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Analytical Methods

Presence of the biocide *ortho*-phenylphenol in canned soft drinks in the United States and Germany

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ABSTRACT

Canned soft drink samples obtained from the United States and from Germany were analyzed for the biocidal (and potentially carcinogenic) compound *ortho*-phenylphenol (OPP). Sampling locations were Baltimore (Maryland), Yonkers (New York), Pasadena (California), and Munich (Germany). OPP was detected in 49 out of the 55 samples analyzed, with concentrations generally at the μg/l level. The highest OPP value was measured at 16.9 μg/l in a lemon flavored cola. A mean concentration of 2.9 μg/l was computed for OPP in all samples, assuming values of zero for those samples in which OPP was not detected. OPP was detected in soft drinks obtained from all regions sampled, suggesting that contamination of canned beverages by OPP may be a widespread phenomenon.

1. Introduction

Contamination of food is a very important issue for the food industry, as negative reports associated with a specific brand or type of product can result in precipitous declines in sales. Packaging materials may contain a variety of chemical compounds capable of migrating to packed food (Lau & Wong, 2000). Several factors contribute to such migrations, although the exact cause of migration has seldom been traced. Exactly what migration occurs depends first on the identity and concentration of any chemicals present in the packaging material. Other important parameters are the nature of the food, along with the conditions of contact. In addition to its chemical nature, the properties of the packaging material itself are important considerations. If the packaging material interacts strongly with the food, leaching can give rise to high rates of migration.

The main concern in the past has been in connection with plasticizers that are used to improve the quality of some packaging materials. Most such plasticizers are contained within polymers such as polyvinyl chloride (PVC). The migration of plasticizers from plastics into food has been discussed in several reports (Castle, Mercer, Startin, & Gilbert, 1988; Page & Lacroix, 1995; Startin et al. 1987). Such findings sparked the packaging industry to replace PVC by other polymers, such as polyethylene (PE) or regenerated cellulose not associated with plasticizers (Nerin, Cacho, & Gancedo, 1993). Other important migrants are antioxidants, monomers, oligomers, and their decomposition products.

Especial interest has been paid to migration of contaminants originating from cans used in the food industry (Montanari, Pezzani, Cassarh, Quaranta, & Lupi, 1996; Schaefer, Maß, Simat, & Steinhart, 2004; Vela, Toma, Reiboldt, & Pierri, 1988. Cans are usually coated internally with a very thin polymeric material, typically consisting of epoxy polymers. It has been shown that bisphenol-A diglycidylether (BADGE, 2,2-bis(4-(2,3-epoxypropyl) phenyl) propane), which is a monomer for the production of epoxy resins and derivatives, can migrate into food (Grob, Spinner, Brunner, & Etter, 1999). We reported recently that the biocide *o*-phenylphenol (OPP) was detectable in concentrations as high as to 33.5 μg/l in canned beers from several countries produced in the last 12 years (Coelhan, Bromig, Glas, & Roberts, 2006). OPP was detected in 40 of 60 canned beer samples analyzed. Contrary to BADGE, OPP is not a component of any polymer chain. We found that it was not contained in the coating, but rather was present in the sealing polymeric material placed on the inner side of the can end. In the United States, five companies are the major producers of cans used for drinks (CMI, 2003). The number of cans produced for soft drinks increased from 13.200×10^{12} in 1970 to 68.266×10^{12} in 2002. During the same time period, the number of beer cans produced increased from 19.900×10^{12} to 32.210×10^{12}. Total units of can shipments accounted to 41.3×10^{12} in Europe in 2005, split equally between beer and soft drinks (BCME, 2006).

OPP has been widely used as preservative for citrus fruits and vegetables because of its broad efficiency as a biocide against bacteria, mold, and yeast (FAO, 1999). It is also used in households,

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industry, and hospitals to disinfect surface materials. It is used as a preservative in the cosmetics, plastics, leather, textile, and paper industries. It is contained in varying concentrations in a multitude of products under the trade names Chemcide, Cotane Dowicide, Nipacide, Preventol, Torsite, among others.

OPP displays low acute toxicity in animal experiments (Bomhard et al., 2002). Nevertheless, it has been found to cause bladder cancer in male rats after chronic exposure to dietary doses up to 4% OPP (Hiraga & Fujii, 1984). OPP is included on the list of chemicals recognized by the State of California as carcinogens (California EPA, 2007). Although phenolic compounds have been reported to exhibit estrogenic or antiandrogenic activity, OPP does not appear to be included in their number (Paris et al., 2002).

