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Search for the Contamination Source of Butyltin Compounds in Wine: Agglomerated Cork Stoppers

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A possible butyltin contamination source in wine was studied in this paper. Agglomerated cork stoppers, which were produced in Portugal, Spain, and Italy, used in wine bottles were examined. The domestic cork products, cork granules, and mucus used for cork products were also analyzed. The levels of mono- and dibutyltin compounds in corks were found in the range from <0.0024 to 3.3 and from <0.0029 to 6.7 μg of Sn/g, respectively. A low level of tributyltin contamination was also found in 2 of 31 tested samples. The presence of butyltin compounds in agglomerated cork stoppers was confirmed by GC−MS. Experimental results indicated that all overseas agglomerated cork stoppers studied contained mono- and/or dibutyltins. Butyltins were not detected in cork granules, mucus, most of the natural cork stoppers, and domestic agglomerated cork products. The concentrations of mono- and dibutyltins increased with the time in a 30-day experiment, showing that butyltin compounds can leach from agglomerated cork to the wine. When the butyltin concentrations in wine samples were compared with their levels in the corresponding agglomerated cork stoppers, a correlation was found. The potential harm of such food contamination was evaluated by the toxic research of butyltin compounds using *Daphnia* sp. as the experimental model.

Introduction

Organotin compounds are widely used as heat stabilizers, catalyst agents, and biocidal compounds. Disubstituted butyltin stabilizers such as dibutyltin bis(isooctyl mercaptoacetate), or its maleate, dilaurate, along with some monosubstituted analogues can prevent the thermal degradation of PVC plastics (*1*). It was reported in several studies that the leaching of butyltins from PVC and related materials could lead to contamination in food products and drinking water (*2*−*4*). Tributyltin as an active ingredient in tin-based pesticides may also result in severe contamination.

Forsyth et al. found the undetermined source of butyltin contamination in wine samples from different countries. Sixty-one of the 122 tested samples (50%) contained at least one of the butyltins with a concentration range from <0.1 to 160 ng/mL (*5*, *6*). A survey on Chinese wine and liquor samples also indicated the presence of butyltin species ranged from <0.016 to 5.7 and from <0.0022 to 33.3 μg of Sn/L for mono- and dibutyltins compounds (*7*). Furthermore, high levels of butyltins were found in some Canadian wines which were stored and transported by PVC-lined storage tanks (*8*). The dominating species in wine were usually mono- and dibutyltin according to these studies. Common explanations for the sources of the butyltins in wine were the use of non-food-grade PVC piping/containers or cultivating raw materials with butyltins-contaminated irrigation water. However, according to our experiments, a similar low level of butyltins was found in liquor samples stored in glass bottles and in those in plastic bottles (*7*), suggesting that the butyltins that actually leached from the plastic containers were very low. The high frequency of butyltins detection could not result from the "possible usage of polluted irrigation water". Therefore, another contamination source might contribute to the butyltin contamination in wines.

Cork from the Cork Oak has been used as closures for wine bottles since the 17th century. Corks are predominantly manufactured in Portugal and Spain, accounting for 52% and 32% of the world's cork production. In Italy and Morocco, cork production accounts for 10% totally (*9*). To confirm whether agglomerated cork stoppers were the contamination source or not, some unused cork stoppers produced in Portugal, Spain, and Italy were analyzed in this study. Cork stoppers, cork granules, and binding agents for cork granules produced in China were also examined at the same time. The leaching behavior of butyltin compounds in agglomerated cork stoppers was, therefore, studied. The risk assessment of such contamination was carried out by the butyltin acute toxic test to larval *Daphnia* sp.

Experimental Section

Reagents. Monobutyltin trichloride (MBT, 97%), dibutyltin dichloride (DBT, 97%), tributyltin chloride (TBT, 90%), and tetrabutyltin (TeBT, 96%) were obtained from Acros Organics (NJ). Potassium tetrahydroborate (KBH_4, 99.7%) was obtained from Shanghai Chemical Reagent Co. Their stock standards and working solutions' preparation and preservation were described previously (*7*).

