Study of 1,4-Dioxane Intake in the Total Diet Using the Market-Basket Method

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1,4-Dioxane has been classified by the US Environmental Protection Agency and the International Agency for Research on Cancer as a compound that may be carcinogenic in humans. Although there are several reports of 1,4-dioxane being detected in the environment, such as in tap water, there have been few reports on the content of 1,4-dioxane in food. We therefore studied the intake of 1,4-dioxane in food based on the average intake of food in the Kanto area of Japan as reported by the Ministry of Health, Labor and Welfare. The food was cooked in the normal manner and then homogenized in a mixer. A 20 g of sample of the homogenate was added to a solution of the purified water with 0.2 μg of 1,4-dioxane-d$_8$ as a surrogate and the 200 ml azeotropic solution was recovered using the steam distillation method. This solution was applied to a pair of active carbon solid-phase cartridges and the analyte was eluted from each cartridge with dichloromethane. The eluted solution was prepared for gas chromatographic/mass spectrometric analysis by reduction to a volume of 1 ml under a gentle stream of nitrogen. The detection limit of the analysis was 2 μg/kg. We found that the 1,4-dioxane content of 12 food groups ranged between 2 μg/kg and 15 μg/kg. From these results, the total daily intake of 1,4-dioxane was calculated to be 0.440 μg. An intake of this magnitude corresponds to 0.055% of the calculated total daily intake (TDI) (16 μg/kg body weight/day). This study indicates that the amount of 1,4-dioxane intake contributed by food is very low and that this value does not represent a potential problem as it does not raise the risk of carcinogenesis.

Key words —— 1,4-dioxane, total diet, risk

INTRODUCTION

1,4-Dioxane is used extensively as an industrial solvent in dyes, paints, lacquers, varnishes, oils, waxes, and resins and is also added as a stabilizer to chlorinated solvents.[1] 1,4-Dioxane is highly soluble in water, forming an azeotropic mixture, and when discharged into the atmosphere it returns to the surface as rainwater. As a property of its low adsorption to soil, 1,4-dioxane then permeates into the groundwater and causes water pollution over the long term. Therefore 1,4-dioxane has the potential to cause widespread contamination of the environment. There are reports of 1,4-dioxane being detected in river water at levels between 0.1 and 16.0 mg/l, and in groundwater at a maximal concentration of 94.8 mg/l in Japan.[2] Several other studies have also shown high levels of 1,4-dioxane pollution in groundwater.[2–4] As the removal of 1,4-dioxane in water purification systems is difficult, these findings raise concerns regarding chronic exposure to 1,4-dioxane in drinking water.

1,4-Dioxane has been classified as a carcinogenic compound by both the USA Environmental Protection Agency[5] and the International Agency for Research on Cancer (IARC).[6] Long-term oral administration of 1,4-dioxane has been shown to cause tumors in the liver and gallbladder in guinea pigs,[7] and in the nasal cavity and liver of rats.[8–11] 1,4-Dioxane has also demonstrated promoter activity in studies in mice using a two-stage carcinogenic test.[12]

Levels of 1,4-dioxane between 0.2 mg/l and 1.5 mg/l were also detected in tap water samples collected during 1995 and 1996 from six cities in Kanagawa prefecture, Japan.[13] This finding raises the possibility that food may also have become con-

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taminated. Although 1,4-dioxane is now included in the quality standards for drinking water in Japan, there have been few reports on the contents and intake of 1,4-dioxane in food. To safeguard human health, it is important to determine the Japanese intake levels of 1,4-dioxane through food. This paper describes a study on the intake of 1,4-dioxane through food in Japan using the market-basket method.

MATERIALS AND METHODS

Chemicals —— 1,4-Dioxane was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan), 1,4-dioxane-d_8 from Sigma-Aldrich Co. Ltd. (St. Louis, MO, U.S.A.), dichloromethane from Kanto Chemical Industry Co. Ltd. (Tokyo, Japan), ethanol from Katayama Chemical Industry Co. Ltd. (Osaka, Japan), acetonitrile and acetone from Wako Chemical Industry Co. Ltd. (Osaka, Japan), and the antifoaming agent silicon TAS730 from Toshiba Silicon Co. Ltd. (Tokyo, Japan). All solvents were of the highest reagent grade. Purified water was prepared using a Milli-Q water purification PSS20 system (Millipore Corp., Bedford, MA, U.S.A.).