The purpose of this work was to evaluate the presence of OPP in canned soft drinks purchased in the United States and (to a lesser extent) in Germany. To our knowledge, this represents the first investigation of OPP in canned soft drinks.

1.1. Samples

The total number of samples was 55. Of 55 samples, eight were purchased and analyzed in Germany; the remaining samples were all purchased within the United States. Canned beverages were purchased at retail stores in Maryland (Baltimore), New York (Yonkers), California (Pasadena), and in Munich, Germany (Table 1). For purposes of comparison, some bottled soft drinks were analyzed too. We readily acknowledge that the canned soft drink samples in this study do not constitute a representative sampling of either soft drink production or sales throughout the Unites States or Germany as a whole. Rather, the soft drinks samples reflected those available in several commercial outlets in both countries between March 2005 and May 2006.

1.2. Analysis

Analytical methods were essentially the same as previously employed in the analysis of OPP in beer (Coelhan et al., 2006), with only slight modifications. A 50 ml of sample and 500 ng of deuterated OPP dissolved in 100 μl of acetonitrile were mixed in a stoppered 100 ml (nominal volume) mixing cylinder (actual volume of the cylinder was about 127 ml). Next, 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. The top organic phase was transferred quantitatively into a 100 ml beaker and allowed to evaporate in a hood. The residue was transferred with three aliquots of 1 ml acetonitrile into a test tube (100 mm × 12 mm id) with a Teflon-lined screw cap. To that solution, 200 μl of a 10% potassium carbonate solution, 2.7 ml of MilliQ water, and 40 μl of neat pentafluorobenzyl bromide (PFBBr) were added in succession. After closing the tube 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. The cyclohexane phase was transferred using a 100 μl microliter pipette into a sample vial and was analyzed by GC/MS.

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.

2. Results and discussion

Measured concentrations of OPP in soft drinks are provided in Table 1. OPP was detected in 49 of the 55 samples analyzed, with concentrations typically at the μg/l level. The highest OPP value was measured at 16.9 μg/l in a lemon flavored cola, followed by iced tea at 10.3 μg/l, both purchased within the United States. The lowest measured OPP concentration was 0.3 μg/l.The mean concentration of OPP in all samples, calculated using zero for the six samples in which OPP was not detected, was 2.91 μg/l. OPP was not present in any of the bottled soft drink analyzed ($n = 7$).

The ubiquity of OPP in canned soft drinks accords with its presence in canned beer; we detected OPP in 30 of 34 canned beer samples from 2005, while 20 of 39 samples dating from 1993 to 2004 were contaminated with OPP (Coelhan et al., 2006). The high frequency with which we encountered OPP in canned beer led us to suggest it is widely used as a biocide in the canned beer industry. Apparently its use is not restricted to beer as a beverage. We detected OPP in analyzing the same product purchased at different locations and canned at different facilities, indicating that the problem of OPP in canned soft drinks is not a regional one.

Table 1
Concentrations (μg/l) of OPP determined in soft drinks

Speciation	Place of Purchase	Company	Concentration[a]
Cola	Baltimore	1	1.7/2.7
Lemonade	Baltimore	2	1.4/4.4
Diet cola	Baltimore	2	2.6
Lemonade	Baltimore	3	2.5/4.4
Diet cola	Baltimore	1	1.6/3.3
Cola	Baltimore	2	3.8/3.6
Grape soda	Baltimore	4	nd[b]
Green iced tea	Baltimore	4	0.4
Iced tea	Baltimore	4	nd
Cola	Baltimore	5	2.2
Lemonade	Baltimore	6	2.7
Iced tea	Baltimore	7	10.3/6.7
Lemonade	Baltimore	8	6.0
Fruit drink	Baltimore	9	3.8
Cola	Baltimore	9	4.6
Black cherry soda	Baltimore	9	1.1
Grape drink	Baltimore	9	8.6
Ginger ale	Baltimore	9	0.6
Orange drink	Baltimore	9	5.7
Lemonade	Baltimore	9	0.3
Selzer water	Baltimore	10	1.1
Lemon selzer water	Baltimore	10	3.9
Diet cola	Pasadena	1	2.0
Lemonade	Pasadena	2	1.8
Diet cola	Pasadena	2	2.1
Lemonade	Pasadena	11	3.3
Cola	Pasadena	2	2.3
Lemonade	Pasadena	12	1.7
Cola	Pasadena	1	1.9
Ginger ale	Yonkers	13	1.2
Lemonade	Yonkers	3	4.4
Lemonade	Yonkers	13	2.2
Iced tea	Yonkers	14	3.2
Soda	Yonkers	13	2.4
Diet lemonade	Yonkers	6	1.8
Lemonade	Yonkers	6	1.8
Orange soda	Yonkers	2	5.1
Cola	Yonkers	1	2.3
Diet cola	Yonkers	1	2
Lemonade	Yonkers	15	2.8
Lemonade	Yonkers	6	2.1
Cola	Munich	2	4.5/4.3
Cola (similar to above but with a different lot number)	Munich	2	5.5
Lemonade	Munich	2	4.9
Lemon flavored cola	Munich	2	16.9
Energy drink	Munich	16	5.5
Cola	Munich	1	nd
Diet cola	Munich	1	nd