The Grignard reagent of *n*-propylmagnesium bromide (*n*-PrMgBr, 2 M) was laboratory-prepared according to the standard synthetic methods (*10*) and stored under dry conditions. All of the other solvents and reagents used were of analytical reagent grade or better. The glassware was decontaminated overnight in 1:1 nitric acid solution before being reused.

Instrumentation. A Shimadzu (Kyoto, Japan) GC-9A gas chromatograph coupled with a laboratory-modified flame photometric detector using quartz surface-induced tin emission (QSIL-FPD) (*11*, *12*) was used to perform quantitative analysis of butyltin species. An Agilent GC 6890/MS 5973N was used to confirm the presence of butyltin compounds in agglomerated cork stoppers. The optimized chromatographic and MS conditions were controlled as in the description in the literature (*7*). The SPME manual device with 100 μm poly(dimethylsiloxane) (PDMS) coated fibers was obtained from Supelco Inc. (Supelco, Bellefonte, PA).

Samples. Unused wine and drug cork stoppers (four batches of samples), cork granules (four batches), and the binding agents for agglomerated cork (three samples including waterproof mucus, bone glue, and the polyurethane mucus) produced in China were obtained from different domestic cork factories. The unused overseas wine cork stoppers including five batches of natural cork (one-piece cork), seven batches of agglomerated cork, and four batches of technical cork (agglomerated cork with two disks of natural cork applied on both bases) were imported from Spain,

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Portugal, and Italy. One native cork plank sample was purchased from retail market. The cork products and mucus were held hermetically in glass vials under room temperature before analysis. The technical corks were divided into agglomerated and natural parts and analyzed butyltins, respectively, to reveal their possible contamination differences.

Used agglomerated cork stoppers were the closures of domestic wine samples purchased from markets. The butyltin levels of the wines were previously determined (*7*).

Several imported wine samples that were produced and bottled from Spain, Portugal, France, and Italy were purchased from supermarkets. Wine samples were kept unopened at room temperature until analysis.

Analytical Procedure. Liquid−liquid extraction combined with Grignard derivatization described previously (*13*) was used for the analysis of tri-, di-, and monobutyltin compounds in the cork and in the mucus samples. Briefly, a suitable amount of shattered cork or mucus samples was mixed well with 2 mL of the internal standard solution of TeBT. The sample preparation was followed by acidification with THF−HCl solution, extraction with 0.01% tropolone−hexane, concentration by a rotary evaporator, propylation with Grignard reagent, and purification by anhydrous Na_2SO_4, florisil, and silica gel. The concentrated sample solution was finally analyzed by GC−QSIL-FPD. The GC oven temperature program of 100 °C (1 min hold) to 150 °C (3 min hold) at 5 °C/min could offer the baseline separation of propylated butyltin compounds.

Headspace solid-phase microextraction (HS-SPME) after in-situ hydride derivatization, a previous optimum method, was used for liquid wine samples' pretreatment (*14*, *15*). When the study of the leaching of butyltins from unused agglomerated cork stoppers to the blank wine sample was carried out, suitable dilution of the wine samples was required before analysis. The GC oven temperature program here was controlled as 55 °C (1 min hold) to 150 °C (3 min hold) at 10 °C/min to obtain the separation of butyltin hydrides.

Standard calibration was performed in a way similar to that described above. The cork samples were replaced by 10 mL of butyltin standard solutions at different levels in the liquid−liquid extraction, and the detection limits were MBT, 0.0024 μg of Sn/g; DBT, 0.0029 μg of Sn/g; and TBT, 0.0018 μg of Sn/g. In the SPME technique, a volume of 50 mL of butyltin standard mixture was used instead of wine samples.

