BOTTLE MATERIAL AND CLEANSING PROCEDURES OF INFANT FEEDING BOTTLES

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Abstract. The cleanliness of feeding bottles is vital for child health. Although machine cleansing of bottles in the food industry has been established, mechanical and manual cleansing methods are highly variable. This study was undertaken to determine the differences in the cleanliness of bottles that were cleaned using various combinations of bottle materials [glass and polypropylene (PP)], rinsing water volumes (1/3, 1/2, and 2/3 capacity of a bottle), and sustained shaking times (5 seconds and 20 seconds). Total organic carbon (TOC) and conductivity measurements were respectively used to evaluate the rinsed quantities of organic and inorganic formula residue from feeding bottles. The results indicated that glass bottles filled with rinsing water to 2/3 of their capacity showed the most efficient cleansing performance. However, the PP bottles exhibited a relatively poor cleansing result, particularly for organic cleanliness. The organic residue tends to accumulate on the PP bottle interior because of the aggregation of compounds with similar properties. The shaking time hardly influenced the cleanliness. The glass bottle was superior to the PP bottle in both organic and inorganic cleanliness, and organic constituents were more difficult to rinse from the bottle than the inorganic constituents were.

Keywords: bottle cleanliness, bottle material, infant formula

INTRODUCTION

Although breastfeeding infants and young children is well-recognized and recommended (Kramer and Kakuma, 2002), bottle-feeding is still widely used during the neonatal period or for those incapable of breastfeeding cases, for example, the infants of women infected with HIV (Ma *et al*, 2009). The cleanliness of feeding bottles is vital for infant health and hygiene in hospitals, nursing centers, and households. Improperly cleansing of a bottle causes milk or food to remain inside. Consequently, bacteria reproduce in a two-log level in the bottle (Palcich *et al*, 2009; Winthrop and Homestead, 2012). Particularly in developing countries, contaminated infant formula and other weaning foods may cause diarrhea in children. This accounts for 25%-33% of all deaths in children under-five years old (Redmond *et al*, 2009; Weisstaub and Uauy, 2012). Children in food-insecure households may also be at-risk of poorer health

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(Ramsey *et al*, 2011; Byrd-Bredbenner *et al*, 2013; Korir, 2014).

Effective procedures for cleansing reusable bottles have been developed in the food industry (Kuhlman, 1998; Rogener *et al*, 2002; Franz *et al*, 2004). The effectiveness of this cleansing procedure depends on several factors, such as, bottle shape, bottle material, formula constituents, washing water quantity, washing time, and auxiliary appliances (Meister *et al*, 2012; Krehula *et al*, 2013). Tawfik *et al* (1997) indicated that certain highly hydrophobic compounds remain in bottles even after washing and can migrate to the subsequent product contained in the bottle. Therefore, the remaining constituents may be highly associated with the chemical interaction between the constituents and the bottle materials (Choodum *et al*, 2007; Ceretti *et al*, 2010).

Because they are versatile, light, sturdy, reusable, and cost-efficient, plastic bottles, rather than conventional glass bottles are increasingly being used in the food and beverage industry. Resistance to the uptake of substances is essential for the plastic materials used in reusable bottles. Devlieghere *et al* (1997a) evaluated various bottle materials and cleansing parameters, and they found that none of the different washing conditions could be used to remove all absorbed chemicals from the polymeric resins. Commercially available plastic refillables (PET and PC) demonstrated the proper chemical rinsability (Santos *et al*, 2010). However, glass bottles demonstrated the best rinsing characteristics under all tested conditions (Devlieghere *et al*, 1997b). The examined materials were ranked in the following order, from highest to lowest, according to their microbial rinsability when optimal cleansing procedures were applied: glass > PET > PC > PP = PVC > HDPE.

The machine cleansing of reusable bottles in the food and beverage industry has been extensively established. However, the results may not pertain to bottle cleansing procedures conducted by people, because the conditions between mechanical and manual cleansing are highly variable. The latter is generally used in households and the nurseries of hospitals. In addition, studies on bottle-machine cleansing have typically analyzed inorganic residuals by using conductivity as a cleanliness indicator; however, the organic residuals in bottle cleansing processes are susceptible to bacterial enrichment and, therefore, are more crucial for infant health.

