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Estimation of ethanol and methanol exposure through jarred fruit purees

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Abstract

Introduction. Chemicals in foods enter the human body from early life likely posing chronic toxic health risks in the future. This study aimed to estimate the exposure to ethanol and methanol in children consuming an acceptable daily amount of fruit purees.
Methods. Different fruit purees were purchased and measured for methanol and ethanol by using HS-GC. The exposure dose of these alcohols was calculated based on a consumption of 125-250 g of fruit purees in children weighing 7, 12 and 16 kg.
Results. The highest methanol was found in carrot-apple puree (29.07 mg/dL) and ethanol in peach-banana puree (42.07 mg/dL). Daily methanol exposure was estimated between 4.54 and 6.06, and ethanol between 6.57 and 8.76 mg/kg bw.
Conclusions. Our results show higher exposure doses of methanol and ethanol than allowable ones (methanol 2 and ethanol 6 mg/kg/day) in children consuming fruit purees. This should be handled as a public health risk and further comprehensive studies should be enrolled on the chronic toxic effects of food-derived alcohols. Besides, food-derived exposure to toxic chemicals from early life should be more questioned by physicians (in assessing chronic diseases), and related authorities should establish a sustainable, safe, and healthy food production policy.

Key words
- children
- ethanol
- fruit puree
- methanol

INTRODUCTION

Human beings are exposed to various chemical substances through nutrition from the mother's womb. In addition to genetic and phenotypic effects, chemicals taken into the body for a long time, albeit in low amounts, can affect the health status of people throughout their lives. The most important of these are chemicals that are exposed during infancy and childhood when development and growth are rapid. In the healthy and balanced diet of children, ready-made complementary foods mainly based on fruits and vegetables (e.g., jarred baby foods and fruit purees) are often preferred and most of them are consumed from the 4-6 months of life [1]. Infants (0-9 months) require three times more energy than an adult (30-59 years) and eat more food per kilogram of body weight (bw) than adults which make them more vulnerable to toxic effects of hazardous chemicals [2].

Industrial food products designed for infants and children available on markets are regulated by some directives in several countries especially in European Union (EU) to monitor dietary exposure to potentially toxic chemicals in children. These directives include use of additives and materials (coming into contact with foods), presence of contaminants and other toxic substances (e.g., pesticides), and measures about hygiene [3]. However, no provisions about methanol and ethanol as well as some endocrine disrupters in baby foods are available.

Ethanol can be found in foods as a natural fermentation product produced by the action of alcohol dehydrogenase from pyruvate under hypoxic conditions, as well as added to foods as a solvent, preservative and flavoring agent in the food industry. Alcoholic beverages contain more than a certain ratio (>3-5%, v/v) which must be stated on product labels. If the ethanol content is below this specified rate, these products are considered soft drinks or non-alcoholic foods and no labeling about the alcohol content is mandatory [4, 5]. Ethanol is also a commonly used excipient in medications and although its safety limits in pediatric formulations are regulated, some countries question replacing them with alcohol-free formulations since higher ethanol levels than proposed by international guides were found on some pediatric formulations' leaflets [6].

Present data on the effect of ethanol in children is based mainly on acute poisoning cases or studies enrolled in adults or animals. Ethanol may cause acute poisoning symptoms in children at a level of 0.3 g/kg and death at 3 g/kg. There is inadequate data about the effect of low dose ethanol exposure in pediatric population. Committee for Human Medicinal Products (CHMP) proposed not to use traditional herbal medications in children younger than two years, and

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suggested a critical blood alcohol concentration (BAC) of 0.125 g/L at which psychomotor impairment can appear, and calculated a max ethanol dose of 1.5 g for 6 years old (20 kg) child [7]. The blood ethanol should not exceed 1 mg/dL (or 0.01 g/L or a dose of 6 mg/kg) in children less than 6 years old. According to CHMP (questions and answers on ethanol in the context of the revision of the guideline on "Excipients in the Label and Package Leaflet of Medicinal Products for Human Use") French Medicines Agency and Food and Drug Administration (FDA) suggested not to use ethanol in pediatric medicines unless necessary and the World Health Organization (WHO) recommended an ethanol limit less than 0.5% in over the counter products prepared for children under 6 years old [8].

