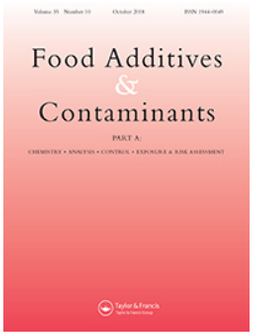

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Styrene in foods and dietary exposure estimates

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ABSTRACT

Low levels of styrene may be found in foods as a result of possible migration from polystyrene-based food packaging and as a result of its formation during the biodegradation of a wide variety of naturally occurring compounds with structures similar to styrene. In this study, composite food samples from a recent (2014) Canadian Total Diet Study were analysed for styrene, and levels of styrene in samples of most food types were low in general with a few exceptions (e.g. 4934 ng/g in herbs and spices). Dietary exposures to styrene were estimated for different age-groups based on the occurrence data and the food consumption data for all persons, and they are 0.17–0.38 µg/kg body weight/day for children and 0.12–0.16 µg/kg body weight/day for adults, similar to air intakes (0.085–0.27 µg/kg body weight/day). Thus, for the general population, both food and air contribute similar portions of the total daily intake of styrene for all age groups. However, for the smoking population, intakes from cigarettes are still the major route of exposure to styrene.

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Styrene; total diet; food; dietary intake; exposure; headspace; SPME; GC-MS

Introduction

Styrene is an important industrial chemical and is used mainly for the production of polystyrene (PS) and styrene copolymers (Tang et al. 2000). Polystyrene is the second most widely used polymer in food packaging (Nerin et al. 1998), with expandable PS (EPS) being used for beverage cups and as trays for packaging meat, poultry, cheese, fruits and vegetables, and high impact PS (HIPS) packaging being used for beverage cups, yogurt, sandwich clamshells packaging, etc. Residual styrene monomer present in polystyrene can migrate into foods (Withey 1976; Murphy et al. 1992; Jickells et al. 1993; Ehret-Henry et al. 1994; Gramshaw and Vandenburg 1995; Lickly et al. 1995; Tawfik and Huyghebaert 1998), thus styrene can be detected at low levels in foods (Withey 1976; Withey and Collins 1978; Steele et al. 1994; Nerin et al. 1998). Styrene concentrations of 550 ppb (µg/kg) in avocado (Fleming-Jones and Smith 2003) and 111.2 µg/kg in ready-to-eat meals (Vinci et al. 2015) have been reported. Styrene can also be formed during the biodegradation of a wide variety of naturally occurring flavouring compounds with structures similar to styrene (e.g. cinnamic acid, cinnamic aldehyde, cinnamyl acetate, cinnamyl alcohol, cinnamyl benzoate, cinnamyl cinnamate), and has been detected in cinnamon at levels as high as 39.2 mg/kg (Steele et al. 1994) and 524 mg/kg (Lafeuille et al. 2009).

Title 21, Part 165 of the United States Food and Drug Administration's (U.S. FDA) Code of Federal Regulations (CFR) specifies a maximum allowable styrene concentration of 0.1 mg/L in bottled water (UDFDA 2017). In addition to affecting sensory properties at low levels in foods (Ehret-Henry et al. 1994), exposure to styrene may also be a health concern; styrene has been classified as possibly carcinogenic to humans (Group 2B), on the basis of limited evidence in animals and humans, by the International Agency for Research on Cancer (IARC 2002).

The previous Canadian assessment of human exposure to styrene was conducted through the Government of Canada's Priority Substances List (PSL) initiative more than 20 years ago (Government of Canada 1993). Due to the limitations of the available data on concentrations in the environment, the principal route of exposure to styrene for the general population of Canada could not be clearly identified. Because of the volatility of

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styrene, ambient air and indoor air were estimated to contribute a substantial portion of the total daily intake for all age groups. Contribution from food in that assessment was considered to be an overestimate as dietary intakes were calculated using analytical detection limits. Styrene was not detected in food samples from the only survey conducted in Canada in the early 1990s and considered in the PSL assessment (Government of Canada 1993) and therefore concentrations in food were assumed to be equivalent to the detection limits.