[a] Results for different cans but same lot number separated by slash.
[b] nd: not detected.

Fig. 1A displays a total ion chromatogram (TIC) of a flavored drink, along with ion traces for *m/z* 169 and 350, and the average full scan mass spectrum of the peak with a retention time of 16.09 min (corresponding to that of OPP–PFB). The TIC exhibits numerous peaks, many of which are present at high intensity relative to the peak for OPP–PFB. The ion *m/z* 169 has the second greatest abundance after base peak *m/z* 181 in the mass spectrum of OPP–PFB. Displaying the ion trace of *m/z* 169 (Fig. 1B) reveals that many compounds possess this ion. A peak with a retention time of 16.09 min, corresponding to OPP–PFB, is now clearly discernable. The molecular ion for pentafluorobenzylated OPP is at *m/z* 350; displaying this ion trace (Fig. 1C) reveals only a single peak in the ion chromatogram, at 16.09 min. The full scan mass spectrum of this peak (Fig. 1D) reveals ions characteristic of OPP–PFB: m/z 115, 141, and 350. It also reveals some additional peaks (*m/z* 105, 133, 187, 212, and 243), indicative of some co-eluting constitutent or constituents that we were unable to identify. We do not believe such co-elution affects our quantification of OPP, as the ratio of the peak areas of the ions *m/z* 350, 169, and 141 remained essentially constant within all our samples (and within of 20% of the values obtained in standards).

Chromatograms obtained for other samples were less complex. For example, the TIC of a cola sample (Fig. 2A) exhibited many fewer peaks than the lemon flavored cola. The single ion chromatogram for *m/z* 169 (Fig. 2B) revealed only a few peaks, of which the peak corresponding to OPP–PFB represents one of the major signals. In the full scan mass spectrum of this peak (Fig. 2C) only ions generated from OPP–PFB and deuterated OPP–PFB are observed. For comparison, full scan spectrum of pure OPP derivative is included in Fig. 2D.

OPP concentrations were found to vary from can to can even for samples analyzed of a single brand of soda with the same production date and lot number. This finding is not unexpected, as it accords with our observations with canned beer samples (Coelhan et al., 2006). Migration from sealing materials into drinks may depend on several factors, including OPP concentration in the sealing