Methanol is a commonly used industrial solvent, and a raw material for the production of several compounds such as formaldehyde, acetic acid, and methyl tertiary butyl ether. It is also frequently utilized as a sample extraction solvent in routine or research laboratories. Dietary methanol is consumed via fruits and fruit-based products, vegetables, fermented beverages and aspartame (artificial sweetener). It is present in plants as both free alcohol and a major component of pectin. Pectin is a gelling agent and contains methyl esters of polygalacturonic acid. The utilization of methyl esterase during industrial food processing leads to the release of methanol by the decomposition of methyl esters. The European Food Safety Authority (EFSA) suggested a reduction of the maximum permitted level (10 g/L) of pectin in baby foods, and moreover, the Scientific Committee on Food (SCF) suggested not to use pectin in baby foods [9]. Daily ingestion of 90% methylated pectin (2,000-2,600 mg/kg bw) via foods can induce significant exposure to methanol (91.6-119.4 mg/kg bw) which may threaten an infant's health. It is considered that all methanol is transformed to formaldehyde and then to formaldehyde acetal which may lead to an increase in the intracellular formaldehyde level at a rate of 70% by 91.6 mg/kg per day methanol. This amount of formaldehyde exceeds the upper limit of natural level (blood steady state concentration: 2.25 mg/L in rats, 2.6 mg/L in human; intracellular level: 12 mg/L in human) [9, 10]. Methanol is also produced endogenously during normal metabolism including the amino acid and methanol metabolism, one carbon pool, P450-linked demethylations, and lipid peroxidation and exist in all body fluids due to its water solubility [10, 11]. It is claimed that methanol is a neurodevelopmental toxicant leading to autistic disorders if ingested by pregnant women, where this toxicity is particularly associated with formaldehyde [12]. Formaldehyde is defined as a class I carcinogen. It can be built in various parts of the body (blood vessels, brain, heart, breast, skin, bone) by the action of aldehyde dehydrogenase 1 (ADH 1) [13]. Formic acid is produced rapidly from formaldehyde (half-life: ~1 min) by the activity of formaldehyde dehydrogenase and transformed to carbon dioxide through the action of formyl-tetrahydrofolate (THF) synthetase and formyl-THF dehydrogenase where THF (coenzyme of folic acid) deficiency may lead to formic acid accumulation and then acidosis [14]. Thus, the oxidation of formic acid varies among individuals (even in different ages) and species (twice as slow in humans compared to rats) depending on the availability of folate [15, 16].

In this study, it was aimed to evaluate whether infants and children are at risk of food-derived chemicals, such as methanol and ethanol. Hence, ethanol and methanol ingredients of jarred fruit purees were measured, and the daily exposure amounts of these alcohols were estimated.

MATERIAL AND METHODS

Chemicals

All chemicals and reagents used in laboratory works were of analytical grade. Methanol and ethanol as standard solutions, and n-propanol as internal standard solution were obtained from Merck. Ultrapure water was obtained from Medical Industrial Systems Minipure (MES Mp) water system (Turkey).

Food samples

Different jarred fruit purees (n=12) under different brand names (n=4, each 125 g) were purchased from supermarkets. The jars were held at room temperature (max: 1h) until opening for alcohol measurements and closed and put into the refrigerator after sampling.

Sample preparation and instrumental analysis

All fruit puree samples (800 µL) were mixed with internal standard (200 µL from 40 mg/dL n-propanol) in a 20 mL headspace vial and analyzed using headspace gas chromatography – flame ionization detector (HS-GC-FID). The instrumental properties of the device are presented in *Table 1*. Every batch (12 samples) of

Table 1
Instrumental properties of headspace gas chromatography

Equipment	Agilent, 7890B GC and 7694E headspace (HS)
Detector	Flame ionization detector (dual detector)
Carrier gas	Helium
Detector gas	Hydrogen and dry air
Column	DB-ALC1 and DBA-ALC2 (30 m / 0.320 mm / 1.80 µm, 30 m / 0.320 mm / 1.2 µm)
Mode	Split (ratio 20:1)
Injection time	1 min
Injection temperature	250 °C
Vial temperature	70 °C
Loop temperature	80 °C
Transfer line temperature	90 °C
Pressure build-up time	3 min
Detector temperature	260 °C
Analysis time	6 min
Thermostatic temperature	80 °C
Thermostation time	15 min

HS: headspace; GC: Gas chromatography; DB-ALC1: Alcohol analysis coloumn1; DB-ALC2: Alcohol analysis coloumn2.

the analysis involved negative and positive control samples at the beginning and at the end. Each sample was measured twice and the mean value was obtained.