Acute Toxic Test of Butyltins to *Daphina sp*. The larval *Daphnia* sp. less than 18 h after oviposition were separated for the acute butyltin exposure experiment. These test animals were raised in standardized conditions without any disease and parasite. No food was supplied throughout the experiment. Six levels of three kinds of butyltin compounds (TBT, 0, 1.5, 3.0, 6.0, 12.0, and 24.0 μg of TBT/L; DBT, 0, 50, 100, 200, 400, and 800 μg of DBT/L; MBT, 0, 125, 250, 500, 1000, and 2000 μg of MBT/L) were respectively established for the exposure tests. Four groups at each level were set for repetition, and five larval *Daphnia* sp. were used per treatment. Movement inhibition was observed, and 24 h EC_{50} was calculated by the probability graphic method.

Three typical cork samples including cork granule (China), natural cork (Portugal), and agglomerated cork (Spain), which represented uncontaminated and contaminated cork samples, were cut into pieces. Five grams of swarf was immersed in 100 mL of water for 24 h. The leaching liquors after filtration were used as the exposure solutions for larval *Daphnia* sp. to evaluate the possible toxic effects caused by the contaminants in corks.

Results and Discussion

Qualitative Analysis of Agglomerated Cork Stoppers. Unused cork products and mucus were analyzed quantitatively by GC−QSIL-FPD after propylated Grignard derivatization coupled with liquid−liquid extraction. According to the standards' retention time, the butyltin compounds in the tested samples were identified primarily. GC−MS analysis of agglomerated cork stoppers further confirmed the presence of the butyltins. Identification of *m*/*z* ions in spectra was given in Table 1. Good matching was obtained between the detected *m*/*z* ions and theoretic spectra by pattern analysis.

TABLE 1. EI-MS Fragments Based on ^{120}Sn

butyltripropyltin		dibutyldipropyltin	
m/*z*	origin	*m*/*z*	origin
120	Sn^+	120	Sn^+
163	$SnC_3H_7^+$	163	$SnC_3H_7^+$
177	$SnC_4H_9^+$	177	$SnC_4H_9^+$
206	$Sn(C_3H_7)_2^+$	206	$Sn(C_3H_7)_2^+$
220	$Sn(C_4H_9)(C_3H_7)^+$	220	$Sn(C_4H_9)(C_3H_7)^+$
249	$Sn(C_3H_7)_3^+$	235	$Sn(C_4H_9)_2^+$
263	$Sn(C_4H_9)(C_3H_7)_2^+$	263	$Sn(C_4H_9)(C_3H_7)_2^+$
		277	$Sn(C_4H_9)_2(C_3H_7)^+$

Quantitative Analysis and Possible Contamination Source Study. Butyltin compounds were quantified in 28 cork products and 3 binding agents, wherein 12 of the samples contained detectable butyltins which were listed in Table 2. Butyltins contamination was found in all of the agglomerated wine corks made in Portugal and Spain and in the agglomerated parts of the Italian technical wine corks. Similar to the results of wine analysis, the principal organotin compounds present in the corks were mono- and dibutyltins. Their concentrations were from 0.016 to 3.3 and from 0.63 to 6.7 μg of Sn/g, respectively. Tributyltin compound was seldom detected. Only one Italian agglomerated cork and one Chinese agglomerated cork had very low levels of tributyltin contamination with concentrations of 0.0027 and 0.0022 μg of Sn/g. From the analysis of the Italian corks, lubrication has no effect on the butyltins' contamination. High levels of butyltins were found both in the lubricated and in the nonlubricated agglomerated parts (samples 9 and 10 in Table 2). The bleached samples (samples 11 and 12 in Table 2) contained relatively low levels of butyltins, which indicated that the process of bleaching could decrease the butyltins concentration sharply. The dibutyltin could not be detected in the bleached agglomerated parts, and the concentrations of monobutyltins decreased by at least 30 times as compared with those in corks with natural color. The bleaching agents may result in degradation of the organotin compounds by an oxidation mechanism.

Butyltin compounds were not detected in all of the tested cork granules, most of the natural cork, and the Chinese agglomerated cork products. Only one Spanish natural one-piece cork (sample 19) was detected to contain a very low level of monobutyltin compound (0.017 μg of Sn/g). No butyltin compounds were detected in the domestic waterproof mucus, bone glue, and polyurethane mucus.

