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Migration of Nonylphenol from Plastic Containers to Water and a Milk Surrogate

JORGE E. LOYO-ROSALES,[†] GEORGINA C. ROSALES-RIVERA,[‡,||] ANIKA M. LYNCH,[§] CLIFFORD P. RICE,[⊥] AND ALBA TORRENTS*,[†]

Environmental Engineering Program, Department of Civil and Environmental Engineering, University of Maryland, College Park, Maryland 20742, Area de Química, FES-Zaragoza, UNAM, Prol. Plutarco Elias Calles y Batalla del 5 de Mayo s/n, Mexico, 09239, DF, Mexico, Science Department, Northwest High School, 13501 Richter Farm Road, Germantown, Maryland 20874, and Environmental Quality Laboratory, ANRI, ARS/USDA, 10300 Baltimore Avenue, Beltsville, Maryland 20705

Nonylphenol (NP) is used as an antioxidant and plasticizer in some plastic products. After the discovery of its endocrine-disrupting potential, concern over human exposure to this chemical has increased. Recently, a group in Germany estimated the average daily intake of NP from food (7.5 μg/day), excluding water. In the present study, NP, octylphenol (OP), and their respective ethoxylates (1−5) were measured in spring water bottled in three different types of plastic (HDPE, PET, and PVC). NP was present in water from HDPE and PVC containers, at 180 and 300 ng/L respectively, which represent 4.8% and 8% of the value calculated by the German group assuming a consumption of 2 L of water per day. OP was found in water from HDPE extracts in lower amounts, 12 ng/L, and neither the NP- nor the OP-ethoxylates were detected in any of the samples. Attempts to measure these compounds in tap water were unsuccessful, probably because reaction with residual chlorine results in the formation of chlorinated byproducts. Migration of NP from HDPE containers to a milk surrogate was also evaluated; results indicate that the amounts of NP leaching into milk might be similar to those in bottled water.

KEYWORDS: Nonylphenol; bottled water; migration; plastic; LC/MS/MS; HDPE; PET; PVC

INTRODUCTION

After the serendipitous discovery in 1991 of the estrogenic properties of *p*-nonylphenol (NP) by Soto et al. (*1*), NP and other related compounds, such as octylphenol (OP), and their ethoxylated derivatives (nonyl- and octylphenol ethoxylates, NP*n*EOs and OP*n*EOs, respectively, where *n* indicates the number of ethoxylate units) have been the subject of different studies addressing their toxicological properties and their ubiquitous presence in the environment (e.g., *2*, *3*). Due to their uses as industrial and heavy-duty surfactants, they are introduced to the environment mainly from wastewater discharges. Therefore, most of the studies have focused on their presence in aquatic media and their impact on aquatic biota (*3*).

According to Talmage (*4*), one of the main industrial uses of alkylphenol ethoxylates is in plastic production; the production of tris(nonylphenyl)phosphite (TNPP), an antioxidant used in plastics, demands approximately 10% of the total NP used in the U.S. (*5*). It is also acknowledged that some compounds present in plastic packaging have the capacity to migrate into foods and several studies have addressed this phenomenon using specific examples of plastic and chemicals of concern (e.g., *6*−*8*). Both the presence of NP and NP*n*EOs in plastics and the widespread distribution of these compounds in the environment prompted Guenther et al. (*9*) to estimate the average daily intake of NP from food by German consumers. They analyzed a wide variety of food products for NP content. Although they found NP in all their samples (ranging from 0.1 to 19.4 μg/kg of fresh weight), the amount of NP in the foodstuff did not correlate with the packaging material or the fat content of the food, suggesting that NP is introduced to food from a variety of sources, one of them being plastic wrapping and/or containers.