Calibration and validation of the method

Standard (pure) ethanol and methanol solutions (diluted with distilled water) at increasing concentrations between 1-100 mg/dL were used for drawing the calibration curves of ethanol and methanol. Matrix based calibration was not possible because of no available reference standard materials for fruit purees. But we prepared a sample pool of fruit purees from different jars and used it as quality control (QC) sample in validation studies. Validation of the method was evaluated by using three different (low, mid, and high concentrated) QC samples by means of linearity (r^2), sensitivity (limit of detection, LOD, limit of quantification, LOQ), repeatability (relative standard deviation, RSD), accuracy (bias), recovery and carryover based on the guideline related to method validation [17]. Repeatability and accuracy were tested by 5 runs in a day (intra-day), and every day up to 5 days (inter-day). Sensitivity was determined by ten-time measurements of the lowest quality control sample in the same batch. Selectivity was evaluated by the runs of mixed alcohol (ethanol, methanol, acetone, isopropyl alcohol, and toluene) spiked QC and blank samples (non-spiked QC and standard solution samples) each with three repeats. Recovery was calculated from the measurements (n=3) of QC and standard samples for three different concentrations. Before using the pooled puree sample its initial (existing) alcohol content was measured and considered during the final calculations. Carryover was tested by repeated analysis of high concentrated (above and near the highest calibration point) spiked samples and a blank sample (n=3) consecutively, where the blank sample should not show any acceptable peak for targeted analytes.

Statistics

Alcohol ingredients of fruit puree samples were determined by HS-GC software tool according to validated calibration curve of the method. Further calculations were performed using MS Excel program. Average daily intake of alcohols via fruit purees for kids weighting between 7 and 16 kg (~4 month-4 years old) was estimated by considering a consumption of one (125 g) and two jars (250 g) of fruit purees daily. Daily exposure amounts of methanol and alcohol were presented as mg/kg bw.

RESULTS

Acceptable values were achieved by method validation studies. The mean bias value was 2.74% for methanol, 4.10% for ethanol, and RSD was 1.36% for methanol, 1.53% for ethanol after intra- and inter-day analysis of QC samples (low, mid, and high concentrated, n=5). LOD and LOQ were found 0.47 and 1.58 mg/dL for methanol, 1.34 and 4.5 mg/dL for ethanol respectively. The method was linear between 2.96 and 94.8 mg/dL with a calibration coefficient (r^2) of 0.9995. No carryover was observed after a high concentrated sample analysis, as well as no interference after analyzing multiple alcohols in the same sample, all analytes appeared separately in their own scheduled time (retention time). Recovery was found between 82 and 112% for three different concentrations. Methanol and ethanol contents of fruit purees, the total methanol and ethanol ingredient of each fruit puree jar and estimated daily exposure levels of children are summarized in *Table 2* and *Table 3*. It is supposed that small kids around 7 kg or 4-6 months old may consume one jar (125 g) of fruit puree in a day, whereas older kids around 12 and 16 kg or 3-4 years old may consume at least two jars daily. Considering these conjectures small kids may consume methanol between 8.01 and 36.34 mg/day (1.14-5.19 mg/kg bw) and ethanol between 6.48 and 52.59 mg/day

Table 2
Methanol ingredients of fruit purees and estimated daily exposure level by consumption of one or two jars based on different body weights (bw)

