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Benzene in infant carrot juice: Further insight into formation mechanism and risk assessment including consumption data from the DONALD study

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ABSTRACT

Benzene was previously detected as a heat-induced contaminant in infant carrot juices. This study shows that carrot juice contains substances such as β-carotene, phenylalanine or terpenes that may act as precursors for benzene formation during food processing. As benzene exposure has been associated with childhood leukaemia and other cancers, this study aimed to provide a quantitative risk assessment. To accomplish this, we used measured food consumption data from the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study, along with survey data on benzene in different juice categories. The calculated exposures for infants between 3 and 12 months were low, with averages between 1 and 10 ng/kg bw/day, resulting in a margin of exposure above 100,000. The exposures were judged as unlikely to pose a health risk for infants. Nevertheless, carcinogenic contaminants should be reduced to levels as low as reasonably achievable. The focus should be set on improving the sterilization conditions.

1. Introduction

Recently, we became concerned about benzene in infant nutrition, specifically in infant carrot juices. Many of these juices contain higher concentrations of benzene than are found in any other beverage group, with an average content above the EU drinking water limit of 1 μg/L (Lachenmeier et al., 2008). Our results were recently confirmed by a consumer magazine survey that tested a number of baby foods (Hansen, 2009).

Benzene is one of the food contaminants with the highest level of evidence for carcinogenicity (Lachenmeier, 2009). Active as well as passive smoking, automobile exhaust and driving or riding in automobiles are postulated as the most important pathways for benzene exposure (Wallace, 1996). Inhalatory exposure to benzene has been associated with increased risk for childhood leukaemia and other cancers (Brosselin et al., 2009; Crosignani et al., 2004; Knox, 2005; Steffen et al., 2004; Whitworth et al., 2008). The inhibition of topoisomerase II is one potential mechanism underlying the genotoxic and carcinogenic effects of benzene (Eastmond et al., 2005), and it is also suspected to contribute to certain childhood leukaemias (Anderson, 2006). Leukaemia is a major form of childhood cancer and data indicate that developed countries have a gradually increasing incidence of leukaemia (Pallapies, 2006). Since high levels of benzene metabolites are frequently reported among children and non-smoking workers without occupational exposure, Johnson et al. (2007) hypothesized that there may be significant sources of benzene that have hitherto been unidentified.

The current study was conducted with the aim of providing a quantitative risk assessment of benzene in infant carrot juices. The intention was to determine if nutritional benzene exposure from these types of sources during childhood could explain the previous metabolite findings or the increased cancer incidence. Furthermore, we conducted research into the mechanisms of formation of benzene during carrot juice processing. The results will be important in guiding manufacturers' efforts towards reducing this contamination.

2. Materials and methods

2.1. Chemical analysis

Benzene was analyzed using a validated headspace-gas chromatography/mass spectrometry (HS-GC/MS) procedure with a deuterated internal standard, previously described in detail (Lachenmeier et al., 2008). The procedure was amended by additionally analyzing toluene, *p*-xylene and *o*-xylene in the selected ion monitoring (SIM) mode, in conjunction with benzene. Benzene: *m/z* 78 as target ion and *m/z* 77 as qualifier ion; benzene-d_6: *m/z* 84 as target ion and *m/z* 82 as qualifier ion. Toluene: *m/z* 91 as target ion and *m/z* 92 as qualifier ion. *p*-/*o*-Xylene: *m/z* 91 as target ion and *m/z* 106 as qualifier ion. Toluene-d_8 as internal standard for toluene and *p*-/*o*-xylene: *m/z* 98 as target ion and *m/z* 100 as qualifier ion. In addition, the HS-GC/MS procedure was used to analyze composition of volatiles using full-scan mode (*m/z* 35–600).

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Amino-acid composition was determined using a LC 3000 Amino-Acid Analyzer (Eppendorf, Hamburg, Germany); for further details on this methodology, see Triebel et al. (2007).

