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Effect of temperature on the release of intentionally and non-intentionally added substances from polyethylene terephthalate (PET) bottles into water: Chemical analysis and potential toxicity

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ABSTRACT

The purpose of this study was to investigate the impact of temperature on the release of PET-bottle constituents into water and to assess the potential health hazard using *in vitro* bioassays with bacteria and human cell lines. Aldehydes, trace metals and other compounds found in plastic packaging were analysed in PET-bottled water stored at different temperatures: 40, 50, and 60 °C. In this study, temperature and the presence of CO_2 increased the release of formaldehyde, acetaldehyde and antimony (Sb). In parallel, genotoxicity assays (Ames and micronucleus assays) and transcriptional-reporter gene assays for estrogenic and anti-androgenic activity were performed on bottled water extracts at relevant consumer exposure levels. As expected, and in accordance with the chemical formulations specified for PET bottles, neither phthalates nor UV stabilisers were present in the water extracts. However, 2,4-di-tert-butylphenol, a degradation compound of phenolic antioxidants, was detected. In addition, an intermediary monomer, bis(2-hydroxyethyl)terephthalate, was found but only in PET-bottled waters. None of the compounds are on the positive list of EU Regulation No. 10/2011. However, the PET-bottled water extracts did not induce any cytotoxic, genotoxic or endocrine-disruption activity in the bioassays after exposure.

1. Introduction

Today, the most common polymer used for the bottling of drinking water is polyethylene terephthalate (PET). Since migration can occur between packaging and foodstuffs, consumers may be exposed to the potentially harmful chemicals (additives, un-reacted monomers, and processing aids) used in manufacturing the packaging. These intentionally-added substances (IAS) are listed and controlled by European Regulation No. 10/2011 and do not pose any risk to humans. However, over 50% of compounds migrating from food contact materials are non-intentionally added substances (NIAS) (Grob, Biedermann, Scherbaum, Roth, & Rieger, 2006). Indeed, Mittag and Simat (2007) reported that 98% of the toxicity evidenced by several epoxy coating migrates was due to NIAS and/or reaction products. European Regulation No. 10/2011 concerning plastics and multilayers recently became more strict, stating that "the risk assessment of a substance should cover the substance itself, relevant impurities and foreseeable reaction and degradation products in the intended use" (EU, 2011).

PET is characterised by a limited range of additives and low diffusion of potential migrants in the polymer matrix (EFSA, 2011b). However, in PET-bottled waters non-polymer origins of NIAS also exist, namely the water itself, the bottling process, disinfection agents and environmental pollutions.

Furthermore, PET can be degraded due to several exposure factors under normal conditions of use (heat and UV light). In addition, certain physicochemical properties of bottled water, such as inorganic composition, carbonation or bacterial presence, influence

Abbreviations: BHT, butylated hydroxytoluene; BHET, bis(2-hydroxyethyl) phthalate; DBP, dibutyl phthalate; DEHA, di-2-ethylhexyl adipate; DEHP, di-2-ethylhexyl phthalate; DEP, diethyl phthalate; DHT, dihydrotestoterone; DiBP, di-isobutyl phthalate; DMP, dimethyl phthalate; DMSO, dimethyl sulfoxide; 2,4-dtBP, 2,4-di-tert-butylphenol; ECCVAM, European Center for the Validation of Alternative Methods; EFSA, European Food Safety Authority; IAS, intentionally added substances; LOQ, limit of quantification; NIAS, non-intentionally added substances; SML, specific migration limit.

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the leaching of constituents from PET bottles into water. It has also been established that the migration of several compounds (formaldehyde, acetaldehyde and Sb) from PET packaging to water is a thermally activated process (see review in Bach, Dauchy, Chagnon, and Etienne (2012)). However, little or nothing is known about the release of other NIAS (Grob et al., 2006) from the PET bottles into water and the final effect in terms of toxicity of all the migrated substances.

Over the last few years, certain studies have reported finding chemical mixtures with cytogenotoxic effects and endocrine disruption activity in PET-bottled water (see review and comments in Bach et al. (2012)). Toxic effects, and especially endocrine disruption, could be attributed to a "cocktail effect" due to compound mixtures (Muncke, 2009). However, migration studies of PET-bottled water rarely combine chemical analysis with toxicological assessments. Therefore when bioassays demonstrate positive responses, analytical data to identify the responsible compounds are always lacking and conclusions are difficult to draw.

The current European Regulatory framework states that an individual toxicological evaluation for substances used in the manufacturing of food contact materials is required. However, potential interactions (dose additivity, synergism, supra-additivity, etc.) between compounds may also occur at very low doses. These two points (low doses and interactions) are not often taken into account and represent new paradigms in toxicology. Furthermore, another current challenge is the development of analytical methods able to detect a wide range of analytes present in bottled water at very low levels (see review in Diduch, Polkowska, and Namieśnik (2011)).