Since 1969, the Canadian Total Diet Study (TDS) has been playing an important role in monitoring the presence of various chemical contaminants in the Canadian food supply and generating occurrence data for human exposure assessments. Recently, food samples from the 2014 Canadian Total Diet Study were analysed for styrene and various volatile organic compounds (VOCs). The purpose of this paper is to report and discuss in depth the occurrence data of styrene and to estimate updated dietary exposures for different age groups of the Canadian population. This will determine the significance of dietary exposure to styrene for the general population of Canada.

Materials and methods

Materials and reagents

Sodium chloride (> 99.0%) was obtained from VWR (Mississauga, Ontario, Canada). Styrene-d8 (99.2 atom % D) and other labelled volatile organic compound (VOC) standards were purchased from C/D/N Isotopes Inc. (Pointe-Claire, Quebec Canada), and the following chemicals were purchased from Sigma-Aldrich (Mississauga, Ontario, Canada): methanol (99.8%), and EPA VOC Mix 2 in methanol containing styrene and other VOCs at 2000 µg/mL. The 100 µm polydimethylsiloxane (PDMS) SPME fibre was purchased from Supelco (Bellefonte, PA, USA). The 20-mL SPME crimp amber glass vials were purchased from Labsphere (Quebec, Canada).

Stock composite standard solution of deuterated VOCs was prepared in methanol at a concentration of 100 ng/µL. Composite standard solutions in methanol with deuterated VOCs at ca 2 ng/µL and native VOCs at different concentrations (ca 0, 0.2, 1, 3, 5, 8 and 10 ng/µL) were prepared by adding 200 µL stock solution of deuterated VOCs (100 ng/µL) and 0, 1, 5, 15, 25, 40 and 50 µL EPA VOC Mix 2 to 10 mL methanol. Composite standard solutions in water with deuterated VOCs at ca 4 ng/mL and native VOCs at different concentrations (ca 0, 0.4, 2, 6, 10, 16 and 20 ng/mL) were prepared by adding 20 µL stock solution of deuterated VOCs (2 ng/µL) and native VOCs (0, 0.2, 1, 3, 5, 8 and 10 ng/µL) to 10 mL water. All solutions were stored at 4°C.

Sample collection and preparation

Food samples were collected from four different stores in Winnipeg, Canada, over a 5 week period in 2014. The foods were prepared as for consumption according to the established procedures (Dabeka and Cao 2013), and individual samples of each type of food were combined into a total of 159 different food composites. Composites for foods that can be consumed both raw and cooked (e.g. cauliflower, carrots, broccoli, tomatoes, spinach) were prepared as a mixture of the raw and cooked (1:1). The food composites covered a variety of food categories including dairy products, meat, poultry, fish, cereal, vegetable, fruit, beverages and other miscellaneous foods. As part of the standard preparation procedures, kitchen staffs do not use any perfumes, hair spray, cologne, fragrant soap, make-up or talcum powder. Stainless steel or glass vessels were used for all processing. Drinking water was used for food processing. Food composites were stored frozen in 250-mL glass jars at −20°C until analysis.

About 5 g of NaCl (pre-heated at 650°C overnight and then stored at 200°C) was weighed into a 20-mL amber glass vial then cooled to 4°C. For most of the samples, about 1 g of sample was weighed into the 20-mL vial, but smaller amounts were weighed for samples containing fat due to matrix effects. Each sample was spiked with internal standard solution, followed by adding 10 mL of deionised water. The vial was capped and then vortexed for 10 – 15 s to speed up dissolution of NaCl and mixing of the sample with water. All vials containing samples were placed on a metal tray, cooled with ice and samples were spiked

within 15 s to minimise any loss of target compounds during sample preparation.