To have a complete assessment of the daily human intake of NP, it is also necessary to include the amounts ingested from water. There has been a significant increase in the consumption of bottled water in the past decade; the market, with a value of 5.2 billion dollars, is projected to grow as much as 30% each year (*10*). Bottled water is usually sold in plastic containers, normally poly(ethylene terephthalate) (PET) and high-density polyethylene (HDPE); poly(vinyl chloride) (PVC) is also used in smaller quantities. There are few studies (most of them

* Address correspondence to this author. Phone: (301) 405-1979. Fax: (301) 405-2585. E-mail: alba@eng.umd.edu.
[†] University of Maryland.
[‡] Area de Química, FES-Zaragoza, UNAM.
[||] On sabbatical leave at the University of Maryland, College Park.
[§] Science Department, Northwest High School.
[⊥] Environmental Quality Laboratory, ANRI, ARS/USDA.

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Japanese) addressing the migration of NP from plastic bottles to water. Toyo'oka and Oshige (*11*) reported the presence of NP in mineral water from PET bottles in concentrations that ranged from 19 to 78 ng/L, but were unable to conclude if the presence of NP in the water was due to leaching from the plastic.

The objectives of this work were (1) to evaluate the presence of NP, OP, and their respective ethoxylates in bottled water to estimate daily human intake from water and (2) to investigate whether the origin of these compounds is the plastic bottle.

MATERIALS AND METHODS

Reagents. NP was obtained from Schenectady International, Schenectady, NY (purity ≥95%, CAS Registry Number 84852-15-3), and OP from Aldrich, Milwaukee, WI (97% purity, CAS Registry Number 140-66-9). NP2EO was an R&D product from Aldrich. NP1EO, NP3EO, NP4EO, NP5EO, and the OP*n*EOs ($n = 1$ to 5) were purified in the laboratory by flash chromatography on silica gel from commercial mixtures as described elsewhere (*12*): NP1EO from Surfonic N-10 (Huntsman Chemicals, Austin, TX); NP3EO, NP4EO, and NP5EO from POE(4) nonylphenol (Chem Service, West Chester, PA); and the OP*n*EOs ($n = 1$ to 5) from POE(3) and POE(5) *tert*-octylphenol (Chem Service). Purity of the standards was assessed by high-performance liquid chromatography with fluorescence detection (HPLC-F) and was above 99% in all cases, except for the octylphenol monoethoxylate, OP1EO (94%). Identity of the compounds was confirmed by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). A mixture of *n*-NP (Lancaster Synthesis, Windham, NH) and *n*-nonylphenol triethoxylate (*n*-NP3EO) synthesized by Ferguson (*13*) was used as an internal standard for quantitation. Ethanol was HPLC/spectrophotometric grade, 200 proof (Aldrich); dichloromethane (DCM) and methanol were high purity, pesticide grade from Burdick & Jackson (Honeywell International Inc., Muskegon, MI). Deionized (18.2 megohm·cm), carbon-free water (DI water) was obtained in the laboratory using a NANOpure water purification system (Barnstead International, Dubuque, IA). Anhydrous Na_2SO_4, granular powder, was purchased from Mallinckrodt Baker Inc. (Paris, KY); both sodium hypochlorite (available chlorine ≥4%) and ammonium acetate (99.99+%) were from Aldrich.

Bottled Water Samples. Spring water jugs (1 gallon) were acquired from three different local stores and brought to the laboratory. Water in HDPE and PET containers was from the same bottler, although the water originated from different springs in the U.S.; water in PVC containers was sourced from a different brand. Special attention was taken to choose all the bottles for a single experiment from the same lot to minimize variation among samples. The water jugs were stored at room temperature and in the dark until analysis, which occurred within 48 h of acquisition.

Extraction. Extraction of the analytes from the milk surrogate was performed by liquid−liquid extraction with DCM, whereas extraction from water was done by solid-phase extraction (SPE) as described below.

The procedure for liquid−liquid extraction was as follows: two 500-mL aliquots from each sample were measured and poured into two 1-L separation funnels. Each aliquot was extracted three times with 50 mL of DCM. DCM extracts from both aliquots were mixed together and dried by passing the liquid through approximately 50 g of Na_2SO_4. DCM was exchanged to methanol in a rotary evaporator and volume reduced to approximately 5 mL. Methanol extracts were transferred to 15-mL graduated tubes and the volume was reduced to 0.5 mL under a gentle nitrogen stream, after which 0.5 mL of water was added. The extracts were then filtered through an Acrodisc LC 13-mm syringe filter containing a 0.2 μm PVDF membrane (Pall Gelman Laboratory, Ann Arbor, MI). Both syringe and filter were then rinsed with 0.5 mL of a 50:50 v/v methanol:water mixture that was added to the extract. Finally, volume was adjusted to 1.5 mL with the methanol:water mixture. Recoveries (average of three determinations ±SD) were NP 78 ± 3%, NP1EO 80 ± 9%, NP2EO 89 ± 11%, NP3EO 106 ± 12%, and NP4EO 110 ± 13%.

