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Determination and Levels of the Biocide *ortho*-Phenylphenol in Canned Beers from Different Countries

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A method was developed for the determination of the biocide *ortho*-phenylphenol (biphenyl-2-ol; OPP) in beer, using deuterated OPP as an internal standard. A new liquid−liquid extraction procedure, employing acetonitrile, diethyl ether, and *n*-pentane, afforded rapid phase separation. The evaporated extract was derivatized with pentafluorobenzyl bromide in a water−acetonitrile mixture that was buffered with potassium carbonate, followed by extraction of the derivative into cyclohexane and analysis by gas chromatography−mass spectrometry in electron ionization mode. The method enables the detection of OPP in 50 mL of beer at concentrations as low as 0.1 μg/L and provides a linear range of quantification of 0.5−40 μg/L. Samples from 61 beers canned over the past 12 years and sold in 27 countries were analyzed for OPP. In 40 of them, the target compound was present at concentrations of 1.2−40 μg/L. Our investigations indicate that the ends of the cans, which contain sealing material presumably treated with OPP, are responsible for this contamination.

KEYWORDS: Food contamination; biphenylol; antiseptics; antimicrobials; pentafluorobenzylation

INTRODUCTION

The contamination of food by toxic chemicals is a public health concern and is a leading cause of international trade problems. A number of food contamination episodes, such as recent findings of acrylamide (*1*), perfluorooctanoic acid (PFOA), and perfluorooctane sulfonate (*2*) in foodstuffs, highlight the need for improved monitoring of chemical residues and contaminants. As several industrially prepared nonfood products are often used in direct or indirect association with food production, it is important to consider their potential for contaminating food.

Among the most important routes for food contamination is through migration of packaging material constituents into foods (*3*). Contamination sources and routes are expected to vary greatly and may occur before, during, or after food processing. Monomers or small polymeric chains, plastic stabilizing agents, or plasticizers are especially likely to contaminate food in this manner, and previous instances have been well documented (*3*−*5*). Such incidents may only represent the "tip of the iceberg"; it is likely that many more foodstuff contaminants remain to be discovered than are known at present. Some contaminants may be of human health concern, and substantial quantities may be ingested on a daily basis. Identifying new contaminants is, however, complicated by the complexity of the chemical composition of food.

Beer represents a particularly challenging analytical matrix, owing to the presence of numerous compounds with very different molecular sizes, physical, and chemical properties that typically originate from malted grains and hops. Among other constituents, beer contains many polyphenols (e.g., anthocyanogens, tannins, and catechins) in varying amounts. In stark contrast to such naturally occurring phenolic compounds, *ortho*-phenylphenol (OPP) is not a natural component of any food. This compound has been widely used as a preservative for citrus fruits and vegetables because of its broad efficacy as a biocide against bacteria, molds, and yeast (*6*). OPP is also used in households, industry, and hospitals to disinfect surface materials. It is used as a preservative in the cosmetics, plastics, leather, textile, and paper industries. OPP is also applied in mushroom farms for pest control (*7*). OPP exhibits low acute toxicity in animal experiments (*8*), although it has been found to cause bladder cancer in male rats after chronic exposure to dietary doses up to 4% OPP (*9*).

Numerous studies have reported the occurrence of OPP in agricultural products (*10*, *11*), in biological matrixes (*12*, *13*), in disinfectants (*14*), and in environmental samples (*15*, *16*). Most previous studies used either high performance liquid chromatography with UV (*14*), electrochemical (*10*), or mass spectrometric (*11*, *12*, *15*) detection or gas chromatography with mass spectrometric detection (*13*, *15*, *16*). At present, no method exists for the analysis of OPP in beer.

In this work we present a GC/MS method for the determination of OPP in beer in the low ppb range and report on OPP levels in canned beers from several countries. The method is

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based on a liquid−liquid extraction procedure using acetonitrile−diethyl ether−*n*-pentane, followed by derivatization with pentafluorobenzyl bromide. To our knowledge, this is the first study on the occurrence of OPP in canned beers.

