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Migration of formaldehyde and acetaldehyde into mineral water in polyethylene terephthalate (PET) bottles

M. MUTSUGA, Y. KAWAMURA, Y. SUGITA-KONISHI, Y. HARA-KUDO, K. TAKATORI, & K. TANAMOTO

National Institute of Health Sciences, Tokyo, Japan

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Abstract
The levels of formaldehyde (FA) and acetaldehyde (AA) in polyethylene terephthalate (PET) bottles and in commercial mineral water are reported. All the water samples bottled in Japan contained detectable levels of FA (10.1–27.9 $\mu g l^{-1}$) and AA (44.3–107.8 $\mu g l^{-1}$). Of 11 European bottled water samples, eight did not contain either FA or AA, while the remaining three had detectable levels of FA (7.4–13.7 $\mu g l^{-1}$) and AA (35.9–46.9 $\mu g l^{-1}$). In three North American bottled water samples, two contained FA (13.6 and 19.5 $\mu g l^{-1}$) and AA (41.4 and 44.8 $\mu g l^{-1}$), and one did not. Regardless of the region of origin, all the sterilized water samples contained FA and AA, whilst in contrast, none of the unsterilized water without carbonate contained FA or AA. Of the carbonated water samples, three contained FA and AA, and one did not. When fortified with FA and AA, the commercial water sample without otherwise detectable FA and AA was able to reduce levels, although the commercial water sample containing FA and AA could not. The presence of bacteria in the commercial water samples was investigated using an ATP-based bioluminescent assay and heterotrophic plate count method. The commercial water without FA and AA contained heterotrophic bacteria, whilst the commercial water with FA and AA did not contain detectable bacteria. It is suggested that in this case both FA and AA migrated from PET materials, but were subsequently decomposed by the heterotrophic bacteria in the unsterilized water.

Keywords: *Polyethylene terephthalate (PET), commercial mineral water, formaldehyde, acetaldehyde, heterotrophic bacteria.*

Introduction

Acetaldehyde (AA) has been reported as being present in polyethylene terephthalate (PET) bottles (Dong et al. 1980; Wyatt 1983; Duflos et al. 1993; Linssen et al. 1995) and bottled mineral water (Nijssen et al. 1996; Sugaya et al. 2001; Dabrowska et al. 2002; Nawrocki et al. 2002; Ewender et al. 2003; Hirayama et al. 2003). AA was reported to migrate from the PET plastics, resulting in an undesirable slightly sweet and fruity taste in the mineral water, particularly in the case of carbonated mineral water (Nijssen et al. 1996; Dabrowska et al. 2002; Nawrocki et al. 2002). On the other hand, there are only a few reports of formaldehyde (FA) in PET bottles and bottled water (Villain et al. 1994; Sugaya et al. 2001; Ewender et al. 2003; Hirayama et al. 2003).

The determination of the AA content of PET is generally carried out using headspace gas chromatography (HS/GC). In contrast, the determination of FA in PET samples using HS/GC is difficult because FA is generated by the heating of PET in the headspace sample. Previous papers reported an analytical method for FA and AA in PET products (Mutsuga et al. 2003). In this method, the PET samples are not heated, allowing the accurate measurement of free FA without decomposition of the PET samples. The levels of FA and AA were measured in PET products including bottles for mineral water (Mutsuga et al. 2005). The findings were that most of the PET products contain FA to the same extent as AA.

In the present study, the content of FA and AA in PET bottled commercial water and the bottle material were determined, and subsequently the origin

Correspondence: M. Mutsuga, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo, Japan. E-mail: mutsuga@nihs.go.jp

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and disappearance of FA and AA in commercial water was studied.

Materials and methods

Sample

Twenty PET-bottled commercial mineral water samples were purchased in Japan between April 2003 and March 2004; six were bottled in Japan, 11 were bottled in Europe and three were bottled in North America.