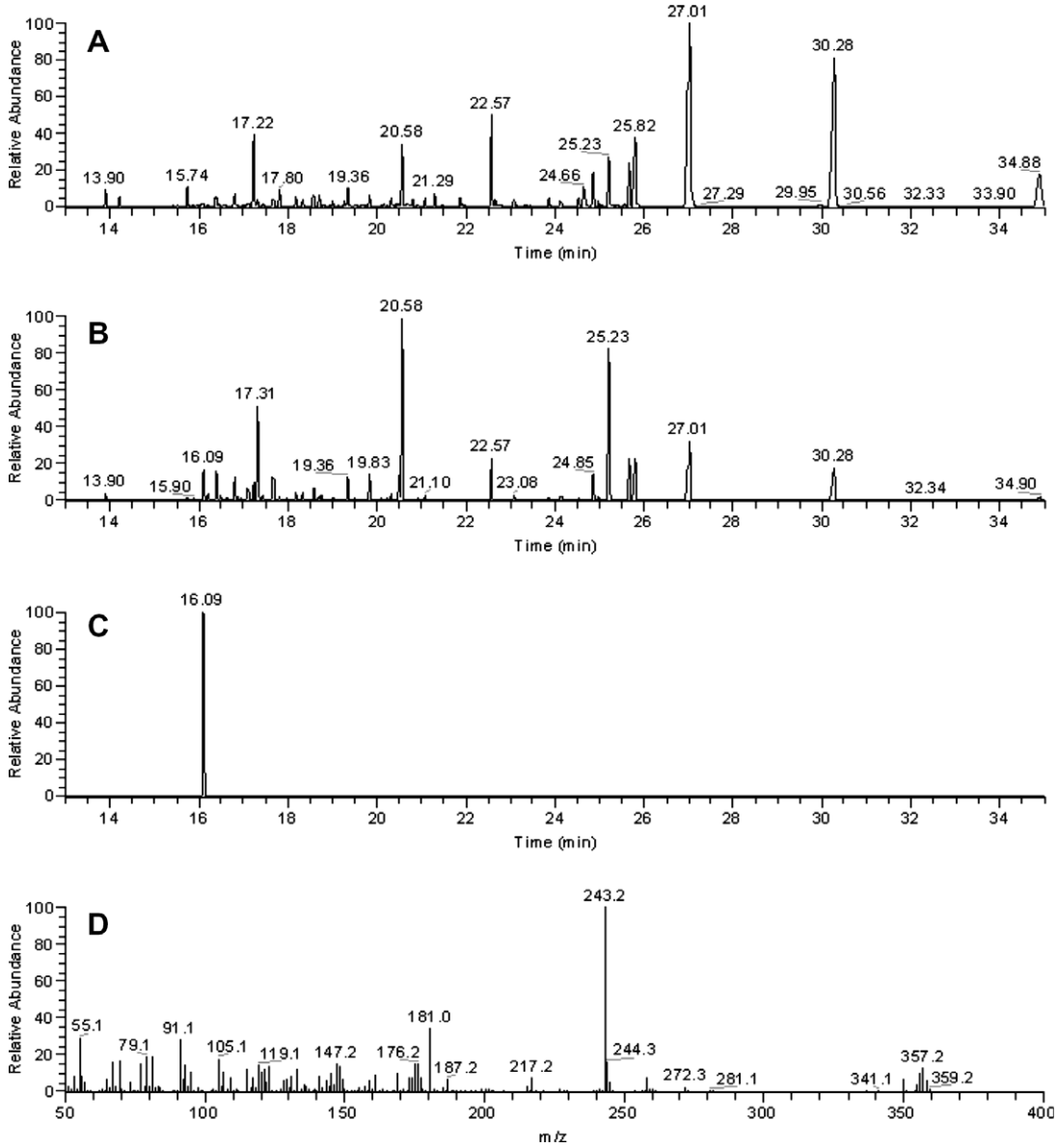

Fig. 1. Total ion chromatogram of pentafluorobenzylated lemonade sample (A), ion traces of pentafluorobenzylated OPP for ions *m/z* 169 (B), *m/z* 350 (C) and full scan mass spectrum of the peak at the retention time of OPP derivative (D).

