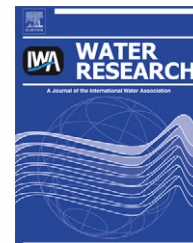




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Chemical compounds and toxicological assessments of drinking water stored in polyethylene terephthalate (PET) bottles: A source of controversy reviewed

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ARTICLE INFO

Article history:

Received 27 July 2011

Received in revised form

21 November 2011

Accepted 22 November 2011

Available online 6 December 2011

Keywords:

Bottled water

Mutagenicity

Genotoxicity

Endocrine disruptors

NIAS

ABSTRACT

A declaration of conformity according to European regulation No. 10/2011 is required to ensure the safety of plastic materials in contact with foodstuffs. This regulation established a positive list of substances that are authorized for use in plastic materials. Some compounds are subject to restrictions and/or specifications according to their toxicological data. Despite this, the analysis of PET reveals some non-intentionally added substances (NIAS) produced by authorized initial reactants and additives.

Genotoxic and estrogenic activities in PET-bottled water have been reported. Chemical mixtures in bottled water have been suggested as the source of these toxicological effects. Furthermore, sample preparation techniques, such as solid-phase extraction (SPE), to extract estrogen-like compounds in bottled water are controversial. It has been suggested that inappropriate extraction methods and sample treatment may result in false-negative or positive responses when testing water extracts in bioassays. There is therefore a need to combine chemical analysis with bioassays to carry out hazard assessments.

Formaldehyde, acetaldehyde and antimony are clearly related to migration from PET into water. However, several studies have shown other theoretically unexpected substances in bottled water. The origin of these compounds has not been clearly established (PET container, cap-sealing resins, background contamination, water processing steps, NIAS, recycled PET, etc.).

Here, we surveyed toxicological studies on PET-bottled water and chemical compounds that may be present therein. Our literature review shows that contradictory results for PET-

List of abbreviations: AA, acetaldehyde; APEOs, polyethoxylated nonylphenols; BBP, benzylbutyl phthalate; BHET, bis(hydroxyethyl) terephthalate; BHT, butylated hydroxytoluene; BPA, bisphenol A; DBP, dibutyl phthalate; DiBP, di-iso-butyl phthalate; DEG, diethylene glycol; DEHP, di-2-(ethylhexyl) phthalate; DEHA, bis-2-ethylhexyl adipate; DEP, diethyl phthalate; DMSO, dimethyl sulfoxide; DMT, dimethylterephthalate; DOP, di-n-octyl phthalate; EEC, European economic community; EEQs, estradiol equivalents; GC–MS, gas chromatography–mass spectrometry; HDPE, high density polyethylene; HULYs, human blood lymphocytes; IPA, isophthalic acid; LDH, lactate dehydrogenase; MEG, ethylene glycol; NIAS, non-intentionally added substances; NP, 4-nonylphenol; OP, octylphenol; PA, polyamide; PC, polycarbonate; PhA, phthalic acid; PVC, polyvinylchloride; RPE, relative proliferative effects; Sb₂O₃, antimony trioxide; SEC–HPLC, size exclusion chromatography–high performance liquid chromatography; SML, specific migration limits; SPE, solid-phase extraction; SPME, solid-phase micro-extraction; SODIS, solar water disinfection; TPA, terephthalic acid; TDI, tolerable daily intake; TNPP, tris(nonylphenyl) phosphite; TOC, total organic carbon; YES, yeast estrogen screen.

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doi:10.1016/j.watres.2011.11.062

bottled water have been reported, and differences can be explained by the wide variety of analytical methods, bioassays and exposure conditions employed.

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1. Introduction

Polyethylene terephthalate (PET) is a semi-crystalline polymer belonging to the family of polyesters. It is the most favorable packaging material for drinking water. PET bottles have been marketed for the last four decades and they have gradually replaced polyvinylchloride (PVC) and glass bottles on the markets. In Europe, packaged water sales account for 44% of the market volume for non-alcoholic drinks in 2009, with an average individual consumption of 105 L per year (EFBW, 2011).

1.1. The synthesis of PET

The prepolymerization of dimethylterephthalate (DMT) or terephthalic acid (TPA) with ethylene glycol (MEG) is the first industrial step in the synthesis of PET. Both reactions generate low-weight oligomers and an intermediate compound named bis(hydroxyethyl)terephthalate (BHET). After this step, a second polycondensation is carried out with an Sb-, Ge- or Ti-based catalyst (ILSI, 2000; Fakirov, 2002).

During PET manufacturing, several degradation and decomposition reactions can occur. High temperatures and the presence of oxygen in the PET melt process can promote thermo-mechanical and thermo-oxidative reactions (Romão et al., 2009a). Also, PET hydrolysis can be induced by the

presence of water during the melt process (Zhang and Ward, 1995; Paci and La Mantia, 1998).

PET thermal degradation generates sub-products such as oligomers and diethylene glycol. Volatile organic compounds such as carbon monoxide, aldehydes (formaldehyde, acetaldehyde, benzaldehyde), C₁–C₄ aliphatic hydrocarbons, aromatic hydrocarbons (benzene, toluene, ethylbenzene and styrene), esters (vinyl benzene, methyl acetate), methanol, and acetophenone were identified in PET samples subjected to temperatures between 200 and 300 °C (Dzięcioł and Trzeszczyński, 2000). In addition, 1,3-dioxolane and 2-methyl-1,3-dioxolane as thermal degradation products in PET bottles (Franz and Welle, 2008).

PET thermal stability depends on the type of co-monomers used for its production (Holland and Hay, 2002b). Concerning bottle-grade PET, co-polymerization with diethylene glycol (DEG) and isophthalic acid (IPA) is usually carried out to minimize polymer thermal crystallization during the production of preforms and the blow-molding process. Both co-monomers reduce the size of spherulites and, as a result the final container is transparent (Holland and Hay, 2002a). Indeed, glass-like transparency is a valued commodity for drinking-water bottles.

1.2. The manufacture of PET bottles for drinking water

Injection blow-molding is the preferred process for manufacturing PET bottles. Amorphous preforms are

obtained by processing PET granules. Preforms are stretched by a blow-molding process to achieve bi-axially oriented bottles. The barrier properties and the diffusion rate are directly affected by the degree of crystallinity and the orientation of polymer chains in PET bottles. The selection of an adequate blow temperature around 20 °C above the PET glass transition temperature (T_g) is essential to achieve these properties (Tadmor and Gogos, 2006).

