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Risk assessment for the Italian population of acetaldehyde in alcoholic and non-alcoholic beverages

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ABSTRACT

Acetaldehyde is a naturally-occurring carcinogenic compound, present in different food items, especially in alcoholic beverages. The aims of this study were to measure acetaldehyde concentration in different beverages consumed in Italy and to estimate the potential cancer risk. The analytical procedure was based on headspace solid-phase microextraction (SPME) and gas chromatography–mass spectrometry (GC–MS), using the isotopic dilution method. The margin of exposure (MOE) approach of the European Food Safety Authority (EFSA) was used for risk characterisation. The highest concentrations (median, min–max) were detected in grappa samples (499, 23.4–1850 mg/l), followed by fruit-based liqueurs and spirits (62.0, 5.23–483 mg/l) and wine (68.0, 18.1–477 mg/l); the lowest were detected in gin (0.91, 0.78–1.90 mg/l). The lowest MOE was estimated for high wine consumers (69). These results suggest that regulatory measures and consumer guidance may be necessary for acetaldehyde in beverages.

1. Introduction

Acetaldehyde (ethanal, CH_3CHO, CAS number 75-07-0) is a volatile compound which belongs to the large family of aldehydes, with a fruity aroma at low levels that turns into a pungent irritating odour at high concentrations (Miyake & Shibamoto, 1993).

Alcoholic beverages contain acetaldehyde in variable amounts. It is also naturally found in many non-alcoholic beverages and in foods (e.g., bread, coffee, ripe fruits) (Uebelacker & Lachenmeier, 2011), as well as in the environment, since it originates from the metabolism of plants. Acetaldehyde is also industrially produced and used as a flavouring (Feron, Til, Vrijer, Woutersen, Cassee, & van Bladeren, 1991).

Experimental data have shown that the main route of human exposure to acetaldehyde is consumption of alcoholic beverages, followed by cigarette smoking and flavourings used in several foods (Homann, Tillonen, & Salaspuro, 2000; Lachenmeier, Kanteres, & Rehm, 2009; Linderborg, Salaspuro, & Väkeväinen, 2011). Another source of exposure can be the release from polyethylene terephthalate (PET) in food packages and beverage bottles (Mutsuga, Kawamura, Sugita-Konishi, Hara-Kudo, Takatori, & Tanamoto, 2006). In alcoholic beverages acetaldehyde may be formed by yeast fermentation, acetic acid bacteria, or oxidation of ethanol and phenolic compounds (Liu & Pilone, 2000). Acetaldehyde levels vary considerably depending on fermentation conditions, such as temperature, O_2 levels, pH, SO_2 levels and yeast nutrient availability (Ebeler & Spaulding, 1998).

In humans acetaldehyde is a metabolite of ethanol, which is oxidised in the liver to acetaldehyde and then to acetate by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), respectively (Yokoyama & Omori, 2005). In addition, marked amounts of acetaldehyde are instantly produced in the oral cavity, from ethanol after a single sip of a strong alcoholic beverage, by salivary microbes (Homann, Jousimies-Somer, Jokelainen, Heine, & Salaspuro, 1997; Miyake & Shibamoto, 1993).

Acetaldehyde associated with the consumption of alcoholic beverages has been recently classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) (Secretan et al., 2009). Cell cultures and animal experiments have shown its carcinogenicity, since it is able to cause point mutations and to form covalent bonds with DNA (Cheng et al., 2003; Fang & Vaca, 1997; Hecht, McIntee, & Wang, 2001; Noori & Hou, 2001; Wang, McIntee, Cheng, Shi, Villalta, & Hecht, 2000). Furthermore, epidemiological studies have suggested that acetaldehyde present in alcoholic drinks or formed endogenously from ethanol oxidation by the oral microflora, is a carcinogen especially in the oral cavity, the oesophagus and the upper digestive tract (IARC, 1999). Thus, acetaldehyde outside of alcohol metabolism could be considered an additional cancer risk (Lachenmeier & Monakhova, 2011; Seitz & Stickel, 2009).

Very limited information is available in the literature about the potential impact of acetaldehyde in foods and beverages on public health. However, Lachenmeier et al. (2009) have developed the

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first quantitative risk assessment for acetaldehyde in alcoholic beverages, using the European Food Safety Agency (EFSA) approach for the risk assessment of genotoxic and carcinogenic substances (EFSA, 2005). The authors concluded that alcohol consumption is a direct source of acetaldehyde exposure, which in conjunction with the other sources, results in a cancer risk requiring intervention.

In the present study we measured acetaldehyde in different alcoholic and non-alcoholic beverages consumed in Italy. The aims were: (1) to evaluate acetaldehyde levels in different beverage groups, (2) to investigate which factors contribute to acetaldehyde contamination in beverages, and (3) to assess direct exposure to acetaldehyde from beverages in Italian consumers.

2. Materials and methods

2.1. Sampling

A "convenience" sampling of selected beverages was carried out by the staff of the Environmental Health Sciences Department of IRCCS-Istituto di Ricerche Farmacologiche Mario Negri. One hundred and forty-three samples were taken mainly among drinks the personnel had at home, with the aim to cover the main categories of alcoholic beverages, normally consumed in Italy. Each sample was collected in a glass vial of 2 ml completely filled, in order to avoid the migration of acetaldehyde into the head space. Wines and beers were taken from bottles just opened, whereas almost all the spirits from bottles already opened. For each beverage selected information, including alcohol by volume, date and site of purchase and date of opening, was collected. Samples were stored at a temperature of 4 °C until analysis.