Consequently, the purpose of this study was to (1) determine the effects of cleansing variables on cleanliness, (2) examine the interactions between bottle materials and cleansing variables, and (3) suggest an optimal usage and manual cleansing combination for feeding bottles from the perspective of infant health.

MATERIALS AND METHODS

Participants

We recruited eight university student volunteers (two women and six men) as technical assistants to perform the bottle cleansing experiments. Six of the participants were right-hand dominant, and two were left-hand dominant.

Experimental bottles and preparation of the formula milk solution

Infant formula from a manufacturer that provides infant formula to more than 20% of the Taiwanese market was purchased from retail stores in Taipei, Taiwan. The samples were milk-based powders that were packaged in metal containers. Glass and polypropylene (PP) bottles having the identical geometry, and size, and weights

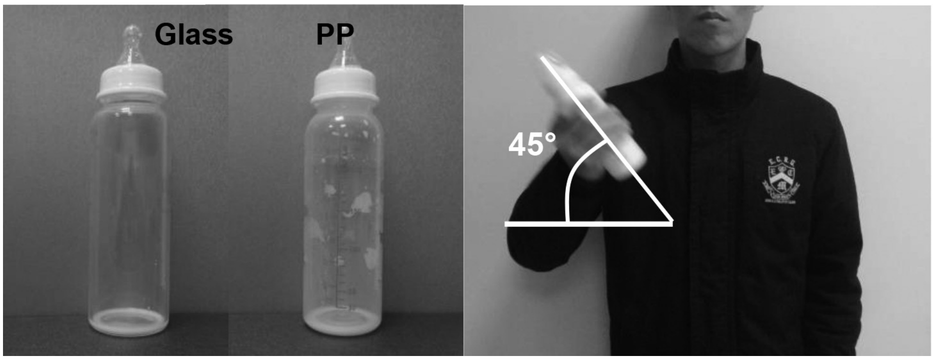

Fig 1–Glass and PP bottles with identical geometry, and the shaking gesture used in this study.

of 176.91 g and 54.57 g, respectively, were purchased from a retail shop (Fig 1).

To simulate the preparation of the formula milk solution in a household and to precisely quantify the residue remaining in a bottle, the recommended preparation of the formula milk solution printed on the exterior of the container was generally followed, but quantitatively measured according to weight. The ratio of formula powder to water in the solution was 1:5 according to weight (precise to 0.01 g), representing the recommended ratio of one spoonful of formula powder to 30 ml of water. The stock formula milk solution was prepared in a 2 liter glass beaker. Distilled and deionized water (1,000 g; conductivity = 0.54 μmho/cm) was poured into a beaker and gradually heated to 60°C. Subsequently, 200 g of formula powder was added and stirred at 100 rpm to form a homogeneous solution that was used in subsequent experiments.

Preliminary experiments were conducted to test the average bottle-feeding residue, and the results indicated that the feeding residue was approximately 4 ml after 240 ml of this milk solution remained after a normal feeding. Therefore, 240 ml of stock formula milk solution was added to the feeding bottle and shaken to wet the entire interior of the bottle. Finally, 236 ml of formula milk solution was poured out to simulate the bottle condition after feeding.