The SPE method is described in detail elsewhere (*14*). Briefly, ENV+ SPE cartridges (Isolute ENV+, 500 mg, 6 mL; International Sorbent Technology Ltd., Hengoed, UK) were prerinsed sequentially with 18 mL of DCM, 12 mL of acetone, and 12 mL of DI water in a vacuum manifold before passing the entire contents of the water jugs through them. The cartridges were then dried for 2 h by drawing air through them under vacuum and eluted sequentially with 12 mL of DCM, 12 mL of methanol, and 12 mL of acetone. The collected solvents were evaporated under a gentle nitrogen flow and exchanged for methanol to a final volume of 0.5 mL. Water was added and the solution was filtered as described above for liquid−liquid extraction. Recoveries (average from two determinations ±SD) from spiked bottled water in PET containers using SPE extraction were NP 87 ± 9%, NP1EO 90 ± 8%, NP2EO 90 ± 4%, NP3EO 97 ± 8%, NP4EO 99 ± 20%, NP5EO 92 ± 17%, OP 76 ± 4%, OP1EO 94 ± 4%, OP2EO 100 ± 2%, OP3EO 99 ± 7%, OP4EO 110 ± 15%, and OP5EO 130 ± 16%.

LC/MS/MS Analysis. After extraction, the samples were analyzed by LC/MS/MS as described by Loyo-Rosales et al. (*14*). A Waters 2690 XE separations module (Waters Corp., Milford, MA) coupled to a benchtop triple quadrupole mass spectrometer with an electrospray interface (Quattro LC, Micromass Ltd., Manchester, UK) was used for the analysis. Chromatographic separation was achieved with an MSpak GF-310 4D column, 4.6 × 150 mm (Shodex, Shoko Co., Tokyo, Japan) at 60 °C; injection volume was 10 μL. The mobile phase was composed of solvents A (50:50 v/v 10 mM ammonium acetate in DI water: methanol) and B (100% methanol). Initial conditions were 100% A; the amount of B was increased to 90% in 20 min, held for 8 min, and increased to 100% in 2 min. The column was then stabilized for 20 min at 100% A; total run time was 60 min. Flow rate was set at 0.2 mL/min and all of the eluent was allowed into the MS. Source parameters were the following: capillary voltage 3.5 kV in electrospray positive (ES+) and −2.9 kV in electrospray negative (ES−); extractor voltage 3 and 2 V, respectively; RF lens 0.1 V in both modes; source and desolvation temperatures 140 and 400 °C. Nitrogen was used as nebulizer and desolvation gas (∼80 and 600 L/hr, respectively). The photomultiplier was set at 650 V. Acquisition was done in the multiple-reaction monitoring mode (MRM) in ES+ for the first 25 min of the run and then switched to ES− for 10 min. For NP the parent ion was 218.9 *m*/*z* and its fragment 132.8 *m*/*z*; cone voltage was set at − 40 V, and collision energy to 30 eV. The reader is referred to Loyo-Rosales et al. (*14*) for the ions, cone voltages, and collision energies used for the rest of the compounds. Analyte concentrations were calculated by the internal standard method with *n*-NP and *n*-NP3EO as ES− and ES+ internal standards, respectively. Six-point calibration curves were prepared in 100% A; analyte concentrations ranged from 20 to 700 ng/mL for OP, NP, OP1EO, and NP1EO, and from 6 to 200 ng/mL for the rest of the NP*n*EOs and OP*n*EOs ($n = 2$ to 5). Peak integration and quantitation were performed automatically with MassLynx 3.5 and 4.0 (Micromass Ltd, Manchester, UK).

