Removal of Endocrine Disruptors in Milk by Circulation Through Polydimethylsiloxane Tubing

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Received 7 October 2005; accepted 9 January 2006
DOI 10.1002/app.24128
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A simple circulating system was developed, in which an aqueous solution, cow milk, or human milk was circulated through hydrophobic polymeric tubing to remove the endocrine disruptors from the solution by sorption into the tubing. The effect of circulating parameters, such as tube length, circulating time, and flow rate, against the removal ratio (R) of endocrine disruptors was investigated. R of 1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH, lindane) increased with the length of the hydrophobic polymeric tubing, circulating time, and flow rate when cow milk containing 1 ppm γ-HCH was circulated through polydimethylsiloxane tubing. The R values of several endocrine disruptors with different octanol–water distribution coefficients (log P_{ow}) was investigated for γ-HCH and dichlorodiphenyltrichloroethane in an aqueous solution, cow milk, and human milk. A similar trend for R versus log P_{ow} of the human milk to that of the aqueous solution and cow milk was observed. The R values of the endocrine disruptors decreased in the following order: Aqueous solution > Human milk > Cow milk. Stable micelles in cow milk disturbed the shift of the endocrine disruptors from the milk micelles into the hydrophobic tubing. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 3634–3640, 2006

Key words: bioengineering; membranes; polysiloxanes; rubber; separation techniques

INTRODUCTION

Human milk is an important food source for infants due to its unique immunological and nutritional properties. Several medical studies have reported that breastfeeding infants have the advantage of protection against infectious disease[1–3] and allergies[4] and enhanced development and intelligence.[5] However, human milk is known to contain trace amounts of endocrine disruptors, such as polychlorinated biphenyls (PCBs), chlorinated dibenzo-*p*-dioxin, dichlorodiphenyltrichloroethane (DDT), and 1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH; lindane).[6–9] The concentrations of PCBs and dioxins in human milk in several countries are so high that the breastfed infant's intake exceeds the tolerable daily intake.[6–9] However, no method for the removal of endocrine disruptors from human milk has been reported, with the exception of that reported in our preliminary study.[10]

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Contract grant sponsor: High-Tech Research Center Project, Ministry of Education, Culture, Sports, Science, and Technology, Japan.
Contract grant sponsor: Asahi Glass Foundation.
Contract grant sponsor: Iwatani Foundation.
Contract grant sponsor: Salt Science Foundation.

Previously, the preferential sorption of chemical contaminants, such as dibenzo-*p*-dioxin, biphenyl, 1,2-dibromo-3-chloropropane, 2-sec-buthylphenyl-methylcarbamate, 2,2-dimethyl-1,3-benzodioxol-4-yl-methylcarbamate (bendiocarb), *n*-buthylbenzene, diethylphthalate (DEP), dibutylphthalate, and γ-HCH, onto hydrophobic flat-sheet membranes to remove chemical contaminants in water was reported.[10] Endocrine disruptors, such as polychlorinated dioxins, DDT, PCBs, and agricultural chemicals, have hydrophobic characteristics similar to hydrophobic estrogen; therefore, these chemical contaminants are expected to leach from the water phase into the hydrophobic membranes.[10–12] The removal ratio (R) of γ-HCH in human milk was investigated previously[10] and was found to be significantly lower than that in an aqueous γ-HCH solution. Furthermore, it was suggested that a large absorbance capacity of the hydrophobic polydimethylsiloxane (PDMS) membranes was necessary to completely remove the endocrine disruptors with high octanol–water distribution coefficients (P_{ow}'s): γ-HCH, PCBs, and polychlorinated dibenzo-*p*-dioxin. This was thought to be because these chemical contaminants were difficult to remove from lipid and/or protein micelles in the milk (milk micelles) into the hydrophobic membranes after the membranes came into contact with milk containing chemical contaminants.