Fruit puree	Methanol mg/dL	Methanol in one jar (mg)	Daily exposure mg/kg bw (7 kg)	Methanol in two jars (mg)	Daily exposure mg/kg bw (12 kg)	Daily exposure mg/kg bw (16 kg)
1 (plum 1)	7.96	9.95	1.42	19.90	1.66	1.24
2 (peach and banana)	15.35	19.19	2.74	38.38	3.20	2.40
3 (apple)	9.12	11.40	1.63	22.80	1.90	1.43
4 (peach and apple)	10.55	13.19	1.88	26.38	2.20	1.65
5 (apple and banana)	10.05	12.56	1.79	25.13	2.09	1.57
6 (mixed fruits)	7.43	9.29	1.33	18.58	1.55	1.16
7 (apple 2)	6.41	8.01	1.14	16.03	1.34	1.00
8 (banana, mandarin and apple)	7.37	9.21	1.32	18.43	1.54	1.15
9 (plum 2)	6.85	8.56	1.22	17.13	1.43	1.07
10 (apple and pear)	17.67	22.09	3.16	44.18	3.68	2.76
11 (carrot and apple)	29.07	36.34	5.19	72.68	6.06	4.54
12 (apple and peach)	11.51	14.39	2.06	28.78	2.40	1.80

Table 3
Ethanol ingredients of fruit purees and estimated daily exposure level by consumption of one or two jars based on different body weights (bw)

Fruit puree	Ethanol mg/dL	Ethanol in one jar (mg)	Daily exposure mg/kg bw (7 kg)	Ethanol in two jars (mg)	Daily exposure mg/kg bw (12 kg)	Daily exposure mg/kg bw (16 kg)
1 (plum 1)	18.89	23.61	3.37	47.23	3.94	2.95
2 (peach and banana)	42.07	52.59	7.51	105.18	8.76	6.57
3 (apple)	ND	-	-	-	-	-
4 (peach and apple)	5.18	6.48	0.93	12.95	1.08	0.81
5 (apple and banana)	11.59	14.49	2.07	28.98	2.41	1.81
6 (mixed fruits)	7.78	9.73	1.39	19.45	1.62	1.22
7 (apple 2)	5.53	6.91	0.99	13.83	1.15	0.86
8 (banana, mandarin and apple)	8.03	10.04	1.43	20.08	1.67	1.25
9 (plum 2)	15.15	18.94	2.71	37.88	3.16	2.37
10 (apple and pear)	15.70	19.63	2.80	39.25	3.27	2.45
11 (carrot and apple)	ND	-	-	-	-	-
12 (apple and peach)	14.18	17.73	2.53	35.45	2.95	2.22

ND: not detected; -: below the limit of quantification (LOQ).

(0.93-7.51 mg/kg bw), older kids may consume methanol between 16 and 73 mg/day (1.34-6.06 mg/kg bw for 12 kg and 1-4.54 mg/kg bw for 16 kg kids) and ethanol between 12.95 and 105.18 mg/day (1.08-8.76 mg/kg bw for 12 kg and 0.81-6.57 mg/kg bw for 16 kg kids). The highest methanol ingredient was measured in carrot-apple puree (29.07 mg/dL) in which ethanol was not detected, followed by apple-pear (17.67 mg/dL), peach-banana (15.35 mg/dL), and apple-peach (11.51 mg/dL) purees. The highest ethanol was measured in peach-banana (42.07 mg/dL), followed by plum 1 (18.89 mg/dL), apple-pear (15.70 mg/dL), apple-peach (14.18 mg/dL) and apple-banana (11.59 mg/dL) purees. It seems that apple containing purees involve methanol (more content of pectin), whereas banana containing purees involve ethanol (more fermentation during ripening) primarily.

DISCUSSION

Acute methanol exposures of human can pose visual problems (from blurred vision to blindness), neurological symptoms (persistent motor dysfunction), metabolic acidosis, and dermatitis through dermal contact. Chronic methanol exposure (oral or inhalation) can cause gastric problems, visual disturbances, conjunctivitis, blindness, headache, giddiness, nausea, and insomnia in humans [18-20]. When these symptoms are forefront and methanol exposure is evident by the individual's statement necessary treatment and preventive measures can be taken. But chronic methanol intake, in low doses, through nutrition especially beginning from an early age may lead to some health problems or may contribute to the emergence of chronic diseases in older ages where the effect of food-derived methanol is not considered. The industrial production of food, the degradation of pectin in fruits and vegetables, and the hydrolysis of aspartame, a sweetener, release methanol which may pose a potential health risk to human most notably infants and children [9, 21]. A study suggested that the mothers of autistic-born children were exposed to higher dietary methanol (142.31 mg/week) than mothers of non-autistic children indicating intrauterine toxicity of methanol [22].