Migration of NP from Plastic Bottles to Water. This procedure was based on U.S. Food and Drug Administration (FDA) recommendations for the estimation of substance migration from packaging materials to food (*15*). The original water contained in 15 HDPE, 15 PVC, and 6 PET jugs was either extracted by using the SPE method and analyzed as described above or discarded, after which all the jugs were rinsed three times and then filled to the top with DI water. The mouths of all the jugs were covered with Teflon tape before capping them to prevent contact with the caps. The water from 3 HDPE, 3 PVC, and 2 PET jugs was immediately extracted by the SPE method and analyzed as described above (time 0). The remaining bottles were stored in a controlled-temperature growth chamber (PGR 15; Controlled Environments, Ltd., Winnipeg, Canada) at 40 °C and analyzed at different intervals: for HDPE and PVC, three bottles for each type of plastic were analyzed after 48, 120, 240, and 360 h; for PET, two bottles were analyzed after 120 and 240 h.

Migration of NP from Plastic Bottles to Milk Surrogate. This procedure was also based on FDA guidelines (*15*). The original water from 27 HDPE jugs was discarded, after which all the jugs were rinsed three times with DI water and then filled to the top with a 10% v/v ethanol solution. The mouths of all the jugs were covered with Teflon tape before capping them to prevent contact with the caps. Three jugs were extracted with DCM and analyzed as described above (time 0). Half of the bottles were stored in a controlled-temperature growth

Table 1. Concentrations of NP and OP Found in Spring Water Bottled in Three Different Plastic Types (HDPE = high-density polyethylene; PET = poly(ethylene terephthalate); PVC = poly(vinyl chloride))

	concn,[a] ng/L	
sample	NP	OP
HDPE av ($n = 6$)	180	12
HDPE SD	53	2.8
HDPE RSD, %	29	23
PET av ($n = 6$)	ND	BQL
PVC ($n = 12$)	300	BQL
PVC SD	44	NA
PVC RSD, %	15	NA

[a] ND: not detected. BQL: <8 ng/L.

chamber (PGR 15; Controlled Environments, Ltd., Winnipeg, Canada) at 40 °C, and half at 20 °C. Three bottles from each temperature were analyzed after 48, 120, 240, and 360 h.

RESULTS AND DISCUSSION

Bottled Water Analysis. Spring water bottled in HDPE, PET, and PVC containers was extracted using the SPE method above, and the extracts were analyzed for NP, OP, and their respective ethoxylates ($n = 1$ to 5). Results are summarized in **Table 1**. NP was found in HDPE and PVC containers, whereas OP was present in all three types of container materials, albeit at lower concentrations. The variation (expressed as RSD) in NP and OP concentrations among HDPE samples, 29% and 23%, respectively, reflects differences among bottles in the lot rather than variability of the analytical method. Variation in recovery experiments of spiked water was consistently lower—below 10% for both compounds (data not shown).

From the results above, it follows that the amount of NP ingested from water depends on the nature of the container. Assuming an average consumption of 2 L of bottled water per day, a person would ingest an average of 360 ng/day of NP if all water intake came from HDPE jugs; this represents 4.8% of the average daily intake value for NP calculated by Guenther et al. (*9*), 7.5 μg/day. This percentage could increase to 8% if the water was from PVC jugs. Although there are no data available for daily intake of NP from food in the U.S., a comparison of these values to the contributions of the 24 different food groups reported by Guenther et al. (*9*) in Germany suggests that water from HDPE and PVC containers could represent one of the most important individual sources of NP, only behind sausages, apples, and tomatoes. In contrast, water from PET containers would not significantly increase NP ingestion.

Tap Water Analysis and the Effect of Hypochlorite on Analyte Recovery. Attempts were made to measure the analytes in laboratory tap water, but the analysis of spiked samples revealed that NP and OP were not recovered, while recoveries for the ethoxylates (75 to 110%) were approximately 20% lower than those for bottled spring water. No NP*n*EOs or OP*n*EOs were detected in the tap water samples analyzed.

