Bull. Environ. Contam. Toxicol. (2005) 74:373–379

DOI: 10.1007/s00128-004-0594-7

Bulletin of Environmental Contamination and Toxicology

Phthalate Levels in Baby Milk Powders Sold in Several Countries

K. Yano,[1] N. Hirosawa,[2] Y. Sakamoto,[2] H. Katayama,[3] T. Moriguchi,[1] K. Asaoka[4]

[1] Department of Chemistry, Saitama Medical School, 981 Kawakado, Moroyama, Iruma-gun, Saitama 350-0496, Japan
[2] Department of Biomedical Research Center, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-gun, Saitama 350-0495, Japan
[3] Saitama Medical College, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-gun, Saitama 350-0495, Japan
[4] Department of Cellular and Molecular Biology, Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan

Received: 15 August 2004/Accepted: 17 November 2004

Phthalates are present everywhere in homes and in the environment, and human exposure to them is widespread and at higher levels than previously anticipated (Blount et al., 2000; Silva et al., 2004; Kato et al. 2004; Hauser et al, 2004). We studied phthalate levels in the blood of wild and breeding monkeys in Japan demonstrating importance of the environmental factors for uptake of phthalates in these animals (Asaoka et al., 2000). Furthermore, we investigated phthalate levels in the same kinds of beverages that were sold in Japan and Korea, discussing some factors for different pollution of phthalates between Japan and Korea (Yano et al., 2001). These findings demonstrate that distributions of phthalates vary not only in different localities within a country but also in different countries.

Phthalates have received a great amount of public attention from various aspects since some of them are considered to be endocrine disruptors (Latini et al., 2000; Lovekamp-Swan et al., 2003). Although the risk of phthalates to humans has not been proved, several studies suggest their endocrine disrupting properties. Phthalates may be related to the recent decline in the male ratio (Vartiainen et al., 1999), the decreasing values of sperm motility (Fredricsson et al., 1993), the earlier pubertal maturation observed in young American girls (Herman-Giddens et al., 1997), the premature breast development in young Puerto Rican girls (Colón et al., 2000), a shorter pregnancy duration (Latini et al., 2003), and reduction in intelligence and increase in risk of cancer (Melnick, 2001).

The infant exposure of phthalates in baby milk powders has become a great issue (MAFF, 1996; Scowen P, 1996; Latini 2000), since the infants have a low body weight, take milk as the sole source of nutrition in the early months, and are more sensitive to a variety of chemicals than the adults. Thus, we decided to evaluate the amounts of di(n-butyl)phthalate (DBP) and di(2-ethylhexyl)phthalate (DEHP) in the baby milk powders in different countries.

MATERIALS AND METHODS

Baby milk powders (27 samples) were purchased from supermarkets or local open markets in several cities in Europe, America, Canada, and Asia during 2001-2002 and collected in the Department of Chemistry, Saitama Medical School. These

Correspondence to: K. Yano

samples were opened at the same time in the same place for evaluation of their phthalate levels. All solvents and water with high purity grade for analysis of phthalates and deuterated phthalates, di(n-butyl)phthalate (DBP-d4) and di(2-ethylhexyl)phthalate (DEHP-d4), were purchased from Wako Pure Chemical Industries, LTD (Osaka, Japan). All glassware was heated at 220°C overnight before use.

To 2 g of each sample in a test tube with a glass cap, was added 1 ml of a mixture of DBP-d4 (1.058 μg) and DEHP-d4 (1.212 μg) in acetonitrile as the internal standards, and the tubes were kept in a refrigerator overnight. To each tube, was added 4.5 ml of acetonitrile saturated with hexane, and it was sealed with a glass cap. The mixture was stirred with a MIXER N-80M (Nissinrika, Tokyo, Japan) for 3 min and treated with an ultrasonic washer US-3 (Iuchi, Osaka, Japan) for 20 min. The resulting mixture was centrifuged at 4000 rpm for 20 min. The upper solution separated from the solid was filtered through a Minisart-RC (Sartorius AG, Gőttingen, Germany), and the solution (2 ml) was washed with 0.5 ml of hexane saturated with acetonitrile once.