MATERIALS AND METHODS

Beer samples analyzed in Germany had been previously sent to our institute for analysis of parameters apart from OPP and were stored at 0 °C prior to analysis. The samples analyzed at the Johns Hopkins University were purchased at local stores and were analyzed immediately. Care was exercized when collecting these samples to ensure that they were taken from a variety of sources, representing several different producers and beer types, including some beers of the same brand and type but with different production dates.

OPP, potassium carbonate, 2,3,4,5,6-pentafluorobenzyl bromide ((PFB)Br), D_2O (99.9%), and D_2SO_4 were purchased from Aldrich. Sodium chloride pa, HPLC grade acetonitrile, *n*-pentane (residue analysis grade), and diethyl ether were obtained from Merck. Diethyl ether was distilled over potassium hydroxide to remove the stabilizer 2,6-di-*tert*-butyl-4-methylphenol (BHT) and was then stored over potassium hydroxide in a refrigerator. All experiments were conducted using deionized water that was produced using a MilliQPlus water purification system.

Deuterated OPP was synthesized according to the following procedure: 10 mg of OPP, 100 μL of D_2O, and 20 μL of D_2SO_4 were placed in a glass tube (300 mm × 12 mm od × 10 mm i.d.) that had been sealed at one end, after which the open end of tube was sealed in a Bunsen burner flame. The sealed tube was heated at 180 °C in a GC oven for 24 h. After cooling of the tube to room temperature, one end was cut off and the contents were extracted 3 times with 10 mL of *n*-pentane. The combined extract was washed with 5 mL of distilled water, was dried over anhydrous sodium sulfate, and was then evaporated gently to dryness with an N_2 stream.

Extraction and Derivatization. Each beer sample was analyzed twice. The beer was degassed for 15 min in an ultrasonic bath. A 50 mL sample was then placed in a stoppered 100 mL (nominal volume) mixing cylinder (actual volume of the cylinder was about 127 mL). Next, 100 μL of acetonitrile (containing 500 ng of deuterated OPP as an internal standard) was added, and the sample was mixed. At this point, 25 mL of acetonitrile, 20 mL of diethyl ether, and 10 mL of *n*-pentane were added, and again the contents were mixed vigorously, with careful ventilation. Note that 100 mL volumetric flasks can also be used for extraction of beer, provided that the volumes of beer and solvents are each reduced by 20%. After phase separation, the top organic phase was transferred quantitatively into a 100 mL beaker using a 30 mL glass pipet. The extract was allowed to evaporate in a hood over the course of 3−4 h. The residue was transferred with 3 aliquots of 1 mL acetonitrile into a test tube (100 mm × 12 mm i.d.) with a Teflon-lined screwcap. To that solution, 200 μL of a 10% potassium carbonate solution, 2.7 mL of MilliQ water, and 40 μL of neat PFBBr were added in succession. After the tube was closed tightly, the sample was heated at 100 °C in a GC oven for 1 h, after which the reaction mixture was cooled to room temperature. The derivative was extracted from the reaction mixture by vortexing the sample several times after adding 200 μL of cyclohexane. After phase separation, the cyclohexane phase was transferred using a 100 μL pipet into a sample vial and was analyzed by GC/MS.

Unused can ends from three different manufacturers were also tested for their ability to leach OPP. This was done by placing two can ends face to face in a 400 mL beaker, followed by adding 50 mL of acetonitrile (containing 10 μg of deuterated OPP) so that the can ends were completely covered by the acetonitrile. The beaker was covered with aluminum foil and was left overnight for extraction. The next day the extraction was continued in an ultasonic bath for 30 min. The extract was passed through a glass filter, and 3 mL was analyzed using the procedure described above.