Despite the good barrier properties of PET, packaging applications require even lower gas permeability. One recent development is the blending of immiscible lamellar polyamide (PA) phases within the PET. This reduces the permeability of O₂ and CO₂ by a factor of two or more. A hexanedioic acid polymer with 1,3-benzenedimethanamine (called MXD6) is used as barrier material in PET bottles (FSA, 2007). However, this PA layer can generate NIAS (Franz and Welle, 2008).

Additives such as plasticizers and antioxidants are not necessary for PET bottles, and colorants are added in small quantities. Acetaldehyde (AA) scavengers are used in PET bottles intended for mineral water to minimize AA content of the melt-processed polyester. The current use of AA scavengers is described in US patent 6274212 (Rule et al., 2001). Anthranilamide is particularly preferred because of its low cost, efficiency and ease of incorporation into PET.

Nowadays, the recycling of PET bottles is a common environmentally friendly procedure, and is used to reduce plastic waste and to reprocess plastics for other applications. In Europe, Regulation No. 282/2008 sets the guidelines for the recycling of plastics for food-contact applications. A variety of recycling technologies have been developed for plastic packaging (EFSA, 2011). PET bottle to bottle recycling processes have been established worldwide due to their huge potential. A rigorous review of this technology has recently been published by Welle (2011).

1.3. European regulations for plastic food-contact materials

European Regulation No.1935/2004 (EU, 2004) covers 17 groups of different materials. It states that food-contact materials should not transfer its constituents to food in quantities that could incur a human health risk, cause an unacceptable change in the composition of the food or bring about deterioration in the food organoleptic characteristics. This regulation is accompanied by specific measures depending on the type of material.

Food-contact plastic materials are covered by the recent Regulation No. 10/2011 (EU, 2011). This regulation establishes the list of compounds authorized for use in plastic formulation on a positive list. The conformity of a plastic material intended to come in contact with food is based on migration tests. The overall migration limit should not exceed 10 mg of the total constituents released for dm² of packaging surface. Specific migration limits (SML) established according to toxicological data is provided for some substances. The main limitation of this regulation concerns impurities and breakdown products generated by authorized initial reactants and additives (NIAS). Furthermore, the new regulation specifies that: “the notion of the risk due to the substance concerns the substance itself, the impurities of this substance and any reaction or degradation products”.

Numerous studies have investigated the interaction of PET bottles in contact with drinking water to provide analytical and toxicological data. Chemical mixtures with estrogenic activity have been reported for PET-bottled water. The presence of NIAS has been designated as a source of this toxicological effect (Evandri et al., 2000; Leivadara et al., 2008). Further, it has been suggested that stresses applied to a material during its production can change its chemical structure and generate degradation products, which in turn may have estrogenicity (Yang et al., 2011). In some cases, the origin of substances found in bottled drinking water has not been clearly established and remains to be elucidated.

Here, our main purpose was to provide a compilation of all known and still controversial data which have been reported regarding PET-bottled drinking water. The chemical compounds, the experimental conditions, the toxicological approaches, and the source of water pollution will be discussed to clarify the relevance of PET packaging as a source of genotoxic and estrogen-like compounds.

2. Substances investigated in PET and PET-bottled water

2.1. PET monomers and oligomers

Regulation No. 10/2011 specifies SMLs for MEG, IPA and TPA of 30 mg/kg, 5 mg/kg, and 7.5 mg/kg, respectively. A review of the literature did not turn up any recent studies of diffusion of these compounds into PET-bottled water. Although, many migration tests of these compounds from PET into distilled water are conducted every year worldwide as part of necessary compliance, the migration of monomers (TPA, IPA, MEG) from PET into distilled water is so low that the results are never published because the lack of practical interest. Only Mutsuga et al. (2005) have reported levels of oligomers ranging from 4.9 to 8.7 µg/kg in PET bottle-grade, but no recent migration studies of oligomers from PET into water have been published.

2.2. Traces of metals

Inorganic species may be present as residues from the catalysts or additives used to produce PET. Antimony trioxide (Sb₂O₃) is the most important catalyst used in the synthesis of this polyester (EU, 2008). PET typically contains Sb levels in the range of 170–300 mg/kg (Nishioka et al., 2002; Westerhoff et al., 2008; Keresztes et al., 2009). However, only a small fraction of the Sb contained in PET is released into the water (Welle and Franz, 2011). Other metals such as Co, Cr, Fe and Mn, with 27 mg/kg, 0.1 mg/kg, 1.3 mg/kg and 0.3 mg/kg, respectively have been found in PET bottles (Westerhoff et al., 2008). Their relatively low levels observed in polymeric material, compared to Sb, explain why so few studies have been done on migration of these metals into bottled water.

All the reviewed studies agree that Sb is the most relevant element leached from PET bottles. The SML prescribed for Sb is 0.04 mg/kg (EU, 2011). Recently, Welle and Franz (2011) reported that the worst case of exposure to Sb due to diffusion never reached the SML stipulated in European packaging regulations. The data from all reviewed studies on Sb migration

into PET-bottled water, collected in Table 1, never exceeded this SML.

The Sb leaching from PET into water increases rapidly during the first storage period and then the migration reaches a “steady state” (Keresztes et al., 2009). Our literature review shows that Sb diffusion is a thermally activated process. Sb concentrations were found to have increased by 90% on average in 48 brands of bottled drinking water after a period of 6 months at room temperature (Shotyk and Krachler, 2007). Also, a significant effect of temperature on the release of Sb has been shown (Westerhoff et al., 2008; Keresztes et al., 2009; Cheng et al., 2010; Bach et al., 2011).

Keresztes et al. (2009) demonstrated that the Sb dissolution rate into water is higher into sparkling water than into still water, due to the lower pH of carbonated water. The higher release of Sb due to low pH has also been observed (Cheng et al., 2010). In contrast, PET exposure to sunlight appears to be less significant than other factors in Sb migration (Keresztes et al., 2009; Cheng et al., 2010).