The acetonitrile solution (1μml) was injected into a GCMS-QP5050 (Shimadzu, Kyoto, Japan) equipped with a ZB-5 column (0.25 mm i.d. x 30m, 0.25μm film thickness) (Phenomenex, Torrance, USA). The injection port and interface were kept at 260°C. The oven temperature was maintained at 50°C for the first 1min and then raised to 15°C/min to 270°C keeping it for 5 min. Each phthalate was determined comparing the peak area with the corresponding isotopic standard in GC/MS. All blank values were averaged and the averaged value was subtracted from the detected phthalate values. For phthalate detected in the blank tests, the limit of detection was determined as three times of the standard deviation of blank values. The limit of quantification was set as twice of the limit of detection.

RESULTS AND DISCUSSION

The 27 baby milk powders consisted of 3 brands obtained in Japan, 3 in Taiwan, 4 in Vietnam, 2 in Thailand, 2 in Indonesia, 2 in Hong-Kong, 2 in Turkey, 2 in UK, 1 in Germany, 2 in USA, and 4 in Canada, but their production countries were not always the same countries in which the milk powders were sold. The production countries were Japan (3 brands), Taiwan (3 brands), Vietnam (1 brand), Turkey (1 brand), UK (1 brand), Germany (1 brand), Spain (1 brand), the Netherlands (3 brands), New Zealand (2 brands), Denmark (1 brand), Ireland (2 brands), and USA (7 brands). Thus, in Japan, in Taiwan, in USA, and in Germany, all samples were produced in their own countries, but in Canada, in Hong-Kong, in Indonesia, and in Thailand, they were imported from other countries. In UK, in Vietnam, and in Turkey, some of them were produced in their own countries but others were imported from other countries.

Typical GS-MS chromatograms of a baby milk extract are shown in Fig.1, in which **A** shows a total ion chromatogram, and **B** and **C** show mass chromatograms of m/z 149 and m/z 153, respectively. As seen in Fig.1 (**B**), there were small peaks in addition to DBP and DEHP, but those peaks were not identified since they were not always found in all samples. Each sample was analyzed three times independently, and the average values were used for an evaluation of phthalate levels. The results are summarized in Fig.2 (**A**). We found that all samples contained both DEHP and DBP, but the amounts of DEHP (range 34 to 281 ng/g) were much higher than DBP (range 15 to 77ng/g) in every country. These values were in an agreement with the amounts of DBP (range <20-85ng/g) and DEHP (range <50-196ng/g) in German milk powders (Gruber et al. 1998).

The DEHP level was highest in the milk powders sold in Turkey (281ng/g), followed by in Japan (218ng/g), in UK (180ng/g), in Thailand (172ng/g), and in Vietnam (123ng/g). On the other hand, the DBP level was highest in the milk powders sold in Japan (77ng/g), followed by in Taiwan (51ng/g) and in Indonesia (32ng/g). The phthalate levels in the other countries were not much different. The observed differences in the phthalate levels might be due to the coatings of the milk cans and lids. Thus, we studied the containers by infrared spectroscopy finding that they were coated with epoxy resin, polyethylene resin, or poly vinyl chloride resin (data not presented). However, we could not find any evidence that the phthalate levels were related to these coatings.

Next, we compared the phthalate levels in the samples concerning their production countries. The results are shown in Fig.2 (**B**). The DEHP level in the milk produced in Turkey was found to be highest but the DBP level was lowest among all the samples. We do not know the reason for the observed high phthalate, but it may be due to the processing equipments including plasticized tubing, films, surface coatings, gaskets, liners or seals for container caps used in the food industry or raw cow milk (Castle et al., 1990). When comparing the total phthalate levels of the 3 brands of milk powders from Japan, Taiwan and the Netherlands, Japanese milk powders contained about twice amounts of the phthalates than Taiwanese or Dutch ones. This finding supports the previous finding that Japanese beverages are approximately twice more polluted by phthalates than the corresponding Korean beverages (Yano et al., 2002). The high phthalate pollution of Japan may be due to the loose regulation on environmental residue of phthalates by the Japanese government compared to the USA or European governments (Katase, 2001).