2.2. Volatiles and amino acids as possible benzene precursors

This experiment was designed to study the decomposition of carrot juice during heating, with the aim of identifying substances that might be precursors of benzene. The heating experiment used a D-optimal algorithm similar to that of a preliminary heating experiment described previously (Lachenmeier et al., 2008). The D-optimal algorithm chooses an ideal subset of all possible combinations, thus significantly reducing the number of required experiments compared to standard design types (see Lachenmeier et al. (2008) for details on experimental design).

The carrot juice used in this experiment was freshly prepared from organic carrots using a juice extractor (centrifuge-type, Starmix, Reichenbach, Germany). The heating temperature was studied at four levels (unheated, 100 °C, 125 °C, or 150 °C) and the heating time was varied at three levels (30 min, 60 min, or 120 min). All juices were analyzed for volatile composition by HS-GC/MS and for amino-acid composition with the amino-acid analyzer.

The experimental designs and calculations were performed using the Design Expert V6 Software Package (Stat-Ease Inc., Minneapolis, MN, USA). The experiments were evaluated using Analysis of Variance (ANOVA) to find the significance of variables and their interactions in the models. The models were checked for consistency by examining lack of fit and possible outliers.

2.3. Benzene formation from pure precursor substances in simulated carrot juice

The identified precursors (pinene, limonene, carene, carotene (β-carotene), phenylalanine, tyrosine, and tryptophan) were purchased as pure substances of the highest degree of purity available (Sigma–Aldrich, Taufkirchen, Germany). These were prepared at concentrations of 1000 mg/l and were heated at 150 °C for 2 h in a simulated carrot juice matrix to confirm their capacity to form benzene. A known benzene precursor, benzoic acid, was included as a control. As ascorbic acid was previously attributed to having a positive influence on benzene formation, we also used ascorbic acid in pure form as well as mixed with the other compounds. The simulated carrot juice matrix was prepared according to the average composition of commercial carrot juice, as described by Otteneder (1982). For this, glucose (7.2 g/kg), fructose (7.1 g/kg), sucrose (31.5 g/kg), malic acid (1.9 g/kg), citric acid (0.3 g/kg), iron (1 mg/kg as sulphate), and copper (0.2 mg/kg as sulphate) were added to deionized water and the pH was adjusted with NaOH to pH 4.

The heated precursor solutions were analyzed for benzene, toluene and xylenes using HS-GC/MS in the SIM mode. The pure precursor standard substances, as well as the unheated simulated carrot juice matrix, were also analyzed for benzene. Some of the pure substances contained trace amounts of benzene. Therefore, all presented results were corrected for the benzene concentration in the solution prior to heating.

2.4. Approach for risk analysis

Analysis was conducted according to the harmonized approach of the European Food Safety Authority (EFSA) for the risk assessment of genotoxic and carcinogenic substances (EFSA, 2005). The EFSA has developed, and recommends, an approach known as the margin of exposure (MOE). This approach uses the doses of substances at which low but measurably harmful responses occur as reference point values and then compares these with relevant substance-specific dietary intake estimates in humans, taking into account differences in consumption patterns. The Benchmark Dose (BMD), derived by mathematical modelling within the observed range of experimental data, is recommended as a standardized reference point. To obtain the MOE, the Benchmark Dose Lower Confidence Limit (BMDL) of 10% was taken. The BMDL is an estimate of the lowest dose that is 95% certain to cause no more than a 10% effect. MOEs are then calculated by dividing the reference point, i.e., the BMDL, by the estimated human intakes.

For benzene, BMDL values are available from human epidemiology and various animal experiments. The US EPA (2003) has provided BMDL dose–response modelling of different long-term animal experiments (rats, mice) conducted by the NTP (National Toxicology Program, 1986). Reduction in lymphocyte count was selected as the critical effect. The resulting BMDL values for a 2-year observation range were 0.95–1.74 mg/kg bodyweight (bw)/day (male rat) and 2.32–18.55 mg/kg bw/day (male mouse). The US EPA also provided a BMDL value of 1.2 mg/kg bw/day, which was derived from a human occupational inhalation study (Rothman et al., 1996) by extrapolation to the oral route of exposure, and which was also based on lymphocyte count (US EPA, 1999). As the values from human epidemiology were in reasonably good agreement with the animal experiments, the EPA decided to use the human epidemiology BMD modelling for their evaluations and established an oral reference dose of 4.0×10^{-3} mg/kg bw/day. In the present study, we also chose to use this value for risk assessment.

