

# The Analysis of Laboratory and Consumer Water Sources for the Presence of BPA and Phthalates

Patricia Atkins, Thomas Mancuso and Vanaja Sivakumar • SPEX CertiPrep, Metuchen, NJ 08840

## Introduction

Bottled water is a multi-billion dollar a year business with projected growth to over \$168 billion in sales worldwide by 2012<sup>(1,2)</sup>. It has gone from a designer fad in the 1980's and 1990's to a mainstay of the world consumer experience. In 2007, the average American drank over 29 gallons of bottled water. Overall, the U.S. consumed over 8.8 billion gallons of bottled water<sup>(2)</sup>. Bottled water has become the second largest consumed beverage in the country, behind carbonated beverages<sup>(2)</sup>. The main reasons that Americans gave for drinking bottled water were they were first substituting bottled water for other beverages and second, they were concerned about the safety of their tap water<sup>(3)</sup>. In many countries, especially developing nations, consumers buy bottled water as a safe alternative to their existing water sources<sup>(4)</sup>. The regulation and monitoring of bottled waters in developing countries can be non-existent or less stringent than regulations in more developed nations.

In the United States, the regulations regarding the quality of municipal water sources and the quality of bottled water are governed by two different agencies. The Environmental Protection Agency (EPA) is responsible for the regulation of municipal water while the Food and Drug Administration (FDA) governs the bottled water regulations<sup>(5-10)</sup>. The FDA has established "Standards of Identity" to differentiate the various bottled water sources: artesian water (aquifer or well water), ground water, mineral water (containing more than 250 ppm total dissolved solids), purified water (products of distillation, deionization and/or reverse osmosis), spring water and municipal water<sup>(11)</sup>.

While the growth of the bottled water industry indicated a worldwide increase in the consumption of bottled water, there are often many costs - financial, ecological and etiological - that are now being addressed in the scientific community and the court of public opinion. The financial costs of bottled water can be great in comparison to the fairly cheap option of U.S. municipal water. The cost for production of bottled water can be as high as \$2 a bottle compared to municipal water that costs a few cent for the same volume<sup>(12)</sup>. The majority of the cost of bottled water is in the production of the bottle and its associated materials (i.e. label, cap, packaging)<sup>(13)</sup>. The environmental costs of bottled water include the use of fossil fuels, energy, transportation, carbon dioxide release, production wastes, and solid refuse.

Bottled water has been advertised around the world as a healthy alternative to other bottled drinks and municipal tap water. Many consumers believe that by choosing bottled water they are choosing a healthier alternative<sup>(3)</sup>. Certain environmental and watchdog groups suggest that bottled water may be more contaminated than municipal water sources as they are governed under less stringent guidelines than those same municipal water sources<sup>(14)</sup>. The Kansas Department of Health and Environment, and other research groups, found traces of various chemicals, including hormone disrupters and carcinogens, in more than half the samples of commercial bottled water tested. In some of these samples it was found that the levels of some of their target compounds exceeded government safety levels<sup>(4)</sup>. Over the past decade, concerns over phthalates and BPA have been highlighted in the scientific community and world press with increasing frequency<sup>(15-28)</sup>. The intense focus on these chemicals has called into question what we consume and how we consume it. The new "popular" wisdom is based on a plethora of studies and "facts" from which recommendations are made regarding which types of bottles, bottled water and conditions to avoid in order to reduce exposure to harmful chemicals.

Potentially there are many sources of organic pollutants in consumer water supplies starting with exposure to pollutants at the aquifer, spring or other water source and continuing through any processing or refinement of the water whether that is in a water treatment center, factory or bottling facility. The greater the external influences on the water, the greater the chance for organic contamination. Questions have been raised if the additional process of bottling water increases the leaching of phthalates and BPA from the bottling materials. The plastic containers for most bottled waters are made of polyethylene terephthalate (PET) or high-density polyethylene (HDPE), which do not contain BPA. But, other plastic containers bearing a plastic identification code (PID) of '7', such as polycarbonate containers, can potentially contain BPA.

Phthalates are a group of esters of 1,2-benzenedicarboxylic acid, primarily used in the production of plastic compounds and plastic containing products. Phthalates are found in consumer and personal care products as binders and coatings for fragrances and colors<sup>(29,30)</sup>. Since the beginning of the first plastic production in the mid to late 1800's, phthalate production has increased to 3.5 million metric tons per year<sup>(31)</sup>. Widespread use of these compounds has led to increased exposure and ingestion of plastic compound residues. In the environment, phthalates have been detected in house dust<sup>(31)</sup> and wastewater<sup>(41, 42)</sup>. Human exposure studies have found detectable levels of phthalates and their by-products in human tissue and urine<sup>(43, 44)</sup> from a variety of sources: inhalation, absorption and digestion<sup>(30, 31, 45-47)</sup>. Studies over the past ten years have linked phthalates to conditions such as childhood asthma<sup>(31)</sup>, reproductive disorders and genetic effects<sup>(16, 32-36)</sup>, obesity<sup>(37)</sup>, and diabetes<sup>(20, 37, 38)</sup>. Concerns over phthalates have caused the U.S. to restrict the use of certain phthalates in children's toys starting in 2009<sup>(21, 39)</sup>. Many other countries, including the European Union member countries, Mexico and Japan, have either restricted or banned the use of certain phthalates in children's toys<sup>(40)</sup>.

BPA, or bisphenol A, is a building block of several essential polymers and polymer additives. BPA is found in various applications including toys, bottles, medical and dental devices, coatings, adhesives, epoxy resins, CDs, and DVDs. Sixty-five to seventy percent of the BPA produced is used in making polycarbonate bottles<sup>(52)</sup>. Production of BPA is now over 3.7 million metric tons a year<sup>(52)</sup>. Some of the first studies that suggested the potential toxicity of BPA were conducted on rats in the 1930's. These experiments showed that BPA exhibited endocrine disrupting properties when fed to rats<sup>(48, 49)</sup>. Since these first studies, the concern over the potential toxicity of BPA has increased<sup>(46, 50, 51)</sup>. The EPA has set a guideline of 50 µg/kg/day human exposure limit<sup>(5, 52)</sup>, but studies by a number of researchers suggest that levels as low as 0.025 µg/kg/day can have permanent adverse health effects<sup>(53-55)</sup>. Studies claim that BPA leaches into food and drink products via contact with plastic containers<sup>(15, 56, 57)</sup>.

The public concern over these potentially dangerous plasticizers and additives have spawned a growing market for phthalate and/or BPA free products. Consumer watchdog groups and environmental advocacy websites promote policies urge consumers not to reuse disposable plastic water bottles and limit the temperature and environmental exposure or prepackaged drinking water<sup>(3, 13, 58-60)</sup>. Common "wisdom" tells the consumer not to expose plastic containers containing phthalates and/or BPA (i.e. water bottles, sports bottles, reusable food containers) to extreme temperatures for the fear of increased leaching of these compounds into water and food sources<sup>(12, 15, 57, 61, 70)</sup>. Bottled water and sports bottle manufacturers, on the other hand, continue to maintain that the levels of phthalate and/or BPA leaching into their products are insignificant<sup>(47, 52, 63)</sup>.

The goal of this study was to examine the currently debated topics regarding BPA and phthalate exposure in consumer water sources such as: **1)** Are BPA and/or phthalates present in commercial bottled water? **2)** Does the exposure of commercial bottled water to summer temperatures increase the leaching of BPA or phthalates into that water? **3)** Are the levels of phthalates and BPA in municipal or filtered water samples significantly different than the levels found in bottled water?