Assuming that a bodyweight of a new-born infant is 3 kg and a daily intake volume of the aqueous milk is 700 mL, we calculated daily intakes of DEHP and DBP from the Turkish and Japanese milk powders that contained the highest amounts of DEHP and DBP, respectively. The former was 16.1μg/kg bodyweight/day and the latter was 2.5μg/kg bodyweight/day. Since the European Commission Scientific Committee has set the tolerable daily intakes (TDI) for DEHP of 37μg/kg bodyweight/day and DBP for 100μg/kg bodyweight/day (MAFF, 1996), the obtained values were corresponding to 43.5% and 2.5% of TDI, respectively. Since the amounts of phthalates in other samples were lower than the above values, all baby milk powders investigated here may not be any risk. Babies, however, have a low body weight and take the milk powders as the sole source of nutrition in the early months. It is therefore right that manufactures should be seeking to identify the source and trying to reduce the phthalate levels in the milk powers even though they were not harmful (Scowen, 1996). Further research of the milk powders is required to establish the reason for any sources of phthalates present in the powders

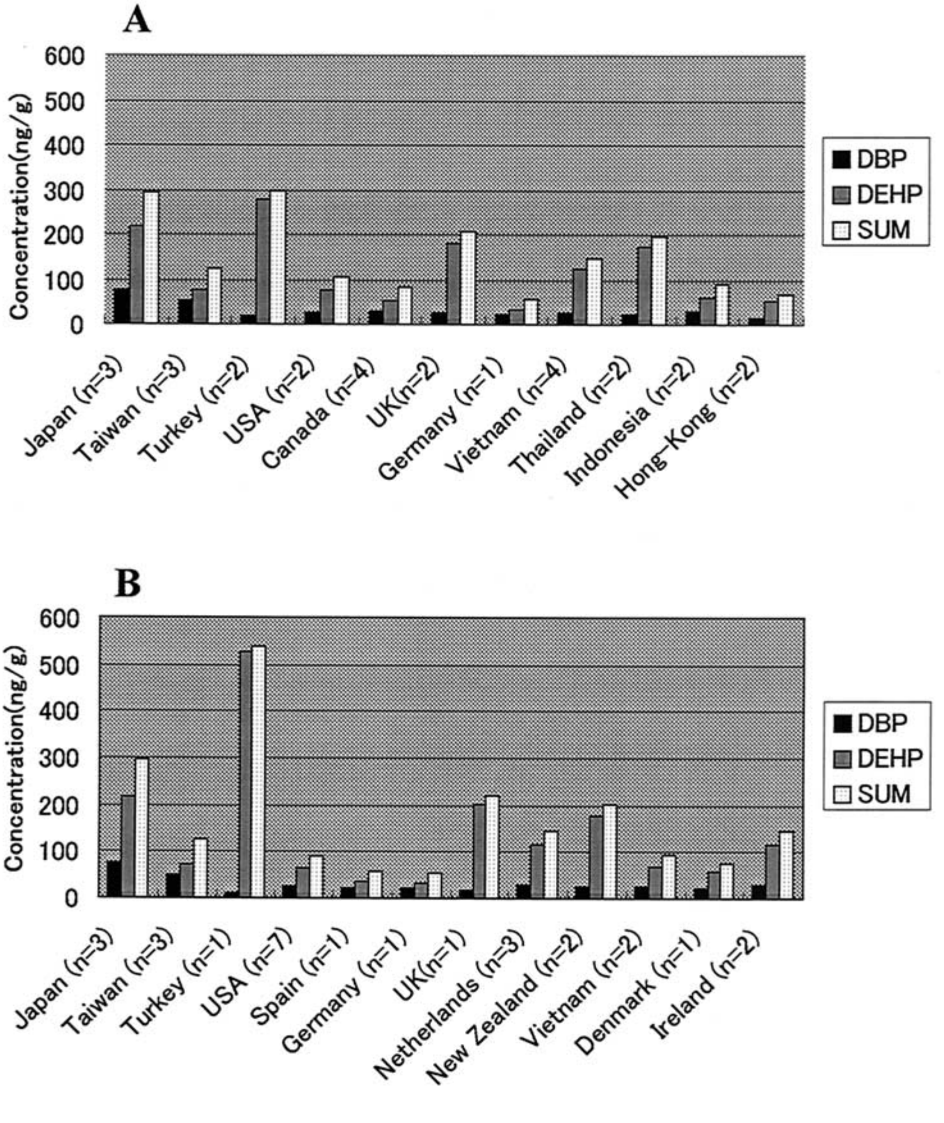

Figure 2. Concentration of phthalates in baby milk powders in different countries. Phthalate levels in baby milk in consumer (**A**) and production (**B**) countries.

