Study of 1,4-Dioxane Intake in the Total Diet

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(Received April 25, 2005; Accepted May 9, 2005)

1,4-Dioxane is a newly added compound to the water quality standards in Japan that were revised in 2003. In order to estimate the contribution of 1,4-dioxane in drinking water to the total exposure in humans, it is necessary to take into account the quantity of the compound in food. In an earlier study, we measured the intake of 1,4-dioxane in food based on the average consumption of food in the Kanto area.[1] The total daily intake of 1,4-dioxane was calculated to be 0.440 μg. In the present study, we investigated the intake of 1,4-dioxane from food by sampling meals from 3 days from 3 homes in 9 prefectures, respectively. 1,4-Dioxane was extracted from 20 g of homogenates of mixed meals using the steam distillation, concentrated by a solid phase cartridge and then measured using gas-chromatography/mass spectrometry. The detection limit of the analysis was 2 μg/kg. No 1,4-dioxane was detected in 26 samples, while 3 μg/kg was detected in one sample. In this sample case, the daily intake of the 1,4-dioxane was calculated as 4.5 μg that represented 0.56% of the total daily intake (TDI) (4.5 μg/{16 μg/kg body weight/day × 50 kg}).

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

INTRODUCTION

1,4-Dioxane has been classified as a carcinogenic compound by both the USA Environmental Protection Agency[2] and the International Agency for Research on Cancer (IARC).[3] Long-term oral administration of 1,4-dioxane has been shown to cause tumors in the liver and gallbladder in guinea pigs,[4] and in the nasal cavity and liver of rats.[5–8] Studies in mice using a two-stage carcinogenic test have demonstrated 1,4-dioxane also has promoter activity.[9]

In 2003, 1,4-dioxane was added to the revised water quality standards in Japan. The compound is used extensively as an industrial solvent and is also added as a stabilizer to chlorinated solvents.[10] 1,4-Dioxane escapes to the aquatic environment and after discharging into the atmosphere returns to the surface as rainwater. As a result of its low adsorption to soil, 1,4-dioxane then permeates into the groundwater causing long term water pollution. As a consequence, 1,4-dioxane has the potential to cause widespread contamination of the environment and it is therefore important when evaluating exposure to the compound that every potential route of contamination is taken into account. Although there are several reports of 1,4-dioxane being detected in the environment,[11–13] there have been few reports on the content of 1,4-dioxane in food. Levels of 1,4-dioxane between 0.2 and 1.5 mg/l were detected in tap water samples collected between 1995 and 1996 from six cities in Kanagawa prefecture, Japan,[14] this finding raises the possibility that food may also have become contaminated. As there have been few reports on the contents and intake of 1,4-dioxane in food, in order to safeguard human health in Japan it is important to determine the intake levels of 1,4-dioxane in food.

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In an earlier study we measured 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.[1] The total daily intake of 1,4-dioxane was calculated to be 0.440 μg. An intake of this magnitude corresponded to 0.055% of the calculated total daily intake (TDI) of 16 μg/kg body weight/day. In this paper, we extended these investigations by measuring the intake of 1,4-dioxane from meals.

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. (Tokyo, Japan), dichloromethane from Kanto Chemical Industry Co., Ltd. (Tokyo, Japan), ethanol from Katayama Chemical Industry Co., Ltd. (Osaka, Japan), acetonitrile and acetone from Wako Pure 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 —— Standard solutions of 1,4-dioxane and 1,4-dioxane-d_8 were prepared in dichloromethane from stock solutions of 1 mg/ml 1,4-dioxane and 10 mg/ml 1,4-dioxane-d_8 as described previously.[1]

Preparation of Food Samples —— Samples of meals from 3 days were collected daily from 3 homes in 9 prefectures. The samples of the three meals cooked each day were added to samples of between-meal snacks and drink, followed by mixing and homogenization. The homogenates were then stored in glass bottles with silicon seals and kept frozen at –20°C until analyzed.

Extraction of 1,4-Dioxane —— 1,4-Dioxane was extracted from 20 g of each mixed meal homogenate using steam distillation as described previously reported.[1] Briefly, the samples were added to 150 ml of purified water and 100 μl of 2 μg/ml 1,4-dioxane-d_8 solution and then extracted, followed by concentration using a solid phase cartridge.