## 2 Experimental

### 2.1 Reagents & Standards

#### 2.1.1 Reagents

HPLC grade water, LCMS grade water, dichloromethane ( $\text{MeCl}_2$ ) and acetone were purchased from major chemical distributors. All of the solvents were contained in glass containers with plastic caps. The other reagent materials, such as the sodium sulfate, sodium hydroxide and hydrochloric acid, were also purchased from major chemical distributors in ACS reagent grades. The solid reagent materials were contained in plastic containers with plastic caps. The sodium chloride was purchased from a local distributor. The sodium chloride was contained in a coated cardboard container.

#### 2.1.2 Reagent Preparation

The solid chemicals for this experiment were contained in original manufacturer's plastic containers. In order to produce a blank with the least amount of outside contaminants, the solid reagents were rinsed with approximately 1-2 mL of dichloromethane ( $\text{MeCl}_2$ ) per gram of solid material. The solids were placed in a funnel lined with filter paper and rinsed with the  $\text{MeCl}_2$ . After the  $\text{MeCl}_2$  had drained, the solids were placed in a 210 °C oven for 10-30 minutes to evaporate any remaining solvents.

Two 60 mL aliquots of the  $\text{MeCl}_2$  rinses were collected from each of the solids washed to determine if phthalates were eluting from the solid materials. At the end of the experiment, pre-cleaned solids were rinsed a second time with  $\text{MeCl}_2$ . This  $\text{MeCl}_2$  rinse was also collected into two 60 mL aliquots for each solid. The "pre-cleaned" and "post-cleaned" solid rinses were analyzed for BPA and phthalates.

A 50% by weight solution of sodium hydroxide and LCMS grade water was prepared.

#### 2.1.3 Standards

The following standards from SPEX CertiPrep, Inc. were used in this experiment: phthalate ester standard (1,000  $\mu\text{g}/\text{mL}$ ) in hexane (cat # 8061-X) (see Table 1 for target phthalates in mix); bisphenol A (1,000  $\mu\text{g}/\text{mL}$ ) in acetone (cat # S-509); deuterated internal standard mix (2,000  $\mu\text{g}/\text{mL}$ ) in  $\text{MeCl}_2$  containing: 1,4-dichlorobenzene-d4, acenaphthalene-d10, chrysene-d12, naphthalene-d8, perylene-d12, and phenanthrene-d10 (cat # CLPS-I90); surrogate standard mix (4,000  $\mu\text{g}/\text{mL}$ ) in  $\text{MeCl}_2$ :benzene containing: 2,4,6-tribromophenol, 2-fluorobiphenyl, 2-fluorophenol, nitrobenzene-d5, p-terphenyl-d14, and phenol-d6 (cat # CLPS-SC4).

Table 1. Target Compounds for Laboratory and Consumer Water Study.

Name	Abbreviation	Retention Time	Ions	Structural Formula	CAS No.
Dimethyl phthalate	DMP	7.57	163, 77, 164, 76	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	131-11-3
Diethyl phthalate	DEP	8.43	149, 177, 150, 65	C <sub>12</sub> H <sub>14</sub> O <sub>4</sub>	84-66-2
Diisobutyl phthalate	DIBP	10.28	149, 57, 41, 223	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	84-69-5
Di-n-butyl phthalate	DBP	10.8	149, 150, 29, 41, 57	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	84-74-2
Di(2-methoxyethyl) phthalate	DMEP	10.98	59, 58, 45	C <sub>14</sub> H <sub>18</sub> O <sub>6</sub>	117-82-8
Diisohexyl phthalate	DIHxP	11.33	149, 43, 85, 150	C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>	146-50-9
Di-n-pentyl phthalate	DNPP	11.66	149, 43, 150, 41, 29	C <sub>18</sub> H <sub>26</sub> O <sub>4</sub>	131-18-0
Bisphenol A	BPA	11.83	213, 228, 119, 214, 91	C <sub>15</sub> H <sub>16</sub> O <sub>2</sub>	80-05-7
Di-n-hexyl phthalate	DNHP	12.36	149, 43, 41, 29, 150	C <sub>20</sub> H <sub>30</sub> O <sub>4</sub>	84-75-3
Butyl benzyl phthalate	BBP	12.42	149, 91, 206, 65, 104	C <sub>19</sub> H <sub>20</sub> O <sub>4</sub>	85-68-7
Hexyl 2-ethylhexyl phthalate	H2EHP*	12.66	149, 43, 251	C <sub>22</sub> H <sub>34</sub> O <sub>4</sub>	75673-16-4
Di(2-n-butoxyethyl) phthalate	DBEP	12.75	149, 57, 56, 101, 85	C <sub>20</sub> H <sub>30</sub> O <sub>6</sub>	117-83-9
Di(2-ethylhexyl) phthalate	DEHP	12.95	149, 167, 279, 71	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	117-81-7
Dicyclohexyl phthalate	DCP	12.96	149, 167, 55, 150, 249	C <sub>20</sub> H <sub>26</sub> O <sub>4</sub>	84-61-7
Di(n-octyl) phthalate	DNOP	13.53	149, 279, 43, 57	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	117-84-0
Dinonyl phthalate	Bisoflex DNP	14.22	149, 293, 71, 57, 43	C <sub>26</sub> H <sub>42</sub> O <sub>4</sub>	84-76-4

\* Abbreviation created for reference only in this study, no known abbreviation found in literature

### 2.1.4 Standard Preparation

Working standard solutions of the phthalate esters, bisphenol A and surrogate standard mixes were created at the 100 µg/mL level and prepared with MeCl<sub>2</sub>. A combined standard mix at the 20 µg/mL level prepared with MeCl<sub>2</sub> was created using the bisphenol A standard, the phthalate ester standard and the surrogate mix standard, All standards were stored at 4 °C.

### 2.2 Glassware Preparation

For all of the samples, 60 mL pre-cleaned VOA glass vials with Teflon-lined septa were used to collect the extracts. The vials and caps were rinsed first with acetone followed by MeCl<sub>2</sub>. The vials were then dried in a 210 °C oven overnight. The caps were allowed to air dry.

Glassware, which made contact with samples, was washed with a series of solvents starting with tap water with laboratory glassware cleaner followed by rinsing with tap water 20 times. The tap water wash was followed by a DI water wash, then with two acetone rinses. The final rinse was with MeCl<sub>2</sub>. Small glassware items were placed in a 210 °C oven to dry for up to an hour and then cooled before using. Larger glassware items, such as the separation funnels, were inverted and allowed to air dry for several hours before use.

### 2.3 Instrumentation and Analytical Conditions

An HP5890 gas chromatograph coupled to a 5972 mass selective detector was used for the analysis of the samples. The GC/MS was equipped with an HP7673 GC/SFC injector. The analytes were separated on a CV-5 capillary column (3.0 m x 0.25 mm x 0.25 µm, Chromatography Associates). The GC oven temperature program was set to an initial temperature of 55 °C for one minute, and raised to 200 °C at 20 °C/minute and held for one minute, then raised to 310 °C at 30 °C/minute and held for a final six minutes.

The MS was operated in electron impact ionization in the scan mode with a sampling rate of 2.7 scans/second, and a scan range of 35-450 m/z. This signal to noise ratio was 3:1. The list of targeted ions for each analyte is summarized in Table 1. The presence of at least two predominant ions and a corresponding retention time was considered a confirmation of identity. The GC/MS interface and MS source were both 280 °C. The injected volume of sample extract was 1 µL.

Chemstation B.02.05 and Enviroquant G1701 BA Ver. B.01.00 were used for the data collection and analysis of the samples.

The initial pH of water samples was determined using a Thermo Orion pH meter model 720. The pH of water samples during extraction was determined using pH paper.