The above results indicate that agglomerated corks are the possible contamination source for butyltin compounds. Butyltin contaminants might be introduced into such corks during their manufacturing procedure.

Leaching of Butyltins from Agglomerated Wine Cork to a Blank Wine Sample. A blank red wine sample without detectable butyltins was used to study the leaching behavior of butyltin compounds in the agglomerated cork to investigate the possibility of butyltin contamination in wines. Portions of 0.5 g of shattered and unused agglomerated wine cork with butyltins (MBT, 3.3 μg of Sn/g, DBT, 1.6 μg of Sn/g, TBT, not detected) were immersed in 30 mL of blank wine. The mixtures were stored hermetically in nitrogen-charged glass vials at room temperature for a series of planned days. The

TABLE 2. Concentrations of Butyltin Compounds in Tested Unused Cork Products and Mucus Samples

sample	sample	country of origin	mean level[a] ± SD[b] (μg of Sn/g) MBT	DBT	TBT
1	agglomerated wine cork (using waterproof mucus)	China	0.0061 ± 0.00004	nd[c]	0.0022 ± 0.00006
2	agglomerated wine cork	Portugal	1.5 ± 0.02	1.5 ± 0.03	nd
3	agglomerated wine cork	Portugal	1.1 ± 0.05	1.3 ± 0.07	nd
4	natural wine cork	Spain	0.017 ± 0.0004	n.d.	nd
5	agglomerated wine cork	Spain	1.4 ± 0.04	3.6 ± 0.1	nd
6	agglomerated wine cork	Spain	0.72 ± 0.01	0.63 ± 0.01	nd
7	agglomerated wine cork	Spain	3.3 ± 0.02	1.6 ± 0.01	nd
8	agglomerated wine cork	West Europe	0.70 ± 0.01	0.95 ± 0.01	nd
9	agglomerated part of technical wine cork[d] (color nature, nonlubricated)	Italy	1.3 ± 0.05	4.2 ± 0.2	nd
10	agglomerated part of technical wine cork (color nature, lubricated)	Italy	1.2 ± 0.07	6.7 ± 0.1	nd
11	agglomerated part of technical wine cork (bleached, nonlubricated)	Italy	0.016 ± 0.0004	n.d.	nd
12	agglomerated part of technical wine cork (bleached, lubricated)	Italy	0.041 ± 0.003	n.d.	0.0027 ± 0.00008

[a] $n = 5$. [b] Standard deviation. [c] Not detected. The concentrations were lower than the detection limits (Grignard derivatization method): MBT, 0.0024 μg of Sn/g; DBT, 0.0029 μg of Sn/g; TBT, 0.0018 μg of Sn/g. [d] Technical wine cork is the agglomerated cork stopper onto both sides of which two disks of natural cork are applied.

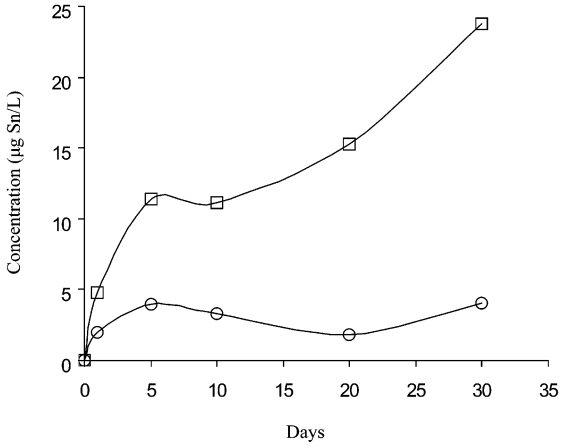

FIGURE 1. Leaching of butyltin compounds from agglomerated cork stoppers to a blank wine sample. (□), MBT; (○), DBT.

concentrations of the analytes in wine were determined by HS-SPME after hydride derivatization. Figure 1 showed the migration trend of mono- and dibutyltins from the cork to the wine within the immersion time course of 30 days. The concentration of monobutyltin in wine increased with the time until it reached 24 μg of Sn/L after a 30-day period. Dibutyltin in the wine increased in the first 5 days and then maintained around 4 μg of Sn/L in the rest of the testing period. According to the results above, butyltin compounds could definitely leach from the agglomerate corks to wine, and those polluted corks should likely contribute to butyltin contamination in the corresponding wine.