Analysis. HRGC/MSD measurements in Germany were performed using a model MSGOLD instrument from Perkin Elmer, equipped with a 50 m Optima-5 MS column (J&W, 0.25 mm i.d., 0.25 μm film thickness), at a carrier gas flow rate (He) of ∼1.5 mL/min. The chromatographic conditions were as follows: splitless/split injection (split open after 30 s), temperature program: 100 °C (3 min), 10 °C/min to 250 °C (30 min). Injection port temperature: 250 °C. Transfer line temperature: 250 °C. Injection volume: 2 μL. The analysis of U.S.-purchased beers was performed using a ThermoQuest (San Jose, CA) Trace 2000 GC/MS system. A DB-5MS (J&W) 30 m length × 0.25 mm i.d. × 0.25 μm phase thickness column was used to effect chromatographic separations. The flow rate (He) was ∼1.5 mL/min. The GC temperature program was 105 °C for 1 min, 8 °C/min to 285 °C, followed by a 10-min hold at 285 °C. Mass spectra were obtained in electron ionization mode (70 eV). Ions selected were *m*/*z* 141, 169 (monitoring ions) and *m*/*z* 350 (quantitation ion) for OPP and *m*/*z* 357 for the deuterated OPP internal standard.

Validation of Method. Triplicate (50-mL) samples of bottled beer, which were found to be free of OPP, were fortified with 0.4−40 μg OPP/L beer and 20 μg deuterated OPP/L beer. Analysis was performed as described above. Detection of three characteristic masses at the correct retention time and with the same ion abundance (±15%) as displayed by the standard was considered as a valid identification criterion according to European Union regulations (*17*).

RESULTS AND DISCUSSION

As preliminary attempts to isolate OPP from beer using solid-phase extraction were unsatisfactory, efforts were expended to develop a liquid−liquid extraction method. Ethyl acetate has typically served as the solvent of choice for extraction of beer (*18*, *19*). Other solvents, such as dichloromethane, a pentane−dichloromethane mixture, and Freon 11, have also been used (*20*−*22*). A problem often encountered in liquid−liquid extraction of beer is that phase separation takes place slowly, and some solvents form relatively stable emulsions.

Because none of the organic solvents tested were found to be appropriate in isolation, we explored extraction using solvent mixtures. Diethyl ether (DEE) as an extraction solvent, in conjunction with acetonitrile (AcN) or isopropyl alcohol (i-PrOH), afforded rapid phase separation. The best results were achieved with DEE/AcN (20/25, mL/mL) or DEE/i-PrOH (20/20, mL/mL) when 50 mL of beer was extracted. Phase separation took place very quickly, resulting in sharp and contrasting phases. Ultimately, a DEE/AcN mixture was chosen because extraction yields of OPP were about 30% higher than in the DEE/i-PrOH mixture.

After extraction, the organic phase had a volume of about 30 mL and contained small amounts of water (up to 0.7 mL). To reduce the amount of water present, *n*-pentane was used as an additional extraction solvent. Addition of *n*-pentane also reduced the time required for solvent evaporation. Another benefit was the disappearance of some peaks in the chromatogram, most likely due to the suppressed coextraction of some beer components (**Figure 1**). The solvent mixture i-PrOH−DEE took almost twice as long to evaporate as the AcN−DEE mixture, even with the addition of *n*-pentane.

We found pH adjustment prior to extraction to be unnecessary, as the pH of beer (at approximately ∼4.4−4.5) is low enough for quantitative extraction of OPP. We noted that phase separation was slightly slower at pH ≈ 2, produced by adding 150 μL of 85% H_3PO_4 to 50 mL of beer. More important, the resulting chromatograms at pH 2 displayed additional peaks, which raised our detection limit for OPP.