Headspace solid phase micro-extraction and instrument conditions

An Agilent 6890 gas chromatograph (GC) coupled to a 5973 mass selective detector (MSD) was used for analysis. The GC-MSD was equipped with a MultiPurpose Autosampler (MPS 2) from Gerstel (Baltimore, MD, USA) set-up in SPME operation mode. At the beginning of analysis, the sample vial was transported from the tray to the agitator held at 30°C. After incubating for 1 min, the PDMS SPME fibre was inserted through the septum into the headspace. Vial penetration depth was set at 25 mm. Agitation speed was set at 250 rpm. After the extraction for 10 min, the SPME fibre was inserted into the GC injector fitted with a 0.75 mm I.D. liner for desorption. The injector temperature was set at 250°C. Injection penetration depth was set at 65 mm, and the SPME fibre was desorbed for 5 min in splitless mode. Analytes were separated on a DB-624 capillary column (60 m x 0.25 mm x 1.4 μm, Agilent Technologies). The GC oven temperature programme was set at an initial temperature of 50°C for 5 min, raised to 250°C at 10°C/min, and held for 5 min. The flow rate of the helium carrier gas was 1.2 mL/min.

The MSD was operated with electron impact ionisation in selected ion monitoring (SIM) mode. The ions selected for the native and labelled VOCs can be found elsewhere (Cao et al. 2016), and they were m/z 104 and 78 for styrene, and 112 for styrene-d8. The GC-MSD interface and the MS system source temperature were 260 and 230°C, respectively.

Quantitation and quality control

The calculation of concentrations in samples was based on the isotope dilution method. Confirmation of compound identity was based on the retention time and the ion ratio of the analytical standard. For each batch of analysis, each sample was analysed in four replicates, two of which were spiked with both native and deuterated VOCs to check accuracy, and the other two were spiked with deuterated VOCs only to determine VOC concentrations in the sample. Three blank samples (water) were also included in each batch. Some VOCs were not detected in blanks while blank levels of other VOCs were low: 0.028 ng for styrene. Results for all samples were corrected for method blanks.

Each new PDMS SPME fibre was initially cleaned by heating at 260°C for 30 min prior to use. This fibre was also cleaned after analysis of each sample by heating in the injector (250°C) for an additional five minutes after desorption of the exposed fibre. Carryover was not observed.

Dietary intake estimates

Dietary intakes of styrene for different age-sex groups of children and adults were calculated by multiplying the average concentration of styrene in a food composite by the average consumption rate for all persons of that food product for various age groups, obtained from the 2004 Canadian Community Health Survey (CCHS), Cycle 2.2 (Statistics Canada 2004). The total styrene exposures for each age category were then calculated by summing the individual intakes from each food composite. Estimates were converted to a body weight basis using measured and self–reported body weight data from the 2004 CCHS.

Results and discussion

The headspace SPME GC-MS method has been previously validated for styrene and other VOCs (Cao et al. 2016). Linearity of the instrument and the headspace SPME method was demonstrated using six standard solutions (0.4–20 ng/mL) and R^2 values for the styrene calibration plots were better than 0.999 for the calibration plots of styrene. The average recoveries for styrene were 103 ± 0.24% for water spiked at 5 ng/ml, and 104 ± 1.4% for water spiked at 20 ng/ml. Since styrene was detected in method blanks, at about 0.028 ng, the method detection limit (MDL) for styrene was calculated as 10 times the standard deviation of method blanks and also 10 times the signal to noise ratio for each food sample, and the higher value was taken as the MDL for styrene in this food sample. The MDLs for styrene for the

159 different food composite samples ranged from 0.023 to 10.7 ng/g with an average of 1.1 ng/g.

A total of 159 different composite food samples from the 2014 TDS were analysed for styrene and other VOCs, and styrene concentrations in these food samples are shown in Table 1. Each result is the average of two replicate analyses, with an average relative difference of 8.6% for all samples, and with an average accuracy of 94.8% of recoveries from all samples.

Table 1. Concentrations (ng/g) of styrene in composite food samples from 2014 TDS.