Herein, hydrophobic polymeric tubing was selected as an absorbent for endocrine disruptors due to its

higher surface area compared with the polymeric flat-sheet membranes. A simple circulating system was developed, in which an aqueous solution, cow milk, or human milk was circulated through the hydrophobic polymeric tubing to remove the endocrine disruptors from human milk. This system worked by sorption onto the hydrophobic polymeric tubing. The effect of circulating parameters, such as tube length and circulating speed, against R was investigated, and R of several endocrine disruptors, including γ-HCH and DDT, in the aqueous solution, cow milk, and human milk with different log P_{ow} values were investigated.

EXPERIMENTAL

Materials

PDMS tubing (diameter = 2.15 mm × 4.2 mm; Tokyo Rika Kikai Co., Ltd.) and three types of rubber tubing (Taigon, Farmade, and Furlan; diameter = 2.15 mm × 4.2 mm; Tokyo Rika Kikai) were used. We pretreated the tubing by circulating 2-propanol through the tubing twice for 12 h, with fresh 2-propanol for each procedure, at 25°C. After the tubing was rinsed, it was subsequently dried *in vacuo* for 4 h at 160°C. Untreated tubing was also used as a control.

Model endocrine disruptors were purchased from Wako Pure Chemicals Industries, Ltd., and are listed together with their physical properties in Table I. Scheme 1 shows a schematic of the endocrine disruptors. Cow milk was purchased from Meiji Milk Products Co., Ltd. Human milk was obtained from a 33-year-old woman volunteer living in Tokyo. Other chemicals were of reagent grade and were used without further purification. Ultrapure water was used throughout the experiment.

Sorption and desorption experiments

A quantity of 25 mL of 1 ppm of endocrine disruptors (see Table I) in the aqueous solution, cow milk, or human milk was circulated for 0.5–24 h at 25°C through the tubing (1–4 m) with a perister pump (MP-1000, Tokyo Rika Kikai), as illustrated in Figure 1. After the sorption experiments, ultrapure water was circulated through the tubing for 1 h. A quantity of 25 mL of 2-propanol at 25°C was subsequently circulated through the tube for 3 h as a desorption experiment. The endocrine disruptors in the aqueous solution, cow milk, or human milk shifted to the hydrophobic tubing during the sorption procedure and again shifted into the 2-propanol solution during the desorption procedure. The concentration of each endocrine disruptor in 2-propanol [C_d (ppm)] was analyzed with a gas chromatograph/mass spectrometer (GCMS-QP5050A, Shimadzu Co.). The R values of the endocrine disruptors in the desorption experiment were obtained as follows:

$$R(\%) = C_d/C_b \times 100 \quad (1)$$

where C_b was the initial concentration of endocrine disruptors solution. C_b was 1 ppm in the current investigation.

Gas chromatography/mass spectrometry (GC–MS) equipment

Aliquots (1 μL) of the endocrine disruptor solution were analyzed from a splitless injection with a gas chromatograph/mass spectrometer equipped with a type AOC-20 autosampler and a DB-1 capillary column (0.25 mm, *i.d.* = 30 m, J&W Scientific Co.). The injection and detection temperatures were set at 250°C. The column temperature was programmed as follows: 60°C for 2 min, heating at 20°C/min up to 250°C, and maintenance at 250°C for 10 min.