## **2.4 Sample Collection & Treatment**

Identical sets of three commercial bottled water brands were purchased in November, 2008 at a local retail store. The bottled water samples were in plastic containers with plastic screw caps. The plastic identification code on the bottles was "1", indicating the bottles were composed of PET. The bottled water sources, as stated by the packaging, were as follows: Brand A) purified public water sources, USA; Brand B) spring water, France; and Brand C) spring waters located in Maine, USA. One set of bottled water was left at ambient laboratory temperature for the entire experiment. A second set of identical bottled water was placed in an incubating oven set at 60 °C for one week. This was to simulate exposure to temperatures reached inside vehicles during hot summer days<sup>(64)</sup> in an effort to determine whether high temperatures cause an increase in phthalates or BPA. The heated bottles of water were allowed to cool for a day before the samples were measured out into 500 mL aliquots for testing. There was no apparent loss of water volume found between the heated and the room temperature bottles.

Water samples were taken from municipal tap water, and two point-of-use (POU) drinking water systems supplied by municipal tap water lines, to compare the composition of consumer water sources to the bottled water sources. POU system A is a dispensing unit for municipal water and has no filtration or sanitization functions. POU system B utilizes a UV sanitation system and a compressed carbon filter. The POU drinking water systems were sampled at the start of the day when the systems had not been in use for over twelve hours (i.e. stationary systems) and then sampled again after more than 2 L of water had been flushed through the system and dispensing apparatus. The stationary water samples were tested to determine if they contained higher levels of phthalates and BPA than the subsequent flushed water samples. All of these water samples were obtained on the day of extraction and decanted into clean glass containers immediately prior to use.

Several sources of laboratory water were also tested for phthalates. Samples were taken from a laboratory DI water storage bottle, a DI tap connected to a DI filtration source, a bottle of HPLC grade water, and a bottle of LCMS grade water. Two samples of the DI laboratory water from the source tap were taken first after standing without use for more than twelve hours and again after more than 2 L of water were flushed through the system.

In addition to the bottled water, municipal water, and laboratory water samples, several blanks and recovery samples were created using LCMS water. BPA and separate phthalate recovery samples were created by spiking 1 mL of the 100 µg/mL working standards into 500 mL of LCMS water.



## 2.5 Extraction Procedure

A modified method based on BPA extraction by del Olmo *et al.*<sup>(65)</sup> was followed. The del Olmo extraction describes primarily an acid extraction for BPA. This acid extraction was followed by an added base extraction in this experiment.

500 mL of each sample were measured into a glass Class A graduated cylinder that had been cleaned using the procedure previously described (Section 2.2). The water was transferred to a previously cleaned and dried separation funnel. 20 mL of each water sample were transferred to a 40 mL VOA vial for pH analysis.

15 g of cleaned NaCl were added to 500 mL of each water sample and shaken until dissolved. Three drops of hydrochloric acid (37% ACS grade) were added to each flask and shaken. The pH of the water was tested on pH paper. HCl was added drop-wise until the resulting pH of the water measured below pH 3.

The samples were extracted using two 30 mL aliquots of MeCl<sub>2</sub>. To each 30 mL aliquot of MeCl<sub>2</sub> was added 0.5 mL of surrogate standard (100 µg/mL). The first 30 mL aliquot of MeCl<sub>2</sub> was added to the separator funnel and shaken for 30 seconds to one minute. The organic phase was collected into a 60 mL VOA vial. The second aliquot of 30 mL of MeCl<sub>2</sub> was added to the funnel and the process repeated. The organic phase was dehydrated using “cleaned” Na<sub>2</sub>SO<sub>4</sub>.

1 mL of NaOH (50% by weight with LCMS water) was added to the extraction funnel and shaken. The pH of the water was tested with pH paper. Base was added until the pH of the water measured from 9-10. The extraction procedure for the base phase of the samples was the same as the acid phase extraction. The organic phase was collected and dehydrated in the same method as previously described. The acid and base phase extracts of each sample were combined and concentrated to 1 mL in a thermal evaporation unit using heat and ultra high purity nitrogen gas.

Blanks and recovery standard samples were extracted in the same manner as all water samples. Blanks consisted of LCMS grade water extracted at several different points in the sampling schedule. Dichloromethane solvent blanks were taken directly from the solvent bottle. Dichloromethane extraction blanks consisted of 60 mL of MeCl<sub>2</sub>, which was extracted in the same manner as the rest of the water samples. Recovery samples consisted of separate aliquots of 500 mL of LCMS grade water spiked with 1 mL of the working BPA or phthalate mix standards (100 µg/mL). All of the recovery samples were extracted in the same method as the water samples.

All concentrated extracts were spiked with 10 µL of the CLPS-I90 internal standard mix (2,000 µg/mL) prior to being analyzed by GC/MS.

## 3 Results and Discussion

### 3.1 Phthalate Contamination in the Laboratory

The largest difficulty in examining phthalate esters in any material is that there are numerous sources of external contamination. The obstacles facing a researcher in regards to external phthalate contamination have been well documented by Tienpont, *et al.*<sup>(29)</sup>, Leivadara *et al.*<sup>(4)</sup> and Cao<sup>(66)</sup>. Contamination from the solvents, reagents, glassware, and extraction procedures must be reduced as much as possible to obtain accurate phthalate levels.

The solid reagents obtained for this study were contained in plastic containers and subject to phthalate contamination. Sources of possible contamination of the reagents could be from processing, purification and/or packaging of the materials. Efforts to find phthalate-free packaging were unsuccessful. In an effort to reduce phthalate contamination, the solid reagents were rinsed with MeCl<sub>2</sub> and dried in a 210 °C oven.

The initial MeCl<sub>2</sub> rinses of the NaCl and Na<sub>2</sub>SO<sub>4</sub> samples showed a significant difference in the amount of phthalates recovered between rinses (Table 2). The first rinse of Na<sub>2</sub>SO<sub>4</sub> contained fourteen phthalates. Subsequent rinses showed decreasing numbers and amounts of phthalates (Figure 1). The initial NaCl rinses had fewer types of phthalates but an increased concentration of those phthalates when compared to the Na<sub>2</sub>SO<sub>4</sub> rinses (Figures 2a & 2b). By the third and fourth rinses, the levels of phthalates and BPA in the NaCl samples were not detectable (Figure 3). The most prevalent phthalates found in both the Na<sub>2</sub>SO<sub>4</sub> and NaCl washers were: diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBP), di(2-n-butoxyethyl) phthalate (DBEP), and di(2-ethylhexyl) phthalate (DEHP).

Table 2. Phthalate and BPA Levels From Solid Reagent Rinses (ppb).

