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Investigation of the genotoxicity of substances migrating from polycarbonate replacement baby bottles to identify chemicals of high concern

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ABSTRACT

Due to the worldwide concern that bisphenol A might act as an endocrine disruptor, alternative materials for polycarbonate (PC) have been introduced on the European market. However, PC-replacement products might also release substances of which the toxicological profile – including their genotoxic effects – has not yet been characterized. Because a thorough characterization of the genotoxic profile of all these substances is impossible in the short term, a strategy was developed in order to prioritize those substances for which additional data are urgently needed. The strategy consisted of a decision tree using hazard information related to genotoxicity. The relevant information was obtained from the database of the European Chemicals Agency (ECHA), *in silico* prediction tools (ToxTree and Derek Nexus™) and the *in vitro* Vitotox® test for detecting DNA damage. By applying the decision tree, substances could be classified into different groups, each characterized by a different probability to induce genotoxic effects. Although none of the investigated substances could be unequivocally identified as genotoxic, the presence of genotoxic effects could neither be excluded for any of them. Consequently, all substances require more data to investigate the genotoxic potential. However, the type and the urge for these data differs among the substances.

1. Introduction

Bisphenol A (BPA) is often used as a starting material to manufacture epoxy resins and polycarbonate (PC) plastics. Polycarbonates, a group of transparent thermoplastic polymers, have many applications including the fabrication of some food contact materials (FCMs), like infant feeding (baby) bottles, cups, etc (EFSA, 2015). Reports on the migration of BPA from PC into food together with studies identifying BPA as an endocrine disruptor have resulted in a worldwide concern about the application of BPA in FCMs (Alonso-Magdalena et al., 2012; Nam et al., 2010; Palanza et al., 2008; Talsness et al., 2009). In 2011, the European Commission decided to prohibit the use of BPA in the manufacture of PC baby bottles in the European Union on the basis of the precautionary principle (European Union, 2011a). As a result of this decision, a wide variety of alternative materials for PC has been introduced on the European market. Examples include, amongst

List of abbreviations: BaP, benzo[a]pyrene; BPA, bisphenol A; CLP, Classification, Labelling and Packaging; ECHA, European CHemicals Agency; 4-NQO, 4-Nitroquinoline 1-oxide; PC, polycarbonate; REACH, Registration, Evaluation, Authorisation and Restriction of Chemicals; SA, structural alert; S/N, signal to noise ratio.

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others, polypropylene, polyamide, polyethersulphone and a co-polyester under the trade name Tritan™, but also non-plastics, such as silicone (Onghena et al., 2014; Simoneau et al., 2012). However, BPA-free polymers might also release substances of which the toxicological profile has not yet been (completely) characterized. These migration products include (i) residual starting products due to incomplete polymerisation, (ii) additives that are not chemically linked to the polymeric structure and (iii) products resulting from degradation of the polymer (Bittner et al., 2014). In Europe, substances used as monomer or additive in plastic baby bottles should be in accordance with commission regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. Consequently, only substances included in the European Union positive list (Annex I) of the regulation can be used and migration should be below the specific migration limit, if available (European Union, 2011b). In contrast, no specific regulation exists for non-intentionally added substances migrating from plastics (e.g. degradation and reaction products with unknown chemical identity) or substances migrating from non-plastic FCMs, such as silicones. In 2012, Simoneau et al. reported on the migration of substances not included in the EU positive list from baby bottles used as substitutes for PC (Simoneau et al., 2012). Recently, migration of substances not authorised by the EU legislation for plastic FCMs from PC-replacement baby bottles was confirmed by Onghena et al. (2014, 2015). More data on the human exposure to and the toxicological properties of these substances are urgently needed to evaluate the toxicity and risks associated with PC-replacement products.

Genotoxicity is an important toxicological endpoint as genetic alterations in somatic and germ cells have been associated with serious health effects including cancer, degenerative diseases, reduced fertility and inherited diseases (Erickson, 2010; Hoeijmakers, 2009; Kong et al., 2012). Consequently, results of genotoxicity tests are key elements in risk assessment of chemicals in general, including those present in food and feed (EFSA, 2011). Also, for substances intended to be used as starting product or additive in plastic FCMs in the EU, genotoxicity data are required, regardless the level of migration (Barlow, 2009). A battery of three *in vitro* genotoxicity tests should be performed including (i) a gene mutation test in bacteria; (ii) an *in vitro* mammalian cell gene mutation test and (iii) an *in vitro* mammalian chromosome aberration test. If any of these tests yields a positive or equivocal result, further genotoxicity tests, including *in vivo* assays, may be required to elucidate the genotoxic potential of the substance (EFSA, 2008). Substances known to be genotoxic are only allowed for use in plastic FCMs under the condition that they do not migrate into food in amounts that are detectable by an agreed sensitive method. In practice, concentrations in food should be below 10 μg/kg (Barlow, 2009).

