposed to PVC during transportation. In wines bottled at the origin, Forsyth and co-workers have found concentrations similar to those obtained in the present study; the source of the contamination was not discussed.

Presently there is not an official tolerable daily intake (TDI) value for DBT, although 5  $\mu\text{g}$  of Sn/kg of body weight has been advanced by Summer and co-workers (15) as a proposed TDI. An adult weighing 60 kg who drinks 1 L of wine containing 0.15  $\mu\text{g/L}$  as Sn of DBT (the highest concentration found in this survey) would intake 0.0025  $\mu\text{g/kg}$  of body weight, which is 2000 times lower than the referred proposed TDI. For a wine containing 138  $\mu\text{g/L}$  as Sn of DBT (10), drinking 1 L would imply an intake of 2.3  $\mu\text{g/kg}$  of body weight for an adult of 60 kg. This intake value poses high concern because, considering the sum with all other possible sources (dietary or household items related), the proposed TDI may be exceeded. History shows that TDI values tend to be lowered over the years, which means that lower values than that proposed by Summer and co-workers may be expected to be established in the future by national and international organizations. Therefore, it may be advisable and safer to produce wine (and other drinks and food) with as low butyltin and other organotin concentrations as possible. For that reason, the eventual sources of the butyltin compounds found in the Portuguese wines were investigated.

**Search for Possible Sources of DBT Residues Found in Table Wines from the Douro Region.** The three table wines from Douro, mentioned in **Table 4**, containing 0.10–0.15  $\mu\text{g/L}$  as Sn of DBT, were produced by the same winery, which used modern but as natural as possible procedures (see **Figure 1**). In collaboration with the enologists of this winery, different plastic materials and oak wood that have been in contact with the must during vinification or the wine were identified and, whenever possible, samples were collected to be studied for their potential release of DBT. In parallel, during the wine manufacturing, samples collected at the output of different steps of vinification were also analyzed for the presence of DBT.

Among the various materials studied (see *Release of Butyltins from Winery Plastics and Oak Wood*), only the high-density polyethylene, used for handling the wine in one of the earliest steps of vinification, released measurable amounts of DBT either to red wine (at 40 °C, but not at room temperature) or to Port wine (at either room temperature or 40 °C). **Figure 2** illustrates, for a red wine, the GC-MS chromatogram obtained for the wine before (**Figure 2A**) (the absence of a peak at 35.7 min indicates undetectable DBT) and after (**Figure 2B**) exposure of that wine to high-density polyethylene. In the last case, the DBT peak occurred, its identification being confirmed by its mass spectrum (**Figure 2C**), which matched that obtained for a DBT standard.

These results are compatible with those obtained for the samples collected through the vinification process. Must pre-



**Figure 2.** GC-ITMS chromatograms showing the diethyl-DBT peaks obtained for a red wine before (A) and after (B) exposure to high-density polyethylene at 40 °C. The mass spectrum recorded at the diethyl-DBT peak is also presented (C).

pared in the laboratory with grapes collected by us in polyethylene bags presented a nondetectable DBT concentration, which indicated negligible contamination in the vineyard. However, at the output of the fermentation vat and at the subsequent steps, DBT contamination was already detected in the product (see **Figure 1**), indicating that the contamination occurred in an early step of the vinification process. Therefore, the available information about the DBT contamination in this case study suggests that possible DBT sources are the plastic materials used for manipulating must or wine in the early stages of vinification.

**Conclusion.** A survey of butyltin compounds in Portuguese wines showed that 14% of the 57 analyzed samples contained measurable DBT or MBT (only 1 sample). Some high-density polyethylene materials used in the process of vinification may be the main sources of this contaminant. Therefore, this topic deserves further investigation, and it is recommended that potential release of butyltin compounds from plastic materials to be used in wineries be tested.