Reagents

Formaldehyde solution (37%), hydrochloric acid for precision analysis grade (36%) and sodium sulfate were purchased from Sigma Aldrich Japan (Tokyo, Japan). Acetaldehyde was purchased from Aldrich Chemical Co., Inc. (Milwawkee, WI, USA). 2,4-Dinitrophenylhydrazine (DNPH) hydrochloride for HPLC labelling grade, formaldehyde 2,4-dinitrophenylhydrazone (FA-DNPH) and acetaldehyde 2,4-dinitrophenylhydrazone (AA-DNPH) were purchased from Tokyo Kasei Kogyo Co, Ltd (Tokyo, Japan). Trifluoroacetic acid, potassium carbonate and dichloromethane for dioxin analysis grade were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Acetonitrile for high-performance liquid chromatography (HPLC) grade were purchased from Merck Co., Inc. (Darmstadt, Germany). Sterilized water was prepared by autoclaving the commercial mineral water in glass bottles.

The CheckLite-HS kit (ATP-based bioluminescent assay kit, containing luciferin-luciferase reagent, ATP-eliminating reagent (ATPase) and ATP-releasing reagent), and CheckLite ATP standard (ATP standard solutions kit) were purchased from Kikkoman International (Chiba, Japan). The LIVE/DEAD BacLight bacterial viability kit (stains mixture of SYTO 9 and propidium iodide) was purchased from Molecular Probes (Eugene, OR, USA), and R2A agar was purchased from Difco Laboratories (Detroit, MI, USA). The membrane filter (0.45 μm, i.d. 13 mm and 0.22 μm, i.d. 47 mm) used was Millex-LH and contains nitrocellulose (Millipore). The carbon membrane filter (0.2 μm, i.d. 19 mm) used was from Track-Etch Membrane (Whatman, ME, USA).

Apparatus

FA and AA were quantified using an HPLC system consisting of the Shimadzu LC-10A (Shimadzu Co., Kyoto, Japan).

Bioluminescence was measured using a Lumitester C-100N (Kikkoman International). Direct counting of bacteria was performed with an epifluorescence microscopy OLYMPUS BX60 (Olympus Co., Tokyo, Japan).

Preparation of standard solution

Stock solution (100 μg ml^{-1} each FA and AA) was prepared by dissolving 70.0 mg FA-DNPH and 50.9 mg AA-DNPH in 100 ml acetonitrile. Standard solutions with which to calculate curves were prepared by stepwise dilution with the acetonitrile/water (1:1) ranging in concentration from 0.05 to 5 μg ml^{-1} for FA and AA.

Analytical procedure for mineral water

Commercial water (100 ml) was collected in 200 ml glass flasks, 5 ml DNPH/hydrochloric acid solution (1 mg ml^{-1}) added and then derivatized for 2 h at room temperature. Approximately 3.7 g potassium carbonate were added slowly with agitating to adjust the pH to approximately 3. The solution was transferred into a separatory funnel and derivatives extracted with dichloromethane (25 ml × 2). The dichloromethane layers were collected and added about 2 g sodium sulfate and filtrated. The filtrate was evaporated completely under reduced pressure. The residue was dissolved in 2 ml acetonitrile.

Analytical procedure for PET bottle material

PET bottle material (0.5 g) was cut into small pieces and placed in a 5 ml centrifuge glass tube equipped with a glass plug, after which 2.5 ml DNPH/trifluoroacetic acid solution (1 mg ml^{-1}) was added and the mixture left overnight for dissolution and derivatization. Dichloromethane (10 ml) was then added and approximately 12 ml potassium carbonate solution (0.2 g ml^{-1} dissolved in sterilized water) was added to adjust the pH to 7, when the solution changed to a thick yellow liquid. The precipitates were removed by filtration under reduced pressure and washed with dichloromethane (2 × 10 ml). The filtrate and washes were combined and transferred to a separatory funnel. The dichloromethane layers were separated from the aqueous layer, which was subsequently extracted using dichloromethane (10 ml). The dichloromethane layers were collected and dried via the addition of sodium sulfate and evaporation. The residue was dissolved in 2.5 ml acetonitrile and diluted to 5 ml with water, then filtered using a membrane filter (0.45 μm).

HPLC condition

Column: TSKgel ODS-80Ts (4.6 mm i.d. × 250 mm) (Tosoh Co., Tokyo, Japan), guard column: stainless column (1.0 mm i.d. × 45 mm)

packed with ODS (Fuji Silysia Chemical Ltd., Aichi, Japan), flow: 1 ml min^{-1}, column temperature: 50°C, injection volume: 20 µl, mobile phase: 55% acetonitrile/water, detection: UV (360 nm).