GC/MS Analysis —— Gas-chromatography/mass spectrometry (GC/MS) analysis was carried out using an Agilent 6890/5973N instrument (Agilent Technologies Inc., Palo Alto, CA, U.S.A.) instrument with an SPB-624 capillary column (60 m $\times$ 0.25 mm i.d. $\times$ 1.4-μm film thickness) (Sigma-Aldrich Co., Ltd.), as reported previously.[1] 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. A calibration curve was prepared from the ratio of the peak height of 1,4-dioxane and 1,4-dioxane-d_8.

RESULTS AND DISCUSSION

Detection Limit in Food Samples

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

Recovery Test of 1,4-Dioxane

The concentration of 1,4-dioxane in the purified water used in the analysis was less than 0.04 μg/ml. After the addition of 0.2 μg of 1,4-dioxane and 1 μg of 1,4-dioxane-d_8 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. The recovery rate of 0.2 μg of 1,4-dioxane was between 99 and 111% in the 12 food groups.[1] These results indicate that extraction of the compound from any food was efficient and met the requirements for this study.

Content of 1,4-Dioxane in the Mixed Meal Samples

The extraction of 1,4-dioxane from each of the 20 g prepared mixed meal samples was carried out according to the method described in the MATERIALS AND METHODS. Table 1 shows the content of 1,4-dioxane in the 27 mixed meal samples. 1,4-Dioxane was not detected in 26 of these samples but was detected in the remaining sample of a meal which collected on the first day from home C in the Nagano prefecture. The content of 1,4-dioxane detected in this sample was 3 μg/kg. Based on data reported by the Ministry of Health, Labor and Welfare the total weight of meals consumed each day is approximately 1.5 kg. Therefore, in the case of the positive sample the daily intake of the 1,4-dioxane was calculated to be 4.5 μg.

Table 1. Content of 1,4-Dioxane in the Mixed Meal Samples

Sample Site	Home Name	Day	Content (μg/kg)	Sample Site	Home Name	Day	Content (μg/kg)
Hokkaido	A	1st day	ND	Nagano Prefecture	A	1st day	ND
		2nd day	ND			2nd day	ND
		3rd day	ND			3rd day	ND
	B	1st day	ND		B	1st day	ND
		2nd day	ND			2nd day	ND
		3rd day	ND			3rd day	ND
	C	1st day	ND		C	1st day	3
		2nd day	ND			2nd day	ND
		3rd day	ND			3rd day	ND
Miyagi Prefecture	A	1st day	ND	Hyogo Prefecture	A	1st day	ND
		2nd day	ND			2nd day	ND
		3rd day	ND			3rd day	ND
	B	1st day	ND		B	1st day	ND
		2nd day	ND			2nd day	ND
		3rd day	ND			3rd day	ND
	C	1st day	ND		C	1st day	ND
		2nd day	ND			2nd day	ND
		3rd day	ND			3rd day	ND
Tokyo	A	1st day	ND	Kagawa Prefecture	A	1st day	ND
		2nd day	ND			2nd day	ND
		3rd day	ND			3rd day	ND
	B	1st day	ND		B	1st day	ND
		2nd day	ND			2nd day	ND
		3rd day	ND			3rd day	ND
	C	1st day	ND		C	1st day	ND
		2nd day	ND			2nd day	ND
		3rd day	ND			3rd day	ND
Aichi Prefecture	A	1st day	ND				
		2nd day	ND				
		3rd day	ND				
	B	1st day	ND				
		2nd day	ND				
		3rd day	ND				
	C	1st day	ND				
		2nd day	ND				
		3rd day	ND				

There is evidence that long-term oral administration of 1,4-dioxane in rodents causes hepatic and nasal cavity tumors in rodents,[8–12] and accordingly the IARC has classified 1,4-dioxane as a group 2B carcinogen.[6] With regard to a cancer endpoints, a TDI of 16 μg of 1,4-dioxane/kg body weight/day has been calculated by applying an uncertainty factor of 1000 to the level of 16 μg/kg body weight/day at which no adverse effects were observed in a long-term study of drinking water in rats.[15,16] This uncertainty factor incorporates 100 for inter- and intraspecies variation and 10 for nongenotoxic carcinogenicity. An intake of 4.5 μg of 1,4-dioxane corresponded to 0.56% of the TDI (4.5 μg/{16 μg/kg body weight/day $\times$ 50 kg}). As this proportion was the highest value in this investigation, we consider all other proportions would be equal to or less than 0.56%. 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 carcinogenicity.

Acknowledgements This work was supported by Grants-in-Aid from the Ministry of Health, Labor and Welfare of Japan.

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