Retention Time	Compound	MeCl <sub>2</sub> rinse of Na <sub>2</sub> SO <sub>4</sub>				MeCl <sub>2</sub> rinse of NaCl			
		1st	2nd	3rd*	4th*	1st	2nd	3rd*	4th*
7.57	DMP	0.00	0.00	0.00	0.00	7.33	0.00	0.00	0.00
8.43	DEP	4.50	0.00	0.00	0.00	241.00	135.17	0.00	0.00
10.28	DIBP	9.67	0.00	0.00	1.83	67.17	65.67	0.00	0.00
10.8	DBP	16.17	1.83	0.00	0.00	331.67	322.83	0.00	0.00
10.98	DMEP	14.83	5.83	0.00	0.00	0.00	0.00	0.00	0.00
11.33	DIHxP	12.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11.66	DNPP	13.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11.83	BPA	0.00	17.67	0.00	0.00	0.00	0.00	0.00	0.00
12.36	DNHP	12.50	0.00	0.00	0.00	29.17	30.00	0.00	0.00
12.42	BBP	16.50	7.33	0.00	0.00	79.00	78.50	0.00	0.00
12.66	H2EHP	13.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.75	DBEP	15.00	0.00	0.00	0.00	432.50	439.83	0.00	0.00
12.96	DCP	8.33	7.17	0.00	0.00	77.83	0.00	0.00	0.00
12.95	DEHP	17.50	10.33	0.00	0.00	141.50	154.50	0.00	0.00
13.53	DNOP	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14.22	Bisoplex DNP	8.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Total ppb</b>		<b>172.50</b>	<b>50.17</b>	<b>0.00</b>	<b>1.83</b>	<b>1407.17</b>	<b>1226.50</b>	<b>0.00</b>	<b>0.00</b>
<b># of Compounds</b>		<b>14</b>	<b>6</b>	<b>0</b>	<b>1</b>	<b>9</b>	<b>7</b>	<b>0</b>	<b>0</b>

\* Materials had been rinsed and baked at 210 °C prior to obtaining 3<sup>rd</sup> and 4<sup>th</sup> rinses

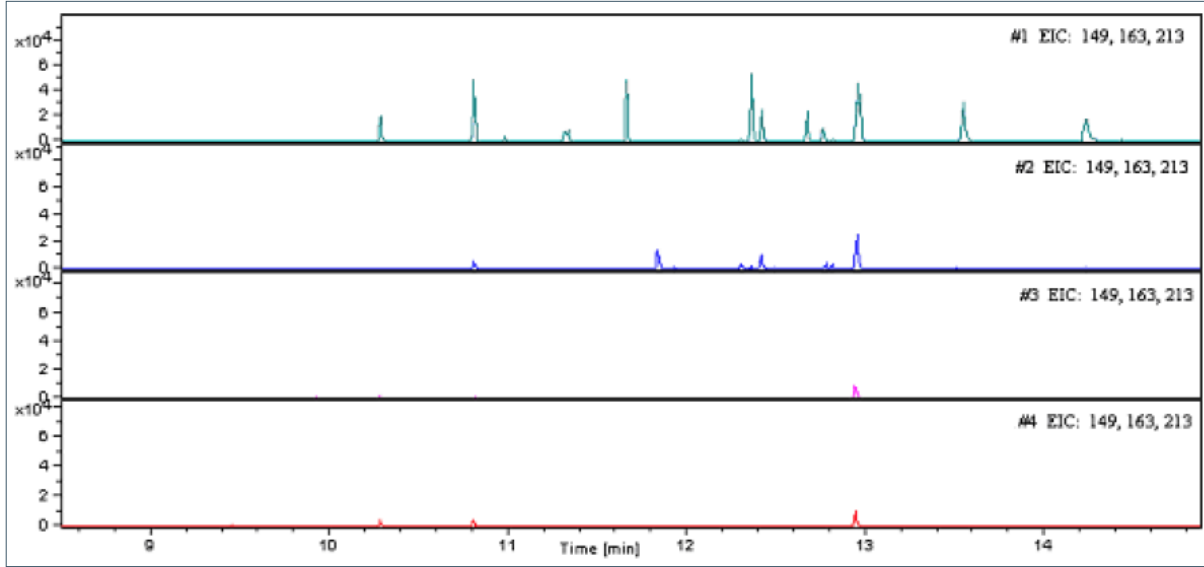


Figure 1. Extracted Ion Chromatograms (EIC) of BPA & Phthalates of  $\text{Na}_2\text{SO}_4$  Rinses.

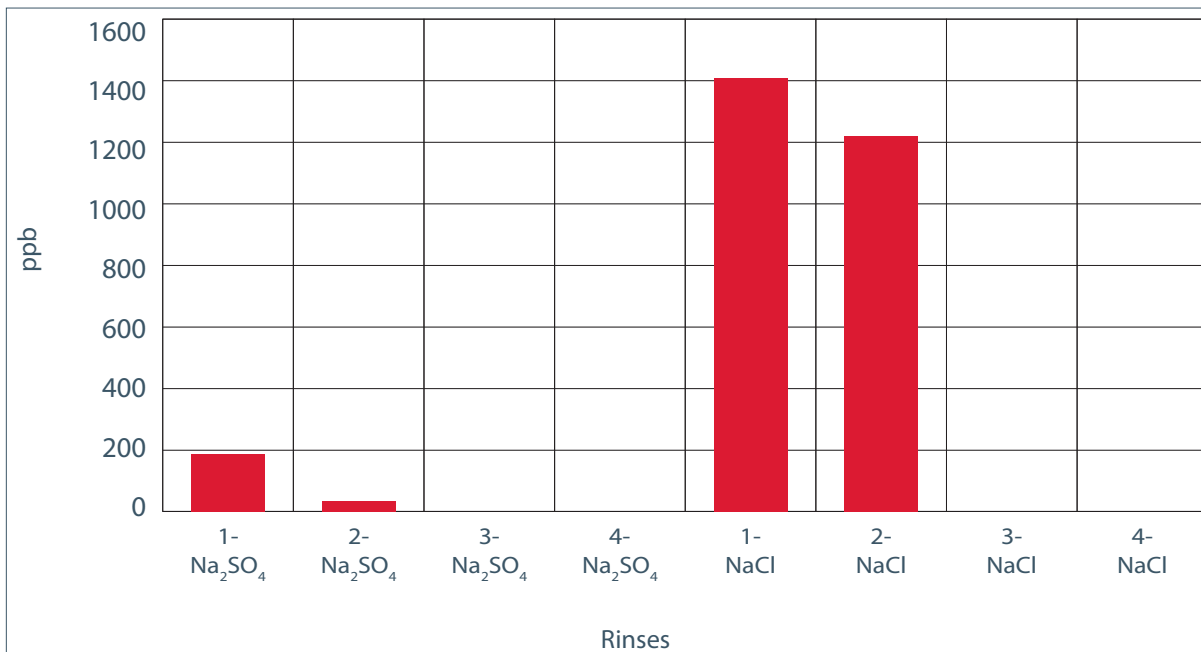


Figure 2a. Comparison of Total Concentration (ppb) of BPA & Phthalates Detected in Solid Reagent Rinses.



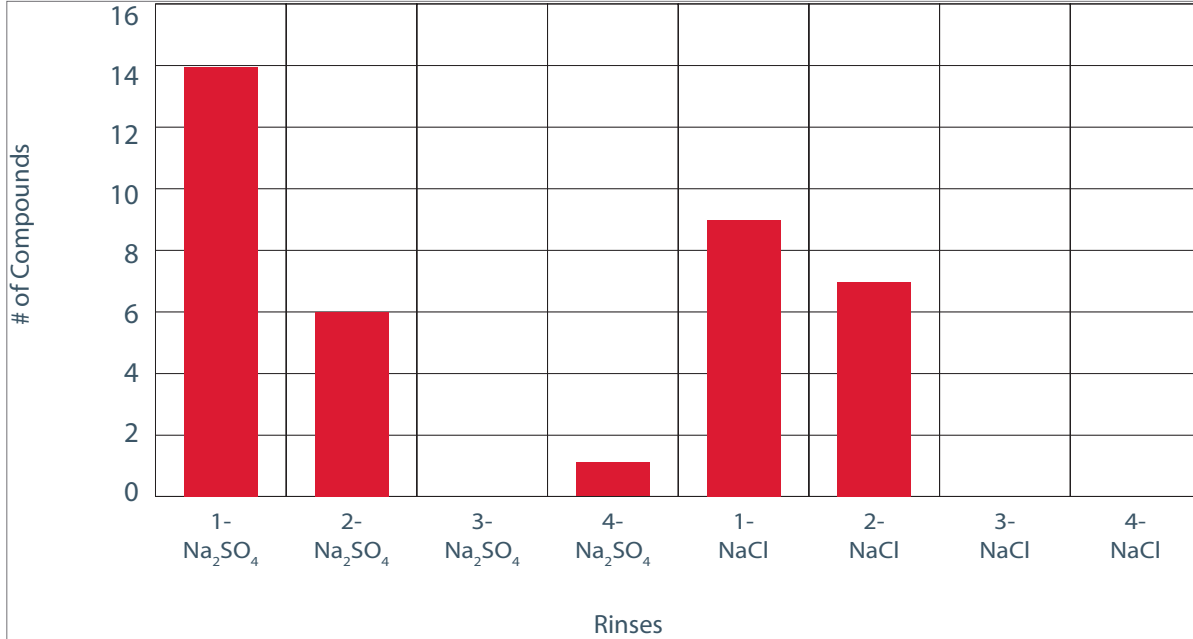


Figure 2b. Comparison of Total Number of BPA & Phthalates Detected in Solid Reagent Rinses.