Contradictory conclusions have been reached regarding the effect of bottle color on Sb migration. Whereas Westerhoff et al. (2008) detected an increase in Sb concentration in clear PET compared to colored ones, Reimann et al. (2010) concluded the opposite.

The contact surface area has also an effect on Sb release from PET to water. With identical contact conditions, it has

been shown that higher bottle volumes release lower levels of Sb (Keresztes et al., 2009; Welle and Franz, 2011).

No significant traces of inorganic elements other than Sb in PET-bottled water subjected to different conditions (low pH, outdoor sunlight irradiation, in-car storage, cooling, heating and microwave treatment) have been observed (Cheng et al., 2010; Reimann et al., 2010). More metals can leach from glass (Ce, Pb, Al, and Zr) than from PET bottles into water (Reimann et al., 2010).

2.3. Carbonyl compounds

Formaldehyde and acetaldehyde are the most relevant carbonyl compounds migrating from PET bottles. They are generated by thermo-mechanical and thermo-oxidative degradation of PET (Kovarskaya et al., 1968; Romão et al., 2009b). The migration of acetaldehyde into bottled water has been widely investigated. However, only a few studies have been devoted to study presence of other carbonyl compounds (formaldehyde, propanal, butanal, etc.) in PET-bottled water.

2.3.1. Carbonyl compounds in PET raw material, preforms and bottles

Formaldehyde and acetaldehyde amounts in the PET wall depend on the chemical quality of the raw material, the molecular weight of the polymer and on the manufacturing

Table 1 – Results of antimony (Sb) migration from PET into bottled water.

Exposure temperature (°C)	Exposure conditions	Simulant	Other parameters	Concentration mean (µg/L)	Concentration range (µg/L)	References
Refrigerated	24 h, darkness	Ultrapure water	–	–	0.846 ± 1.652	Cheng et al. (2010)
Refrigerated	37 days	Groundwater	–	–	0.05 ± 0.017	Shotyk et al. (2006)
Refrigerated (2 °C)	150 days	Ultrapure water	Water bottled in hard PET	0.003	–	Reimann et al. (2010)
Refrigerated (2 °C)	150 days	Ultrapure water at pH = 6.5	Water bottled in soft PET	0.025	–	Reimann et al. (2010)
Refrigerated (2 °C)	150 days	Ultrapure water at pH = 3.5	Water bottled in hard PET	0.0085	–	Reimann et al. (2010)
Refrigerated (2 °C)	150 days	Ultrapure water at pH = 3.5	Water bottled in soft PET	0.027	–	Reimann et al. (2010)
r.t.	24 h, darkness	Ultrapure water at 100 °C	–	–	3.243–1.652	Cheng et al. (2010)
r.t.	24 h	Microwave heated ultrapure water	–	–	0.391–10.51	Cheng et al. (2010)
r.t.	6 months	Groundwater	–	0.566	–	Shotyk et al. (2006)
r.t.	7 days, darkness	Ultrapure water at pH = 4	–	–	<0.02 to 3.794	Cheng et al. (2010)
22 °C	3 months	Commercial water	–	0.226 ± 0.160	–	Westerhoff et al. (2008)
22 °C	<1 year	Still mineral water	–	0.26 ± 0.160	–	Keresztes et al. (2009)
22 °C	<1 year	Sparkling mineral water	–	0.40 ± 0.22	–	Keresztes et al. (2009)
40 °C	10 days	Aqueous simulant	–	–	<0.03	Nishioka et al. (2002)
40 °C	45 °C	Spring still water	–	2.0	–	Bach et al. (2011)
40 °C	45 days	Carbonated mineral water	–	3.5	–	Bach et al. (2011)
80 °C	7 days	Commercial water	–	–	14.4	Westerhoff et al. (2008)
–	7 days, sunlight	Ultrapure water	–	–	<0.02 to 4.611	Cheng et al. (2010)
–	7 days, in-car storage	Ultrapure water	–	–	<0.02 to 3.08	Cheng et al. (2010)

r.t.: room temperature.

technology used (production of granules, preforms and bottle-blowing temperature) (Sugaya et al., 2001; Mutsuga et al., 2006; Choodum et al., 2007; Pinto and Reali, 2009). Mutsuga et al. (2005) found acetaldehyde levels in PET bottles from Japan, Europe and North America in ranging from 8.4 to 25.7 mg/kg, from 5.0 to 13.1 mg/kg and from 9.1 to 18.7 mg/kg, respectively. Traces of formaldehyde have also been found, ranging from 0.8 to 3.0 mg/kg, from <0.5 mg/kg to 1.6 mg/kg, and from <0.5 mg/kg to 1.2 mg/kg in the same Japanese, European and North American bottles, respectively. The highest levels of formaldehyde and acetaldehyde in Japanese bottles were attributed to the difference in formulations and in packaging production.

No traces of other carbonyl compounds have been found in bottle-grade PET.

2.3.2. Studies of migration of carbonyl compounds into PET-bottled water

The occurrence of carbonyl compounds in PET-bottled drinking water will be reviewed with respect to the significant factors affecting migration. The results of these studies are collected in Table 2.

SML values (EU, 2011) have only been established for formaldehyde and acetaldehyde: 15 mg/kg and 6 mg/kg, respectively. Propanal, butanal and acetone appear in positive list, but specific SMLs are not indicated. It is worth to note that none of the reviewed studies on PET-bottled water reached these values (see Table 2).

2.3.2.1. Influence of the manufacturing technology and bottling process. The different steps in the bottling process and bottle caps appear to be a source of pollution for some carbonyl compounds into bottled water. The monitoring of a production line of carbonated water showed the appearance of formaldehyde, acetaldehyde, propanal, nonanal, glyoxal, and methylglyoxal in ozonated water used to disinfect the bottles (Dabrowska et al., 2003). Also, polypropylene caps have been implicated as a source of carbonyl compounds, and particularly acetone, in bottled water, but with a less effect than PET packaging. To date, only Nawrocki et al. (2002) and Dabrowska et al. (2003) have detected acetone in PET-bottled water and they indicate that acetaldehyde and acetone are equally important compounds migrating into bottled water.