Derivatization conditions in this work are slightly different than in our recent work involving analysis of pharmaceuticals and personal care products (including OPP) (*23*). Specifically, twice as much carbonate buffer is used in the present work, as this provided increased sensitivity for analysis of beer. Dipotassium hydrogen phosphate was tested as an alternative buffer to carbonate. However, carbonate led to better results because

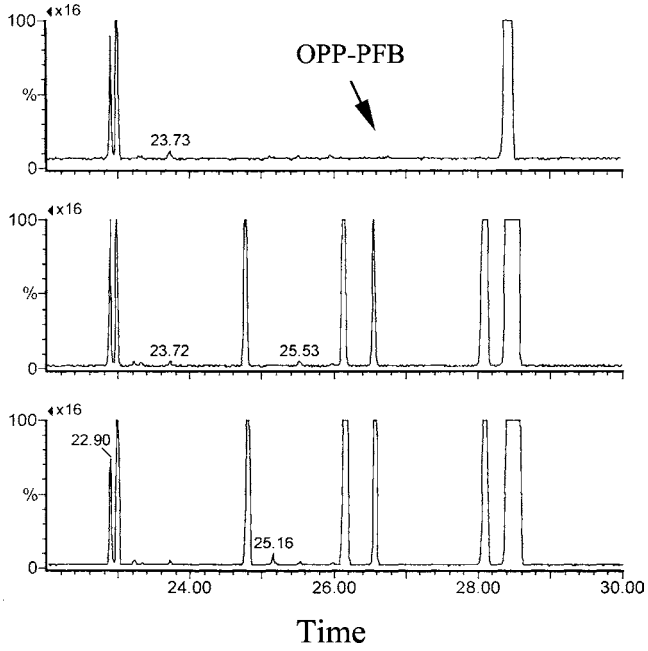

Figure 1. Selected-ion (*m*/*z* 169) chromatograms of a sample of bottled beer obtained using different extraction solvents: AcN/DEE/*n*-pentane mixture (top); AcN/DEE mixture (middle); i-PrOH/DEE/*n*-pentane mixture (bottom).

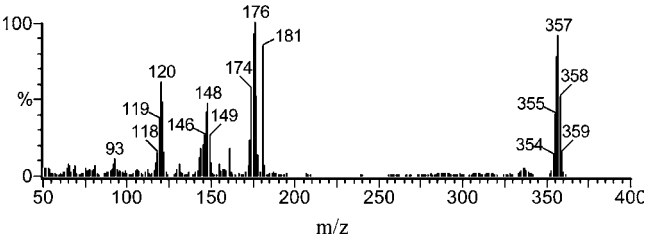

Figure 2. Mass spectrum of deuterated OPP after derivatization with PFBBr.

of fewer interferences and higher sensitivity. During the derivatization reaction, pentafluorobenzyl esters of either carbonic acid or phosphoric acid were formed as major products when either carbonate or phosphate was used as a base. However, because they had different retention times and masses, the analysis of OPP was not influenced.

When a 50-mL sample of beer was analyzed, a detection limit of 0.1 μg/L was achieved at a signal/noise ratio of 3. The range of linearity was determined to be between 0.4 and 40 μg/L, with a correlation coefficient R of 0.999 ($n = 6$). The average relative recovery of OPP from fortified beer samples was determined as being $\geq$95%. Relative standard deviation was 3.95% and 6.1% for 0.4 μg of OPP/L beer and 40 μg of OPP/L beer, respectively.

Inclusion of deuterated OPP as an internal standard proved critical to the success of our method. Without labeled OPP, it was not possible to obtain reproducible and accurate results. The internal standard used was a mixture of deuterated *o*-phenylphenol isotopomers with degrees of deuteration ranging between d_2 and d_{10} (**Figure 2**). The most abundant molecular ion after derivatization of the labeled OPP was *m*/*z* 357, corresponding to the pentafluorobenzyl ether derivative of OPP-d_7. An advantage of using a mixture is that, in case of interferences, masses other than *m*/*z* 357 can be selected for detection. The masses *m*/*z* 169 and 350, which originated from the pentafluorobenzyl ether derivative of unlabeled OPP, were