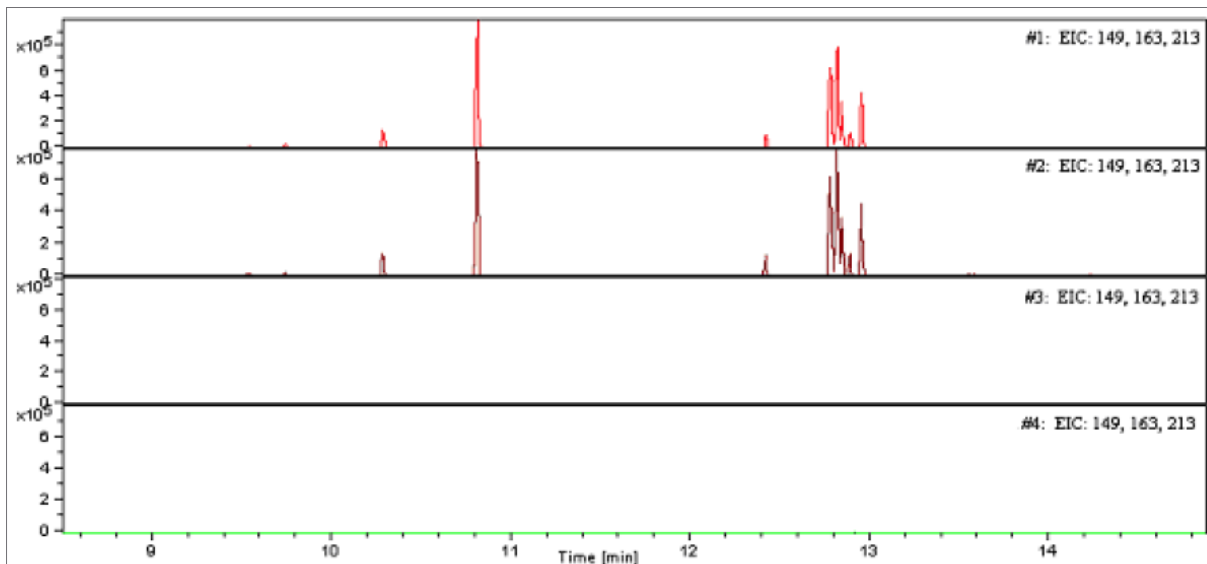


Figure 3. Extracted Ion Chromatograms (EIC) of BPA & Phthalates of NaCl Washes.

The analysis of the MeCl<sub>2</sub> solvent blank and the MeCl<sub>2</sub> rinses of the NaOH solid showed that there were no detectable phthalates in either set of samples. The MeCl<sub>2</sub> extraction blanks contained no detectable BPA or phthalates.

The results highly suggest that all chemical reagents should be examined as possible phthalate sources before using these materials for phthalate and BPA analysis. Dichloromethane, used as a solvent in this experiment, appeared to be free of the target compounds of interest and was an acceptable extraction process blank. In addition, the NaOH used in the base phase extraction appeared to be free from phthalate contamination, possibly due to the affinity of phthalate esters and BPA for acidic matrices. Bosnir *et al.*<sup>(67)</sup> showed that there was higher migration of phthalates into acidic soft drinks than into mineral water highlighting the acid affinity of these target analytes.

Compounds such as the sodium chloride and the sodium sulfate appeared to be highly contaminated with a wide range of different types of organic contaminants. The level of these contaminants could definitely alter the ability of a researcher to obtain accurate phthalate and BPA levels.

### 3.2 Laboratory Water Samples

Phthalate contamination is widespread in the laboratory environment, making it nearly impossible to find water blanks free of phthalates. Five sources of laboratory water were tested for use as blanks. Three of these samples came from a laboratory de-ionized water system and two samples came from reagent grade bottled waters. The water from the de-ionized source was tested after sitting in the lines overnight and after flushing over 2 L of water (as described for the consumer POU systems). In addition to the flowing DI water, samples of water were taken from a laboratory carboy made of HDPE (High Density Polyethylene) containing water from this same source. The DI water in the carboy had been exposed to the carboy for an indeterminate amount of time, however, the time exposed was no less than three weeks. A sample of municipal tap water was taken to compare to the laboratory water samples.

The highest levels of total concentration of phthalates and total number of phthalates were found in the HPLC grade water. The lowest total concentration of phthalates was found in the LCMS grade water. The lowest total number of separate phthalates was found in the municipal tap water. DEHP and BBP were found to have the highest concentration of all of the phthalates in the laboratory water samples. Many researchers have reported that DEHP is one of the phthalates found to migrate into food and beverage sources<sup>(4, 30, 66-68)</sup>. The HPLC grade water was the only sample with a detectable amount of BPA (Table 3).

Table 3. Phthalates and BPA Concentrations Found in Laboratory Water Sources (ppb).

Compound	HPLC	LCMS	DI Source Flushed	DI Source Stationary	DI Bottle	Municipal Tap
DEP	6.28	0.18	0.00	0.30	0.50	0.00
DIBP	3.52	0.16	0.88	1.36	0.52	0.00
DBP	16.72	0.00	0.00	0.00	0.54	0.00
BPA	3.16	0.00	0.00	0.00	0.00	0.00
BBP	44.74	0.20	2.32	0.63	0.47	1.29
DCP	1.00	0.00	0.00	0.00	0.00	0.00
DEHP	15.60	0.63	5.92	26.41	2.44	1.94
<b>Total c (ppb)</b>	<b>91.02</b>	<b>1.17</b>	<b>9.12</b>	<b>28.70</b>	<b>4.47</b>	<b>3.23</b>
<b>Total # of Compounds</b>	<b>7</b>	<b>4</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>2</b>

The laboratory water sources showed widespread contamination by a large number of phthalates as well as BPA. All water samples had a total concentration of target compounds of over 1 ppb. The differences in analyte levels in the water samples shows that the researcher must be cognizant of the goal of the study when selecting a water blank since the reagent grade water may not have the lowest phthalate variety or concentration available.

### 3.3 Consumer Water Sources: Bottled Water, Municipal Water and Filtered Water

#### 3.3.1 Comparison of Consumer Water Sources at Room Temperature

Each of the commercial bottled water brands had less than 10 ppb total phthalates at room temperature. None of the bottled water samples contained detectable amounts of BPA. The total number of phthalates found in the bottled water ranged from two to six different phthalates. In comparison, the municipal tap water had less than 4 ppb total concentration of phthalates and two different detectable phthalates. The municipal water did not contain detectable amounts of BPA. See Table 4 for target compounds detected in consumer water sources.