2.3.2.2. Influence of contact time, temperature, pH, and CO₂ of bottled water. Authors agree that the diffusion of formaldehyde and acetaldehyde is affected by temperature, storage time, and carbonation of water and its resulting lower pH in bottled drinking water (Nawrocki et al., 2002; Dabrowska et al., 2003; Ewender et al., 2003; Bach et al., 2011). However, Dabrowska et al. (2003) suggest that the pressure exerted by CO₂ on the PET wall promotes diffusion. However, Bach et al. (2011) have recently demonstrated that the CO₂ levels in sparkling water do not affect the migration of acetaldehyde from PET into bottled water.

In some diffusion studies, various authors have observed that acetaldehyde and also, formaldehyde in commercial still water and carbonated water disappears over time. Several hypotheses have been formulated to explain this occurrence.

An old study suggests that oxygen or traces of metal ions in still mineral water could promote the degradation of acetaldehyde over time and forms acetic acid, acetic anhydride, peracetic acid and trimer paraldehyde (Nijssen et al., 1996). However, this explanation is not convincing because anhydrides cannot form in aqueous solutions. Mutsuga et al. (2006) demonstrated the presence of heterotrophic bacteria in still water and these bacteria can decompose formaldehyde and acetaldehyde. Recently, Bach et al. (2011) reported that high mineralization of still water could prevent the release of acetaldehyde from PET. For carbonated water, Nawrocki et al. (2002) linked acetaldehyde disappearance to the gradual loss of dissolved CO₂ because bottles are not sufficiently gas-tight after long-term storage (8–9 months).

2.3.2.3. Influence of exposure to sunlight. Contrasting conclusions on the influence of the amounts of formaldehyde and acetaldehyde following exposure to sunlight have been drawn. Nawrocki et al. (2002) observed an increase in formaldehyde, acetaldehyde, and also acetone in carbonated water stored in PET bottles exposed to sunlight over a period of time. In contrast, Wegelin et al. (2001) observed the same level of formaldehyde in unexposed bottled water as in samples subjected to the maximum irradiation rate (548 kWh/m²).

2.4. Plasticizers

The addition of plasticizers (phthalates and adipates) to plastic packaging is widespread to improve their softness and flexibility, especially in PVC (FSA, 2007; Cao, 2010).

Further, phthalates in food-contact materials are subject to strict regulations and are not thought to be used for manufacturing PET bottles. However, they have been found in PET material and in PET-bottled water.

We reviewed studies that report the amounts of phthalates and DEHA detected in PET-bottled water. The results of these studies are collected in Table 3.

It is important to note that in all reviewed studies, phthalate esters and DEHA did not exceed the SML values (EU, 2011) of 0.3 mg/kg, 30 mg/kg, 1.5 mg/kg and 18 mg/kg for DBP, BBP, DEHP and DEHA, respectively.

The major problems of phthalates lie in various sources of possible contamination. Phthalates may come from bottling lines (Higuchi et al., 2004), cap-sealing resins (Hirayama et al., 2001), or water treatment facilities (Leivadara et al., 2008; Montuori et al., 2008). Also, background pollution in the laboratory analyzing phthalates may also be a source (Fankhauser-Noti and Grob, 2007). Moreover, in recent studies, no traces of phthalates (DMP, DBP, BBP, DEHP) were detected into water after incubation at 40 °C for 10 days (Ceretti et al., 2010; Guart et al., 2011).

However, previous reports have claimed that phthalates can come from PET bottles, but convincing explanations have never been offered. Comparing the results of analyses of bottled water before and after storage, Casajuana and Lacorte (2003) concluded that poor storage conditions (10 weeks outdoors at temperatures of up to 30 °C) increases the concentrations of DBP, BBP and DEHP in bottled water. Montuori et al. (2008) found that the concentrations of phthalates (PhA, DMP, DEP, DiBP, DBP and DEHP) in samples bottled in PET were 20

Table 2 – Results of carbonyl compounds migration from PET into bottled water.

