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Assessing human exposure to phthalic acid and phthalate esters from mineral water stored in polyethylene terephthalate and glass bottles

Abstract

Phthalic acid and phthalate esters are of growing interest due to their significant usage and their potential toxicity. Polyethylene terephthalate (PET) and glass are both widely used materials for bottled drinking water. In this study, phthalic acid (PhA), bis(2-ethylhexyl) phthalate (DEHP), dimethyl phthalate (DMP), diethyl phthalate (DEP), diisobutyl phthalate (DiisoBP) and dibutyl phthalate (DBP) were analyzed in a large number of Italian bottled water samples. These samples showed different concentrations of phthalates being are nearly twenty times higher in samples bottled in PET than the ones from glass bottles with total levels of phthalates of 3.52 and 0.19 $\mu\text{g L}^{-1}$ respectively. However, the observed levels do not represent a significant exposure pathway when considering the US Environmental Protection Agency (EPA) reference dose [an estimate of a daily oral exposure to the human population (including sensitive sub-groups) that is likely to be without an appreciable risk of deleterious effects during a lifetime]. Also, no significant correlation was found between the phthalate concentrations and the physicochemical properties of the different water samples, apart from the still/sparkling water parameter for the PET samples. In this instance, slightly higher concentrations were observed for the PET bottled still water samples than for the sparkling water samples although no explanation has been found yet.

Key words: Bottled water, Polyethylene terephthalate, Phthalic acid, Phthalate esters, SPME, EI GC-MS.

Introduction.

The bottled water industry in European countries and North America has expanded over the last 30 years, and it is also increasing rapidly in many developing countries (FDA, 2003). Consumption reached 155 L per capita per year in Italy, 136 L in Mexico, 112 L in France, 123 L in Belgium, 99 L in Germany, 98 L in Spain, 97 L in Switzerland and 63 L in the United States in 2000/2001 (Potera, 2002 and Thurman *et al.*, 2002).

In Italy, as in many developed countries, polyethylene terephthalate (PET) is widely used as a container for commercial bottled water, and its use is also increasing rapidly due to its lower production costs in comparison to glass containers (Petrelli *et al.*, 2006). PET is synthesized by reacting ethylene glycol (C₂H₆O₂) with either terephthalic acid or its methyl ester catalysed by antimony oxide. The reaction is carried out under vacuum at high temperatures to achieve high molecular weight polymers. Some studies have shown PET decomposition and phthalate migration in the absence of an accurate temperature and humidity control during PET synthesis (Castle *et al.*, 1989 and Calà *et al.*, 2003). Other studies have shown that water, PET bottled, can release phthalate additives used in the plastic molding process especially in critical conditions of use (e.g. long storage times) (Sauvant *et al.*, 1995 and Biscardi *et al.*, 2003) as already shown for PVC (Hakkarainen, 2003).

Phthalates display a variety of toxic effects in animal studies including decreased fertility in females (Biscardi *et al.*, 2003), fetal defect (Saillenfait *et al.*, 2001) and reduced survival of offspring (Gray *et al.*, 2000), altered hormone levels (Thompson *et al.*, 2004), uterine damage (Seidlova-Wuttke *et al.*, 2004) and male reproduction abnormalities such as reduced sperm production and motility (Sharpe *et al.*, 1995), Sertoli cell damage (Heindel and Powell, 1992), Leyding cell tumors (Jones *et al.*, 1993), cryptorchidism and hypospadias which may be manifestations of one condition termed as “testicular dysgenesis syndrome” (Skakkebaek *et al.*, 2001). The effects of human exposure to phthalates have not been fully studied (Colon *et al.*, 2000; Health Care Without Harm, 2002; Duty *et al.*, 2003b). Long latency periods between relevant exposures and health impacts, unquantified exposures, and subtle effects that are difficult to detect are added difficulties to the few