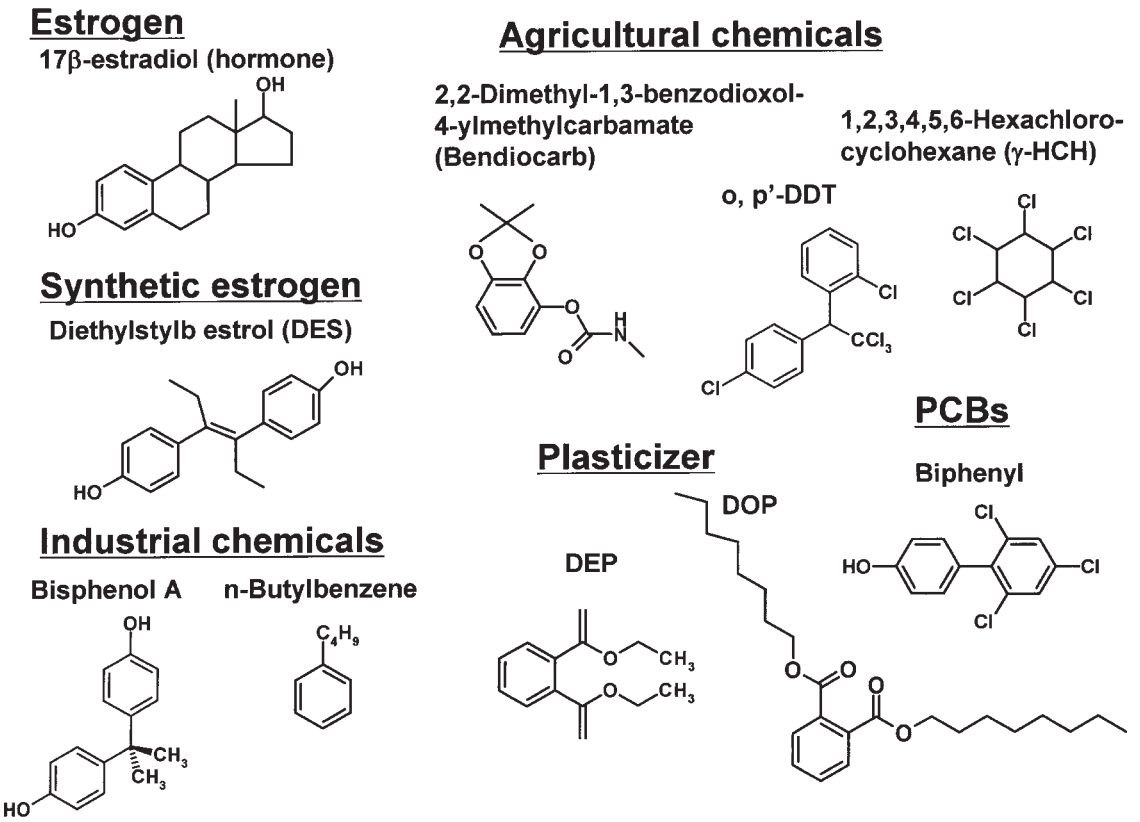
RESULTS AND DISCUSSION

Selection of optimal sorption materials

Several different types of hydrophobic polymeric tubing were evaluated for the optimal removal of endocrine disruptors from the aqueous solution, cow milk, and human milk. The leakage of contaminants from the tubing with a desorption procedure was first investigated. In the desorption procedure, 2-propanol was circulated through the tubing for 3 h at 25°C. Figure 2 shows the GC–MS spectra of effluent into 2-propanol from untreated Tigon tubing, untreated

TABLE I
Properties of the Endocrine Disruptors

Endocrine disruptors	Molecular weight	Use	Water solubility (mg/L)[11–16]	log P_{ow}[11–16]
Biphenyl	154.2	Insecticide	0.0089	3.16
2,2-Dimethyl-1,3-benzodioxol-4-yl methylcarbamate (Bendiocarb)	223.2	Pesticide	260	1.70
DEP	222.2	Plasticizer	1080	2.42
DOP	390.6	Plasticizer	3	4.89
Lindane (HCH)	290.8	Insectcide	8	4.26
DDT	354.5	Pesticide	0.010	6.91



Scheme 1

Furlan tubing, untreated PDMS tubing, and pretreated PDMS tubing. Dioctylphthalate (DOP) and di(2-ethylhexyl)phthalate were found to leak from untreated Tigon tubing. This was confirmed by a significant peak at 38 min in the GC–MS spectra of the 2-propanol. This was thought to be because Tigon tubing is made of poly (vinyl chloride) containing DOP and/or di(2-ethylhexyl)phthalate.