The greatest variability of phthalate concentration, presence of BPA, and total number of target compounds was found in the Point-of-Use (POU) filtered water systems (Figure 4a and 4b). The water samples collected from the filtration and sanitization system (POU-B), having been unused for up to twelve hours (stationary samples), contained both the highest concentration and the highest number of target compounds. The concentration and level of target compounds was found to be lower in the samples taken after the same system had been flushed with several liters of water (flushed samples). A measurable amount of BPA was also detected in both the flushed and stationary filtered water samples. In contrast, the POU water dispensing system, with no discernible filter or sanitization system (POU-A), has some of the lowest levels of target compounds compared with the rest of the consumer source samples. BPA was not detected in the samples from the dispensing system.

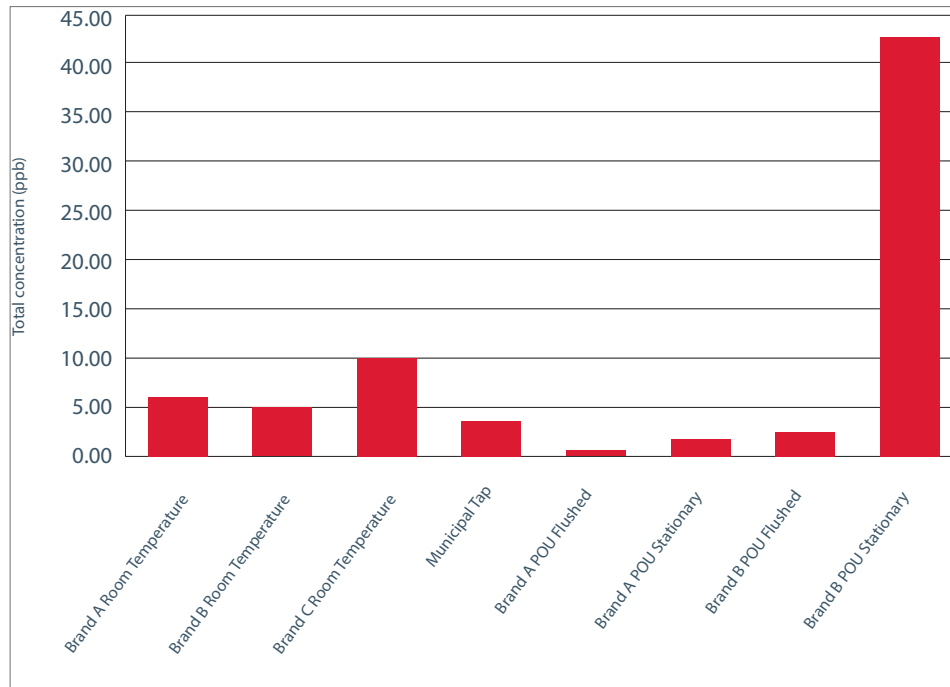


Figure 4a. Comparison of Total Concentration of Target Compounds in Consumer Water Sources at Room Temperature.

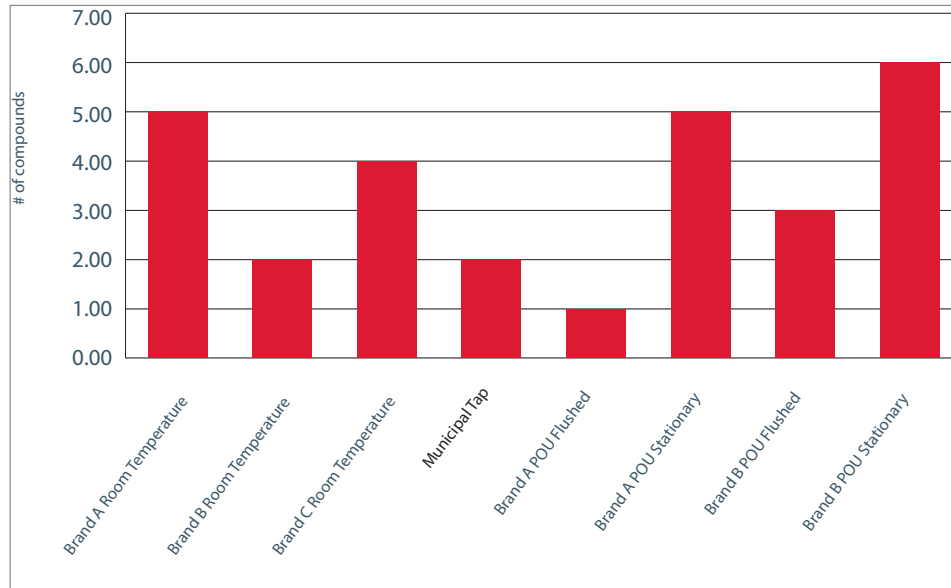


Figure 4b. Comparison of Total Number of Target compounds in Consumer Water Sources at Room Temperature.

### 3.3.2 Comparison of Consumer Bottled Water Sources After Exposure to Heat

Samples of the commercial bottles water held at 60 °C temperatures for one week. The samples were then analyzed to determine if the heat exposure increased the BPA and phthalate levels in the heated bottles. The total concentration of target compounds in the heated commercial water bottle samples did not show a significant increase over the corresponding room temperature samples (Figure 5a). All of the bottled water samples had total concentrations of target compounds of less than 12 ppb. The heated samples had a small increase in the total number of target compounds as compared with the corresponding room temperature samples. The largest change was an increase from two identified phthalates to five identified phthalates for Brand A (Figure 5b).

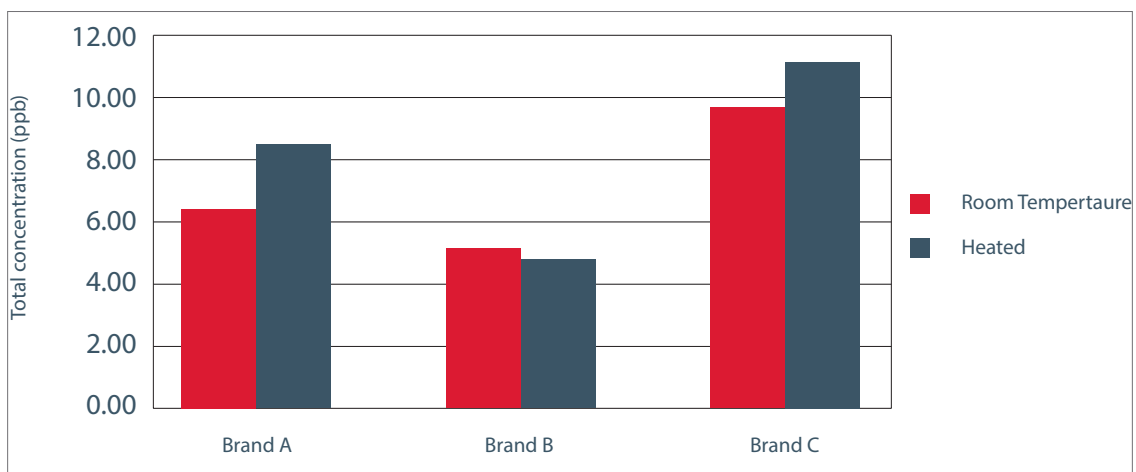


Figure 5a. Comparison of Compound Levels in Room Temperature vs. Heated Commercial Bottled Water.