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3 existing epidemiological studies of phthalate toxicity in humans (Health Care Without
4 Harm, 2002). In one of these few human studies, phthalates were investigated as a cause of
5 precocious puberty in young Puerto Rican girls (Colon *et al.*, 2000). In this study, the
6 serum levels of phthalates obtained from 41 girls with premature appearance of breast
7 tissue were compared with 35 controls. Phthalate esters were detected in 68% of the cases
8 and in the 17% of the controls and they were found significantly lower levels of phthalates
9 than the cases. For the bis(2-ethylhexyl)phthalate (DEHP), the average concentration was
10 70 ppb in the controls compared to 450 ppb in the cases. Even if the study conclusions were
11 limited due to small population size and the possibility of contaminated serum samples, the
12 association between phthalates and premature thelarche is biologically plausible. Two
13 studies present the first human data which demonstrate that phthalates are associated with
14 increased DNA damage in sperm (Duty *et al.*, 2003a; Duty *et al.*, 2003b). Finally, a recent
15 study concludes that monobenzyl phthalate (MBzP) exposure was significantly associated
16 with a 10% decrease in follicle stimulating hormones (FSH) concentration in adult men
17 (Duty *et al.*, 2005). Furthermore, phthalates are chemicals of concern also due to their large
18 production volume and to a non negligible human intake which has been estimated, for
19 example, ranging from 2 to 10 µg/kg bw/day of dibutyl phthalate (DBP) for the U.S.
20 population (NTP-CERHR, 2006) and from 3 to 30 µg/kg bw/day for DEHP (Latini, 2005).
21 In order to assess the safety issues of food containing phthalates their intake can be
22 compared to the reference doses (RfD) defined by the US-EPA. The RfD being an estimate
23 of a daily oral exposure to the human population (including sensitive subgroups) that is
24 likely to be without an appreciable risk of deleterious effects during a lifetime. European
25 Union has included several phthalates as priority substances for evaluation (European
26 Union, 2006) and dibutylphthalate has been proposed by the Committee on the Environment,
27 Public Health and Food Safety to be included as a priority substance in the Water
28 Framework Directive 2000/60/EC (European Union, 2007).

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51 Solid-phase microextraction (SPME) is a solvent free pre-concentration technique, which
52 has recently been applied to the extraction of phthalates from aqueous matrices (Kelly and
53 Larroque, 1999). However, liquid-liquid extraction (LLE) (Jobling *et al.*, 1995; Castillo
54 and Barcelo, 1997) with dichloromethane or hexane, and solid-phase extraction (SPE)
55 (Jobling *et al.*, 1995; Holadova and Hajslova, 1995; Castillo and Barcelo, 1997; Castillo *et*
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3 *al.*, 1998) are usually applied. Nevertheless, a source of error of particular concern for
4 phthalic acid and phthalate esters determination is their high levels in the procedural blanks
5 originating from laboratory plastics, solvents and polymeric sorbents from the pre-
6 concentration techniques (Durand and Barcelo, 1993; Castillo *et al.*, 1998). In this way, the
7 US Environmental Protection Agency (EPA) reports that DEHP, along with other common
8 phthalate esters, cannot be accurately or precisely measured at concentrations below $2 \mu\text{g L}^{-1}$
9 ¹, due to high blank levels when the conventional methods LLE or SPE are employed
10 (Lawrence, 1995), and at the same time the EPA has established a maximum concentration
11 limit (MCL) in drinking water of $6 \mu\text{g L}^{-1}$ in its National Primary Drinking Water
12 Regulations (NPDWR) (EPA, 1991). Nowadays, phthalates are not considered in the
13 European Union drinking water regulations even if, dibutylphthalate has been proposed by
14 the Committee on the Environment, Public Health and Food Safety to be included as a
15 priority substance in the Water Framework Directive 2000/60/EC (European Union, 2007).
16 Recent studies show that detection limits well below this level can be achieved when using
17 SPME coupled to GC-MS (Peñalver *et al.*, 2000; Alzaga *et al.*, 2003). In this way, SPME
18 diminishes the risk of contamination in the extraction of phthalates since it is a solvent free
19 technique and it minimizes the materials use, which can be potentially polluted with
20 phthalates (*e.g.* SPE cartridges, solvents). At present, there are different published papers in
21 which the suitability of SPME phthalate esters extraction from water is shown (Moder *et*
22 *al.*, 1998) and even coupled to GC-MS (Peñalver *et al.*, 2000; Peñalver *et al.*, 2001; Suzuki
23 *et al.*, 2001; Alzaga *et al.*, 2003;) and to LC-UV (Kayali *et al.*, 2006). However, in these
24 studies only a small number of bottled water samples were analyzed in order to evaluate the
25 developed methodology. Recently, stir bar sorptive extraction (SBSE) technique has been
26 used to analyze phthalates in water obtaining lower limits of detection than with SPME due
27 to its higher volume of polymeric phase compared to SPME but as a drawback, it needs a
28 dedicated analytical instrumentation (Serodio *et al.*, 2003; Serodio *et al.*, 2006).
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51 The aim of this study was to determine the phthalic acid and phthalate esters content in
52 Italian mineral water both bottled in glass or in PET bottles. Moreover, the relevance of
53 bottled water consumption in human exposure to phthalic acid and phthalate esters will be
54 assessed. For this reason, phthalic acid (PhA) as the main degradation product of diesters,
55 bis(2-ethylhexyl) phthalate (DEHP), dimethyl phthalate (DMP), diethyl phthalate (DEP),
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3 diisobutyl phthalate (DiisoBP) and dibutyl phthalate (DBP) have been analyzed in Italian
4 samples of commercial mineral water stored in PET and in glass bottles using SPME in
5 combination with GC-MS. To the best of our knowledge this study represents the first
6 survey in which phthalic acid and phthalate esters, in an extensive sample pool, were
7 investigated by using SPME and GC-MS.
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12 13 14 **Material and methods.**