Preparation of Standard Solutions —— A stock solution of 1,4-dioxane (1 mg/ml) was prepared in dichloromethane, and a stock solution of 1,4-dioxane-d_8 (10 mg/ml) was prepared in ethanol. The stock solution of 1,4-dioxane-d_8 was then diluted with dichloromethane to a final concentration of 40 μg/ml. The 1,4-dioxane stock solution was diluted with dichloromethane and used to prepare the working standard solutions containing 1,4-dioxane-d_8 0.2 μg/ml in the concentration range of 0.04–1 μg/ml. A solution of 1,4-dioxane-d_8 2 μg/ml was prepared in acetonitrile and was added to samples to determine the recovery rate of the analytical procedure.

Preparation of Food Samples —— The food ingredients were purchased at a general market in Setagaya-ku, Tokyo, Japan in April and May 2000. The quantity of each food item was determined on the basis of the results of the average intake of food in the Kanto area reported by the Ministry of Health, Labor and Welfare (Table 1), using six times the weight of each food group for preparation. Food was cooked in a manner similar to that used in normal homes and was then homogenized in a mixer (Hamilton Beach/Proctor-Silex, Inc., Washington, NC, U.S.A., Model 911). Food that was difficult to homogenize was made uniform by the addition of purified water. In the preparation and cooking of the food, utensils made from wood, aluminum, fluororesin iron, and plastic were used and included items such as a chopping block, pots, and pans. These utensils were washed in a manner similar to that used in normal homes, and tap water was used for cooking. The food homogenates of each group were stored in glass bottles with silicon seals and were kept frozen at –20°C until analyzed.

Extraction of 1,4-Dioxane —— A 20 g of sample of each homogenate was placed in a 500 ml eggplant-type flask, followed by the addition of 150 ml of purified water and 100 μl of 2 μg/ml 1,4-dioxane-d_8 solution. Two hundred milliliters of aqueous solution was recovered from the sample mixture using the steam distillation method. This solution was then passed through a pair of active carbon solid-phase cartridges equilibrated with 20 ml of dichloromethane, 30 ml of acetone, and then 40 ml of purified water. Extraction of the water samples was carried out at a flow rate of 10 ml/min. After the water sample had passed through the cartridges, the cartridges were washed with 5 ml of purified water at the same flow rate. Dried nitrogen gas was then passed through the cartridges for 30 min, and the analyte was eluted from the each cartridge, with the cartridge for the water adsorption attached at the bottom with dichloromethane 3 ml. The cartridge for water adsorption was washed with dichloromethane 20 ml prior to use. The eluted solution was then reduced to a volume of 1 ml under a gentle stream of nitrogen for gas chromatographic/mass spectrometric (GC/MS) analysis.

GC/MS Analysis —— An Agilent 6890/5973N (Agilent Technologies Inc., Palo Alto, CA, U.S.A.) instrument was used for the GC/MS analysis, with separation carried out on a SPB-624 capillary column (60 m × 0.25 mm i.d. × 1.4-μm film thickness) (Sigma-Aldrich Co. Ltd.). Helium was used as the carrier gas with a column flow rate of 1 ml/min in the constant flow mode. The column temperature was kept at 60°C for 1 min, then programmed to increase by 5°C per minute to 130°C and then 20°C per minute to 230°C. Pulsed splitless injection was used with a pulse pressure of 400 kPa (1 min). The ion source temperature was kept at 230°C with the mass spectrometer operated in the EI mode. In the selected ion monitoring (SIM) mode, the monitoring ions were 58 and 88 for 1,4-dioxane and 64 and 96 for 1,4-dioxane-d_8. The injection volume was 2.0 μl. A calibration curve was prepared from the ratio of the peak height of 1,4-dioxane and 1,4-di-