2.2. Analytical procedure

The analytical procedure used in this work refers to methods reported in previous studies (Wang, O'Reilly, & Pawliszyn, 2004; Ling, Deng, & Zhang, 2008) and based on head-space solid-phase microextraction (SPME) and on isotopic dilution using acetaldehyde-d_4 as internal standard.

Samples preparation consisted of a dilution of variable amounts of alcoholic or non-alcoholic beverages (10–50 μl) in 3 ml of tap water in headspace vials of 10 ml. After addition of 300 ng of the internal standard solution (6 μl of a 50 ng/μl solution), acetaldehyde was then derivatised into a thermally stable and less polar compound by adding 20 μl of *o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA). Samples were heated to 60 °C with a hotplate and then extracted using SPME, i.e., exposing a 2-cm triphasic divinylbenzene/carboxen/polydimethylsiloxane fibre (Supelco, Bellefonte, PA) into the headspace for 10 min. After extraction, the fibre was introduced into the gas chromatograph injector, where analytes were thermally desorbed, separated and identified (Müller, Fattore, & Benfenati, 1997).

2.3. Instrumental analysis

Instrumental analysis was performed by gas-chromatography coupled to mass spectrometry (GC–MS) with an Agilent GC5890-MSD5975C-system, in selected ion monitoring (SIM) mode. Acetaldehyde was quantified using the 209 and 213 ions, for native and deuterated internal standard, respectively. A Varian CP-Select 624 CB chromatography column (60 m, 0.32 mm I.D., 1.80 μm film thickness) was used. The GC oven temperature program was: 80 °C for 1 min, rate 15 °C/min to a final temperature of 220 °C, held for 5 min. Helium was used as a carrier gas at a flow-rate of 0.8 ml/min. The injector was operated in splitless mode and temperature was set to 250 °C. Transfer line temperature was maintained at 280 °C. The linearity of the instrumental response was in the range of 0–600 ng/ml with average regression coefficients (ARC) of 0.9995 and limit of detection (LOD) of 0.2 ng/ml. The repeatability, expressed as average coefficient of variation (CV), was 18% (intra-day) and 13% (inter-day).

2.4. Statistics

Data were evaluated using the software Statistica 6.1 (StatSoft, Inc., Tulsa, OK). Spearman's rank correlation analysis was used to investigate the correlation between alcohol content and acetaldehyde concentration. One-way analysis of variance (ANOVA) and Kruskal–Wallis post-hoc test were used to assess differences in acetaldehyde concentrations across beverage categories. Statistical significance was assumed at probability level <0.05.

2.5. Risk assessment

In order to estimate human exposure to acetaldehyde, data provided by the National Research Institute for Food and Nutrition (INRAN) (Leclercq, Arcella, Piccinelli, Sette, Le Donne, & Turrini, 2009) on consumption of the main beverage categories in the Italian population, considering only consumers, were used. The average daily dose of acetaldehyde was calculated by multiplying concentrations found in beverages for the corresponding daily consumption and divided by the average body weight.

For risk characterisation, we applied the EFSA's margin of exposure (MOE) approach, which considers possible safety concerns arising from the presence in food of substances which are both genotoxic and carcinogenic (EFSA, 2005). The MOE is defined as the ratio between the dose at which a small but measurable adverse effect is first observed in experimental studies, like the no-observed-adverse-effect level (NOAEL) or the lower confidence limit of the benchmark dose (BMDL) (Crump, 1984), and the level of human exposure to the substance considered. In this study a BMDL of 56 mg/kgBW per day corresponding to a 10% increase in cancer incidence as derived in experimental studies (Lachenmeier et al., 2009), was used for the MOE calculation.

3. Results

3.1. Acetaldehyde levels in beverages

Table 1 shows the median value and range of acetaldehyde concentration levels in alcoholic and non-alcoholic beverages for each beverage category. Beers, non-alcoholic beverages, vodka and gin have lower acetaldehyde content than wines, liqueurs and other spirits. Acetaldehyde levels spanned more than three orders of magnitude, ranging from a minimum of 0.78 to a maximum of 1850 mg/l, measured in a gin and in a grappa sample, respectively.

Fig. 1 shows concentrations (mean ± SD) of acetaldehyde calculated for all beverage groups. Highest concentrations were detected in grappa samples (561 ± 539 mg/l), followed by fruit-based liqueurs and spirits (119 ± 140 mg/l), sparkling wines (117 ± 55 mg/l) and rum (106 ± 65 mg/l), whereas the lowest were detected in gin (1.20 ± 0.61 mg/l), fruit smoothies (4.40 ± 3.11 mg/l) and tea (4.95 ± 5.11 mg/l).

The Kruskal–Wallis test showed significant differences of acetaldehyde concentration between the beverage categories analysed. In particular, grappa showed significant differences with beers, herb- and spice-based liqueurs and spirits, gin, and non-alcoholic beverages; beers also showed significant differences, with red, white and sparkling wines and with fruit-based liqueurs and spirits; finally, sparkling wines showed significant differences with