15 16 17 *Study area and sampling.*

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19 The survey was conducted in Italy and a variety of commercial bottled water samples were
20 collected. Commercial bottled water samples included different water type, such as spring
21 water, mineral water, light water (low mineralization water) and sparkling water from
22 miscellaneous commercial brands. The sampling of each commercial brand was carried out
23 twice, packed in polyethylene and in glass bottles. A total of 71 commercial brands coming
24 from 16 different Italian regions were collected. Therefore, 142 samples, 71 packed in PET
25 and 71 in glass containers were analysed.
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33 34 *Sample preparation and analysis*

35 Water samples (5 mL) were placed in a 7 mL glass vial and pH was adjusted at pH 2 by
36 using HCl and were continuously stirred using a magnetic stirrer. Immersion SPME was
37 carried out using the polydimethylsiloxane- divinylbenzene (PDMS/DVB) fibre from
38 Supelco (Bellefonte, PA, USA) adapting an already reported methodology (Peñalver *et al.*,
39 2001). After an extraction of 20 min at 25°C the fibre was thermally desorbed at 250°C (3
40 min splitless time) in the GC-MS standard split/splitless injector. The analysis was carried
41 out using a quadrupole GC-MS QP5050A Shimadzu (Kyoto, Japan) with a GC-MS
42 (version 1.1, data acquisition software), working in the electron impact mode at 70 eV. A
43 SPB 20 (20% diphenyl 80% dimethylpolysiloxane) (60 m, 0.25 mm ID) coated with a 0.25
44 µm film thickness column was used. The gas chromatographic conditions were as follow.
45 The initial oven temperature was 50°C for 2 min, then programmed from 50°C to 250°C at
46 10°C min⁻¹ with a final holding time of 30 min. MS transfer line and ion source were kept
47 at 250°C. Acquisition was carried out in the single ion monitoring mode using two
48 characteristic ions for each target analyte. Compound identification was carried out
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3 comparing their retention times with standards, using two characteristic ions and their ratio
4 for each target analyte. Furthermore, for the samples presenting higher concentrations,
5 target analytes identity was confirmed in full-scan mode (m/z range from 60 to 350).
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7 Quantification was done by using the external calibration method showing linear
8 correlations with $R^2 > 0.98$ for all the target analytes from 0.01 to $1 \mu\text{g L}^{-1}$.
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14 Data analysis was performed with the statistical software SPSS (release 13.0, SPSS Inc.,
15 Chicago, IL, USA). All data was presented as the mean \pm SD. The level of significance was
16 set at $P \leq 0.05$.
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22 Procedural blanks were carried out and used for the calculation of the detection limits
23 (LOD). They ranged from $0.01 \mu\text{g L}^{-1}$ for the DBP to $0.08 \mu\text{g L}^{-1}$ for phthalic acid and
24 quantification limits (LOQ) ranged from $0.02 \mu\text{g L}^{-1}$ for DEHP to $0.1 \mu\text{g L}^{-1}$ for phthalic
25 acid.
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30 31 32 **Results & Discussion**