#### ACKNOWLEDGMENT

We thank the Port Wine Institute (Porto) for providing the Port wine samples and Douro's collaborate winery for supplying materials, grapes, wine samples, and valuable information.

#### LITERATURE CITED

- (1) Hoch, M. Organotin compounds in the environment—an overview. *Appl. Geochem.* **2001**, *16*, 719–743, and references cited therein.
- (2) Organotin stabilisers in PVC—Assessment of risks and proposals for risk reduction measures. Summary of Report 6/00; National Chemicals Inspectorate, Solna, Sweden, 2000.

- (3) Champ, M. A review of organotin regulatory strategies, pending actions, related costs and benefits. *Sci. Total Environ.* **2000**, *258*, 21–71.
- (4) Kannan, K.; Senthilkumar, K.; Giesy, J. Occurrence of butyltin compounds in human blood. *Environ. Sci. Technol.* **1999**, *33*, 1776–1779.
- (5) Takahashi, S.; Mukai, H.; Tanabe, S.; Sakayama, K.; Miyazaki, T.; Masuno, H. Butyltin residues in livers of humans and wild terrestrial mammals and in plastic products. *Environ. Pollut.* **1999**, *106*, 213–218.
- (6) Whalen, M.; Loganathan, B.; Kannan, K. Immunotoxicity of environmentally relevant concentrations of butyltins on human natural killer cells in vitro. *Environ. Res.* **1999**, *81* (2), 108–116.
- (7) De Santiago, A.; Aguilar-Santelises, M. Organotin compounds decrease in vitro survival, proliferation and differentiation of normal human B lymphocytes. *Hum. Exp. Toxicol.* **1999**, *18*, 619–624.
- (8) Okada, Y.; Oyama, Y.; Chikahisa, L.; Satoh, M.; Kanemaru, K.; Sakai, Noda, K. Tri-*n*-butyltin-induced change in cellular level of glutathione in rat thymocytes: a flow cytometric study. *Toxicol. Lett.* **2000**, *117* (3), 123–128.
- (9) Vandebriel, R.; Spiekstra, S.; Hudspith, B.; Meredith, C.; Van Loveren, H. In vitro exposure effects of cyclosporin A and bis-(tri-*n*-butyltin)oxide on lymphocyte proliferation, cytokine (receptor) mRNA expression, and cell surface marker expression in rat thymocytes and splenocytes. *Toxicology* **1999**, *135*, 49–66.
- (10) Forsyth, D.; Weber, D.; Dalglish, K. Survey of butyltin, cyclohexyltin, and phenyltin compounds in Canadian wines. *J. AOAC Int.* **1992**, *75*, 964–973.
- (11) Forsyth, D.; Sun, W.; Dalglish, K. Survey of organotin compounds in blended wines. *Food Addit. Contam.* **1994**, *11*, 343–350.
- (12) Azenha, M.; Vasconcelos, M. A Headspace Solid-Phase Microextraction Gas-Chromatography Mass-Detection Method for the Determination of Butyltin Compounds in Wines. *Anal. Chim. Acta* **2002**, in press.
- (13) Miller, J. C.; Miller, J. N. *Statistics for Analytical Chemistry*; Ellis Horwood: Chichester, U.K., 1984; Chapter 4.
- (14) Carlier-Pinasseau, C.; Lespes, G.; Astruc, A. Validation of organotin compound determination in environmental samples using NaBEt<sub>4</sub> ethylation and GC-FPD. *Environ. Technol.* **1997**, *18*, 1179–1186.
- (15) Summer, K.; Klein, D.; Grelm, H. Ecological and toxicological aspects of mono- and disubstituted methyl-, butyl-, octyl-, and dodecyltin compounds. GSF Forschungszentrum für Umwelt und Gesundheit, 1996.

Received for review November 26, 2001. Revised manuscript received February 6, 2002. Accepted February 7, 2002. M.A. thanks the Portuguese Foundation for Science and Technology for Grant BPD-22106-99.

JF0115544