Standard aerobic plate count

A water sample (100 ml) was filtered through a membrane filter (0.22 µm) under reduced pressure. The filter was then placed upon a tryptone glucose yeast extract agar medium plate. The plates were incubated aerobically at 37°C for 24 h. Colonies were counted and expressed as colony forming unit (CFU) ml^{-1}.

Direct counting of bacteria with fluorescent staining

A water sample (50 ml) was drawn through a carbon membrane filter under negative pressure. The carbon membrane filter was strained by LIVE/DEAD BacLight bacterial viability kit, 1 ml stains mixture prepared according to the manufacture's instructions, and incubated for 20 min in the dark at room temperature. The mixture was removed and filters mounted with low-fluorescence immersion oil on glass microscope slides and observed by epifluorescence microscopy. The number of green cells was counted after viewing 20 microscopic fields.

ATP-based bioluminescent assay

The ATP derived from bacteria was measured by following methods. A 1 ml aliquot of water sample was placed in a tube, an ATP-eliminating reagent (0.1 ml) was added, the solution was mixed and it was incubated for 10 min to remove any extracellular ATP. A total of 0.1 ml of the solution then combined with 0.1 ml of a detergent for lysing cells before the addition of 0.1 ml luciferin-luciferase reagent. The sample was mixed and the amount of bioluminescence measured using a luminometer. The luminescence curves were prepared using the ATP standard solutions (2×10^{-12}–2×10^{-9} mol ml^{-1}). The results are expressed as relative light units (RLU) and ATP concentration.

Heterotrophic plate counting method

Heterotrophic plate counts (HPC) were obtained by plating 100 µl of tenfold dilutions of commercial water samples on R2A agar plates. The plates were incubated aerobically at 22°C for 72 h. Colonies were counted and the arithmetic mean expressed as CFU ml^{-1}.

Result

Analytical procedure

FA and AA in the PET bottle material were measured as described (Mutsuga et al. 2003, 2005). Recoveries of 76.9–101.2% were obtained for a PET pellet sample (0.5 g) spiked with FA (2.5 µg) and AA (2.5 µg). The limits of detection of FA and AA were 0.2 µg g^{-1} for each, based on the linearity of the calibration curve.

The analytical method for FA and AA in the commercial water was improved upon, and recovery rates of 85.7–104.9% were obtained for sterilized water (100 ml) spiked with FA and AA (1 and 10 µg each). The limit of detection of FA and AA were 5.0 µg l^{-1} for each, based on three times the blank value (FA: 1.34 ± 0.11 µg l^{-1} and AA: 0.77 ± 0.04 µg l^{-1}).

Contents of FA and AA in mineral water

The levels of FA and AA in the 20 commercial water samples are shown in Table I. FA and AA ranged from not detected (n.d.) to 27.9 µg l^{-1} and n.d. to 107.8 µg l^{-1}, respectively. All samples bottled in Japan had detectable levels of FA (10.1–27.9 µg l^{-1}) and AA (44.3–107.8 µg l^{-1}). In the European water, three samples had detectable levels of FA (7.8–13.7 µg l^{-1}) and AA (37.2–46.9 µg l^{-1}), while the remaining eight did not. In the North American water, two samples contained FA (13.6 and 19.5 µg l^{-1}) and AA (41.4 and 44.8 µg l^{-1}), while one did not. Most of the samples bottled in Europe and North America contained FA and AA under the detection limits. The detected levels of FA and AA in the Japanese water were higher than in the European or North American samples. There was no relationship between FA-and-AA content and pH or hardness of the commercial water. The samples were classified into two groups: those in which FA and AA were detected in the water (group A), those in which FA and AA were not detected in the water (group B). Group A contained all the sterilized water samples and three carbonated water samples. Group B contained unsterilized water samples without carbonate and one carbonated water sample.