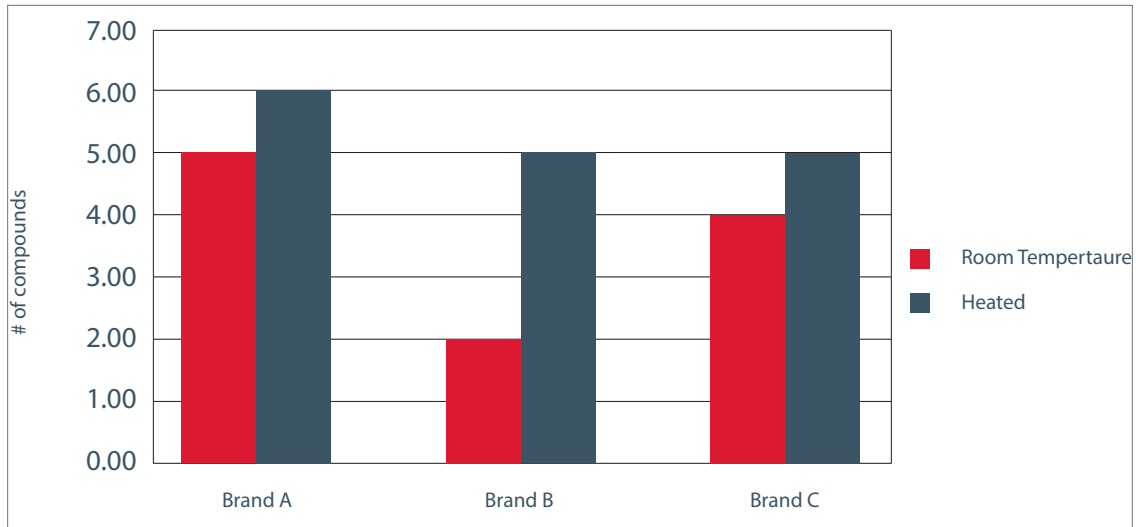


Figure 5b. Comparison of Number of Phthalates and BPA in Room Temperature vs. Heated Commercial Bottled Water.

The levels of DEP and BBP increased slightly in the heated samples of all three brands while the level of DEHP decreased in Brands B and C but remained virtually unchanged in Brand A. Several researchers including Jie *et al.*<sup>(69)</sup>, Casajuana & Lacorte<sup>(70)</sup>, and Leivadara *et al.*<sup>(4)</sup> have found higher concentrations of DEHP in water samples exposed to temperatures below 20 °C than water samples exposed to higher temperatures. These results suggest that DEHP may degrade at higher temperatures<sup>(4)</sup> (see Figure 6).

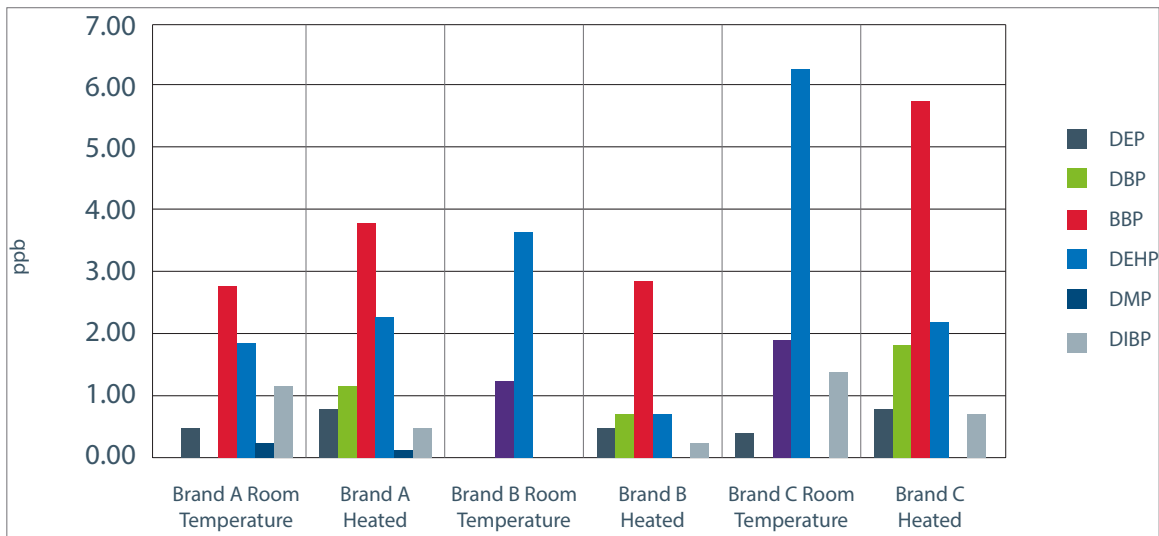


Figure 6. Changes in Phthalate Levels in Bottled Water After Exposure to Heat.

Table 4. Target Compounds Detected in Consumer Water Sources (ppb).

	DMP	DEP	DIBP	DBP	BPA	BBP	DEHP	Bisoflex DNP	Total ppb	Total # target compounds
Brand A Room Temperature	0.16	0.40	1.10	0.00	0.00	2.70	1.88	0.00	<b>6.24</b>	<b>5</b>
Brand A Heated	0.10	0.84	0.40	1.10	0.00	3.74	2.22	0.00	<b>8.40</b>	<b>6</b>
Brand B Room Temperature	0.00	0.00	0.00	0.00	0.00	1.20	3.66	0.00	<b>4.86</b>	<b>2</b>
Brand B Heated	0.00	0.40	0.15	0.61	0.00	2.84	0.64	0.00	<b>4.64</b>	<b>5</b>
Brand C Room Temperature	0.00	0.30	1.28	0.00	0.00	1.88	6.30	0.00	<b>9.76</b>	<b>4</b>
Brand C Heated	0.00	0.82	0.58	1.74	0.00	5.66	2.20	0.00	<b>11.00</b>	<b>5</b>
Municipal Tap	0.00	0.00	0.00	0.00	0.00	1.29	1.94	0.00	<b>3.23</b>	<b>2</b>
Brand A POU Flushed	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.00	<b>0.42</b>	<b>1</b>
Brand A POU Stationary	0.00	0.18	0.18	0.25	0.00	0.60	0.74	0.00	<b>1.95</b>	<b>5</b>
Brand B POU Flushed	0.00	0.00	0.00	0.00	0.09	0.14	2.38	0.00	<b>2.61</b>	<b>3</b>
Brand B POU Stationary	0.00	0.27	0.00	0.33	0.04	0.04	4.41	36.95	<b>42.04</b>	<b>6</b>

All of the consumer water sources had less than 11 ppb of total targeted compounds. The only exception to this observation was the water sample from the stationary POU filtered water source (POU-A) that had a concentration of target compounds over three times higher than any other source. The most prevalent phthalates found in the consumer water sources were BBP and DEHP. In the bottled water industry, the common phthalate, (2-ethylhexyl) phthalate (DEHP), is often used as a gasket for inside the plastic cap of the bottles<sup>(62)</sup>. BPA was not found in any of the bottled water sources or municipal water samples. BPA was only found in the POU filtration and sanitization system samples (POU-B). The water samples from the municipal tap sources had the overall lowest amount of phthalates of all of the consumer and laboratory sources.



### 3.4 Concentrations of Phthalates and BPA in Consumer Water Samples and U.S. Government Regulations

The quality of bottled water is governed under the auspices of the FDA. The FDA, to date, has not defined maximum levels for either BPA or phthalates for bottled water products. On the other hand, municipal and public waters are regulated by the EPA, which has set limits and levels for BPA and many phthalates. One measure of exposure is called the Oral Reference Dosage or RfD. The EPA, on its website, defines the RfD in the following way: “...the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is generally expressed in units of milligrams per kilogram of body weight per day (mg/kg/day)”. The EPA RfD values for BPA and four additional phthalates can be seen in Table 5<sup>(5, 7-10)</sup>. The EPA has another measure of contamination in drinking water called the “Maximum Contamination Level” or MCL. The MCL is the maximum concentration of a chemical that is allowed in public drinking water systems. Only one phthalate (DEHP) is currently measured by the EPA under drinking water standards. The current MCL for DEHP is 6 ppb<sup>(6)</sup>. There is currently no MCL standard listed for BPA.