In fact, the common practice for wine storage is to lie the bottles down so that the wine soaks the corks, so that the bloated corks can keep the wine airproof for a long time. Moreover, a lot of wineries use agglomerated cork stoppers from Portugal, Spain, and so on to seal the wine bottles because of their low cost as compared with the natural one-piece cork. Therefore, the continuous migration of butyltin compounds from agglomerated cork stoppers to the wine occurs directly and easily during storage.

Comparison of Butyltin Compounds in Wine with Those in Cork Stoppers. From the survey on wines carried out formerly (*7*), the levels of butyltin compounds detected in wine samples sealed with metallic caps (with highest levels of MBT 0.31 μg of Sn/L, DBT 0.065 μg of Sn/L, TBT 0.021 μg of Sn/L) and plastic caps (with highest levels of MBT 0.45 μg of Sn/L, DBT 0.84 μg of Sn/L, TBT 0.015 μg of Sn/L) were far lower than the values detected in wine samples sealed with agglomerated cork stoppers (with highest levels of MBT 5.7 μg of Sn/L, DBT 33.2 μg of Sn/L, TBT 13.6 μg of Sn/L). Butyltin compounds in seven oversea wine samples imported from Portugal, Spain, Italy, France, and Australia were analyzed in this study to further testify the relationship of butyltin contamination in wine and the corresponding cork stoppers. Four wine samples from bottles with technical corks and two with natural corks had undetectable levels of butyltins. A wine sample with agglomerated cork had a dibutyltin level of 0.085 μg of Sn/L. In addition, it is interesting to find out that two dry red wine samples with the same brand but sealed with different cork stoppers have different levels of butyltin contamination. The total butyltin compound concentrations were 0.69 and 0.14 μg of Sn/L for wines with an agglomerated cork and with a technical cork stopper.

TABLE 3. Concentrations of Butyltin Compounds in Used Agglomerated Corks

used agglomerated wine cork	mean level[a] ± SD[b] (μg of Sn/g) MBT	DBT	TBT
A	9.8 ± 0.2	0.59 ± 0.01	nd[c]
B	5.3 ± 0.2	0.21 ± 0.007	0.6 ± 0.001
C	3.4 ± 0.1	3.2 ± 0.1	nd
D	2.9 ± 0.1	0.59 ± 0.006	nd
E	0.15 ± 0.001	7.4 ± 0.3	0.082 ± 0.006
F	0.072 ± 0.00055	0.067 ± 0.0004	0.14 ± 0.0015
G	13.1 ± 0.5	0.30 ± 0.01	nd

[a] $n = 5$. [b] Standard deviation. [c] Not detected. The concentrations were lower than the detection limits of the Grignard derivatization method.

Several used agglomerated corks for sealing wine samples were also analyzed. The middle part of the corks that did not contact with the wine was used for analysis. The concentrations (Table 3) showed that the distribution of three butyltin species was similar to that of corresponding wines and those in the unused agglomerated corks. High MBT and DBT concentrations and a relatively lower TBT concentration were detected. The butyltin concentrations in corks from A to E were about 10^3−10^4 fold higher than those in wines when compared at the same unit, while dibutyltin levels in corks F and G were several decade folds higher than those in wine