A large, broad peak at 45 min was observed in the GC–MS spectra of 2-propanol circulated through

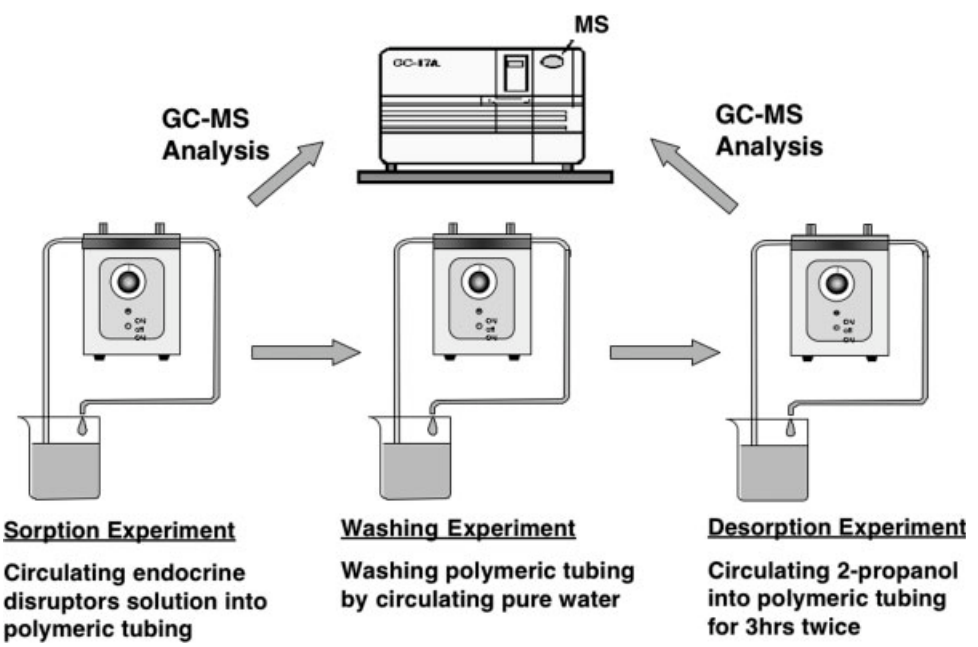


Figure 1 Schematic diagram of the circulating system for the aqueous solution and milk containing chemical contaminants through hydrophobic polymeric tubing.

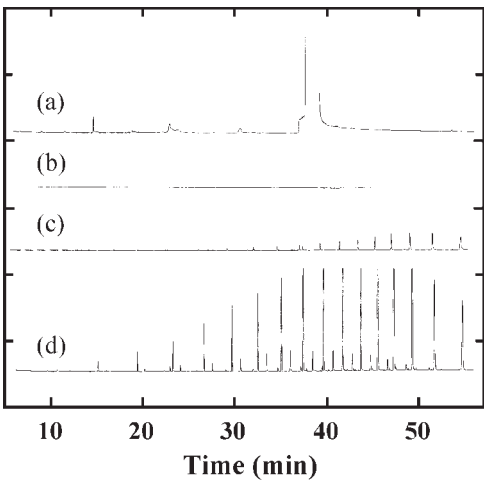


Figure 2 GC–MS spectra of effluent in 2-propanol from untreated (a) Tigon tubing, (b) untreated Furlan tubing, (c) pretreated PDMS tubing, and (d) untreated PDMS tubing. 2-Propanol was circulated through the tubing for 3 h at 25°C before GC–MS analysis.

Farmade for 3 h on desorption (data not shown). This was also attributed to the effluent of plasticizer from the Farmade tube, which was made of an amorphous fluorine polymer. No contaminants were found in the 2-propanol after desorption from untreated Furlan tubing, which was made of a fluorine polymer. Several siloxane oligomers were observed in the GC–MS spectra of the 2-propanol after desorption with untreated PDMS tubing. However, only small peaks attributed to siloxane oligomers were observed in the GC–MS spectra of the 2-propanol used in the desorption from the pretreated PDMS tubing. Pretreatment was found to significantly reduce the effluent of siloxane oligomers from the PDMS tubing.

The siloxane oligomers observed in Figure 2 are the effluent from the PDMS tubing into 2-propanol for the desorption measurements and not into the aqueous solution or human milk. No effluent from the untreated and pretreated PDMS tubing was observed in the aqueous solution from the GC–MS measurements.