Table 1. List of Food in the Total Diet Study

Group	Food Group	Food	Daily Intake (g)
I	Rice	Rice	149.0
	Rice products	Rice vermicelli	4.1
II	Barley	Oatmeal	0.2
	Flour	Wheak flour	8.3
	Bread	Bread	36.1
	Sweet bun	Bean-jam bun	8.6
	Noodles	Japanese wheat noodles	39.2
	Noodles, macaroni	Buckwheat	6.5
	Instant noodles	Instant noodles	3.7
	Grain	Cornflakes	2.5
	Seed	Crushed almonds	2.0
	Sweet potato	Sweet potato	8.6
	Potato	Potato	35.9
	Tubers and roots	Taro	10.4
	Product of tubers and roots	Konjak	13.5
III	Sugar	Granulated sugar	7.6
	Jam	Strawberry jam	1.6
	Candy	Caramel	0.3
	Rice cracker	Rice cracker	2.0
	Cake	Pound cake	4.0
	Biscuit	Biscuit	3.3
	Other snacks	Japanese fried-dough cookies	15.0
		Azuki bean jelly	
		Chocolate	
IV	Butter	Butter	1.3
	Margarine	Margarine	1.7
	Vegetable oil	Soybean oil	10.4
	Animal oil	Lard	0.2
	Mayonnaise	Dressing	5.7
V	Soybean paste	Soybean paste	12.8
	Beancurd (tofu)	Beancurd (tofu)	35.4
	Product of beans	Deep-fried beancurd	6.0
	Soybean products	Freeze-dried beancurd	9.9
	Beans		2.6
VI	Citrus	Navel orange	30.6
	Apple	Apple	26.6
	Banana	Banana	7.7
	Strawberry	Strawberry	0.2
	Fruit	Watermelon	49.0
		Loquat	
		Japanese apricot	
	Juice	Tomato juice	17.0
VII	Carrot	Carrot	24.0
	Spinach	Spinach	19.7
	Green pepper	Green pepper	4.7
	Tomato	Tomato	21.7
	Green and yellow vegetables	Broccoli	30.8
		Celery	
		Okra	

Table 1. Continued

Group	Food Group	Food	Daily Intake (g)
VIII	Japanese radish (daikon)	Japanese radish	36.0
	Onion	Onion	27.7
	Cabbage	Cabbage	24.8
	Cucumber	Cucumber	14.7
	Napa cabbage	Napa cabbage	18.2
	Vegetables	Burdock (gobo root)	44.5
		Beansprouts	
		Eggplant	
	Pickles	Pickles (nozawa-na)	6.5
	Pickled Japanese radish	Fukujinn-zuke	15.5
	Mushrooms	Mushrooms	12.8
	Seaweeds	Green laver	5.6
IX	Soy sauce	Soy sauce	19.9
	Sauces	Ketchup	5.4
	Salt	Salt	1.3
	Seasoning	Sauce	11.6
	Sake	Sake	15.5
	Beer	Beer	64.2
	Liquor	Wine	12.4
	Soft drinks	Soft drinks	69.4
		Tea	
X	Salmon and trout	Salmon	3.3
	Tuna	Tuna	8.8
	Bream and flatfish	Flatfish	8.0
	Horse mackerel and sardine	Horse mackerel	12.5
	Raw fish	Ayu	6.4
		Kisu	
		Halfbeak	
	Cuttlefish, octopus, and crab	Octopus	13.6
	Shellfish	Scallop	4.7
	Salted fish	Salted cod	8.7
	Dried fish	Dried sardine	9.5
	Canned fish	Bonito	2.9
	Cooked fish	Smelt	0.7
	Cooked fish paste	Hannpen	12.2
	Fish product	Fish sausage	0.3
XI	Beef	Beef	21.7
	Pork	Pork	31.9
	Chicken	Chicken	19.8
	Whale		0.0
	Other animal meat	Lamb	0.9
	Ham and sausage	Pork loin ham	11.0
	Eggs	Chicken eggs	38.3
XII	Milk	Milk	116.4
	Cheese	Cheese	2.8
	Daiury products	Yoghurt	21.1
	Others	Sake sediment	5.8

oxane-d_8. Quantitative analysis of the food samples was carried out using methodology identical to that used in the preparation of the calibration curve.[13)]

RESULTS AND DISCUSSION

Detection Limit in Food Samples

The minimum detection level of 1,4-dioxane-d_8 added as an internal standard was 0.04 μg/l (S/N = 10). The minimum detection limit of 1,4-dioxane in the prepared food was calculated to be 2 μg/kg using the following formula: (0.04 μg/l $\times$ 1 ml)/20 g = 0.002 μg/g = 2 μg/kg , in which 1 ml indicates the final volume for GC/MS analysis and 20 g indicates the weight of the food homogenate.

Recovery Test of 1,4-Dioxane

The concentration of 1,4-dioxane in the purified water and tap water used in the analysis and in the preparation of the food samples was less than 0.04 μg/ml. This level represented the minimum detection limit when the analysis was carried out in the manner used for the food samples.