Composite	Conc (ng/g)	Composite	Conc (ng/g)	Composite	Conc (ng/g)
Dairy		**Vegetable**		**Fat and oil**	
Milk, whole	0.73	Baked beans, canned	3.6	Cooking fats and salad oils	< 8.7
Milk, 2%	1.1	Beans, string	1.2	Margarine	< 6.8
Milk, 1%	0.63	Beets	0.16	Mayonnaise	4.1
Milk, skim	0.33	Broccoli	< 0.15	Salad dressing	< 5.7
Evaporated milk, canned	< 0.73	Cabbage	< 0.21	**Baby food**	
Cream	1.8	Carrots	1.4	Cereals, mixed	0.92
Ice cream	1.7	Cauliflowers	< 0.13	Desserts	0.54
Yogurt	19	Celery	0.30	Dinners, cereal + vegetable + meat	0.46
Cheese	4.6	Corn	1.6	Dinners, meat or poultry + vegetable	0.48
Cheese, cottage	0.40	Cucumbers	0.87	Formulae, milk base	0.92
Cheese, processed	1.7	Lettuce	0.37	Formulae, soya base	< 0.43
Butter	18	Mushrooms	0.41	Fruit, apple or peach	0.12
Chocolate milk, 1%	6.8	Onions	< 0.070	Meat, poultry or eggs	< 1.8
Butter milk, 1%	6.1	Peas	< 0.30	Vegetables, peas	< 0.11
Meat		Peppers	2.1	**Fast food**	
Beef, steak	2.7	Potatoes, peeled and boiled	< 0.10	Popcorn, microwave	4.4
Beef, roast	0.92	Potatoes, chips	< 4.7	Frozen entrees	1.0
Beef, ground	6.7	Rutabagas	< 0.14	Pizza	33
Pork, fresh	3.9	Vegetable juice, canned	0.060	French fries	24
Pork, cured	3.4	Tomatoes	0.82	Hamburger	7.0
Veal, cutlets	1.5	Tomatoes and tomato sauce, canned	< 0.12	Chicken burger	9.8
Lamb	< 1.0	Spinach	0.34	Hot dogs	5.8
Luncheon meats, cold cuts	3.8	Asparagus	1.2	Chicken nuggets	15
Luncheon meats, canned	1.5	Brussel sprouts	0.22	Beef chow mien, carry-out	21
Organ meats	0.94	Potatoes, baked with skins	0.23	Fried rice (Chicken and veg)	49
Wieners and sausages	6.0	Corn chips	< 3.4	Prepared Breakfast sandwiches	19
Poultry		**Fruit**		Fast food sandwiches	6.0
Eggs	< 1.4	Applesauce, canned	0.086	**Others**	
Poultry, chicken and turkey	< 0.54	Apples, raw	1.0	Chocolate bars	12
Poultry, liver pate	15	Bananas	0.29	Candy	0.86
Fish		Blueberries	2.2	Gelatine dessert	0.077
Fish, marine	0.60	Cherries	0.070	Honey, bottled	0.59
Fish, fresh water	2.0	Citrus fruit, raw	< 0.080	Jams	0.32
Fish, canned	2.3	Grapes	< 0.090	Peanut butter	19
Shellfish	0.92	Melons	0.43	Puddings	12
Soup		Peaches	0.41	Sugar, white	< 0.14
Soups, meat, canned	0.16	Pears	1.5	Syrup	< 0.062
Soups, creamed, canned	0.23	Pineapple, canned	0.12	Seeds, shelled	11
Soups, broth, canned	0.070	Plums and prunes	0.10	Nuts	595
Soups, dehydrated	< 0.081	Raisins	< 0.79	Chewing gum	12
Cereal		Raspberries	15	Condiments	4.1
Bread, white	34	Strawberries	0.23	Salt	< 0.020
Bread, whole wheat	30	Kiwi fruit	0.18	Baking powder	< 0.18
Bread, rye	21	Apricot	1.3	Yeast	21
Cake	9.8	**Beverage**		Vanilla extract	1.2
Cereal, cooked wheat	0.33	Apple juice, canned	0.12	Herbs and spices	4934
Cereal, corn	0.61	Citrus juice, frozen	0.12	Soya sauce	0.061
Cereals, oatmeal	0.35	Citrus juice, canned	0.19		
Cereals, rice and bran	2.1	Grape juice, bottled	0.10		
Cookies	60	Fruit drinks (cocktails)	0.17		
Crackers	16	Alcoholic drinks, beer	4.0		
Danish, donuts and croissants	42	Alcoholic drinks, wine	0.23		
Flour, white (wheat)	17	Coffee	0.15		
Muffins	41	Soft drinks, canned	0.23		
Pancakes and waffles	5.9	Tea	< 0.039		
Pasta, mixed dishes	1.0	Soy beverage, fortified	< 0.30		
Pasta, plain	< 0.37	Tap water, kitchen	< 0.073		
Pie, apple	63	Tap water, sample area	< 0.030		
Pie, other	34	Water, natural spring	< 0.028		
Rice	0.10	Water, natural mineral	0.070		
Buns and rolls	39				
Breads, other	11				