Considering the large number of substances that can migrate from different types of baby bottles, a complete characterization of the genotoxic profile of all substances is not feasible in the short term. Within this context, a strategy was developed in order to identify chemicals of high concern among the substances for which migration from baby bottles has been reported in literature (Onghena et al., 2014, 2015; Simoneau et al., 2012). The strategy consisted of a decision tree based on hazard information related to genotoxicity retrieved from literature combined with results of *in silico* and *in vitro* methods. Firstly, the database of the European Chemicals Agency (ECHA) was consulted to collect data of previous *in vitro* and *in vivo* genotoxicity tests on the selected compounds. Secondly, the genotoxic potential of these substances was predicted by two *in silico* rule-based programmes, i.e. ToxTree and Derek Nexus™. Thirdly, an *in vitro* screening study on their genotoxicity was performed with the Vitotox® test. Because this rapid indicator test uses only small amounts of the test compound for detecting DNA damage, the test was particularly suited for the present study (Westerink et al., 2009). Finally, all information was combined according to a decision tree in order to classify the substances into three groups, each characterized by a different probability to induce genotoxic effects. Substances included in Annex I of the European Regulation 10/2011 were not considered in the present study as these substances have already been subject to evaluation.

2. Materials and methods

2.1. Chemicals

The positive control substances for the Vitotox® test, i.e. benzo [a]pyrene (BaP) and 4-nitroquinoline 1-oxide (4-NQO), were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). An overview of the 48 substances selected for the current study based on the data from Simoneau et al. (2012) and Onghena et al. (2014, 2015) and their provider is presented in Table 1.

2.2. Data collection from the ECHA database

The ECHA website was consulted in order to collect information on the genotoxic potential of the substances available within the context of the REACH regulation (Registration, Evaluation, Authorisation and Restriction of CHemicals) (European Union, 2006). According to this regulation, the chemical industry must identify and manage the risks linked to the substances they manufacture and market in the EU in the quantity of 1 ton or more per year. In addition, they have to demonstrate to ECHA how the substance can be safely used, and they must communicate the risk management measures to the users. ECHA has to make the information available so that the general public can make informed decisions about their use of chemicals. For this reason, all information on the substances is collected in an ECHA database which can be consulted on the ECHA website (ECHA, 2015). The information collected from the ECHA database within the present study is summarized below.

2.2.1. In vitro and in vivo genotoxicity data for registered substances

Registration of the chemicals under REACH was checked by introducing the CAS number of each compound in the ECHA database. If registered, toxicological information is publicly available in the database. The type and the amount however depend on the quantity and the use of the substance that is produced or imported. Genotoxicity data (i.e. at least the results of a bacterial gene mutation test) are required for all chemicals produced or imported in quantities of more than 1 ton/year.

The available *in vitro* and *in vivo* data were collected for the different genotoxic endpoints (i.e gene mutations, chromosome damage and non-specific genotoxicity). Only data of studies with a Klimisch score of 1 (reliable without restrictions) or 2 (reliable with restrictions) were retained (Klimisch et al., 1997). A final call for each of the endpoints was made based on the available data. In case one of the *in vitro* gene mutation tests was positive, the substance was considered to induce gene mutations *in vitro*. A similar strategy was applied for the other *in vitro* and *in vivo* endpoints (i.e. *in vivo* gene mutations, *in vitro* and *in vivo* chromosomal damage and *in vitro* and *in vivo* non-specific genotoxicity). Substances were classified as 'clearly genotoxic' in case a positive result was observed in one of the *in vivo* genotoxicity tests. If all *in vitro* studies were negative or *in vitro* positive results were not confirmed in an adequate *in vivo* follow-up test, substances were considered 'not genotoxic'. For substances with insufficient or inadequate data, no final conclusion on the genotoxic potential was formulated.