Content of FA and AA in the bottle material and their migration

The levels of FA and AA in the bottle materials were analysed (Table I). The FA and AA levels of the Japanese bottles ranged from 1.3 to 2.9 µg g^{-1} and from 11.5 to 25.0 µg g^{-1}, respectively, while the European and North American bottles ranged from n.d. to 1.6 µg g^{-1} and from 5.2 to 17.1 µg g^{-1}, respectively. The levels of FA and AA in the Japanese bottles were significantly higher than that in European and North American bottles. The explanation for this may be the higher moulding temperatures used in the production of Japanese bottles (thicker bottle walls) and the use of scavengers in European bottles, which minimizes the

Table I. Contents of FA and AA in mineral water.

Region	Sample number	Bottled country	Representation				Bottle colour	Bottle material ($\mu g\,g^{-1}$)		Water ($\mu g\,l^{-1}$)		
			pH	Hardness	Sterilized	Carbonate		Formaldehyde	Acetaldehyde	Formaldehyde	Acetaldehyde	Classification
Japan												
	J-1	Japan	7.4	84	sterilized	–	colorless	2.2	16.7	10.1	44.3	group A
	J-2	Japan	8.3	32	sterilized	–	colorless	2.9	12.1	27.9	60.7	group A
	J-3	Japan	–	30	sterilized	–	colorless	1.6	13.6	15.0	41.1	group A
	J-4	Japan	–	99	sterilized	–	colorless	1.3	25.0	10.6	107.8	group A
	J-5	Japan	–	25	sterilized	–	colorless	2.4	14.0	15.4	66.3	group A
	J-6	Japan	7.1	28	sterilized	–	colorless	1.7	11.5	17.6	46.4	group A
Europe												
	E-1	France	7.0	62	not treated	–	pale blue	n.d.	8.8	n.d.	n.d.	group B
	E-2	France	7.3	309	not treated	–	colorless	1.0	5.5	n.d.	n.d.	group B
	E-3	France	7.2	294	not treated	–	colorless	0.7	7.2	n.d.	n.d.	group B
	E-4	France	7.6	628	not treated	–	colorless	0.7	5.4	n.d.	n.d.	group B
	E-5	France	–	201	not treated	carbonated	green	0.9	6.7	n.d.	n.d.	group B
	E-6	Italy	7.8	161	not treated	carbonated	deep blue	0.6	5.2	7.9	37.2	group A
	E-7	Italy	7.5	740	not treated	carbonated	green	0.9	8.4	13.7	37.8	group A
	E-8	Italy	5.8	609	not treated	carbonated	green	0.5	6.7	7.8	46.9	group A
	E-9	Italy	7.8	161	not treated	–	green	1.0	5.7	n.d.	n.d.	group B
	E-10	UK	–	104	not treated	–	colorless	1.6	7.5	n.d.	n.d.	group B
	E-11	UK	7.8	122	not treated	–	colorless	1.1	5.9	n.d.	n.d.	group B
North America												
	A-1	Canada	–	24	sterilized	–	pale blue	0.9	9.8	13.6	41.4	group A
	A-2	Canada	–	1	–	–	deep blue	n.d.	9.2	n.d.	n.d.	group B
	A-3	USA	–	38	sterilized	–	colorless	1.1	17.1	19.5	44.8	group A

–, No description.
Each value is the mean of three trials.
Bottle material n.d. $< 0.2\,\mu g\,g^{-1}$, water n.d. $< 5.0\,\mu g\,L^{-1}$.

Table II. Migration of FA and AA from PET bottles into water at 40°C.

Bottle	Contents in bottle ($\mu g\,g^{-1}$)		Storage days	Migration level ($\mu g\,l^{-1}$)	
	FA	AA		FA	AA
J-1	2.2	16.7	14	42.6	112.0
			30	55.2	169.6
E-1	0.7	5.4	14	11.4	14.5
			30	14.9	21.1

Each value is the mean of four trails

formation of FA and AA. Some of the bottles were blue or green colour. However, the results for the European bottles showed that the colour of bottles did not affect the FA and AA levels.