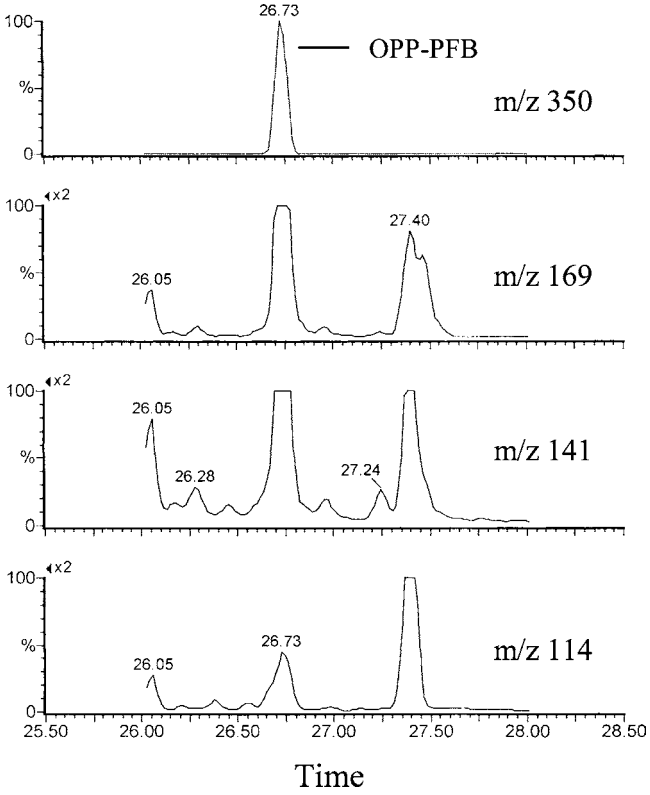

Figure 3. Selected-ion chromatograms of pentafluorobenzyl ether derivative of OPP (OPP–PFB) of a canned beer sample.

not detected when 0.5 μg (the mass that was typically used during sample analyses) of labeled OPP was derivatized. The *m*/*z* 169 ion is the base peak in the mass spectrum of the pentafluorobenzyl ether derivative of OPP. Other important peaks are 115, 141, 181, and the molecular ion at 350, with relative abundances of 46.6, 44.1, 26.3, and 40.7%, respectively. The ion at *m*/*z* 181 derives from the pentafluorobenzyl fragment and is not a specific ion for detection of OPP, as almost every peak displays this ion. In a few samples, especially those with low OPP levels, some minor interferences from unknown compounds were observed at *m*/*z* 115, 141, and to a lesser degree at 169 but not at 350 (**Figure 3**). However, these interferences did not affect identification, as good chromatographic resolution was achieved.

The results for OPP concentrations in different beers are provided in **Table 1**. Of 61 different samples from 27 countries analyzed, OPP was detected in 40 beers at μg/L level concentrations. The highest value was measured at 33.5 μg/L, in a beer from Finland sold in 2001. The lowest measured OPP concentration was 1.2 μg/L. In most samples, concentrations ranged between 5 and 9.9 μg/L (representing 24 cans), while 15 cans had concentrations between 1.2 and 4.9 μg/L (**Figure 4**).

Our results indicated considerable variability in OPP concentration even within a given production lot. When OPP was detected, we analyzed a second can of beer from the same lot (if available); in one case, four cans from the same lot were analyzed. Although the OPP concentration in the second can was very close to the value measured in the first can for nearly half of the pairs of cans analyzed, concentrations varied up to 3-fold higher (or lower) in the other half of the pairs of samples. For example, in the four cans from the same production date and lot number, OPP concentrations were found to be 11.7, 13.6, 15.5, and 26.5 μg/L.