The removal ability of γ-HCH from the aqueous solution and cow milk was evaluated in this circulation method with the hydrophobic polymeric tubing, with the exception of Farmade tubing. This was because the plasticizer effluent from Farmade tubing was too large to evaluate *R* of the endocrine disruptors adequately. Figure 3 shows *R* of γ-HCH after the circulation of 25 mL of the aqueous solution and cow milk containing 1 ppm γ-HCH into 2 m of pretreated PDMS, Tigon, and Furlan tubing for 3 h at 25°C. *R* from the aqueous solution was significantly higher than that from cow milk. This indicates that hydrophobic endocrine disruptors were contained in the lipid and/or protein micelles (milk micelles) and that the milk micelles disrupted the sorption of the endocrine disruptors from milk micelles to the hydrophobic tubing. *R* of γ-HCH from the aqueous solution with PDMS tubing was the highest among the different types of tubing, although *R* of γ-HCH from cow milk with pretreated PDMS tubing was less than 30% under these conditions. Therefore, the PDMS tubing was selected for use in the following experiments.

Improvement of *R* of endocrine disruptors from milk

The optimal conditions for the removal of endocrine disruptors from milk were investigated with the circulation method with cow milk through the PDMS tubing. First, the effect of tube length was investigated by the circulation of the solution of endocrine disruptors. Figure 4 shows the dependence of *R* of γ-HCH from cow milk on the length of pretreated and untreated PDMS tubing after 25 mL of cow milk containing 1 ppm γ-HCH was circulated for 3 h at a flow rate of 90.6 mL/h. *R* of γ-HCH increased with increasing tube length. This was attributed to the increase in the contact time of the endocrine disruptors in the cow milk with the PDMS tubing. No significant difference in *R* with pretreated PDMS tubing and untreated PDMS tubing was observed ($p > 0.05$).

The circulating time of cow milk through the PDMS tubing was also investigated. Figure 5 shows the dependence of *R* of γ-HCH from cow milk on the circulating time of cow milk after 25 mL of cow milk containing 1 ppm γ-HCH was circulated through 2 m of PDMS tubing at a flow rate of 90.6 mL/h. *R* of γ-HCH increased with increasing circulating time. A significant amount of time was required to shift the endocrine disruptors in the milk micelles to the hydrophobic tubing. No significant change in the appearance of the cow milk (no milk coagulation) was observed after

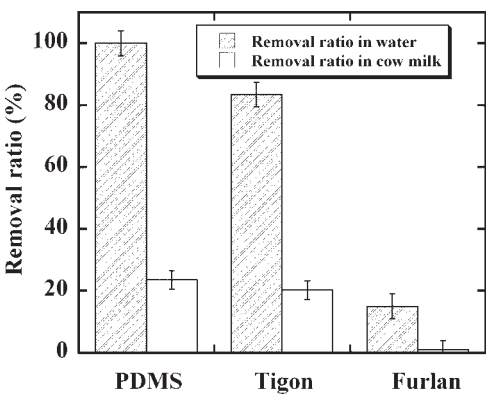


Figure 3 *R* of γ-HCH after the circulation of 25 mL of the aqueous solution and cow milk containing 1 ppm γ-HCH into 2 m of pretreated PDMS, Tigon, and Furlan tubing for 3 h at a flow rate of 90.6 mL/h and 25°C.

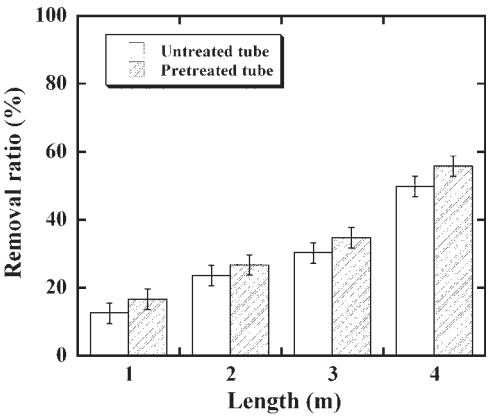


Figure 4 Dependence of R of γ-HCH from cow milk on the length of pretreated and untreated PDMS tubing after 25 mL of cow milk containing 1 ppm γ-HCH was circulated for 3 h at a flow rate of 90.6 mL/h and 25°C.

circulation through the PDMS tubing for 24 h. Furthermore, no difference in R with pretreated PDMS tubing and untreated PDMS tubing was observed ($p > 0.05$).