Because of their phenolic nature, NP and OP undergo chlorine substitution reactions in the presence of hypochlorite, resulting in the rapid formation of diverse chlorinated byproducts (*16*). This might explain why NP and OP are not recovered from spiked tap water, which contains residual amounts of chlorine from the disinfection procedure. To test this hypothesis, sodium hypochlorite (1.2 ppm) was added to DI water and spiked with NP, OP, and their ethoxylates. A reference solution (DI water spiked with the analytes) without hypochlorite was also prepared as reference. All the solutions were stored in the dark for 24 h to allow sufficient reaction time. After this period, all the samples were extracted using the SPE method described above and the extracts analyzed by LC/MS/MS. As expected, NP and OP were not recovered from samples containing NaClO, while there was no significant difference in the recoveries of the NP*n*EOs and the OP*n*EOs between the chlorinated and non-chlorinated samples. It is therefore important to consider the reactivity of NP and OP when measuring NP and OP concentrations in water treated with hypochlorite, which includes not only tap water, but also effluent from wastewater treatment plants, where NP is often measured. The toxicity of the chlorination products is also of importance when conducting risk assessment for these compounds, because they have been found to elicit antiestrogenic effects (*16*).

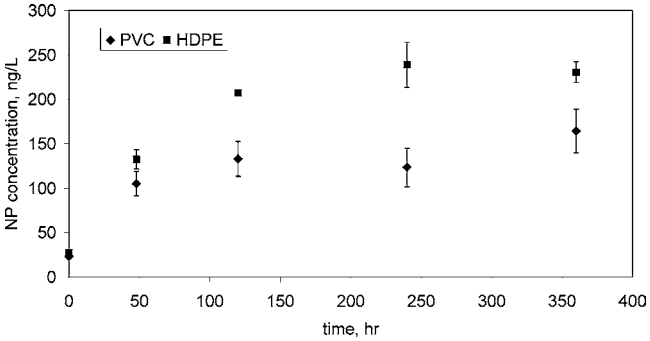

Figure 1. Migration of NP from two types of plastic bottles (HDPE and PVC) to water over time at 40 °C. Error bars represent standard deviation of three determinations.

Migration of NP from Plastic Bottles to Water. The sole presence of NP in bottled spring water is not enough evidence to conclude that this compound migrated from the plastic jugs. NP could have been present in the water itself, or remained as a residue from washing steps during the bottle manufacturing process. Therefore, experiments based on FDA guidelines for migration testing (*15*) were conducted as described in the Experimental Section. Although all samples were analyzed for NP, OP, and their ethoxylates 1 to 5, only NP was found in amounts above quantitation limits (8 ng/L) in water from HDPE and PVC jugs; OP was detected in both types of plastic, but always below quantitation limits (8 ng/L), and no increasing trend was observed in this experiment. Neither NP nor OP was found in extracts from water stored in PET containers, even after 240 h; and none of the ethoxylates were detected in any of the samples, regardless of the nature of the plastic.

Results for NP migration from PVC and HDPE containers are shown in **Figure 1**. The levels of NP increased during the first hours, and tended to stabilize after 120 h at around 140 ng/L for PVC and 230 ng/L for HDPE. Using these values, NP ingestion from bottled water would represent 6% of the daily intake calculated by Guenther et al. (*9*) if all water consumption was from HDPE jugs, and 4% for PVC, in contrast with 5% and 8% calculated above. Interestingly, the amount of NP was higher for HDPE than for PVC in this case, whereas the analysis of the original water contained in these jugs showed higher NP values for PVC. It was also surprising to find lower amounts of NP in the PVC bottles stored at 40 °C than in the original water, which we assumed was stored at room temperature (the actual conditions during storage and transportations are unknown). Both observations might be an indication of exposure to higher temperatures during transport or storage of the PVC bottles, which would result in an increase of NP migration from the plastic to the water. Although these variations could also