Although styrene was detected frequently among all the composite samples analysed (125 of 159 samples or 78.6%), the concentrations of styrene in the composite samples of most food types were generally low with a few exceptions. Styrene was detected at the highest level of 4934 ng/g in the composite sample of herbs and spices which was prepared as a mixture of black pepper, oregano, basil, and cinnamon in equal portions. Styrene can be formed during biodegradation of some naturally occurring compounds with structures similar to styrene and it has been previously detected in cinnamon at levels up to 524 mg/kg (Lafeuille et al. 2009). Therefore, the level of styrene in the herbs and spices composite sample may be attributed to the presence of cinnamon. Assuming styrene is not present or present at low levels in the other herbs and spices (black pepper, oregano and basil) of the composite sample, styrene concentration in the cinnamon could be greater than 20 mg/kg, approaching the level of 39.3 mg/kg reported by Steele et al. (1994). The next highest level of styrene was found in the composite sample of nuts (595 ng/g) which was prepared as a mixture of roasted and unsalted peanuts and walnuts at a ratio of 3:1. The presence of styrene in the roasted nuts is a new observation, and the sources of styrene in roasted nuts are not clear. Further research is planned to investigate the consistency of the occurrence of styrene in roasted nuts by analysing roasted nuts samples from future total diet studies, and to investigate if styrene could be formed during roasting.

Among the dairy food samples, concentrations of styrene in yogurt and butter are slightly higher, at 19 and 18 ng/g, respectively. One of the sources for styrene in yogurt could be migration from its polystyrene packaging. The styrene concentration in yogurt found in this study is similar to those reported by others (Ehret-Henry et al. 1994; Nerin et al. 1998). Styrene was also detected at concentrations in the present study ranging from 20 to 63 ng/g in certain grain-based foods and fast foods such as bread, cookies, muffins, pie, pizza and French fries. The exact source for styrene in these samples is not clear, but it is unlikely to be derived from the Maillard reaction (Goldmann et al. 2009). The presence of styrene in certain baked products, such as cookies and muffins, could be related to the use of cinnamon.

The average dietary exposures to styrene were estimated for different age-groups based on the occurrence data from 2014 TDS and the food consumption data for all persons from the 2004 CCHS, Cycle 2.2 (Statistics Canada 2004) and are shown in Table 2. The per cent of styrene exposure from different food groups to the total dietary exposures was also calculated for different age groups, and the results are shown in Table 3. Dietary exposures to styrene are mainly from dairy (12.9–46.3%), grain-based foods (11.8–39.4%) and nuts (2.9–36.2%).

In general, dietary exposures to styrene are lower for children, ranging from 1.4 μg/day for infants (6–11 months) to 5.7 μg/day for toddlers (2–3 years), and increase for older age groups as exposure estimates range from 8.1 to 11 μg/day for adults, which are similar to the estimated daily intake of

Table 2. Average dietary exposures to styrene for different age groups.

Age groups	μg/day	μg/kg body weight/day
6–11 months	1.4	0.20
1 year	3.3	0.22
2–3 years	5.7	0.38
4–8 years	7.7	0.31
9–13 years	9.5	0.21
14–18 years	10	0.17
19–30 years	11	0.15
31–50 years	11	0.16
51–70 years	11	0.15
71+ years	8.1	0.12

Table 3. Per cent (%) of styrene exposures from different food groups relative to the total dietary exposures of styrene for different age groups.