The flow rate of cow milk and human milk through the pretreated PDMS tubing was investigated. Figure 6 shows the dependence of R of DDT from cow milk and human milk on the flow rate of the milk after 25 mL of cow milk and human milk containing 1 ppm DDT was circulated through 2 m of the pretreated PDMS tubing for 3 h. R of DDT from both cow milk and human milk increased with increasing flow rate. This was also attributed to the increase in the contact time of endocrine disruptors in the milk with the PDMS tubing. R of DDT from human milk was higher than that from cow milk ($p < 0.05$) at a flow rate of milk greater than 90.6 mL/h.

Human milk sometimes showed a phase separation when the milk was left stationary for more than 30 min, whereas cow milk was always homogeneous, even after it was left stationary for several weeks. This was thought to be because the lipid and/or protein micelles in cow milk were more stable than the micelles in human milk. Therefore, the stable micelles in cow milk disturbed the shift of endocrine disruptors from the milk micelles to the hydrophobic polymeric tubing, which resulted in the lower R from cow milk than from human milk.

Removal of several endocrine disruptors

Several model endocrine disruptors are listed in Table I. The following endocrine disruptors were selected, agricultural chemicals, bendiocarb, pesticides, DDT, γ-HCH, model PCBs, biphenyl, plasticizer, DEP, and dibutylphthalate (DBP), and Rs from the aqueous solution, cow milk, and human milk were investigated by the circulation of the solution through 2 m of the pretreated PDMS tubing for 3 h at a flow rate of 90.6 mL/h. Figure 7 shows the relationship between log P_{ow} and R of the endocrine disruptors from the aqueous solution. C_b was 1 ppm. We investigated the removal of endocrine disruptors at $C_b = 1$ ppm in this study, which was a higher concentration than that in human milk found in the order of parts per billion. However, the removal mechanism of endocrine disruptors by the circulation of the human milk through pretreated PDMS tubing was based on the sorption mechanism of Henry's law. Therefore, no concentration dependence of R of endocrine disruptors was theoretically expected.

R of the endocrine disruptors in the aqueous solution was more than 90% at log $P_{ow} \leq 4.5$. However, R of the endocrine disruptors decreased with increasing log P_{ow} at log $P_{ow} > 4.5$. Therefore, highly hydrophobic endocrine disruptors such as DOP and DDT are suggested to be more difficult to remove from aqueous solution than slightly hydrophobic endocrine disruptors such as bendiocarb and DEP.

Figure 8 shows the relationship between log P_{ow} and R of the endocrine disruptors from cow milk. C_b was 1 ppm. R from cow milk and that from the aqueous solution showed a similar trend; the highly hydrophobic endocrine disruptor DDT showed the lowest R. However, R from cow milk was lower than that from the aqueous solution. This was explained as the stable micelles of lipids and/or proteins in the cow milk disrupting the shift of endocrine disruptors from the milk micelles to hydrophobic PDMS tubing in the sorption procedure (the circulating procedures for cow milk through the tubing).

Figure 9 shows the relationship between log P_{ow} and R of the endocrine disruptors from human milk. C_b was 1 ppm. R versus log P_{ow} of human milk to that in the aqueous solution and cow milk showed similar trends. R of each endocrine disruptor followed the following order: R from aqueous solution

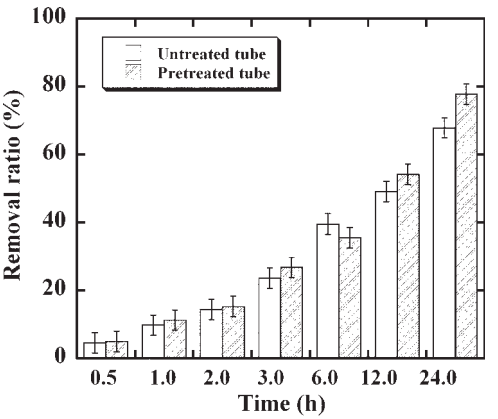


Figure 5 Dependence of R of γ-HCH from cow milk on the circulating time of cow milk after 25 mL of cow milk containing 1 ppm γ-HCH was circulated through 2 m PDMS tubing at a flow rate of 90.6 mL/h and 25°C.