Rinsed-out formula analysis and cleanliness evaluation

Total organic carbon (TOC, OI Analytical 1010) (Environmental Protection Agency, 2009) and conductivity (HACH Sension Conductivity Electrode p/n: 51975-00) measurements were selected as the methods for determining the organic and inorganic constituent concentrations in the rinsed-out water samples. The organic and inorganic cleanliness of the feeding bottles were defined as follows:

$$\text{Organic cleanliness} = \frac{TOC_w \times V_w}{TOC_s \times V_r} \quad (1)$$

$$\text{Inorganic cleanliness} = \frac{Cond_w \times V_w}{Cond_s \times V_r} \quad (2)$$

Where TOC_w and TOC_s represented the TOC concentration (mg/l) in the rinsed-out water sample and the original stock formula milk solution, respectively. V_w(L) and V_r(L) indicated the volume of rinsing water and formula milk solution that remained in a bottle after a feeding was completed, respectively. $Cond_w$ and $Cond_s$ represented the conductivity (mmho/cm) of the rinsed-out water and original stock formula milk solution, respectively. Therefore, both values of organic and inorganic cleanliness ranged from 0-to-1, and a higher value of cleanliness indicated that a bottle was cleaner. The TOC_s and $Cond_s$, were measured as 106.92 g/l and 11.702 mmho/cm, respectively, and V_r was fixed at 4 ml.

Experimental design

In this study, each participant performed 12 combinations of bottle cleansing tasks: two bottle materials (M)×two shaking times (T)×three rinsing water volumes (V). Two bottle materials, glass and PP, which are extensively used in Taiwan, were employed in this study. The shaking times were 5 seconds and 20 seconds. Based on our pilot study, three levels of rinsing water volume were selected, one-third, one-half, and two-thirds bottles capacities. To accurately measure the rinsed formula quantity, 95, 142.5, and 190 ml of distilled water were used to represent the three levels of rinsing water volume. The bottle cleansing performance for each trial was evaluated according to organic and inorganic cleanliness, as defined previously, with values of '0', indicating no cleanliness and '1', indicating completely clean. In the experiment, each participant performed the various testing combinations in a random order.

Experimental procedures

Prior to data collection, participants were requested to watch a 1-min film in which an experienced nursery assistant instructed participants on bottle cleansing (shaking) tasks. Participants then familiarized themselves with the experimental procedures and practiced shaking the bottle for at least 5 minutes before the data were collected. During the experiment, participants were asked to stand and shake the bottle with a 45° angle at an approximate frequency of twice per second (Fig 1). Participants were also requested to shake the bottle in the natural manner. The range of the bottle shaking was approximately 30 cm. When a trial was completed, the rinsed water of the test was sampled and stored at 5°C before further analysis was conducted. In addition, the temperature of the laboratory was controlled at 25°C, and the relative humidity was controlled at 60%.

Statistical analysis

A three-factor experimental design was used to analyze the performance of bottle cleansing, and a three-way analysis of variance (ANOVA) was performed. The dependent variables, organic and inorganic cleanliness, were measured according to the percentage of rinsed formula residue, which ranged from 0-to-1. The independent variables were the two bottle materials, three water volumes, and two shaking times. Each participant was considered as a 'block'. Duncan's multiple range test (MRT) was used for *post hoc* comparison. Experimental data were then analyzed using a statistics program package SPSS® (version 19; IBM, Armonk NY) with a significance level of 0.05. In this study, we also employed a two-way ANOVA to further understand the effects of water volume and shaking time on the cleanliness of the bottles composed of the two materials.

RESULTS

This study aimed to determine the differences in the cleanliness of bottles that were cleaned using various combinations of bottle materials, rinsing water volumes, and sustained shaking times.

Table 1
Means and SDs of organic and inorganic cleanliness of all 12 experimental combinations (n=8).

Variables	Organic cleanliness		Inorganic cleanliness	
	Glass Mean (SD)	PP Mean (SD)	Glass Mean (SD)	PP Mean (SD)
Shaking time=5 seconds				
1/3 bottles	0.345 (0.053)	0.418 (0.052)	0.782 (0.057)	0.791 (0.062)
1/2 bottles	0.422 (0.121)	0.492 (0.046)	0.889 (0.061)	0.896 (0.082)
2/3 bottles	0.707 (0.043)	0.511 (0.115)	0.959 (0.041)	0.898 (0.091)
Shaking time=20 seconds				
1/3 bottles	0.416 (0.068)	0.430 (0.053)	0.785 (0.087)	0.793 (0.098)
1/2 bottles	0.605 (0.095)	0.475 (0.041)	0.905 (0.067)	0.907 (0.090)
2/3 bottles	0.756 (0.133)	0.440 (0.061)	0.953 (0.042)	0.922 (0.098)

Table 2
ANOVA results for organic and inorganic cleanliness data.