Compound Name	Simulant	Exposure temperature	Exposure conditions	Other parameters	Concentration range ($\mu\text{g/L}$)	Concentration mean ($\mu\text{g/L}$)	References
Formaldehyde	Mineral water	–	–	–	<0.5 to 59	–	Sugaya et al. (2001)
	Still water	–	–	Total organic carbon	2.2–64.6	–	Nawrocki et al. (2002)
	Still water	–	–	<2.0 to 2.9 $\mu\text{g/g}$ in PET	<5.0 to 27.9	–	Mutsuga et al. (2006)
	Still water	r.t.	6 days	–	–	7.1 \pm 0.7	Dabrowska et al. (2003)
	Still mineral water	–	63 days, sunlight	–	–	44	Wegelin et al. (2001)
	Still mineral water	–	126 days, sunlight	–	–	1	Wegelin et al. (2001)
	Carbonated water	r.t.	170 days	–	–	60.0 \pm 6.0	Dabrowska et al. (2003)
	Carbonated water at pH = 4.5	r.t.	6 days	–	–	10.5 \pm 1.1	Dabrowska et al. (2003)
	Carbonated water	–	–	TOC	24.6–96.1	–	Nawrocki et al. (2002)
	Carbonated water	–	–	<0.5 to 0.9 $\mu\text{g/g}$ in PET	<5.0 to 13.7	–	Mutsuga et al. (2006)
Carbonated water	–	–	–	–	1.4 \pm 0.1	Mutsuga et al. (2006)	
Acetaldehyde	Mineral water	–	–	–	<0.5 to 59	–	Sugaya et al. (2001)
	Mineral water	–	–	–	<0.5 to 260	–	Sugaya et al. (2001)
	Mineral water	–	–	–	<0.5 to 0.9	–	Sugaya et al. (2001)
	Mineral water	–	–	–	<0.5 to 0.3	–	Sugaya et al. (2001)
	Still water	–	–	TOC	0.9–133.8	–	Nawrocki et al. (2002)
	Still water	–	–	<2.0 to 2.9 $\mu\text{g/g}$ in PET	<5.0 to 107.8	–	Mutsuga et al. (2006)
	Still water	r.t.	6 days	–	–	4.8 \pm 0.5	Dabrowska et al. (2003)
	Still mineral water	–	63 days, sunlight	–	–	3	Wegelin et al. (2001)
	Still mineral water	–	126 days, sunlight	–	–	2	Wegelin et al. (2001)
	Still mineral water	40 °C	10 days	–	<2	–	Ceretti et al. (2010)
	Carbonated water	–	–	TOC	4.7–317.8	–	Nawrocki et al. (2002)
	Carbonated water	–	–	0.5–0.9 $\mu\text{g/g}$ in PET	<5.0 to 46.9	–	Mutsuga et al. (2006)
	Carbonated water at pH = 4.5	r.t.	6 days	–	–	24.6 \pm 2.5	Dabrowska et al. (2003)
	Carbonated water	r.t.	170 days	–	–	78.1 \pm 7.8	Dabrowska et al. (2003)
	Carbonated water	r.t.	5 weeks	CO ₂ content: 3.88 g/L	–	28.0 \pm 2.8	Dabrowska et al. (2003)
Carbonated water	r.t.	5 weeks	CO ₂ content: 4.53 g/L	–	52.0 \pm 5.2	Dabrowska et al. (2003)	
Carbonated water	r.t.	5 weeks	CO ₂ content: 6.40 g/L	–	79.0 \pm 7.9	Dabrowska et al. (2003)	
Carbonated water	40 °C	10 days	–	<2	–	Ceretti et al. (2010)	
Propanal	Mineral water	–	–	–	<0.5 to 0.9	–	Sugaya et al. (2001)
	Carbonated water	–	–	–	–	<0.05	Dabrowska et al. (2003)
Butanal	Mineral Water	–	–	–	<0.5 to 0.3	–	Sugaya et al. (2001)
Nonanal	Still water	–	–	TOC	0.9–11.3	–	Nawrocki et al. (2002)
	Carbonated water	–	–	TOC	0.95–7.9	–	Nawrocki et al. (2002)
	Carbonated water	–	–	–	–	<0.05	Dabrowska et al. (2003)
Glyoxal	Still water	–	–	TOC	–	5.9	Nawrocki et al. (2002)
	Carbonated water	–	–	TOC	–	0.9	Nawrocki et al. (2002)
	Carbonated water	–	–	–	–	<0.05	Dabrowska et al. (2003)
Methylglyoxal	Still water	–	–	TOC	0.9–15.8	–	Sugaya et al. (2001)
	Carbonated water	–	–	TOC	0.8–4.6	–	Sugaya et al. (2001)
Acetone	Still water	–	–	TOC	5.1–107.6	–	Nawrocki et al. (2002)
	Carbonated water	–	–	TOC	16.8–125.6	–	Nawrocki et al. (2002)

r.t.: room temperature; TOC: total organic carbon; n.d.: not detected.

Table 3 – Results of phthalate esters migration from PET into bottled water.

Compound name	Simulant	Exposure temperature	Exposure conditions	Concentration range (µg/L)	Concentration mean (µg/L)	References
DMP	Still water	–	–	<0.012	–	Cao (2008)
	Mineral water	22 °C	30 days	<0.04	–	Bošnjir et al. (2007)
	Water	Up to 30 °C	10 weeks	<0.002 to 0.003	0.002	Casajuana and Lacorte (2003)
DEP	Still water	Refrigerated	–	0.054–0.1	0.077 ± 0.016	Cao (2008)
	Mineral water	22 °C	30 days	<0.04 to 1	0.11	Bošnjir et al. (2007)
	Water	Up to 30 °C	10 weeks	0.082–0.355	0.214	Casajuana and Lacorte (2003)
DBP	Still water	Refrigerated	–	0.08–0.32	0.357 ± 0.606	Cao (2008)
	Mineral water	22 °C	30 days	<0.04 to 50	11	Bošnjir et al. (2007)
	Water	Up to 30 °C	10 weeks	0.020–0.070	0.046	Casajuana and Lacorte (2003)
DiBP	Still water	Refrigerated	–	0.13–0.35	0.225 ± 0.081	Cao (2008)
BBP	Still water	–	–	<0.085	–	Cao (2008)
	Mineral water	22 °C	30 days	<0.005	–	Bošnjir et al. (2007)
	Water	Up to 30 °C	10 weeks	<0.004 to 0.010	<0.004	Casajuana and Lacorte (2003)
DEHP	Dionised water	r.t.	17 h, darkness	0.14–0.24	0.19 ± 0.05	Schmid et al. (2008)
	Dionised water	r.t.	17 h, sunlight	0.10–0.38	0.26 ± 0.10	Schmid et al. (2008)
	Dionised water	60 °C	17 h, sunlight	0.15–0.71	0.36 ± 0.21	Schmid et al. (2008)
	Still water	–	–	0.05–0.093	0.102 ± 0.055	Cao (2008)
	Mineral water	r.t.	3 months	<0.02 to 6.8	–	Leivadara et al. (2008)
	Mineral water	22 °C	30 days	<0.04 to 50	8.8	Bošnjir et al. (2007)
	Water	Up to 30 °C	10 weeks	<0.002 to 0.188	134	Casajuana and Lacorte (2003)
	Mineral water	Up to 30 °C	3 months, sunlight	<0.02	–	Leivadara et al. (2008)
DHP	Still water	–	–	<0.036	–	Cao (2008)
DOP	Still water	–	–	<0.003	–	Cao (2008)
	Mineral water	22 °C	30 days	<0.04	–	Bošnjir et al. (2007)

DMP: dimethyl phthalate; DEP: diethyl phthalate; DBP: dibutyl phthalate; DiBP: di-iso-butyl phthalate; BBP: benzylbutyl phthalate; DEHP: di-2-ethylbutyl phthalate; DHP: dihexyl phthalate; DOP: dioctyl phthalate; r.t.: room temperature.

times higher than in those from glass bottles directly analyzed after purchased. However, no correlation between the physico-chemical water properties and phthalate migration has been found. Nevertheless, phthalate levels detected in still mineral water are slightly higher than in sparkling water.

Several studies have provided data on phthalates levels in bottled water immediately after purchase; the initial levels are frequently lower than 0.4 µg/L (Peñalver et al., 2000; Kayali et al., 2006; Montuori et al., 2008).

The effect of pH and sunlight exposure on phthalate migration has also been studied. Bošnjir et al. (2007) suggest that acidic pH stimulates the diffusion of phthalates. Schmid et al. (2008) and Amiridou and Voutsas (2011) concluded that plasticizers migration in outdoor conditions is not substantial.

