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Phthalates and heavy metals as endocrine disruptors in food: A study on pre-packed coffee products

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ABSTRACT

Phthalate plasticizers and heavy metals are widely recognized to be pollutants that interfere with key developmental processes such as masculinization. We investigated the release of phthalates and heavy metals in coffee brewed from coffee packed in single-serve coffee containers made from different types of materials: metal, biodegradable and plastics. We detected with GC–MS small amounts phthalates, below the tolerated daily risks levels, in all the coffees prepared from the different types of capsules. Specifically, Di (2-ethyl-hexyl)-phthalate and DiBP: Diisobuthyl-pthalate were ubiquitously present despite the high variability among the samples (respective range 0.16–1.87 μg/mL and 0.01–0.36 μg/mL). Whereas, diethyl-phthalate (range 0.20–0.26 μg/mL) and di-*n*-buthyl-phthalate (range 0.02–0.14 μg/mL) were detected respectively in one and three out of the four types of capsule tested. In contrast, we detected by atomic mass spectrometry on mineralized samples heavy metals lead (Pb) and nickel (Ni), in all coffee tested. PB levels (respective range 0.32–211.57 μg/dose) accounted for 42–79%, whereas Ni levels (respective range 166.25–1950.26 μg/dose) accounted for > 100% of the tolerable daily intake. These results add to the already present concerns related to the multiple pathways of human exposure and the ubiquitous presence of these pollutants in consumer products and their long-term effect on human health.

1. Introduction

Over the past two decades, public health has focused on the identification of environmental chemical factors that are able to adversely affect hormonal function, known as endocrine disruptors (EDs) [1]. EDs mimic naturally occurring hormones like estrogens and androgens which can in turn interfere with the endocrine system. As consequence, EDs affect human reproduction as well as human post and pre-natal development. In fact, infants can be affected already at prenatal level due to maternal exposure to ED (reviewed in [2]). Epidemiological studies have reported an overall decline of male fertility and an increase of incidence of diseases or congenital malformations of the male reproductive system [3]. Specifically, it has been observed a decreased sperm count in semen over time which inversely correlates with the incidence of diseases such as testis cancer, cryptorchidism and hypospadias [4]. This trend, known as testis dysgenesis syndrome, was first reported in 1992 by a Danish study that found a 50% decrease in sperm count in the male population across the 1938–1992 period [5]. These reports alarmed both general population and public authorities. In particular, great attention has been given to those chemicals, or their metabolites, that have estrogenic properties or antagonistic effects on the activity of androgen or even inhibiting their production. These compounds have therefore the potential of interfering with important physiological processes, such as masculinization, morphological development of the urogenital system and secondary sexual traits and, not least, bone metabolism [6,7].

There are numerous substances with a recognized anti-androgenic effect, from air and ground pollutants to plasticizers. In the latter category, phthalates are the most investigated compounds as they are employed in virtually all industrial applications and consumer products as additives. Since these compounds are not covalently bound polymers, their exposure to heat over time has the potential to transfer-migrate into food [8,9]. As a consequence, a widespread human and environmental exposure to phthalates has been described, identifying

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ingestion as the main route of administration of these compounds [10]. Phthalates, together with another widely used plasticizer bisphenol A, showed to have a role also in the development of obesity and glucose metabolism disorders [11].

Heavy metals have also been recognized as likely inducers of testicular damage and, to this regard, the toxicity of Cadmium (Cd) as environmental contaminant has been known for several decades [12]. Some industrial activities, such as melting and welding of metals, as well as municipal waste incineration are processes that contribute in the release of heavy metals in the environment. Although the mechanisms of testicular toxicity exerted by heavy metals are still under investigation, the permeation through the blood-testis barrier is acknowledged as a fundamental process [13].

The production and delivery of ready-to-use consumer foodstuff require adequate packaging either in metal or plastic containers that can withstand high temperature used for cooking. Single serve coffee containers has simplified the production of authentic Italian espresso coffee with the added benefit of reducing time and maintain a consistent flavor for each serving. Coffee capsules can be made from different materials and are specifically designed to be used in specific brewing devices. In this brewing procedure, known as subrogation, a limited amount (20–50 mL) of hot water under high pressure (80–90 °C, 8–12 atm) is percolated in a very short time through a ground coffee cake (~7 g). This process produces a concentrated brew generally known as coffee surrogate [14]. Although the use of these containers has been declared to be safe by manufacturers, the actual release of contaminants in food, and in particular of endocrine disruptors, deserves higher attention. In this study, we evaluated the possible release of phthalates plasticizers and of biologically-relevant heavy metals from pre-dosed capsules or pods used for the domestic production of espresso coffee.

2. Methods

2.1. Sample preparation and processing

Four pre-packed coffee types were randomly chosen and purchased in July 2015 at local retail stores. We selected-compared coffee prepared using coffee packaged in a metal (type M), bio-degradable (type BD) and two different types of plastic (type P1 and P2, respectively) capsules.