Sources	DF	Organic cleanliness			Inorganic cleanliness		
		F	p-value	η^2	F	p-value	η^2
Bottle material (M)	1	26.54	0.000	0.237	0.46	0.488	0.009
Water volume (V)	2	39.08	0.000	0.465	32.27	0.000	0.415
Shaking time (T)	1	3.00	0.087	0.046	0.28	0.599	0.007
M×V	2	22.66	0.000	0.278	1.27	0.286	0.019
M×T	1	13.82	0.000	0.092	0.06	0.807	0.000
V×T	2	1.64	0.200	0.037	0.04	0.959	0.002
M×V×T	2	2.21	0.116	0.066	0.13	0.882	0.004

Table 1 shows the organic and inorganic cleanliness data under all 12 experimental combinations when the participants performing the simulated bottle cleansing tests.

ANOVA of cleanliness

Table 2 displays the ANOVA results of organic and inorganic cleanliness. Organic and inorganic cleanliness results indicated markedly different traits among the tested cleansing variables. Bottle material (M) and water volume (V), as well as two-way interactions of M×V and M×T significantly affected the organic cleanness (all $p<0.001$), whereas the inorganic cleanliness depended only on water volumes ($p<0.001$). Notably, the independent variable of sustained shaking time (T) did not influence organic or inorganic cleanliness (both $p>0.05$).

Table 3 illustrates the means and SDs of the cleanliness response as a function of each independent variable when averaged across other variables. The cleansing performance of the glass bottle was superior to that of the PP bottle in the organic clean-

Table 3
Means and SDs of organic and inorganic cleanliness, and Duncan MRT groups for various independent variables.

Variables	n	Organic cleanliness Mean (SD)	Duncan groups[a]	Inorganic cleanliness Mean (SD)	Duncan groups[a]
Bottle materials					
Glass	48	0.549 (0.180)	A	0.879 (0.093)	A
PP	48	0.462 (0.072)	B	0.868 (0.099)	A
Water volumes					
1/3 bottle	32	0.403 (0.065)	A	0.788 (0.074)	A
1/2 bottle	32	0.499 (0.105)	B	0.899 (0.073)	B
2/3 bottle	32	0.604 (0.162)	C	0.932 (0.074)	B
Shaking times					
5 seconds	48	0.487 (0.138)	A	0.869 (0.090)	A
20 seconds	48	0.524 (0.148)	A	0.877 (0.102)	A

[a]Differing letters within any independent variable indicate statistically significant differences between the means (Montgomery, 1991).

Table 4
Means and SDs of organic and inorganic cleanliness, and Duncan MRT groups for glass and PP bottles.

Variables	n	Organic cleanliness Mean (SD)	Duncan groups[a]	Inorganic cleanliness Mean (SD)	Duncan groups[a]
Glass bottle					
Water volumes					
1/3 bottle	16	0.381 (0.072)	A	0.783 (0.072)	A
1/2 bottle	16	0.513 (0.141)	B	0.897 (0.141)	B
2/3 bottle	16	0.732 (0.099)	C	0.956 (0.099)	C
Shaking times					
5 seconds	24	0.498 (0.072)	A	0.876 (0.180)	A
20 seconds	24	0.601 (0.180)	B	0.881 (0.072)	A
PP bottle					
Water volumes					
1/3 bottle	16	0.425 (0.051)	A	0.792 (0.079)	A
1/2 bottle	16	0.484 (0.043)	B	0.901 (0.083)	B
2/3 bottle	16	0.476 (0.096)	B	0.910 (0.092)	B
Shaking times					
5 seconds	24	0.476 (0.085)	A	0.862 (0.092)	A
20 seconds	24	0.447 (0.054)	A	0.874 (0.109)	A

[a]Differing letters within any independent variable indicate statistically significant differences between the means (Montgomery, 1991).