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37 Despite few data being available, the results reported here are compared in Table 1 with
38 bibliographic phthalate concentrations for tap and bottled water. It is important to point out
39 that the scarcity of data on this topic is mainly due to the analytical difficulties in the
40 phthalate determination. In this way, the large number of non-detected samples in the USA
41 National Resources Defence Council study (NRDC, 2006) (DEHP was not detected in
42 98.5% of the samples) is explained by the high detection limits of the applied
43 methodologies (e.g. EPA $2 \mu\text{g L}^{-1}$). However, for the most studied compounds such as
44 DEP, DnBP and DEHP the results found in this study are in agreement with the literature.
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54 The use of SPME GC-MS as analytical technique achieved lower detection limits due to its
55 improved blank level. In this way, lower target analytes concentrations were detected
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3 avoiding the production of a large number of non-detected samples as occurred in the
4 NRDC study (NRDC, 2006).
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10 In Table 2 the quartiles are shown of the target analytes concentrations for the glass and
11 PET bottled water. PET bottled water shows, as expected, a higher content for all
12 phthalates, being PhA, degradation product of the phthalates esters, the most abundant
13 individual compound. Box plots of the different target analytes concentrations depending
14 on bottle material are shown in Figure 1. In this figure, it is evident that higher
15 concentrations are found for all the analysed phthalates in the PET bottled water. However,
16 these differences were confirmed using statistical tools and significant differences (Mann-
17 Whitney test $p < 0.05$) were found between glass and PET for all the compounds. It should
18 be pointed out, that also different phthalate patterns were obtained in the two cases (Figure
19 2). For the PET bottles PhA (69%) and DnBP (10%) are the most abundant compounds and
20 for the glass bottles the most abundant are DiisoBP (25%) and the DnBP (15%). In both
21 cases, the lowest concentrations were found for DEHP, which was always far below the
22 EPA regulation limit of $6 \mu\text{g L}^{-1}$ with a maximum of $0.17 \mu\text{g L}^{-1}$ for PET bottles and of
23 $0.02 \mu\text{g L}^{-1}$ for glass bottles. These results confirm that the use of PET containers is the
24 main cause of higher concentrations of phthalates in bottled water, as the concentration of
25 the sum of the studied compounds is more than twelve times higher in PET than in glass
26 bottled water. The presence of phthalates in glass bottled water could come from the other
27 water processing steps (PVC tubing, storage tanks, filtration steps, cap-sealing). In the case
28 of PET, four different bottle volumes have been sampled (0.75, 1, 1.5 and 2 L) but no
29 correlation was found between the bottle volume and the phthalate content. Moreover, no
30 correlation was found between the phthalates content and several water physicochemical
31 parameters such as conductivity, pH, solid residue, sodium, calcium, potassium,
32 magnesium, chlorine, fluorine, sulphates, carbonates, nitrates and silicates (data taken from
33 the bottle label). Therefore, it could be considered that these parameters are not relevant in
34 controlling the leaching of the phthalates from the bottle to the water.
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57 No significant differences were found between sparkling and still waters when there was no
58 segregation of the data between PET and glass containers. However, when considering PET
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3 bottles alone, DEP was found to be significantly higher in still water (n=55) than in
4 sparkling water (n=16) (p<0.05). Until now, no reason has been found, to explain this
5 result.
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11 From a toxicological point of view, and considering that a MCL is only available for DEHP
12 ($6 \mu\text{g L}^{-1}$ EPA limit for drinking water), the concentrations found are lower. Another way to
13 assess the health impact of phthalates is to study the importance of drinking PET bottled
14 water referred to the EPA available phthalates reference doses (RfD) (EPA, 2006) (0.1
15 mg/kg/day for the DnBP, 0.8 mg/kg/day for the DEP, 2 mg/kg/day for PhA and 0.02
16 mg/kg/day for DEHP). In this way, we will consider a body weight of 70 kg and a daily
17 water consumption of 2 L. In Table 3, it is shown the proportion of the different RfDs,
18 which can be achieved by drinking PET bottled water. The results show that, due to the low
19 phthalate concentrations, PET bottles do not represent any health risk related to the
20 phthalate intake as they do not contribute significantly to the estimated RfD. The maximum
21 expected contribution will be 0.051% of the RfD for the DnBP and only for the most
22 contaminated sample. However, it is important to point out the lack of data on this subject
23 and the need of further studies involving both food analysis and epidemiological research to
24 estimate the phthalates uptake in a comprehensive way.
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39 **Conclusions**