Italian expresso coffees were prepared in the laboratory with HPLC-grade water using compatible system machines. Three espresso coffee machines were used: the first one was suitable for type M and BD capsules, the remaining two machines were suitable for type P1 and P2 capsule respectively. Coffee machines were assessed to elute coffee surrogates at 80 °C. In each preparation a default volume of espresso coffee surrogates, automatically dispensed by the coffee machine which corresponded to approximately 40 mL, was collected. For each coffee machine, prior to collecting samples, five coffees were discarded. Three espresso coffee surrogates from each capsule type were then analyzed in triplicate, for a total of 9 determinations each. Additionally, each capsule type was broken and the outer jacket underwent to the same coffee preparation, in order to address the possible source of phthalates.

Coffee surrogates underwent different processing depending on the type of contaminant assessed. For analysis of phthalates, liquid/liquid (water:dichloromethane) extraction by treatment of each coffee surrogate with dichloromethane (2 × 20 mL) in a separating funnel was performed. Organic extracts were then desiccated under nitrogen stream, solubilized in 1:1 dichloromethane/methanol solution and analyzed with GC/MS.

For analysis of heavy metals, 5 mL of each coffee surrogate and of water extract obtained from the outer jacket capsule, as well as the coffee powder contained in each capsule were treated with 5 mL of 1:1 hydrogen peroxide/ultrapure nitric acid (Pb ≤ 0.005 μg/kg, Pb ≤ 0.005 μg/kg, Pb ≤ 0.005 μg/kg TraceSELECT Ultra, Sigma Chemical Co.) solution and were transferred into a microwave Teflon vessel. Subsequently, samples were mineralized using a speed wave MWS- 3 Berghof instrument (Eningen, Germany).

2.2. GC/MS

The analyses were performed with a 5975C quadrupole mass spectrometer (Agilent Technologies, Milano, Italy) equipped with a 6850 gas chromatograph (Agilent Technologies, Milano, Italy) equipped with. The gas chromatographic conditions used were the following: column DB5 (60 mt, 0.32 mm i.d., 1 μm film thickness; He flow: 1 mL/min; Oven ramp: T1 = 60 °C, R1 = 8 °C/min, T2 = 190 °C (5 min), R2 = 8 °C/min, T3 = 240 °C (5 min), R3 = 8 °C/min, T4 = 315 °C (10 min). Phtalate plasticizers have been firstly identified by comparison with a standard phthalate mixture (Sigma-Aldrich: cod.48741 EPA 606-M phtalate Ester Mix), and by comparison with the NIST library. The standard Phtalate Ester Mix EPA 606-M (Sigma-Aldrich: cod.48741), containsing Benzyl-butyl phthalate (BBP), Bis(2-ethylhexyl)-phthalate (DEHP), Dibutyl phthalate (DBP), Diethyl phthalate (DEP), Dimethyl phthalate (DMP) and, Di-*n*-octyl phthalate (DOP). Diisobuthyl-pthalate (DiBP) was also used as reference standard. For quantification, Di-*n*-butyl phthalate-d4 (Sigma-Aldrich: cod.488763–25 mg) was used as internal standard (IS). Different solutions containing the reference standards at different concentrations (from 20 μg/mL to 0.25 μg/mL) and IS at constant concentration (2 μg/mL) were prepared. For quantification, different solutions containing the standard at different concentrations were prepared mixture (from 20 μg/mL to 0.25 μg/mL) and IS at constant concentration (2 μg/mL). Representative chromatograms of GC analysis on standard phthalate mixture are reported in Fig. 1. The areas relating to chromatographic peaks due to characteristic ions of phtalates, obtained through the reconstructed ion current (RIC) were considered. The ions used were the following: *m/z* 163 for DMP, *m/z* 149 for all the other phthalates and *m/z* 153 for IS. The results are reported as μg of compound per mL of surrogate. Representative chromatograms of GC analysis on real espresso coffee surrogate are reported in Fig. 2.

2.3. Atomic absorption spectroscopy

The content of heavy metals in mineralized surrogates was measured with atomic absorption spectrometry (AA) with the graphite furnace technique under argon at a wavelength of 228.8 nm, and 283.3 nm for Cd, Ni and Pb, respectively (Varian AA Duo Graphite Furnace Atomic Absorption Spectometer, Paloalto, CA). The calibration curves were obtained using known concentrations of standard solutions purchased from Sigma Chemical Co.

2.4. Statistical analysis

All statistical calculations were made by SPSS 23.0 software package for Windows (SPSS Inc., Chicago, IL). To evaluate the significance of differences on plasticizer and heavy metal concentrations, the analysis of variance (ANOVA) was applied with Bonferroni correction for multiple comparison. Statistical significance was set for values of $P < 0.05$.

3. Results

3.1. Quantification of phthalate plasticizers

Results on quantification of phthalate plasticizers in pre-packed coffee capsules are reported in Table 1. Phthalates were detected in any of the surrogate assessed, whether produced from metal, bio-degradable or plastic capsule. However, among the available panel of plasticizers, only DEP, DiBP, DBP and DEHP were detected in at least one sample. All the other phthalate plasticizers were below their