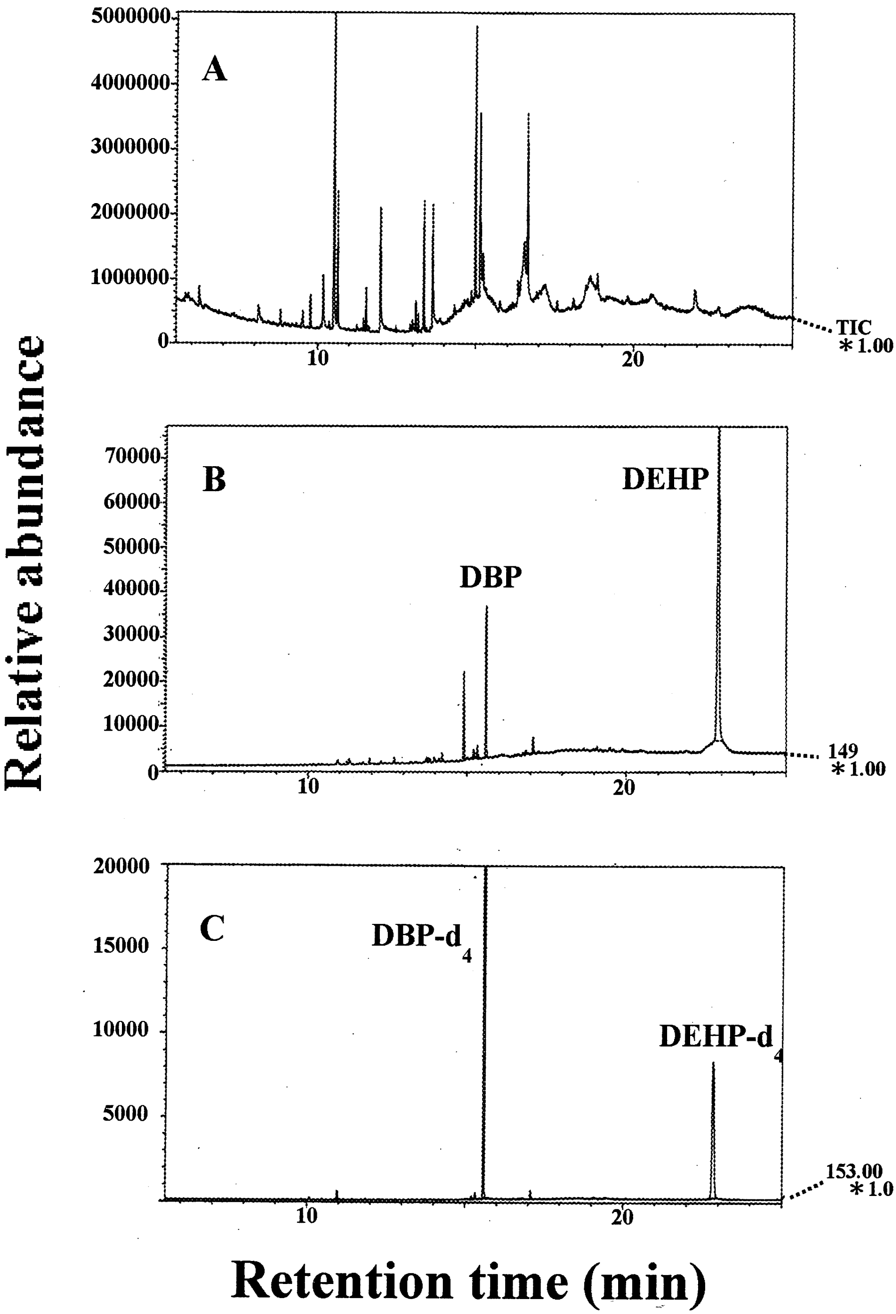

Figure 1. GC-MS chromatograms of baby milk powder extract. **A** shows a total ion chromatograms, and **B** and **C** show mass chromatograms of m/z 149 and 153, respectively.

and for any systematic variation between different brands.

Effects of phthalates on humans have not been proved, but there are several suggestions for their effects on human health. Phthalates can induce the premature breast development (Colón et al., 2000) as well as the earlier pubertal maturation in young girls (Herman-Giddens et al., 1997), a shorter pregnancy duration (Latini et al., 2003), and reduction in intelligence and increase in risk of cancer (Melnick, 2001). Furthermore, they stimulate the proliferation of human breast cells (MCF-7) (Blom et al., 1998). These reports suggest a possibility that constant exposure to phthalates for the long term from the infant period may affect the normal hormonal regulation and normal brain development resulting in breast cancer and attention deficit hyperactivity disorder (ADHD) both of which are rapidly increasing in the industrialized countries.