Regarding the occurrence of DEHA in PET-bottled water, Serôdio and Nogueira (2006) found that DEHA concentrations in bottled water (0.15 µg/L) are slightly higher than in tap water (0.09 µg/L), but this difference is not significant. The effect of sunlight exposure and temperature related to DEHA levels in PET-bottled water has been investigated (Schmid et al., 2008). Slight differences in DEHA amounts in PET-bottled water have been observed in association with high temperatures and in samples from different countries.

In conclusion, the plasticizers present in packaged food do not necessarily mean that these compounds will be present in packaging material (Page and Lacroix, 1995). In addition, it is worth to note that phthalates and adipates are not used in PET. More investigations are needed to improve the accuracy

of the analytical methods and to clarify the entry pathways of plasticizers in the bottling line.

2.5. Antioxidants

Small amounts of these additives can be added to the polymer before it is processed to inhibit or reduce the oxidation of plastic material. However, PET bottles intended for water are usually manufactured without antioxidants (Zweifel, 2001).

2.5.1. Alkylphenols

In food packaging manufacture, alkylphenols, such as 4-nonylphenol (NP), and octylphenol (OP), can be generated by oxidation of tris(nonylphenyl)phosphite (TNPP) (additive) or by degradation of polyethoxylated nonylphenols (APEOs), which are cleaning agents in bottle manufacturing (McNeal et al., 2000; Casajuana and Lacorte, 2003). Alkylphenols are known to be endocrine disruptors (Loos et al., 2007; Baugros et al., 2009). SMLs have not been established for NP and OP.

In the case of PET containers, either NP is not detected (Casajuana and Lacorte, 2003; Fernandes et al., 2008) or the authors doubt whether these containers are a source of alkylphenols (Toyo'oka and Oshige, 2000; Loyo-Rosales et al., 2004).

When NP or OP is found in bottled water, it is always at low levels, ranging from 1 to 20 ng/L (Amiridou and Voutsas, 2011; Guart et al., 2011). Only Li et al. (2010) found NP concentrations ranging from 108 to 298 ng/L in bottled water, but the

material which the water container was made (PVC, PE or PET) was not specified, thus making this study not reliable.

It is important to note that the source of alkylphenols may be the antioxidants in laboratory equipment and materials (vessels, tubes, detergents, etc.), the water itself, or pollution during the container washing steps when the packaging was manufactured. Consequently, the slight amounts of alkylphenols in bottled water cannot be directly attributed to PET.

2.5.2. Butylated hydroxytoluene (BHT)

BHT is a phenolic antioxidant used as a thermostabilizer for PE, PP, polyesters and PVC (Sheftel, 2000).

In all reviewed studies, BHT amounts in PET-bottled water are much lower than the established SML of 3 mg/kg (EU, 2011).

Tombesi and Freije (2002) found BHT amounts ranging from 21 to 38 µg/L in PET-bottled water. Later on, the same research group (Tombesi et al., 2004), again detected BHT, but amounts were 10 times lower than in the first studies. It should be mentioned that their first results are not reproducible. Further, Higuchi et al. (2004) found only BHT in glass-bottled water (2.5 µg/L) concluding that the origin of this compound is the PE bottle caps. This conclusion seems to be logical because this additive may be used in the production of PE.

2.6. UV stabilizers

Light stabilizers based on benzotriazoles are used in the production of PET packaging in some markets (FSA, 2007). The SMLs of Tinuvin 234 and Tinuvin P are set at 1.5 mg/kg and 6 mg/kg, respectively (EU, 2011).

Studies of benzotriazole stabilizers in PET and bottled water are pretty old and are now inaccurate due to the changes in PET manufacture over the years. Further, the migration potential of stabilizers is not noteworthy due to their insolubility into water.

2.7. Lubricants

Lubricants are used for the production of plastic packaging to minimize adhesion, to reduce friction, or to promote the elasticity of plastics (Zweifel, 2001). European Regulation No. 10/2011 authorizes the use of erucamide and oleamide for the manufacture of food-contact packaging. No specific SMLs have been prescribed for these substances. Only old studies have found these two compounds in PET-bottled water (Buiarelli et al., 1993; Monteiro et al., 1996). More recently, Strube et al. (2009) attributed several volatile compounds found in PET-bottled water after sunlight exposure to photo-oxidation products of erucamide present in bottle closures. Unfortunately, the closures material was not analyzed and this hypothesis could not be confirmed.

2.8. Bisphenol A

Bisphenol A (BPA) is a moiety used in the manufacture of PC for food packaging. It is known to be an endocrine-disrupting chemical that may cause harmful effects in animals and probably in humans (Berryman et al., 2004). A total daily intake (TDI) for BPA of 0.05 mg/kg/body weight (EFSA, 2008)

and an SML of 0.6 mg/kg have been established. In the reviewed studies, BPA levels found in bottled water varies greatly and concentrations fall in the range of a few ng/L. Therefore, BPA values calculated for a consumption of 2 L per day for an adult weighting 60 kg does not exceed the TDI.

Furthermore, few publications have been devoted to the investigation of BPA in bottled water, as BPA is not used in the manufacture of PET. BPA concentrations remain constants (Toyo'oka and Oshige, 2000) or increase slightly (Casajuana and Lacorte, 2003) before and after heating PET-bottled water. Therefore, it is difficult to incriminate PET material as a source of BPA.

A recent study (Amiridou and Voutsas, 2011) found low BPA concentrations (up to 4 ng/L) in PET-bottled water before and after sunlight exposure. In contrast, Shao et al. (2005) and Guart et al. (2011) did not detect this compound in PET-bottled water.

It has been suggested that the source of BPA in PET-bottled water could be due to bottle closures (Guart et al., 2011), the water itself (polluted prior to bottling) (Li et al., 2010), or the use of recycled PET (Sax, 2010).

3. Toxicological evaluation of PET-bottled water

Although, food-contact packaging is strictly controlled by European regulations (EU, 2004, 2011), it has been suggested that food packaging may leach estrogenic substances (Muncke, 2009; Yang et al., 2011). Although controversial, PET has been designated as a possible source (Pinto and Reali, 2009; Sax, 2010; Wagner and Oehlmann, 2011).