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43 Due to analytical difficulties in achieving low detection limits, there is a lack of data on the
44 phthalate occurrence in drinking water. The use of SPME and GC-MS as an analytical
45 technique has proven to be suitable for the analysis of phthalates at low concentrations and
46 for a large number of samples. The use of PET bottles has been clearly correlated with the
47 concentration of phthalates in the bottled water. In this way, phthalates concentrations are
48 significantly higher (nearly twenty times) in this water than in glass bottled water.
49 However, the concentrations found do not represent any risk for human health as can be
50 seen by comparing the concentrations found to the existing EPA regulation (only available
51 for DEHP) or by referring to the EPA RfDs. The contribution from drinking water is
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3 always below 0.1% of the RfD for all the target analytes. Therefore, the main conclusion of
4 this study is that Italian bottled water does not represent a relevant ingestion source of
5 phthalates esters and phthalic acid for the population who consume bottled water. Due to
6 similarities in the materials being used and in the technologies in water processing
7 industries all around Europe, similar conclusions to the ones presented here should be
8 expected for the whole European Union.
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14 15 16 **Acknowledgements** 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

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3 **Figure legends**
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7 **Figure 1.** Box plots showing the phthalates concentrations depending on the bottle
8 material.
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12 **Figure 2.** Normalised phthalates patterns for the PET and the glass bottled water.
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Table I. Comparison of bibliographic data on phthalates content in tap and bottled water. Presented results are the mean of the different analysed samples expressed in $\mu\text{g L}^{-1}$.

($\mu\text{g L}^{-1}$)	NRDC, 2006	Casajuana <i>et al.</i> , 2003	Peñalver <i>et al.</i> , 2001	Kayali <i>et al.</i> , 2006	Serodio <i>et al.</i> , 2006	Serodio <i>et al.</i> , 2006	Luks <i>et al.</i> , 2001	Luks <i>et al.</i> , 2001	Current Study
Sample	Bottled water	Bottled water	Bottled water	Bottled water	Bottled water	Tap water	Tap water Poland	Tap water Germany	Bottled water
Samples number	132	20	3	4	1	1	1	1	142
Phtalic acid (PhA)	-	-	-	-	-	-	-	-	1.28
Dimethylphthalate (DMP)	-	0.002	ND	-	ND	0.04	-	-	0.07
Diethylphthalate (DEP)	-	0.254	0.24	-	0.04	0.19	0.16	0.20	0.17
Diisobutylphthalate (DiisoBP)	-	-	-	-	-	-	-	-	0.20
Di-n-butyl- phthalate (DBP)	-	0.047	0.10	-	0.35	0.52	0.64	0.38	0.21
Bis(2-ethylhexyl)phthalate (DEHP)	8.5 ¹	0.164	1.0	ND	0.17	0.06	0.06	0.05	0.02

¹DEHP was only detected in two samples

ND not detected

- not analysed

Table 2. Target analytes quartiles in ($\mu\text{g L}^{-1}$) for glass and PET bottled water.