The aim of this study was to determine the chemical composition of various bottled waters and, in parallel, to perform *in vitro* bioassays to check the potential toxicity of these waters when exposed to high temperatures. The effect of temperature on the migration of aldehydes, trace metals and several other potential migrants present in plastic packaging was monitored in PET-bottled waters. The migration tests were performed under realistic conditions of human exposure according to the EU Regulation migration criteria (1 kg of water in contact with 6 dm^2 of packaging material). The toxicological evaluation of bottled water extracts was carried out using toxicological endpoints of concern at low concentrations. The bioassays retained in this study were the Ames test (using prokaryotes) and the micronucleus assay using P53 competent human cells (HepG2 cell lines) to assess genotoxicity. Gene reporter assays were also performed for endocrine disruption activities (estrogenic and anti-androgenic) using human HepG2 and MBA-MB453-kb2 cell lines. All the assays were chosen for their performance and feasibility and in accordance with EFSA and/or ICCVAM recommendations (EFSA, 2011a; ICCVAM, 2003).

2. Materials and methods

2.1. Bottled water samples and storage conditions

Two French brands of non-carbonated water (brand A) and carbonated water (brand B) bottled in PET and in glass directly purchased from a local store were analysed. Water samples were bottled at the same time and were from identical batches. Three samples were derived from each brand by replacing the commercial water with ultrapure water: non-carbonated water in PET and glass (brand A), ultrapure water in PET (brand A), carbonated water in PET and in glass (brand B), ultrapure water in PET (brand B). Water samples were analysed (i) before the experiments (after 10 days at 20 °C), and (ii) after 10 days of storage at three different temperatures: 40, 50 and 60 °C.

2.2. Solid phase extraction (SPE)

Fourteen compounds, presented in Table 1, were extracted using Oasis HLB glass cartridges (6 cc/200 mg, Waters, Milford, USA). These compounds were previously identified in PET-bottled waters using a preliminary GC–MS screening method (see Additional Data section). Prior to SPE extraction, three internal standards were added to water samples as surrogates (Table 1), namely 2,6-di-tert-butyl-d9-4-methylphenol-3,5-d2, benzophenone-d5 and,di-2-ethylhexyl-phthalate-3,4,5,6-d4 (CDN isotopes, Pointe-Claire, Quebec, Canada) at a concentration of 0.4 μg/L. Carbonated water was degassed by ultrasonication. Cartridges were conditioned with 5 mL of ethyl acetate, methanol and UPLC grade water (Biosolve, Valkenswaard, the Netherlands), and

Table 1
Analytical parameters for the 14 compounds related to plastic packaging. Ions monitored limits of quantification (LOQ) and average recoveries and standard deviations (SD) are indicated.

Compound	Ions[b]	LOQ	% Recovery (SD)	
	(*m*/*z*)	μg/L	0.1 μg/L (0.3 μg/L[c])	0.5 μg/L (1.6 μg/L[d])
Dimethyl phthalate (DMP)	**163**, 77	0.1	107 (12)	100 (7)
2,6-Di-tert-butyl-p-benzoquinone	**177**, 220, 135	0.1	76 (17)	65 (11)
2,6-Di-tert-butyl-d9–4-methylphenol-3,5-d2[a]	**222**, 240	–	57 (6)	66 (4)
Butylated hydroxytoluene (BHT)	**205**, 220, 177	0.1	63 (8)	73 (12)
2,4-Di-tert-butylphenol (2,4-dtBP)	191	0.3	85 (10)	81 (4)
Ethyl-4-ethoxybenzoate	**194**, 121, 166	0.1	102 (15)	97 (10)
Diethyl phthalate (DEP)	149	0.1	114 (13)	95 (15)
Benzophenone-d5[a]	**187**, 110, 82	–	106 (13)	103 (9)
Benzophenone	**182**, 105, 77	0.1	99 (10)	92 (10)
4-Nonylphenol (NP)	**135**, 121, 107	0.1	85 (16)	79 (11)
3,5-Di-tert-butyl-4-hydroxybenzaldehyde (BHT-CHO)	**219**, 191	0.1	56 (10)	65 (13)
Di-iso-butyl phthalate (DiBP)	149	0.1	93 (11)	87 (11)
Dibutyl phthalate (DBP)	149	0.1	100 (13)	87 (11)
2-Ethylhexyl-p-methoxycinnamate	**178**, 161	0.3	44 (11)	45 (6)
Di-2-ethylhexyl adipate (DEHA)	**129**, 111	0.1	46 (8)	41 (5)
Di-2-ethylhexyl-phthalate-3,4,5,6-d4[a]	**153**, 171	–	46 (5)	48 (7)
Di-2-ethylhexyl phthalate (DEHP)	**149**, 167	0.1	60 (5)	60 (4)

[a] Internal standard.
[b] In bold: quantification ions.
[c] For 2,4-di-tert-butylphenol and 2-ethylhexylmethoxycinnamate, average recovery was calculated for a spiked level of 0.3 μg/L.
[d] For 2,4-di-tert-butylphenol and 2-ethylhexylmethoxycinnamate, average recovery was calculated for a spiked level of 1.6 μg/L.