Table 1. Concentrations (μg/L) of OPP in Beer Samples

country	brewery	specification	date of productn	concn[a]
Albania	1	lager	Jun 2005	4.2
Australia	2	0.9% alcohol	Aug 1993	nd
Australia	3	0.9% alcohol	Sep 1993	nd
Austria	4	lager	Jan 2005	9.5/13.5
Czech Rep	5	premium	Feb 2001	nd
PR China	6	nonalcoholic	Nov 1993	17.2
Saudi Arabia[b]/Germany[c]	7	nonalcoholic	May 1994	6.6
Egypt[b]/Germany[c]	7	nonalcoholic	Jan 1993	7.3
Egypt	8	original	May 1999	nd
Egypt	9	export	Nov 1998	nd
England	10	export	July 1998	nd
England	11	stout	Feb 2001	nd
Finland	12	lager	Jan 2001	33.5
Germany	13	nonalcoholic	Mar 2005	nd
Germany	14	Pilsner	Jan 2005	nd
Germany	13	original	Jan 2005	1.5/2.1
Germany	14	original	Jan 2005	2.8/7.6
Germany	14	dark	Jan 2005	6.9/7
Germany	14	Pilsner	Jan 2005	2.1/6.2
Germany	15	original	Jan 2005	1.2/2.9
Germany	16	Pilsner	Oct 2004	5.4/6
Germany	17	Pilsner	Feb 2005	9.2
Germany	18	nonalcoholic	Apr 2002	31.1
Germany	19	Pilsner	Jan 2005	7.9
Germany	19	Pilsner	Sep 2004	9.4
Germany	19	original	Feb 2005	11.7/13.5/15.5/26.2
Greece	20	nonalcoholic	Jun 1995	2.3
Greece	21	lager	May 2001	nd
Holland	22	premium Pilsner	Dec 2004	2.2/2.5
Hong Kong	23	Pilsner	Mar 1998	nd
Indonesia	24	Pilsner	Oct 1999	30.4
Ireland	25	draught	May 1998	nd
Ireland	26	lager	Apr 1998	4.8
Ireland	27	nonalcoholic	Mar 1993	nd
Jordan[b]/Germany[b]	18	premium	Oct 1999	nd
Jordan	28	lager	Jul 1999	nd
Macedonia	29	Pilsner	Aug 1999	17.4
Namibia	30	draught	Jan 2005	8.3/9.1
Namibia	30	draught	Jan 2005	8.4/9.5
Philippines	31	Pilsner	Mar 1998	4.7
Philippines	32	Pilsner	Mar 1998	nd
Philippines	31	lager	Apr 1998	3.8
Russia	33	Pilsner	May 2004	6.7/7.5
Simbabwe	34	lager	Mar 1999	nd
Simbabwe	35	Pilsner	Mar 1999	nd
Slowakia	36	pale lager	Jan 2005	3.0/6.2
Spain	37	Pilsner	May 2001	25.6
Spain	38	dark	Feb 1999	3.6
Spain	38	export	Nov 1997	30.5
Spain	39	premium	Mar 1999	8.1
Spain	40	Pilsner	Sep 1999	20.7
Sweden	41	premium Pilsner	Jan 2005	9.0/9.4
Sweden	42	Pilsner	Jan 2005	11.2
Sweden	43	Pilsner	Apr 1998	12.6
Sweden	44	original	Jul 2000	25.3
Switzerland	45	nonalcoholic	Nov 1993	nd
Thailand	46	draught	Apr 1999	5.1
USA[b]/Holland[c]	17	Pilsner	Mar 2005	14.6
USA[b]/Mexico[b]	47	special	Feb 2005	nd
USA	48	premium	Feb 2005	nd
USA	49	ale	Jan 2005	6.8

[a] Results for different cans resolved by slash. [b] Country of purchase. [c] Country of production; nd = not detected.

The purpose of analyzing older beers was to assess how long OPP has been used in beer cans. The oldest beers analyzed dated from 1993 and were brewed in five different countries by five different breweries (**Table 1**). In two of these beers, OPP was measured at 7.3 and 17.2 μg/L. OPP was found in 20 of 39 beer samples dating from 1993 to 2002, and 15 of 16 samples from 2005 were contaminated with OPP. This indicates a widespread use of OPP in beer cans.