Although a limited number of samples were analyzed, the present study provides an indication of the maximum intake of phthalates from baby milk powders. More research on phthalate analysis of specific foodstuffs would be needed to evaluate phthalate effects on humans.

REFERENCES

Blount BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, Lucier GW, Jackson RJ, Brock JW (2000) Levels of seven urinary phthalate metabolites in a human reference population. Environ Health Perspect 108:979-982

Silva MJ, Barr DB, Reidy JA, Malek, NA, Hodge, CC, Caudill SP, Brock JW, Needham LL, Calafat AM (2004) Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES)1999-2000. Environ Health Perspect 112:331-338

Kato K, Silva MJ, Reidy JA, Hurtz III D, Malek NA, Needham LL, Nakazawa H, Barr DB, Calafat AM (2004) Mono(2-ethl-5-hydroxyl)phthalate and mono-(2-ethyl- 5-oxohexyl) phthalate as biomarkers for human exposure assessment to di-(2-ethlhexl)phthalate. Environ Health Perspect 112:327-330

Hauser R, Duty S, Godfrey-Bailey L, Calafat AM (2004) Medications as a Source of Human Exposure to Phthalates. Environ Health Perspect 112:751-753

Asaoka K, Hagihara K, Kabaya H, Sakamoto Y, Katayama H, Yano K (2000) Uptake of phthalate esters, di(n-butyl)phthalate and di(2-ethylhexyl)phthalate as environmental chemicals in monkeys in Japan. Bull Environ Contam Toxicol 64: 679-685

Yano K, Hirosawa N, Sakamoto Y, Katayama H, Mopriguchi T, Joung KE, Sheen YY Asaoka K (2002) Phthalate levels in beverages in Japan and Korea. Bull Environ Contam Toxicol 68: 463-469

Latini G. (2000) Potential hazards of exposure to di-(2-ethylhexyl)-phthalate in Babies. Biol Neonate 78:269-276

Lovekamp-Swan T, Davis BJ (2003) Mechanisms of phthalate ester toxicity in the female reproductive system. Environ Health Perspect 111:139-145

Vartiainen T, Kartovaara L, Tuomisto J (1999) Environmental chemicals and changes in sex ratio: Analysis over 250 years in Finland. Environ Health

Perspect 107: 813-815
Fredricsson B, Mőller L, Pousette A, Westerholm R (1993) Human sperm motility is affected by plasticizers and diesel particle extracts. Pharmacol Toxicol 72:128-133
Herman-Giddens ME, Slora EJ, Wasserman R.C. Bourdony CJ, Bhapkar MV, Koch GG, Hasemeier CM (1997) Secondary sexual characteristics and menses in young girls seen in office practice: A study from the pediatric research in office settings network. Pediatrics 99:505-512
Colón L, Caro D, Bourdony CJ, Rosario O (2000) Identification of phthalate esters in the serum of young Puerto Rican girls with premature brest development. Environ Health Perspect 108:895-900
Latini G, Felice CD, Presta G, Vecchio AD, Paris I Ruggieri F, Mazzeo P. (2003) In utero exposure to di(2-ethylhexyl)phthalate and duration of human pregnancy. Environ Health Perspect 111: 1783-1785
Melnick RL (2001) Is peroxisome proliferation an obligatory precursor step in the carcinogenicity of di(2-ethylhexyl)phthalate (DEHP)? Environ Health Perspect 109: 437-442
MAFF (1996) Phthalates in infant formulae. Food Surveillance Information Sheet no 83 (London: Food Safety Directorate, Ministry of Agriculture, Fisheries and Food)
Gruber L, Wolz G, Piringer O. (1998) Untersuchung von phthalaten in baby-nahrung (1998) Deutsche Lebensmittel-Rundschau 94:177-179
Petersen JH, Breindahl T (2000) Plasticizers in total diet samples, baby food and infant formulae. Food Add Contam 17: 133-141
Katase T (2001) Environmental residue of phthalates. Kikan Kagaku Sosetsu No 50: 199-205 (in Japanese)
Scowen P (1996) Infant feeding. The facts about the phthalates scare. Professional Care Mother & Child 6:126-127