3.1. Genotoxicity assays

The Ames test was performed by Monarca et al. (1994) on carbonated water kept in PET bottles. The mineral water was concentrated using C18 cartridges after daylight storage (1, 3, 6 months at room temperature). The results for the concentrated extracts showed no mutagenic activity with Salmonella strains TA 98 and TA 100 (with and without S9), whatever the time periods. In parallel, several compounds were detected in distilled water stored in PET bottles, namely acetaldehyde, acetic acid, propanal, terephthalic acid, dimethylterephthalate, phenol-2,6-bis(1,1-dimethylethyl)-4,4-methyl and 1,2-benzenedicarboxylic acid butyl-2-methyl-propyl ester.

Other authors have investigated the genotoxicity of PET-bottled water after exposure to daylight and temperature. Evandri et al. (2000) observed an increase of chromosomal aberrations in *Allium cepa* with PET water samples exposed to direct sunlight for 16 weeks (two-fold induction) and exposed in the dark at 40 °C for 10 days (three-fold induction). Although, these aberrations were attributed to the migration of volatile compounds into PET-bottled water, the chemical analysis of water was not done. Further, the results may be not representative because very few PET brands were tested. Biscardi et al. (2003) performed the *Tradescantia* micronucleus bioassay and the Comet assay with human leucocytes in lyophilized water stored in PET bottles. Only samples stored for 2 months showed an eight-fold increase in the frequency of micronuclei compared to distilled water. Also, significant

DNA damage in human cells was observed only in PET-bottled water collected directly in the bottling plant. It was suggested that the distribution pipelines in the bottling plant were a source of mutagens in mineral water. In parallel, the same authors identified DEHP in the acetone extracts of still and carbonated mineral water in concentrations ranging from 0.4 to 3.2 mg/L after 9 to 10 months of storage. These elevated values of DEHP are clearly a problem of contamination in the laboratory environment or reagents. Furthermore, DEHP is not genotoxic (Dybing, 2002); its presence cannot explain the observed toxic effect; consequently, this study can be disregarded.

In contrast, using human cells (HULYs), Ergene et al. (2008) did not observe any significant effect on sister chromatid exchange for spring water and purified water stored in PET for 8 weeks after bottling, but they noticed a cytostatic effect. Ceretti et al. (2010) found that genotoxic effects may be associated with the mineral and CO₂ content of the water using the *Tradescantia* micronucleus test and *A. cepa* assay. Again these findings should be interpreted with caution because of the small number of samples tested.

Furthermore, media must be buffered to prevent false positive responses in the assay due to a change in extracellular pH. Water conductivity must also be checked to ensure that the physico-chemical properties of the water itself are not of the cause of the positive response.

3.2. Endocrine disruptor assays

Endocrine disruptors are compounds that mimic or antagonize the actions of natural estrogens, and are the most common form of endocrine disruptor activity (ICCVAM, 2003, 2006). These compounds alter the hormone system involved in many biological metabolisms and can produce many health-related problems, such as early puberty in females, reduced sperm counts, altered function of reproductive organs, obesity, altered gender-specific behaviors, and increased rates of some breast, ovarian, testicular, and prostate cancers (Newbold et al., 2004; Della Seta et al., 2006; Patisaul et al., 2009).

Some authors have reported estrogenic activity in mineral water in PET bottles, using bioassays such as the E-screen assay (MCF-7 cell line) and yeast estrogen assay (*Saccharomyces cerevisiae*) expressing the human estrogen receptor α (ER α). Estrogenic activity has also been evaluated using a reproduction test performed with mudsnails, *Potamopyrgus antipodarum* (Pinto and Reali, 2009; Wagner and Oehlmann, 2009; Sax, 2010; Wagner and Oehlmann, 2011). All, these studies suggested the presence of endocrine disruptors in PET-bottled water.

Contamination of bottled water by endocrine disruptors could occur during the different steps of the bottling process, namely untreated groundwater from a spring, supply pipes or the filling and cleaning of containers in the bottling process (Montuori et al., 2008; Wagner and Oehlmann, 2009; Sax, 2010). Furthermore, for Yang et al. (2011) stress (UV radiation and heat) applied to the plastic material could also be a source of endocrine disruptors. Dissolved salts in water have been also pointed out as responsible for estrogenic activity (Criado et al., 2005).

Some authors have suggested several compounds as being the source of hormonal activity. As shown in Section 2.4, phthalates have been detected in PET-bottled water (Casajuana and Lacorte, 2003; Bošnjir et al., 2007; Montuori et al., 2008). However, phthalates are not used as additives in the manufacturing of PET bottles (ILSI, 2000). Furthermore, contamination cannot be excluded in these studies, as they did not prove the absence of phthalates in mineral water and soft drinks before bottling. In any case, even if they had been present individually, the estrogenic activity of these compounds is too weak (Jobling et al., 1995), particularly for DEHP, to explain these data.

As reported in Section 2.2, Sb has been found in PET-bottled water. Sax (2010) mentioned that Sb could be also a source of estrogenicity. Indeed, Choe et al. (2003) observed a high estrogenicity of antimony trichloride (SbCl₃) using the estrogen receptor-dependant transcriptional expression assay and the E-screen test. However, the most common catalyst used in PET synthesis is the Sb₂O₃, not the SbCl₃ (Biros et al., 2002; Duh, 2002).

Recently, Yang et al. (2011) claim that all plastics subjected to “stress” could leach xeno-estrogenic substances, even those that have no estrogenic activity in the initial step (formulation). The estrogenic activity of PET water bottles was evaluated by an E-screen assay using MCF-7 cells. The saline extracts of PET show estrogenic activity (RME response > 15%) in all stress conditions (microwave, sunlight, autoclave). However, no chemical analyses were performed in parallel to identify the compounds involved in the observed effect.

For other authors, estrogenicity may be due to the use of recycled PET (Safa, 1999). Sax (2010) suggests that the DMP concentration in PET-bottled soda detected by Bošnjir et al. (2007) may be due to the use of recycled PET coming from shampoo bottles and intended for bottling of soft drinks.

Furthermore, the extraction efficiency of estrogen-like compounds from bottled water depends on the water preparation techniques (SPE, evaporation) (Wagner and Oehlmann, 2011).