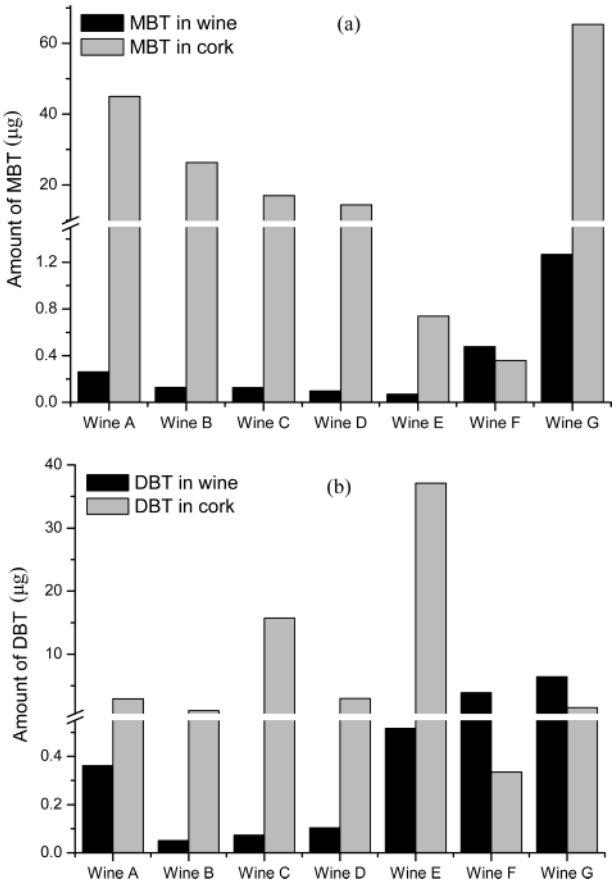

FIGURE 2. Comparison of butyltin compound levels in several wine samples with those in the corresponding agglomerated corks. (a) MBT; (b) DBT.

samples. A correlation of calculated absolute contents of butyltins between a cork stopper (general 5 g for one cork) and its corresponding bottle of wine samples (750 mL) based on their concentrations was shown in Figure 2. The amounts of mono- and dibutyltin in wine samples increased with those in the cork stoppers except for wine samples F and G. The amount of mono- and dibutyltins in wine samples A−E accounted for 0.5−11% of the total butyltins in the wine and cork stopper. The percentage of butyltins in wine samples F and G were, however, up to 57−92%, which indicated a mass of butyltins had migrated from the cork stoppers to its corresponding wines. The results proved that the agglomerated cork is a potential butyltin contamination source in wines. Moreover, agglomerated corks with different levels of butyltin compounds might induce different levels of butyltin contamination in wines. Due to the limitation of our knowledge about the production procedure of the agglomerated corks, the detailed mechanism of butyltin introduction into the corks is still unclear. As the art of making wine is also very complex, butyltin contamination in wine could also possibly be caused by other potential contamination sources. That might explain why low levels of butyltins were also detected in some liquor samples and some wine samples with metallic caps.

Toxicological Assessment of Butyltin Contamination. According to the 24 h EC_{50} values of MBT, 229.97 μg of Sn/L, DBT, 61.86 μg of Sn/L, and TBT, 0.85 μg of Sn/L, it was obvious that butyltin compounds at the level of micrograms of Sn per liter could effectively induce acute movement inhibition of *Daphnia* sp.

The experiment using leaching liquors from three cork samples as the exposure solutions showed there was no difference between the activity of larval *Daphnia* sp. in control and exposure groups during the whole procedure, which might indicate that small amounts of butyltin compounds could be extracted into water because most of the organotin compounds were hydrophobic. Low levels of butyltin compounds leached in the aquatic phase could not effectively induce acute movement inhibition of larval *Daphnia* sp. However, as the solubilities of organotin compounds were different in water and alcoholic solution, the harmful effects of butyltins extracted by wine from contaminated corks should be studied.

Comparison of butyltin levels in contaminated wine samples with the 24 h EC_{50} values was carried out to further assess the possible effect induced by cork contamination. Based on the results in a previous report (*7*), the levels of butyltin compounds in most of the wine samples were lower than the thresholds, except for one dry red wine imported from Spain where 5.7 μg of Sn/L of MBT, 33.3 μg of Sn/L of DBT, and 13.6 μg of Sn/L of TBT were detected. Generally, relatively low amounts of butyltin compounds extracted by wine might not induce the acute deleterious influences on human health. However, much attention should still be paid to the possible harm caused by some special wines with high butyltin contamination. In addition, in view of chronic toxic effects of butyltin compounds, their occurrence in food samples was noteworthy.

Acknowledgments

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