Migration tests were performed using Japanese (J-1) and European (E-1) bottles, in which J-1 bottle contained FA and AA while the E-1 bottles did not. The bottles were washed with sterilized water, then 100 ml sterilized water added, and stored at 40°C for 14 and 30 days. The analysis showed that migration of FA and AA occurred from both bottles to water (Table II). The migration levels of FA and AA depended on the level in the bottle material and duration of storage. Thus, the FA and AA in commercial water was a result of migration from their PET bottles.

Clarification of the disappearance of FA and AA in commercial water

The E-1 bottle showed migration of FA and AA into the sterilized test water, although the commercial water contained in the E-1 bottle was not contaminated with either FA or AA. Thus, it was speculated that unsterilized water might have some capacity to reduce levels of FA and AA.

Thus, 100 ml commercial water samples were transferred to a 200-ml glass bottle with plastic screw cap. Water samples were fortified with 2 μg FA and 10 μg AA and stored in the dark at 37°C for 48 or 96 h (Figure 1 and Table III). Figure 1 shows the time–response curves of FA and AA in E-1 and J-1 water for 12, 24, 36 and 48 h. In E-1 water, FA and AA reduced quickly and reached the 'blank' level after 48 h incubation, whereas the FA and AA in the J-1 water did not decline. Table III shows the recoveries of fortified FA and AA in several kinds of commercial water. Water of group B was able to reduce spiked FA and AA, while water of group A could not. Two carbonated water (E-6 and E-7) produced a small reduction in FA, and it seemed to be more volatile in carbonated water than still water at 37°C. However, water samples (E-1–3) after sterilization in group B did not have the same ability to reduce levels.

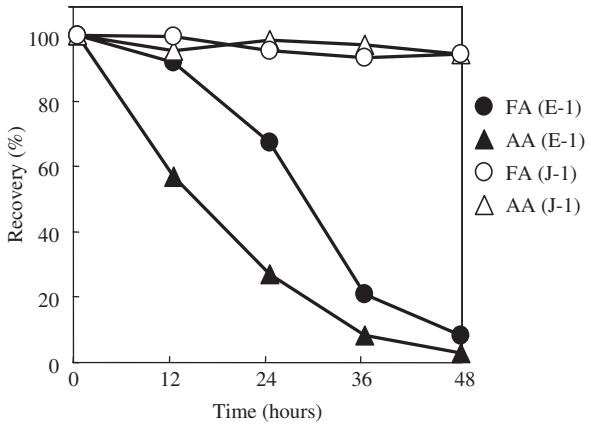

Figure 1. Recovery of FA (2 μg) and of AA (10 μg) fortified to 100 ml E-1 and J-1 water stored at 37°C. Each value is the means of two trials.

Table III. Recoveries of fortified FA and AA in several mineral water samples.

	Sample number	Incubation time (h)	Recovery (%)	
			Formaldehyde	Acetaldehyde
Group A	J-1	48	>95	>95
	J-1	96	>95	>95
	J-3	48	>95	>95
	J-6	48	>95	>95
	E-6	48	78	>95
	E-7	48	83	>95
	A-3	48	95	>95
Group B	E-1	48	<5	<5
	E-2	48	>95	75
	E-2	96	27	<5
	E-3	48	86	5
	E-5	48	>95	10
Sterilized group B	E-1	48	>95	>95
	E-2	96	>95	92
	E-3	48	>95	>95

FA (2 μg) and AA (10 μg) were added to 100 ml mineral water and stored at 37°C for 48 or 96 h.
Each value is the mean of two trials.

According to these results, unsterilized commercial water can reduce the levels of FA and AA in the water. It is suspected that bacteria in commercial water were involved in the reduction in FA and AA.

Confirmation of bacterial activity

At first, the standard aerobic plate count method was performed. However, none of the water samples produced colonies. Therefore, the direct counting method was performed using the LIVE/DEAD BacLight bacterial viability kit, which contained two nucleic acid-binding stains, SYTO 9 and propidium iodide (Venkateswaran et al. 2003). SYTO 9 stained

Table IV. Amount of intracellular ATP in water.