Age groups	Dairy	Meat	Poultry	Fish	Soup	Grain-based	Vegetable	Fruit	Fat	Nuts	Beverage	Baby food	Fast food	Herbs and spices
6–11 months	46.3	0	0	0	0	11.8	0.44	2.07	0	0	0	39.3	0	0
1 year	32.1	3.75	0	0.60	0.14	40.0	1.77	9.53	0.073	2.9	0	1.79	0.081	7.31
2–3 years	32.9	3.46	0	0.37	0.15	35.4	1.58	4.63	0.10	13.4	0.076	1.04	0.11	6.76
4–8 years	26.8	4.13	0	0.36	0.079	39.4	1.41	3.61	0.16	14.5	0.17	0.0088	0.13	9.28
9–13 years	24.2	4.01	0	0.34	0.12	38.3	1.30	3.49	0.27	19.7	0.32	0	0.18	7.71
14–18 years	21.8	4.71	0	0.34	0.10	37.0	1.45	2.37	0.41	20.3	2.18	0	0.14	9.19
19–30 years	17.4	3.88	0	0.38	0.10	29.2	1.66	2.53	0.37	26.5	7.26	0	0.12	10.6
31–50 years	14.5	4.10	0	0.69	0.11	27.5	1.87	2.96	0.35	33.0	6.01	0	0.11	8.92
51–70 years	12.9	3.72	0	0.89	0.13	26.6	2.17	3.56	0.31	36.2	4.81	0	0.083	8.58
71+ years	16.5	3.88	0	1.04	0.22	33.3	2.35	4.14	0.25	35.6	2.38	0	0.030	0.29

6.6 µg/person/day from food packaging presented recently by the Plastics Foodservice Packaging Group in the United States to the US FDA (Plastics Foodservice Packaging Group 2015). On a body weight basis, dietary exposures to styrene for children (0.17 – 0.38 µg/kg body weight/day) are higher than those for adults (0.12–0.16 µg/kg body weight/day). Higher exposure estimates on a body weight basis for younger age groups would be expected as children tend to consume more food per unit of body weight than adults. Dietary exposure estimates to styrene from this study are also comparable with the intake estimates from ambient and indoor air determined in the early 1990s for non-smokers (0.096–0.24 µg/kg body weight/day for 7 months–4 years; 0.107–0.27 µg/kg body weight/day for 5–11 years; 0.096–0.23 µg/kg body weight/day for 12–19 years; and 0.085–0.21 µg/kg body weight/day for 20–70 years) (Government of Canada 1993). This suggests that both food and air (ambient and indoor) may contribute similar proportions of the total daily intake of styrene for all age groups but they are at least an order of magnitude lower than that reported from cigarettes for adults and adolescents (Government of Canada 1993).

In contrast, the study by Tang et al. (2000) found that air intake accounts for the majority of the total intake of styrene (> 90%), estimated at 0.3–0.8 µg/kg body weight/day, compared with food intake at 0.003–0.017 µg/kg body weight/day. It should be mentioned that a daily respiratory intake of 30 m^3 of air was used for air intake estimates by Tang et al. (2000) while different daily respiratory intakes of air (2–23 m^3) were used in the Canadian assessment for different age groups (Government of Canada 1993). However, dietary exposure estimates from this current study are well below, by at least 20-fold, the tolerable daily intake (TDI) of 7.7 µg/kg of body weight established by World Health Organization (WHO 2003), and at least 300-fold lower than the tolerable daily intake of 120 µg/kg body weight per day derived for oral exposure to styrene as part of the PSL critical review (Government of Canada 1993).

In summary, levels of styrene in most of the total diet food samples are very low. Of the TDS samples, styrene was detected at higher levels in some dairy foods, cereal and fast food samples, with the highest levels found in herbs and spices. The current dietary intakes estimated using occurrence data from the 2014 TDS confirm the previous assessment (Government of Canada 1993) that food may also contribute a similar portion to the total daily intake of styrene for all age groups (non-smoking), in addition to air. However, considering the high levels of exposure to styrene (2.86 and 3.51 µg/kg bw/day) for smoking adults and teens estimated in the previous assessment (Government of Canada 1993), intakes from cigarettes are still the major route of exposure to styrene for the smoking population.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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