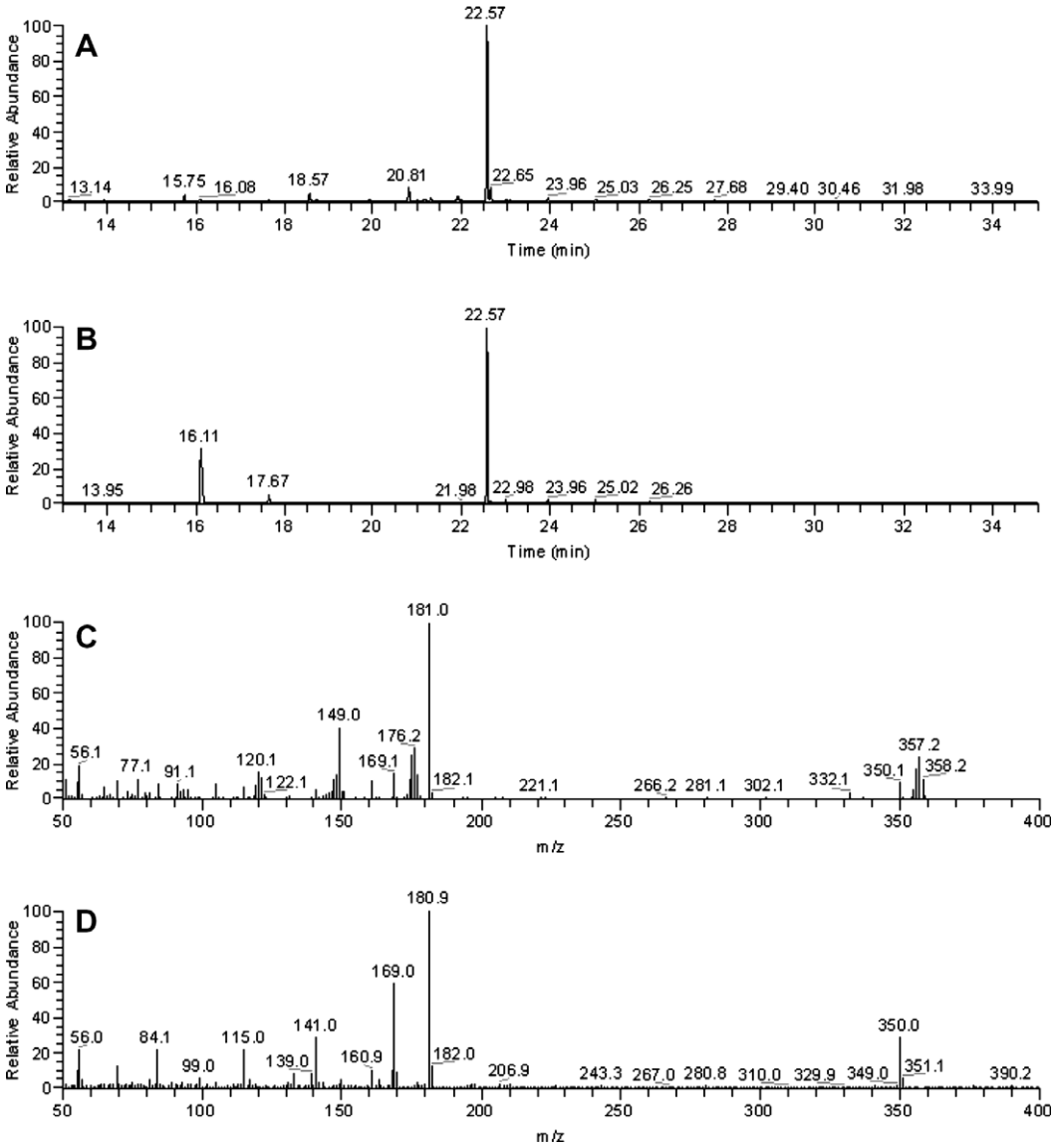

Fig. 2. Total ion chromatogram of a pentafluorobenzylated cola sample (A), ion trace of OPP derivative of *m/z* 169 (B), full scan mass spectra of the peak at the retention time of OPP derivative (C) and pure OPP derivative (D).

material, soda pH, temperature, elapsed time, the presence of constituents within the beverage (such as alcohol or other constituents that may solubilize OPP), whether the surface of sealing polymer is rough or smooth etc. Some of these factors may dictate the nearly 4-fold difference in the mean OPP value (2.9 μg/l) obtained for soft drinks in the present study compared to the mean concentration (10.8 μg/l) we determined in canned beers produced in 2005 (Coelhan et al., 2006). The aqueous solubility of OPP is reported as 0.76 g/kg at pH 5.67 and 20 °C (FAO, 1999). This value is far above the concentrations we have encountered in canned drinks. Consequently, solubility may not represent the limiting factor dictating the rate of OPP migration into canned beverages.

OPP has been detected in wastewater at μg/l levels (Rudel, Melly, Geno, Sun, & Brody, 1998; Ternes, Stumpf, Schuppert, & Haberer, 1998) and in marine sediments (Aguera, Fernandez-Alba, Piedra, Mezcua, & Gomez, 2003). Some fraction of the OPP found in wastewater could originate from contamination of drinks ingested by humans. Very little OPP is excreted in the free form after ingestion by human (Bartels et al., 1988); most is excreted as conjugated forms. Some of the conjugated forms of OPP could conceivably be released during or even prior to treatment of wastewater, although the major fraction of OPP residues excreted by humans probably remains undetected in wastewater. The amount of OPP ingested by human through their consumption of canned drinks could be estimated knowing the number of cans manufactured that contain OPP treated sealing material. It might be instructive to compare this number to the mass of OPP released to wastewater.

It may be reasonably inferred that the reduced consumption of canned beverages has resulted in a reduction of OPP intake by the general population in Germany. The extent of the reduction in human exposure will depend on the occurrence of OPP in canned foods – a topic about which no information exists.

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