In each case, when biological data were provided there was insufficient analytical data to enable us to draw a conclusion.

Pinto and Reali (2009) report low estrogenic activity, but with great variability, in nine Italian brands of PET-bottled water using a yeast estrogen screen (YES) bioassay. The water samples were concentrated using C18 cartridges and the extracts were dissolved in dimethyl sulfoxide (DMSO). Although, higher estrogenic activity was observed in one brand of mineral water (23.1 ng/L estradiol equivalents (EEQ)), in the other brands, hormonal activity was often found to be in the same range as for tap and surface water (15–17 ng/L EEQ).

Another research group, Wagner and Oehlmann (2009) tested 18 brands of PET- and glass-bottled water using the YES assay. Compared to glass, a weak increase in the estrogenic activity in PET-bottled water was observed in three of four brands with a maximum value of 75.2 ng/L of EEQ. The parthenogenetic generation of mud snail embryos (*Potamopyrgus antipodum*) cultured on PET bottles was investigated. Although the differences were not statistically significant, the authors claim that the production of embryos per female increased slightly on PET bottles suggesting that estrogenic contamination comes from PET packaging. However, no correlation was observed with similar brands between both

assays and nothing proved whether the effect was really due to PET or to contamination. Furthermore, the American Chemistry Council (ACC, 2009) reports that PET is not a source of estrogenic compounds.

More recently, Wagner and Oehlmann (2011), in a follow-up of their previous article, reported that C18 cartridges and the evaporation of extracts with DMSO entrap more estrogenic compounds. The authors suggested that volatile compounds retained in DMSO could be the cause of the higher estrogenic activity. Using the E-screen assay, with optimized sample preparation (C18 cartridges and evaporation with DMSO), estrogenic activity in PET-bottled water was three-fold higher than in glass-bottled water. However, it is important to note that this concentration of EEQs with E-screen assay is about a factor 15,000 lower than the estrogenic activity found in their previous paper with the YES assay. Further, other studies using the E-screen bioassay cannot confirm the presence of endocrine activity in PET-bottled water (Böhmler et al., 2006; Bopp et al., 2010; Brüscheweiler, 2011).

Unfortunately, the Wagner and Oehlmann (2011) study did not provide any analytical data, underlying the need to combine chemical analysis with bioassays to clearly identify these compounds and to understand the potential risk of exposure for humans.

Also, using theoretical models of migration with potential xeno-estrogenic candidates such as nonylphenol and BPA, Franz and Welle (2009) rule out PET packaging as being responsible for this observed hormonal activity. They suggest that the estrogenic potency of the endocrine disruptors alone is too low to explain this observed hormonal activity. Consequently, a chemical mixture, or “cocktail effect”, and/or unknown compounds (NIAS) could be the source of the estrogenic activity observed, with low concentrations of endocrine disruptors giving rise to a synergistic effect (Muncke, 2009).

4. Discussion and conclusions

Although food-contact packaging is strictly regulated (EU, 2004, 2011), the detection of contaminants released from plastic materials is controversial, particularly regarding estrogenic activity. As we have highlighted in this review, PET has also been incriminated.

Throughout this review, we have made it clear that studies use different storage conditions to evaluate the migration of compounds from PET into bottled water. Different analytical methods with sensitive detection limits have been employed to identify or/and quantify these substances in a large variety of PET bottles. Since migration depends directly on these factors, it is difficult or even impossible to compare data from these different studies, leading to controversial interpretation of results. The same is true for the toxicological studies performed on bottled drinking water. Depending on the type of assay (yeast, human cell lines, snails, plants systems, etc.) and sample preparation (lyophilized, concentrated, etc.) different conclusions can be drawn. Further, plant systems (*A. cepa*, *Tradescandia*) are not considered as primary screening tools by current international guidelines for mammalian systems, making extrapolation very difficult (Evandri et al., 2000).

The cyto-/genotoxic effects and the endocrine disruptors activities observed *in vitro* by some authors have raised doubts and revealed discrepancies in the debate on quality and the safety of PET-bottled water.

Regarding any potential estrogenic activity, a ‘cocktail’ effect in bottled drinking water with compounds having weak endocrine-disrupting properties and/or water mineral content may explain the reported positive results (Muncke, 2009).

Also, it is important to specify that very few studies combined both chemical water analysis and toxicological evaluation in the same investigation on PET-bottled water.

It is now assumed, and all scientific reports agree, that formaldehyde, acetaldehyde and antimony are related to PET and can migrate into the bottled water depending on certain storage parameters and types of drinking water (Nawrocki et al., 2002; Dabrowska et al., 2003; Mutsuga et al., 2006; Shoty and Krachler, 2007; Westerhoff et al., 2008; Keresztes et al., 2009).

Apart from these well-known compounds, which usually do not pose a human health risk, we conclude that it is necessary to be cautious before claiming that there is a direct link between PET use and the compounds found in bottled drinking water.

PET is the polymer which uses the least additives (ILSI, 2000). Phthalates, alkylphenols, antioxidants, UV stabilizers, lubricants and carbonyl compounds in PET-bottled water could come from several sources, namely bottle closures, transport pipelines, disinfection agents, background pollution in analytical methods and the bottling process itself or even environmental pollution. Their presence in glass-bottled water, as demonstrated by some authors, is another reason to believe that there may be other sources than PET.

Less is known about NIAS (byproducts, impurities, etc.) in PET bottles and these substances can also migrate into bottled drinking water (Skjevraak et al., 2005; Grob et al., 2006; Franz and Welle, 2008; Muncke, 2009). However, NIAS can be present in any food-contact material.

More comparable and reliable information on chemical mixtures and the effect observed in the PET-bottled drinking water is necessary before concluding that there is a potential human health risk. Bioassays are complementary tools of chemical analyses. They are useful as screening tools to detect potential toxic effects due to NIAS and/or chemical mixtures. However, human cells lines are more relevant and the answer should be placed in a physiological context of the organism. The use of these bioassays must also be standardized (CEN-OCDE guidelines, etc.). Further, it is necessary to combine toxicological data and chemical analysis, especially when the responses are positive, and to determine the possible entry pathways and concentration of compounds.

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