According to the data of EFSA [21] on the methanol composition based on pectin contents in different foods, fruit and fruit products contain 531 mg/kg methanol which corresponds to 53.1 mg methanol intake for a child consuming 100 g of these foods. Given the methanol content in fruit purees (3,673 mg) evaluated in this study and fruit products mentioned in the EFSA opinion, the maximum daily methanol consumption would exceed the human MADL (maximum allowable dose level, 23 mg/day) suggested by the Office of Environmental Health Hazard Assessment (OEHHA) (https://oehha.ca.gov/proposition-65/crnr/proposed-specific-regulatory-levels-chemicals-causing-reproductive-toxicity) even if children consume these foods in acceptable quantities.

The EFSA Panel estimated the methanol exposure via baby foods as 43.2 mg/kg bw (in case of 1,084 mg/kg bw/day pectin exposure, 95th percentile) for infants younger than 16 weeks and 50% lower exposure for infants older than 16 weeks. This required them to take precautions on reducing the maximum permissible pectin level in special foods for infants and children in order to prevent toxic exposure to methanol [9]. Methanol consumption via fruit purees corresponds to a maximum of 5.19-6.06 mg/kg bw methanol exposure for children weighing 7-12 kg (16 weeks-4 years); these values are lower than those estimated by the EFSA Panel. However, given that infants or children may ingest different types of foods that contribute to the release of different amounts of methanol in their bodies, exposure may rise up to the amounts specified by EFSA. Moreover, the

individual differences in the metabolism of methanol into its toxic metabolites formaldehyde and formate via aldehyde dehydrogenases along with the availability of folate are also important factors in the chronic toxicity of methanol [16]. ADH, in humans, is the main actor in oxidation of alcohols (ethanol and methanol) especially in low dose exposures. Children below 5 years old have reduced ADH activity. It is claimed that infants have 20-50% of adult ADH activity indicating that some infants may use catalase to eliminate methanol especially in high exposures [23, 24]. But little is known about the metabolism of methanol in infants since catalase has not been observed in adult methanol metabolism. Several studies in rats, mice, and monkeys have shown some cellular effects of methanol, especially in liver and brain tissues after 90 days of chronic exposure [25-28]. Some carcinogenicity studies in animals revealed increased incidence in cancer diseases involving ear duct, bone, and hemolymphoreticular tumors [25] .

The endogenous blood methanol level is considered to be below 0.25 mg/dL. The Environmental Protection Agency (EPA) (https://iris.epa.gov/static/pdfs/0305tr.pdf) recommended an oral reference dose of 2 mg/kg methanol per day for humans. Considering this data, approximately half of the fruit purees evaluated in this study exceed the allowable methanol dose when eaten in specified amounts by children. CPMP used the Widmark equation to make approximate assumptions on pediatric blood alcohol [8]. So, the same formula may also be applied to methanol having similar pharmacokinetic properties. A 12 kg child consuming 72.68 mg of methanol (carrot-apple puree) will have a blood methanol level of around 1.1 mg/dL, 4 times higher than normal, consistent with previous estimated findings (0.76 to 1.11 mg/dL) for fruit and fruit products [29].