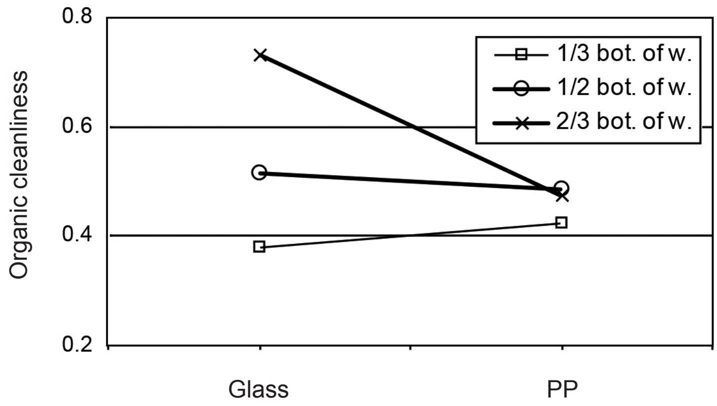

Fig 2–Interaction between the bottle material and the water volume (M×V) on the organic cleanliness.

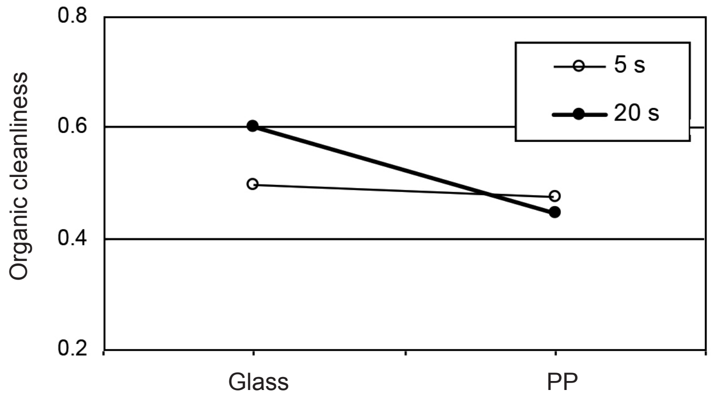

Fig 3–Interaction between the bottle material and the shaking time (M×T) on the collected organic cleanliness.

liness test. The organic cleanliness also increased as water volumes increased. By contrast, inorganic cleanliness did not demonstrate such a distinct association with the tested variables.

Two-way ANOVA results

The two-way interaction of M×V and M×T revealed a significant influence on organic cleanliness (Table 2), which indicates that the bottle material is a crucial variable for bottle cleansing and requires further clarification. As indicated in Table 4, a two-way ANOVA of glass bottles demonstrated that significant differences in organic cleanliness existed because of the main effect of water volume (F2.42=53.94, $p<0.001$, η^2 =0.729) and shaking time (F1.42=13.31, $p<0.005$, η^2=0.250).

The difference in the inorganic cleanliness of glass bottles caused by varying the shaking time was non-significant (F1.42=0.06, $p>0.05$, η^2=0.002), and the effect of water volume remained significant (F2.42=32.49, $p<0.001$, η^2=0.607). As demonstrated in Table 4, both the organic and inorganic cleanliness of glass bottles increased significantly as the water volumes increased. Sustained shaking for 20 seconds flushed more TOC out of the glass bottle than did shaking for 5 seconds, but did not affect conductivity. Regarding PP bottles, water volumes of one-half and two-thirds the bottle capacity enhanced the inorganic cleanliness compared with a water volume of one-third the bottle capacity, and shaking time did not influence either cleanliness indices.

Figs 2 and 3 illustrate the two-way interaction effects on cleanliness. As mentioned previously, the interaction terms were significant only for organic cleanliness. Although the bottle material and water volume affected the organic cleanliness (Table 2), the interaction effect indicated that glass bottles were more sensitive to water volume during cleansing than PP bottles were (Fig 2). A similar trend was observed in the effect of shaking time on cleanliness (Fig 3).