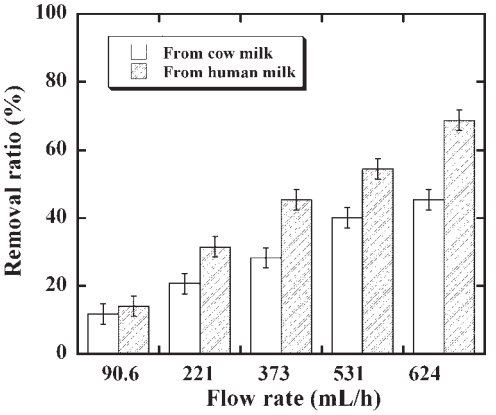


Figure 6 Dependence of R of DDT from cow milk and human milk on the flow rate of milk after 25 mL of cow milk and human milk containing 1 ppm DDT was circulated through 2 m of pretreated PDMS tubing for 3 h at 25°C.

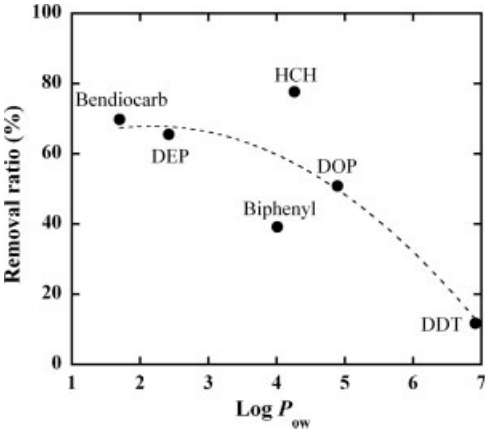


Figure 8 Relationship between log P_{ow} and R of the endocrine disruptors from cow milk. The cow milk containing 1 ppm of endocrine disruptors was circulated through 2 m of PDMS tubing for 3 h at a flow rate of 90.6 mL/h and 25°C.

$>$ R from human milk $>$ R from cow milk. Micelles in human milk were less stable than those in cow milk; therefore, R in human milk higher than that from cow milk. Although R of DDT from human milk was found to be less than 20% by the circulation of human milk through 2 m of pretreated PDMS tubing for 3 h at a flow rate of 90.6 mL/h, R of DDT from human milk was improved as much as 72% by the circulation of human milk through 4 m of pretreated PDMS tubing for 3 h at a flow rate of 624 mL/h.

Sorption of proteins

One of the disadvantages for the application of the removal of endocrine disruptors from human milk for infants by the circulation method with hydrophobic PDMS tubing might be the decrease of some nutrients in the human milk due to their cocurrent sorption into the PDMS tubing. One of the important nutrients in human milk is protein, such as caseins and immunoproteins, to strengthen the immune system of the infants. Therefore, R of γ-globulin and casein into the PDMS tubing was investigated, and the results are listed in Table II. R was found to be less than 1.5% with 1000 ppm of γ-globulin or casein solution by the circulation of the aqueous protein solution through 4 m of pretreated PDMS tubing for 3 h at a flow rate of 624 mL/h. We suggest that the protein molecules were too large to be sorbed into the PDMS tubing.

CONCLUSIONS

Endocrine disruptors, such as γ-HCH and DDT, were efficiently removed from milk by a simple circulation method with hydrophobic PDMS tubing. R

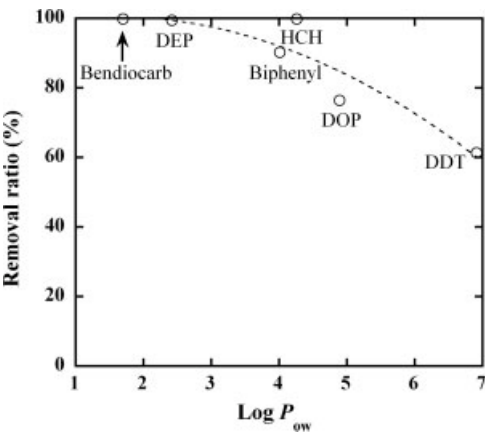


Figure 7 Relationship between log P_{ow} and R of the endocrine disruptors from the aqueous solution. The aqueous solution containing 1 ppm of endocrine disruptors was circulated through 2 m of PDMS tubing for 3 h at a flow rate of 90.6 mL/h and 25°C.