In examining the phthalates and BPA detected, a comparison was made to the current EPA RfD for five compounds: DEP, DBP, BPA, BBP, and DEHP. The phthalates with the highest concentration in room temperature bottled waters were DEHP, DBP and BBP. The highest level of DEHP found was 6.30 ppb. The EPA oral reference dosage (RfD) for DEHP is 2 µg/kg/day, which means a dosage limit for a 170 lb person could be up to 150 µg. A typical serving size of bottled water is the 591 mL bottle. The sample size of this study was approximately the same size as the typical commercial bottled water. The levels of phthalates found in these bottled water samples were far below the governments RfD (see Table 5). The levels of all monitored phthalates and BPA in all of the consumer water sources were below the EPA RfD.

Table 5. EPA RfD Guidelines for Selected Phthalates and BPA Compared to Experimental Results (µg/500 mL Sample).

EPA RfD (µg/kg/day)	DEP		DBP		BPA		BBP		DEHP	
	8 µg	% of RfD found in 500 mL sample	125 µg	% of RfD found in 500 mL sample	50 µg	% of RfD found in 500 mL sample	200 µg	% of RfD found in 500 mL sample	2 µg	% of RfD found in 500 mL sample
For 170 lb person (77.3 kg) (µg/day)	618.4 µg		9662.5 µg		386.5 µg		15460 µg		154.6 µg	
	<b>Total µg</b>		<b>Total µg</b>		<b>Total µg</b>		<b>Total µg</b>		<b>Total µg</b>	
Brand A Room Temperature	0.4	0.03	0	0	0	0	2.7	0.01	1.88	
Brand A Heated	0.84	0.07	0.55	0	0	0	3.74	0	2.22	0.72
Brand B Room Temperature	0	0	0	0	0	0	1.2	0.01	3.66	1.18
Brand B Heated	0.4	0.03	0.31	0	0	0	2.84	0.01	0.64	0.21
Brand C Room Temperature	0.3	0.02	0	0	0	0	1.88	0.02	6.3	2.04
Brand C Heated	0.82	0.07	0.87	0	0	0	5.66	0	2.2	0.71
Municipal Tap	0	0	0	0	0	0	1.29	0	1.94	0.63
POU A Flushed	0	0	0	0	0	0	0	0	0.42	0.14
POU A Stationary	0.18	0.01	0.14	0	0	0	0.6	0	0.74	0.24
POU B Flushed	0	0	0	0	0.09	0.01	0.14	0	2.38	0.77
POU B Stationary	0.27	0.02	0.17	0	0.04	0.01	0.04	0	4.41	1.43

### 3.5 Examination of pH and Compound Levels

A study by Bosnir, *et al.*<sup>(67)</sup> showed that pH might possibly increase the migration of phthalates from plastic bottles into the liquid. The researchers found that the soda samples all had pH levels below three while the mineral water samples had pH levels above five. Their studies determined that the soda samples had higher concentrations of phthalates than the mineral water samples. Their conclusions were that pH could be responsible for the increased leaching of phthalates into the more acidic sodas over the more neutral water samples.

In an attempt to determine if there was a correlation between pH and concentration of phthalates for the bottled water samples, the pH of each of the bottled water samples (both room temperature and heated samples) was measured to determine whether the pH changed after exposure to heat. Several other consumer and laboratory water sources were also measured for pH.

The pH of samples ranged from 6.0 to 8.0. The pH levels and the corresponding compound concentrations can be seen in Table 6. The pH of the bottled water samples was found to be higher in the heated samples than in the room temperature samples. There did not appear to be a significant correlation between changes in pH and number or concentration of target compounds. Further studies would be needed to determine if changes in pH of water samples effects the concentration or number of the target compounds.

Table 6. Selected Water Source pH and Concentration Results.

	pH	Total Target Compound Concentration ppb	# of Target Compounds
LCMS	6	1.17	4
Brand A Room Temperature	6	6.24	5
Brand A Heated	6.5	8.40	6
Brand B Room Temperature	7.4	4.86	2
Brand B Heated	7.6	4.64	5
Brand C Room Temperature	6.4	9.76	4
Brand C Heated	7	11.00	5
Municipal Tap	8	3.23	2
POU A Flushed	7.9	0.42	1
POU A Stationary	7.9	1.95	5
DI Source Flushed	8	9.12	3
DI Source Stationary	7.9	28.70	4

## 4 Conclusions

Since there is a lot of debate on the safety of consumer water supplies it was important to address some of the common points of discussion such as: the exposure of commercial bottled water to extreme temperatures and the cleanliness or purity of bottled water versus municipal source water. There was also the question as to whether commercial filtration systems actually reduced contaminants.

The innate problem associated with the study of phthalates and BPA in water was found to be the numerous potential sources of contamination. It was necessary to limit analyte contamination by attempting to remove phthalate and BPA residue from as many experimental components as possible. The cleaning of reagent solids with  $\text{MeCl}_2$  and subsequent heating appears to dramatically reduce the amount of phthalate and BPA contamination coming from the solid reagents. The choice of laboratory water blanks was also critical to the analytes being measured. Common laboratory water sources such as HPLC grade water or house DI water may be within specifications for their intended and designated use but may be unsuitable for phthalate or BPA analysis.

The first part of our study looked at the phthalate and BPA levels in bottled water. We compared these samples to each other and to samples of other consumer and laboratory waters maintained at room temperature. Looking at the number of compounds of interest and their concentration, it was found that there was no significant difference in levels between the brands of bottled water studied.

The second part of our study was to determine whether our data supported the commonly debated theory that heating bottled water increases the level of phthalates and BPA in the water. Our results did not find any significant increase in the concentration of phthalates in the commercial bottled water after being heated, however, there was a slight increase in the number of phthalates detected. One phthalate, DEHP, appeared to decrease in two of the water brands after heating. Studies cited previously seem to suggest there is a possibility that some phthalates, DEHP in particular, are subject to degradation at temperatures above  $20\text{ }^\circ\text{C}$ <sup>(4, 69, 70)</sup>.

There was little to no statistical difference between phthalate levels in the different brands of bottled water and the municipal water sources. In comparing the other consumer water sources, we found that the levels and numbers of phthalates in municipal tap water were generally slightly lower than the amount and concentration of phthalates found in bottled water. Point-of-Use filtration, sanitization and dispensing systems appeared to have a wide range of variability as to the number and concentration of phthalates depending on the system and the amount of flow through that system. In general, with regard to POU systems, it appeared that allowing the system to void several liters of water was effective in lowering the amount of phthalates and BPA dispensed. The only consumer water source that was found to have BPA was the POU-A. Both the initial stationary samples and the subsequent flushed samples contained similar amounts of BPA (0.04 to 0.09 ppb). These detected amounts were still well below the EPA RfD guidelines.

Further studies would be needed to determine the other possible contaminants in the consumer water sources of this study. No determination was made on the overall safety of any particular water source based on the measurement of one group of potential contaminants.

**DISCLAIMER:** This study was not intended to determine the safety or purity of any water source. Only the compounds listed in this study were examined and quantified. It is possible that there are other compounds contained in these water sources that were not covered by this study. The types and sources of water studied were just representative samples of different types of water and not all encompassing in water types or brands. This study was not intended to advise or warn against any particular brand or water purification method.

References: Available upon request. Please call +1.732.549.7144 or email [USMet-CRMSales@antylia.com](mailto:USMet-CRMSales@antylia.com).

**spex.com**

Phone: +1.732.549.7144 • +1.800.LAB.SPEX  
Fax: +1.732.603.9647  
[spexsales@antylia.com](mailto:spexsales@antylia.com)

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