It is known that acute or chronic exposure to ethanol poses a health risk. It affects various systems and can contribute to different diseases such as cancer, diabetes mellitus, cardiovascular and neurological disorders. The main oxidation pathway of ethanol results in the production of acetaldehyde (toxic, carcinogenic) followed by acetate, through reactions catalyzed by ADH and acetaldehyde dehydrogenase [30]. An alternative way uses cytochrome enzymes where oxygen radicals are the by-product and may contribute to oxidative damage [31]. As in methanol, there are genetic variations in ethanol metabolizing enzymes among people even genders and different ages which clarifies why the same amount of alcohol causes different effects among individuals. Our knowledge on ethanol metabolism and ethanol exposure consequences in children are obtained from poisoning cases or studies enrolled in adults or animals [25, 32]. There are some studies that measured the ethanol contents of various nonalcoholic foods or beverages and estimated the exposure amount and discussed their consequences to remark on the topic that nonalcoholic considered foods or products are not alcohol-free. Ethanol ingredients of flavored beverages and soft drinks were found between 0 and 0.096% w/v. This was explained with that ethanol is a commonly used chemical as a carrier of volatiles and flavoring materials in the production of beverages and with artificial flavoring agents containing ethanol [4]. Various fruit juices such as grape, apple and orange juices were found to contain considerable amounts of ethanol, up to 0.77 g/L, and led to an average ethanol exposure of 10.3 mg/kg bw/day which is higher than the critical dose (6 mg/kg bw) accepted for children under 6 years old [33]. In a recent study, a similar result was also found for fruit juices where the blood ethanol concentration they posed was also estimated. The estimated ethanol exposures in children were above the risky level when they consume certain amounts of fruits or fruit juices [29]. Also, in this study, we found that fruit purees, the most preferred food for small kids in many countries, could lead to an ethanol exposure of up to 8.76 mg/kg bw per day considering a minimum consumption amount in children weighing between 7 and 16 kg (*Table 3*). The approximate blood ethanol level of a 12 kg weighing child consuming 105.18 mg ethanol (peach-banana puree) will be 1.46 mg/dL exceeding the suggested normal level (1mg/dL) [8].

Initially, both methanol and ethanol are oxidized with the same enzyme (ADH) which has a greater affinity (20 folds) for ethanol than methanol. In addition to this, children under 5 years have low ADH activity than adults. So, children are more vulnerable to chronic toxic effects of alcohols than older ones. But considering that methanol (more toxic than ethanol) almost exists with ethanol in most of purees evaluated in the current work, its toxic consequences would be reduced by ethanol (due to high enzyme affinity), which is relatively less dangerous. However, methanol derived from food additives would not be prevented by ethanol and metabolized by entering additional pathways (cytochrome P and catalase) and would play role in the occurrence of chronic health problems in children's future life. Assuming that fruit purees are made from ripe fruits, methanol may accumulate by the action of pectin methylesterase and ethanol by the yeast fermentation of sugars during the ripening of these fruits and then may be transferred to kids by nutrition [14].

CONCLUSIONS

Long-term and continuous consumption of fruits and vegetables, especially industrially produced ones, can create more alcohol exposure (namely methanol and ethanol) than expected contributing to gradual cell and tissue damage, and playing an additive role in the etiology of some chronic diseases (autism, liver diseases, cancer, diabetes, neurological disorders, etc.) along with the presence of metabolic and genetic predispositions (e.g., folate deficiency, ADH variations). In this pilot study made on a limited number of samples ethanol and methanol exposure levels of children (7-16 kg or 4 months-4 years) consuming estimated amounts of different fruit purees were evaluated. The results showed that the maximum methanol consumption was 73 mg/day, exceeding the level suggested by OEHHA, and ethanol consumption 105 mg/day, leading to exceed the blood ethanol level recommended by CHMP. Based on this findings and current evidences [34-36] and the different susceptibility of children to certain chemicals present in food products, significant regulations should be put into effect.

In conclusion, we have to pay attention to what and how much food we and our children eat. In order to produce natural and safe foods, especially for infants, and to eliminate chronic food-borne toxicity, authorities should determine food ingredients, make regulations regarding food additives, preservatives and contaminants, and constantly inspect them. In addition, more comprehensive research on industrial foods for infants and children should be performed in future.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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No potential conflict of interest is declared by the Authors. All Authors agree with the final version of the manuscript and approve its submission.

Authors' contributions

MG designed the work, analyzed and interpreted the results, and was the major contributor in writing the manuscript. BB Bayram purchased the samples, analyzed and interpreted the results.

Consent for publication

Not applicable. Our manuscript does not include individual data in any form (images, videos, personal identity) requiring consent to publish.

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