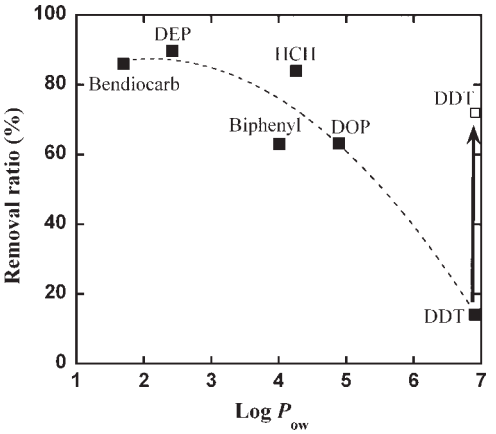


Figure 9 Relationship between log P_{ow} and R of the endocrine disruptors from human milk. The human milk containing 1 ppm of endocrine disruptors was circulated through (■) 2 m of PDMS tubing for 3 h at a flow rate of 90.6 mL/h and 25°C and (□) 4 m of PDMS tubing for 3 h at a flow rate of 624 mL/h and 25°C.

TABLE II
***R* of Proteins from Aqueous Protein Solution by the Circulation Method with 4 m of Pretreated PDMS Tubing for 3 h at a Flow Rate of 624 mL/h**

Protein	Concentration (ppm)	*R* (%)
γ-Globulin	1000	0.3
Casein	1000	1.4

of endocrine disruptors was observed to increase with increasing length of hydrophobic polymeric tubing, circulating time, and flow rate when cow milk containing 1 ppm of γ-HCH or DDT was circulated through PDMS tubing. *R* of the endocrine disruptors was in the following order: Aqueous solution > Human milk > Cow milk. Stable micelles in cow milk disturbed the shift of endocrine disruptors from the milk micelles to the hydrophobic tubing, which resulted in a lower *R* from cow milk than that from both human milk and the aqueous solution. Extremely hydrophobic endocrine disruptors such as DOP and DDT may be removed less efficiently than less hydrophobic endocrine disruptors such as γ-HCH and DEP from both cow milk and human milk. This is thought to be because the more hydrophobic endocrine disruptors tend to remain in the milk micelles and not to shift to the hydrophobic tubing. Furthermore, the stability of the micelles containing endocrine disruptors disturbed the removal of endocrine disruptors from milk. Because micelles in human milk were less stable than those in cow milk, *R* in human milk was higher than that from cow milk.

R of DDT from 25 mL of human milk was as high as 72% by the circulation of the human milk through 4 m of the pretreated PDMS tubing for 3 h at a flow speed of 624 mL/h. The circulation of milk into longer PDMS tubing at a higher flow rate and for longer circulating times was expected to destroy the milk micelles due to higher shear stress and may have accelerated the shift of endocrine disruptors in the milk micelles to the hydrophobic PDMS tubing. Therefore, the most harmful endocrine disruptors present in human milk, such as DDT, PCBs, and polychlorinated dioxin, can be removed with this circulating method with long hydrophobic tubing at a high flow rate and for long circulation times.

γ-Globulin and casein were not sorbed into the PDMS tubing because the protein molecules were too large to be sorbed into the PDMS tubing. Water-soluble vitamins, such as vitamin B_2 and B_{12} and niacin, were not expected to be sorbed into the PDMS tubing. On the other hand, hydrophobic nutrients with low molecular weights, such as hydrophobic vitamins, however, may be removed. Therefore, a solution for obtaining human milk safe for the consumption of infants may be to first treat the milk with the circulating method with hydrophobic PDMS tubing described herein and then supplement it with the nutrients that were removed.

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