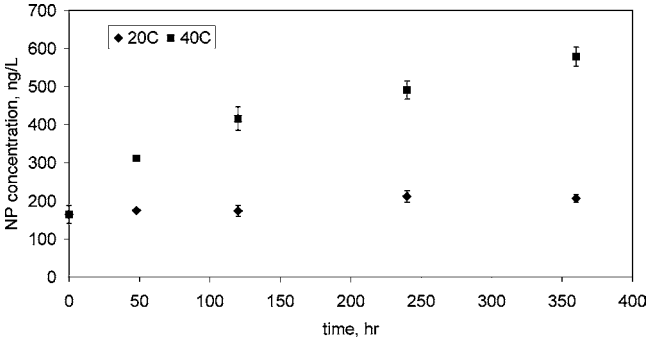

Figure 2. Migration of NP from HDPE bottles to milk surrogate (10% ethanol solution) over time at two different temperatures (20 and 40°C). Error bars represent standard deviation of three determinations.

be caused by other factors (or a combination of them), such as (i) different types of water (DI for migration experiments versus spring water in the original analysis), (ii) storage time before analysis (water in HDPE bottles was analyzed 13 days after being bottled, whereas water from PVC bottles had been in storage for 27 days), and (iii) spring water in the PVC jugs could have contained NP before being bottled.

The FDA guidelines for migration testing of products stored at room temperature claim that testing at 40 °C for 10 days reflects migration levels obtained after 6−12 months storage at 20 °C (*15*). Assuming that bottles are always stored at room temperature (which is very possibly a conservative assumption, especially during the summer months), a better estimation for NP intake from bottled water would require the use of new plastic jugs and the spring water being bottled, whose NP content should be known. In any case, our estimates indicate that bottled water could contribute up to 8% of the daily NP intake estimated by Guenther et al. (*9*).

Migration of NP from Plastic Bottles to Milk Surrogate. HDPE jugs are also used to bottle milk. Because milk contains fat and NP is relatively lipophilic [log K_{ow} = 4.48 (*17*)] it is possible that NP migration from plastics to milk is larger than that to water. To test this, we used a 10% ethanol solution as a surrogate for milk, as suggested in the FDA guidelines (*15*). These guidelines recommend storage at 20 °C to simulate long-term storage of refrigerated foods. We also conducted a test at 40 °C to compare with the results for migration to water. After extraction with DCM, all the samples were analyzed for NP, OP, and their ethoxylates. As found for the water tests, only NP was found in concentrations above quantitation limits, OP was detected but below quantitation limits, and the ethoxylates were not detected. Results for NP are summarized in **Figure 2**. NP concentration increased only slightly with time in the containers stored at 20 °C, whereas it increased 3.5 times after 15 days at 40 °C. Although this increment might be only due to an increase in NP's solubility and diffusion rate from the plastic matrix to the interface with the solvent, an additional consideration is the possible presence of TNPP in the plastic, whose reactions with water and ethanol yield NP. The rates of these two reactions would also increase with temperature and affect the final concentration of NP found in the milk surrogate. As expected, the amount of NP found in the milk surrogate after 15 days at 40 °C, 580 ± 25 ng/L, was higher than that in water, 230 ± 12 ng/L, presumably due to a higher affinity of NP for the milk surrogate.

Averaging the concentration of NP found in the milk surrogate at 20 °C at all times, a value of 186 ± 21 ng/L is obtained, which is very close to the concentration found in the spring water originally contained in the jugs (180 ± 53 ng/L, see **Table 1**). It would appear from these numbers that milk bottled in HDPE may not contribute significantly more NP than water packed in the same containers, probably because milk is normally kept at lower temperatures than water and it is stored for shorter periods of time.

Abbreviations Used: DCM, dichloromethane; DI water, deionized, carbon-free water; HDPE, high-density polyethylene; HPLC-F, high performance liquid chromatography with fluorescence detection; LC/MS/MS, liquid chromatography coupled to tandem mass spectrometry; NP, *p*-nonylphenol; NP*n*EO, nonylphenol ethoxylates (*n* = number of ethoxylate units); OP, octylphenol; OP*n*EO, octylphenol ethoxylates (*n* = number of ethoxylate units); PET, poly(ethylene terephthalate); PVC, poly(vinyl chloride); RSD, relative standard deviation; SD, standard deviation; SPE, solid-phase extraction; TNPP, tris(nonylphenyl)-phosphite.

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