We also investigated the source of OPP in beer cans. Since we could not detect OPP in samples of bottled beer that we analyzed, we examined unused cans and can ends. OPP was present on the inner side of can ends but not in the cans themselves. All can ends (diameter 5.95 cm) had a thin plastic sealing band at the edge of the inner side, which was approximately 3−4 mm wide. At first it was not clear whether OPP was on the whole surface of the can end or only on (or in) the plastic sealing. To distinguish between these possible sources, a disk with a diameter of ca. 4.2 cm was cut out from the middle of the can end, leaving the sealing band on the remaining piece. The pieces had similar surface areas. On analysis, more than 90% of the OPP was found to be on the piece with the sealing material.

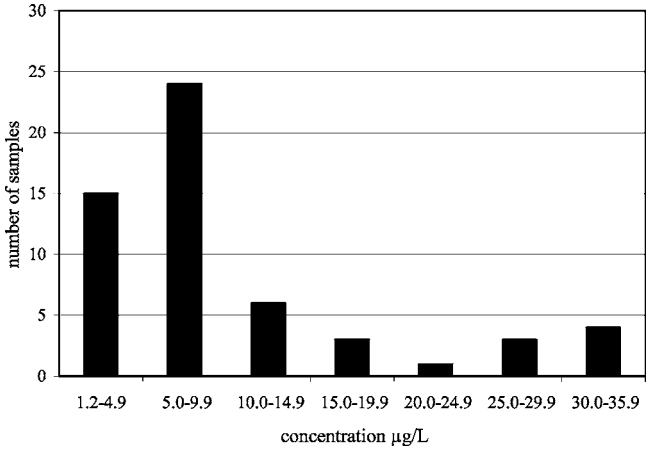

Figure 4. Distribution of OPP levels in beer samples.

SAFETY

Care should be taken during deuteration at high temperature because of risk of explosion of the reaction vessel.

LITERATURE CITED

(1) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Acrylamide: a cooking carcinogen? *Chem. Res. Toxicol.* **2000**, *13*, 517−522.

(2) Begley, T. H.; White, K.; Honigfort, P.; Twaroski, M. L.; Neches, R.; Walker, R. A. Perfluorochemicals: Potential sources of and migration from food packaging. *Food Addit. Contam.* **2005**, *22*, 1023−1031.

(3) Lau, O. W.; Wong, S. K. Contamination in food from packaging material. *J. Chromatogr., A* **2000**, *882*, 255−270.

(4) Helmroth, E.; Rijk, R.; Dekker, M.; Jongen, W. Predictive modelling of migration from packaging materials into food products for regulatory purposes. *Trends Food Sci. Technol.* **2002**, *13*, 102−109.

(5) Skjevrak, I.; Brede, C.; Steffensen, I. L.; Mikalsen, A.; Alexander, J.; Fjeldal, P.; Herikstad, H. Nontargeted multicomponent analytical surveillance of plastic food contact materials: Identification of substances not included in EU positive lists and their risk assessment. *Food Addit. Contam.* **2005**, *22*, 1012−1022.

(6) FAO, Pesticide Management, Evaluations of Pesticide Residues, 1999, available at http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/pes_alp.htm

(7) Davoren, M.; Fogarty, A. M. Ecotoxicological evaluation of the biocidal agents sodium *o*-phenylphenol, sodium *o*-benzyl-*p*-chlorophenol, and sodium *p*-tertiary amylphenol. *Ecotoxicol. Environ. Saf.* **2005**, *60*, 203−212.

(8) Bomhard, E. M.; Brendler-Schwaab, S. Y.; Freyberger, A.; Herbold, B. A.; Leser, K. H.; Richter, M. *o*-Phenylphenol and its Sodium and Potassium Salts: A Toxicological Assessment. *Crit. Rev. Toxicol.* **2002**, *32*, 551−626.