Sample number	Bioluminescent assay: Luminescence (RLU)	Bioluminescent assay: ATP ($\times 10^{-11}$ M)	HPC assay colony count (CFU ml^{-1})
Group A			
J-1	n.d.*1	n.d.	n.d.*2
J-2	n.d.	n.d.	–
J-3	n.d.	n.d.	n.d.
J-4	n.d.	n.d.	–
J-5	n.d.	n.d.	–
J-6	n.d.	n.d.	–
E-6	n.d.	n.d.	n.d.
E-7	n.d.	n.d.	–
E-8	n.d.	n.d.	n.d.
A-1	n.d.	n.d.	–
A-3	n.d.	n.d.	–
Group B			
E-1	1005	14.3	1.5×10^5
E-2	149	1.9	3.2×10^4
E-3	801	11.3	7.8×10^4
E-4	655	9.2	–
E-5	1059	15.0	1.6×10^3
E-9	112	1.4	–
E-10	1003	14.2	4.1×10^4
E-11	3808	54.8	–
A-2	778	11.0	–

n.d.*1 < 100 RLU.
n.d.*2 < 100 CFU ml^{-1}.
Each value is the mean of three trials.
–, Not evaluated.

all cells green, while propidium iodide stained all the cells with damaged membranes red. As observed by epifluorescence microscopy, both viable and dead bacteria were detected. In J-1 water, neither green nor red bacteria were detected. On the other hand, in E-1 water, viable green bacteria were visualized (5×10^4 cells ml^{-1}). This bacterium was presumed to heterotrophic bacterium, which exists widely in the environment, as this bacterium is very small and there have been some reports documenting its existence in commercial water (Mosso et al. 1994; Tsai and Yu 1997; Ramalho et al. 2001; Leclerc and Moreau 2002).

Subsequently, intracellular ATP was measured by the ATP-based bioluminescent assay (Table IV). This method confirmed that none of the water samples in group A contained intracellular ATP. On the other hand, the water in group B showed intracellular ATP concentrations of between 1.4×10^{-11} and 5.5×10^{-10} M. Among the carbonated waters, intracellular ATP was detected in E-5 of group B, but not in E-6–8 of group A. All samples showed correlation between the existence of intracellular ATP and a reduction in FA and AA levels.

The enumeration of heterotrophic bacteria in the commercial mineral water is usually performed using the HPC method (Mosso et al. 1994; Tsai and Yu 1997; Ramalho et al. 2001; Leclerc and Moreau 2002; Venkateswaran et al. 2003). This method was recommended by Council Directive 98/83/EC (1998) for the count of heterotrophic bacteria in commercial water, and was performed in the present study on nine water samples by incubation for 72 h at 22°C on R2A medium. None of the water samples in group A possessed heterotrophic bacteria, while the water samples in group B formed between 1.6×10^3 and 1.5×10^5 CFU ml^{-1}.

Discussion

It has been shown that FA and AA migrated into commercial water from the PET bottle material. In commercial water without bacteria, the levels of migrated FA and AA remain unchanged, whereas in natural mineral water containing heterotrophic bacteria, the migrated FA and AA was decomposed. Of the carbonated water samples, one sample contained bacteria and showed a reduction in FA and AA, while the others had no bacteria and showed no decomposition activity. It was speculated that the existence of bacteria influenced the concentration of carbonate gas.

In the European Union regulations, natural mineral water cannot be treated for the elimination of microorganisms by disinfection or sterilization. The current drinking water guidelines in many European countries are based on recently revised Directive 98/83/EC. The current recommended microbiological standards include HPC limits for private supplies, i.e. no significant increase over normal levels when incubated at 22 and 37°C, and for bottled water within 12 h of bottling, 100 CFU ml^{-1} when incubated at 22°C for 72 h and 20 CFU ml^{-1} when incubated at 37°C for 48 h. In the present study, several European waters contained 1.6×10^3–1.5×10^5 CFU ml^{-1} heterotrophic bacteria. These waters appear to have passed the regulations during the bottling stage, but then bacteria proliferated during transport to Japan.

The potential negative impact to human health from the consumption of treated water containing high HPC levels of bacteria is still being debated. However, until now, no report has documented the decomposition of FA and AA by heterotrophic bacteria. PET bottled commercial water has two problems: the existence of heterotrophic bacteria and the migration of FA and AA, and the close relationship exists between these problems. Thus, it is

necessary to pay sufficient attention to both problems.

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