After the addition of 1,4-dioxane 0.2 μg and 1,4-dioxane-d_8 1 μg to 4 g of the prepared food samples, the recovery rate of 1,4-dioxane was obtained using the method described in the MATERIALS AND METHODS section. Table 2 shows that the recovery rate was between 99% and 111% in the 12 groups. These results indicate there was no problem with the efficiency of extraction when 1,4-dioxane was added to the food samples at a concentration < 0.2 μg/4 g (50 μg/kg).

Content of 1,4-Dioxane in the Food Samples

The extraction of 1,4-dioxane from each 20 g prepared food sample was carried out according to the method described in the MATERIALS AND METHODS section. Table 3 shows that the content of 1,4-dioxane in the 12 food groups was between 2 μg/kg, the detection limit of the analysis, and 15 μg/kg. If the food sample was difficult to homogenize after cooking, an appropriate quantity of purified water was added to achieve homogeneity (*i.e.*, groups I, II, III, and X in Table 4). The weight of the food used for the extraction of 1,4-dioxane before and after cooking was then calculated.

The intake of 1,4-dioxane was calculated based on the average intake of food in the Kanto area as reported by the Ministry of Health, Labor and Welfare. For example, the calculation in group II included a food sample of 1061.6 g that was added to 600.0 g of purified water for cooking and then homogenized. The actual weight of food for extraction was 18.34 g calculated as (20 g/1157.5 g) $\times$ (1061.6 g + 600.0 g) $\times$ {1061.6 g/(1061.6 g + 600.0 g)}. Since the intake of group II food was 175.5 g, the intake of 1,4-dioxane from food in this group was 0.057 μg calculated as (175.5 g/18.34 g) $\times$ 6 μg/kg $\times$ (1/1000). From the results of the content of each group in Table 4, the daily total intake of 1,4-dioxane from food was calculated to be 0.440 μg.

Table 2. Recovery Rate of 1,4-Dioxane Added to Food Samples

Group	Recovery Rate (%)
I	104
II	101
III	105
IV	101
V	106
VI	100
VII	100
VIII	100
IX	99
X	102
XI	104
XII	111

Table 3. Content of 1,4-Dioxane in Food Samples

Group	Content (mg/kg)
I	ND
II	6
III	6
IV	8
V	3
VI	4
VII	3
VIII	8
IX	7
X	5
XI	6
XII	13

ND: not detectable.

Risk from 1,4-Dioxane in Food

There is evidence that long-term oral administration of 1,4-dioxane causes hepatic and nasal cavity tumors in rodents,[8–12)] and accordingly the IARC has classified 1,4-dioxane as a group 2B carcino-

Table 4. Intake of 1,4-Dioxane from Food

Group	Weight before Cooking (g)	Weight of Added Water (g)	Weight after Cooking (g)	Actual Weight of Food for Extraction (g)[a]	Intake of Food (g)	Content in Food (μg/kg)	Intake of 1,4-Dioxane (μg)
I	894.0	1143.3	1715.0	10.43	153.1	ND	0.000
II	1061.6	600.0	1157.5	18.34	175.5	6	0.057
III	202.8	100.0	202.8	20.00	33.8	6	0.010
IV	1114.0		1114.0	20.00	19.3	8	0.008
V	727.9		400.2	36.38	66.7	3	0.006
VI	981.5		981.5	20.00	131.1	4	0.026
VII	605.4		609.0	19.88	100.9	3	0.015
VIII	1237.8		1143.7	21.65	206.3	8	0.076
IX	416.4		416.4	20.00	199.7	7	0.070
X	550.2	300.0	524.0	21.00	91.6	5	0.022
XI	608.9		908.9	13.40	123.6	6	0.055
XII	1019.4		1019.4	20.00	146.1	13	0.095

a) 1 : 20 g was used for extraction.

gen.[6] With regard to a cancer endpoint, a total daily intake (TDI) of 16 μg of 1,4-dioxane/kg body weight/day has been calculated by applying an uncertainty factor of 1000 that incorporates 100 for inter- and intraspecies variation and 10 for nongenotoxic carcinogenicity to the no observed adverse effect level of 16 μg/kg body weight/day, as found in a long-term study involving drinking water in rats.[14,15] The 0.440 μg intake of 1,4-dioxane we measured in our study corresponds to 0.055% of the calculated TDI (0.440 μg/{16 μg/kg body weight/day $\times$ 50 kg}). We therefore conclude that the intake of 1,4-dioxane from food appears to be very low and that this value does not increase the risk of carcinogenicities.

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