(9) Hiraga, K.; Fujii, T. Induction of tumors of the urinary bladder in F344 rats by dietary administration of *o*-phenylphenol. *Food Chem. Toxicol.* **1984**, *22*, 865−870.
(10) Yang, L.; Kotani, A.; Hakamata, H.; Kusu, F. Determination of ortho-phenylphenol residues in lemon rind by high-performance liquid chromatography with electrochemical detection using a microbore column. *Anal. Sci.* **2004**, *20*, 199−203.
(11) Blasco, C.; Font, G.; Manes, J.; Pico, Y. Solid-Phase Microextraction Liquid Chromatography/Tandem Mass Spectrometry To Determine Postharvest Fungicides in Fruits. *Anal. Chem.* **2003**, *75*, 3606−3615.
(12) Ye, X.; Kuklenyik, Z.; Needham, L. L.; Calafat, A. M. Automated On-Line Column-Switching HPLC-MS/MS Method with Peak Focusing for the Determination of Nine Environmental Phenols in Urine. *Anal. Chem.* **2005**, *77*, 5407−5413.
(13) Bartels, M. J.; Brzak, K. A.; Bormett, G. A. Determination of *ortho*-phenylphenol in human urine by gas chromatography−mass spectrometry *J. Chromatogr., B* **1997**, *703*, 97−104.
(14) Thompson, R. D. Determination of phenolic disinfectant agents in commercial formulations by liquid chromatography. *J. AOAC Int.* **2001**, *84*, 815−822.
(15) Aguera, A.; Fernandez-Alba, A. R.; Piedra, L.; Mezcua, M.; Gomez, M. J. Evaluation of triclosan and biphenylol in marine sediments and urban wastewaters by pressurized liquid extraction and solid-phase extraction followed by gas chromatography mass spectrometry and liquid chromatography mass spectrometry. *Anal. Chim. Acta* **2003**, *480*, 193−205.
(16) Rudel, R. A.; Melly, S. J.; Geno, P. W.; Sun, G.; Brody, J. G. Identification of Alkylphenols and Other Estrogenic Phenolic Compounds in Wastewater, Septage, and Groundwater on Cape Cod, Massachusetts. *Environ. Sci. Technol.* **1998**, *32*, 861−869.
(17) Available at http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_221/l_22120020817en00080036.pdf.
(18) Dufour, J.-P.; Wierda, R.; Leus, M.; Lissens, G.; Delvaux, F.; Derdelinckx, G.; Larsen, D. Quantitative analysis of beer aromatic alcohols using stable isotope dilution assay. *J. Am. Soc. Brew. Chem.* **2002**, *60*, 88−96.
(19) Iverson, W. G. Ethyl acetate extraction of beer: quantitative determination of additional fermentation byproducts. *J. Am. Soc. Brew. Chem.* **1994**, *52*, 91−5.
(20) Tressl, R.; Friese, L.; Fendesack, F.; Koeppler, H. Gas chromatographic-mass spectrometric investigation of hop aroma constituents in beer. *J. Agric. Food Chem.* **1978**, *26*, 1422−6.
(21) Wei, A.; Mura, K.; Shibamoto, T. Antioxidative activity of volatile chemicals extracted from beer. *J. Agric. Food Chem.* **2001**, *49*, 4097−4101.
(22) Alvarez, P.; Malcorps, P. Analysis of free fatty acids, fusel alcohols, and esters in beer: an alternative to CS_2 extraction. *J. Am. Soc. Brew. Chem.* **1994**, *52*, 127−34.
(23) Yu, J. T.; Bisceglia, K. J.; Coelhan, M.; Bouwer, E. J.; Roberts, A. L. Occurrence and Removal of Selected Pharmaceuticals and Personal Care Products (PPCPs) in Sewage Treatment Plants. Prsented at the Society of Environmental Toxicology and Chemistry 26th Annual Meeting in North America, Baltimore, MD, 2005.

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