2.5. Dietary intake assessment of carrot juice in infants

The EFSA harmonized approach was also used to conduct the dietary intake assessment (EFSA, 2005). The EFSA recommends that risk assessments provide different exposure scenarios (e.g., for entire or specific groups of populations) along with their inherent uncertainties. Other than the mean and median, intakes from highly exposed individuals (due to high consumption or to average consumption of highly contaminated foods) should be considered as represented by the 90th, 95th, 97.5th and 99th percentiles. Consumption data for carrot juices in infants are currently unavailable in the literature. We therefore provide our own data from the DONALD study (Dortmund Nutritional and Anthropometric Longitudinally Designed study).

The DONALD study is an ongoing, longitudinal (open cohort) study that has been collecting detailed data on diet, growth, development and metabolism between infancy and adulthood since 1985 (Kroke et al., 2004). In short, the starting study sample included infants, children and adolescents recruited from cross-sectional studies conducted in schools and kindergartens ($n \approx 470$). Since 1989, infants have been recruited and followed up longitudinally at least until the age of 18 years.

The regular DONALD assessments include records of dietary intake, anthropometry, urine sampling and medical examination, once a year per study participant $\geqslant$2 years of age and every 3 months during the first year of life.

The DONALD study, which is exclusively observational and non-invasive, has been approved by the International Scientific Committee of the Research Institute of Child Nutrition and the Ethics Committee of the University of Bonn.

For the present evaluation, we analyzed 3-day dietary records of subjects aged 3–12 months in the study period of 1997–2008. This selection resulted in 1566 records from 498 infants (251 boys and 247 girls). Per participant, one ($n = 40$; 8% of the total sample), two (68, 14%), three (170, 34%) or four (220, 44%) 3-day records were available and analyzed. Per study year, between 101 (2007) and 176 (2004) dietary records were available (mean: 131 records per year).

Parents weighed and recorded all foods and beverages consumed using electronic food scales (±1 g) on three consecutive days. Semi-quantitative recording (e.g., number of glasses) was allowed when weighing was not possible. The complete food collection details have been described elsewhere (Kroke et al., 2004). Energy and nutrient intakes were calculated using the in-house nutrient database LEBTAB, which undergoes continuous updating for all new food items. Energy and nutrient content of composite foods, e.g., commercial complementary food, were estimated by recipe simulation using the labelled nutrient contents and ingredients (Sichert-Hellert et al., 2007). For the present evaluation, six different categories of juices were used (Table 1).

3. Results and discussion

3.1. Identification of likely precursors of benzene formation in carrot juice

We had previously detected benzene as a heat-induced food contaminant (Lachenmeier et al., 2008). Because infant juices are

Table 1
Definition of juice categories.

All juices	Including common juices and infant juices from different origin, e.g., fruit juices, mixed juices, juice–tea-mixtures, juice–water-mixtures without added sugars ('Schorlen'). Mixtures of juice, water and any sort of sugar were excluded ('Saftgetränke', 'Saftnektare')
All pure carrot juices	Including only pure common and infant carrot juices without addition of other juices
All carrot juices inclusive carrot juice from recipes	Including carrot juice-ingredients of juice mixtures from common and infant juices
All infant juices	Including juices intended for infants from different origin, e.g., fruit juices, mixed juices, juice–tea-mixtures, juice–water-mixtures without added sugars
All pure infant carrot juices	Including only pure carrot juices intended for infants without addition of other juices
All infant carrot juices inclusive carrot juice from recipes	Including pure carrot juice intended for infants and carrot-ingredients of juice mixtures intended for infants