DISCUSSION

Based on a review of the literature, this study was the first to systematically investigate the cleansing performances of infant feeding bottles by combining various water volumes, shaking times, and bottle materials. Our results demonstrated that glass bottles filled with water to two-thirds their capacity demonstrated optimal cleansing performance.

In this study, only 8 participants were recruited to perform the bottle cleansing tests. This relatively small number may be insufficient, and further studies with greater sample sizes should be conducted. Although each participant was considered to be a block in the ANOVA; however, the individual difference did not exist among participants when performing the bottle cleansing (organic: $F7.42=1.34$, $p>0.05$, $\eta^2=0.002$; inorganic: $F7.42=1.8$, $p>0.05$, $\eta^2=0.006$).

In other words, the bottle cleansing performances were influenced by the cleansing variables, not by the participants. This implies that the results from the manual cleansing tests can also be applied to mechanical bottle cleansing tasks.

The detailed constituents of formula are complex, but can be categorized into organic and inorganic compounds. Organic compounds (*eg*, proteins, carbohydrates, and lipids) are relatively non-polar, insoluble in water, and highly hydrophobic compared with inorganic compounds such as mineral ions. The bottle materials of glass and PP that were examined in this study were also inorganic and organic materials, respectively. Water, a well-known polar molecule, is widely used as a cleansing reagent.

Hydrophobicity is the physical property of a molecule that is repelled from a mass of water. Hydrophobic molecules, such as the organic compounds in formula powder, prefer other non-polar molecules and tend to aggregate in water. To be rinsed, the residual formula constituents should first dissolve in rinsing water.

Solubility is the property of a compound to dissolve in a certain volume of water to form a homogeneous solution. Compounds with high solubility, such as inorganic constituents in formula, dissolve more easily than relatively insoluble organic constituents in the identical volume of water do.

Solubility and hydrophobicity could be respectively regarded as the drag and repellent forces between water and residual formula constituents. Because of the distinct chemical properties and interactions among formula constituents, bottle material, and cleansing water, various cleansing performances were anticipated and evaluated in this study.

That different affinity between formula constituents and bottle materials resulted in different quantities of formula residue remaining in the bottle after a feeding merits further clarification by analyzing the morphology of the bottle interiors. For example, organic constituents displaced water molecules from the region near the bottle interior surface to an extent and, therefore, may be associated with the surface through van der Waals, dipole-dipole, and other weak intermolecular forces, because of the unfavorable free-energy costs of remaining in the aqueous solution.

The cleanliness substantially depended on the residual formula constituents that tend to be soluble in water or be hydrophobic and repelled from water. Both water and inorganic formula constituents are polar compounds; therefore, residual inorganic constituents are soluble in water irrespective of the bottle material ($p>0.05$, Table 3). However, the organic cleanliness between the glass and the PP bottles indicated significant differences.

Finally, the effect of shaking time on cleanliness could determine whether the mass transfer of formula constituents in water controls the cleanliness. The statistical results indicated that the two shaking times (5 seconds and 20 seconds) hardly influence organic and inorganic cleanliness. This indicates that mass transfer is not the dominant control mechanism in the cleansing of residual formula.

In our study, the glass bottle was superior to the PP bottle in both organic and inorganic cleanliness, and organic constituents were more difficult to rinse from the bottle than the inorganic constituents. We therefore suggest that filling glass bottles with water to two-thirds their capacity and shaking the bottles for 20 seconds is the optimal combination for manual cleansing. Furthermore, the participants recruited from the university student group in the study did not affect the bottle cleansing performance. The finding of the optimal cleansing procedure combined with different variables needs to be validated by other populations, and different bottle cleansing gestures is also a matter for further investigation.

ACKNOWLEDGEMENTS

The authors would like to thank Chang PR, Chen LL, Chen TW, Chen TY, Chuang KC, Ho WY, Lee BT, Lee W, Liu TA, Liu YN, Yeh YC, Yu DP, and Yuan CT (alphabetically arranged by surname) for their contributions to the experiment and data processing.

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