(μgL^{-1})	Quartiles (%)	PhA	DMP	DEP	DiisoBP	DBP	DEHP	Total
GLASS	25	<LOD	<LOQ	<LOQ	0.02	0.02	<LOQ	0.13
	50	<LOD	0.02	0.02	0.03	0.04	<LOQ	0.19
	75	<LOD	0.04	0.06	0.06	0.09	<LOQ	0.36
PET	25	1.24	<LOQ	0.14	0.221	0.17	<LOQ	2.11
	50	2.20	0.06	0.22	0.32	0.23	<LOQ	3.52
	75	3.50	0.10	0.35	0.45	0.52	0.02	4.81

Table 3. Minimum, maximum and quartiles values of the water consumption contribution to the RfDs for the PET bottled water.

Reference value (mg/kg/day) (EPA, 2006)	RfD PhA*	RfD DEP	RfD DnBP	RfD DEHP
	2	0.8	0.1	0.02
Min.	<0.001	<0.001	0.003	<0.001
25	0.002	0.001	0.005	<0.001
50	0.003	0.001	0.007	<0.001
75	0.005	0.001	0.015	0.001
Max.	0.010	0.003	0.051	0.008

*For the PhA the RfD of the phthalic anhydre was considered

Figure 1

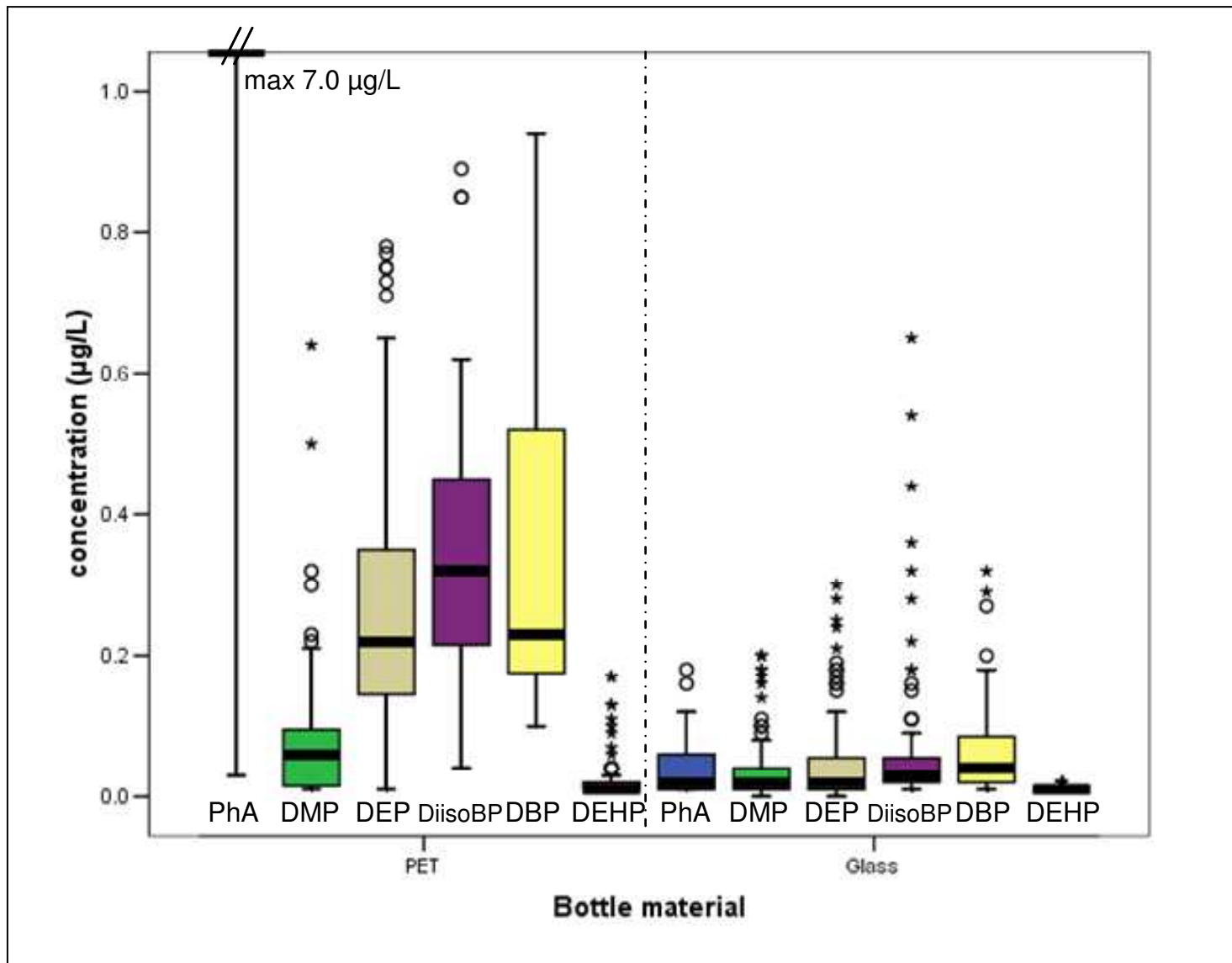
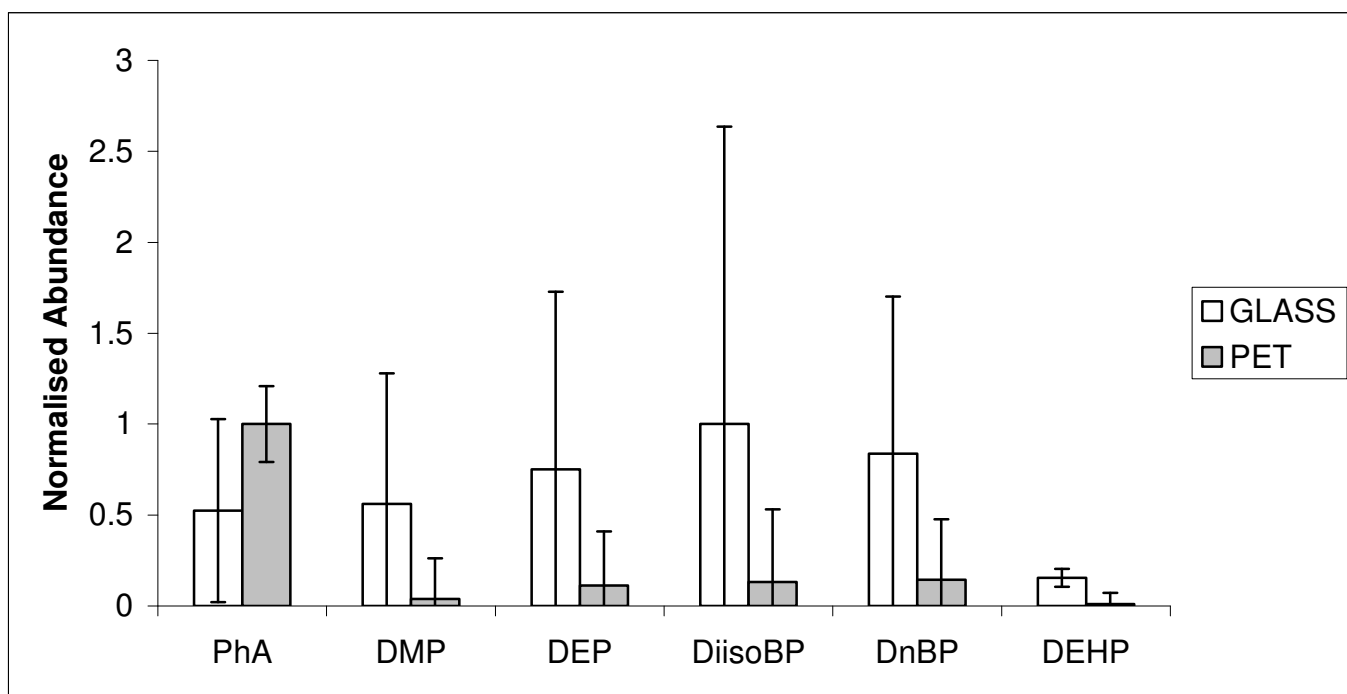


Figure 2.



Review Only