Bull. Environ. Contam. Toxicol. (2002) 68:463–469

DOI: 10.1007/s00128-001-0277-6

Bulletin of Environmental Contamination and Toxicology

Phthalate Levels in Beverages in Japan and Korea

K. Yano,[1] N. Hirosawa,[2] Y. Sakamoto,[2] H. Katayama,[3] T. Moriguchi,[1] K. E. Joung,[4] Y. Y. Sheen,[4] K. Asaoka[5]

[1] Department of Chemistry, Saitama Medical School, 981 Kawakado, Moroyama, Iruma-gun, Saitama 350-0496, Japan
[2] Laboratory of Biomedical Research, Saitama Medical School, 38 Morohongo, Moroyama, Iruma-gun, Saitama 350-0495, Japan
[3] Saitama Medical College, 38 Morohongo, Moroyama, Iruma-gun, Saitama 350-0495, Japan
[4] College of Pharmacy, Ewha University, Seoul 120-750, Korea
[5] Department of Cellular and Molecular Biology, Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan

Received: 3 June 2001/Accepted: 30 November 2001

Considerable numbers of endocrine disruptors (67 compounds) are known today (Japan Environmental Agency, 1998). Among them, phthalates are considered to be in most abundance in our environment. The production of phthalates in Japan was 474 thousand tones in 1998 (Kasozai Kogyo Kai, 1999), around 56 % of which was di(2-ethylhexyl)phthalate (DEHP) used mainly as plasticizers in polyvinyl chloride (PVC) products. Di(n-butyl)phthalate (DBP), about 3% of the total production, is widely used in PVC, nitrocellulose polyvinyl acetate, painting inks, adhesives and lacquers. PVC contains up to 40% phthalates by weight without covalently binding to other ingredients in the PVC (Needham *et al.*, 2000). Thus, significant amounts of DEHP and DBP are easily released from the plastics into the environment during production, use and disposal, resulting in the pollution of food, air, water, soil and homes.

In recent years phthalates have received a great amount of public attention from various aspects since some of them are suspected of possessing endocrine disrupting properties either by acting as hormone mimic or antagonists, or by more indirect mechanisms (Tyler *et al.*, 1998). Although the risk to humans from phthalates is currently theoretical, it has been suggested that increased exposure to phthalates may be partially responsible for the recent decline in the male ratio (Moller, 1996, Vartiainen *et al.*, 1999), for the decreasing values of sperm density (Murature *et al.*, 1987) and motility (Fredricsson *et al.*, 1993), for the earlier pubertal maturation observed in young American girls (Herman-Giddens *et al.*, 1997), and for the premature breast development in young Puerto Rican girls (Colón *et al.*, 2000). Furthermore, at present, it is completely uncertain, but there is a possibility that phthalates themselves or their cocktails with other chemicals may exert profound effects on normal brain development leading to loss of intelligence, mental retardation, and abnormal behavior such as attention deficit hyperactivity disorder (ADHD), which are gradually increasing in the industrialized countries (Robinson *et al.*, 1999; Krimsky, 2000; Rice, 2000).

Recently, we studied phthalate levels in the blood of wild and breeding monkeys in Japan demonstrating the importance of environmental factors for uptake of phthalates in these animals (Asaoka *et al.*, 2000). Since animals and humans mainly uptake these agents through their diets including food contamination and

Correspondence to: K. Yano

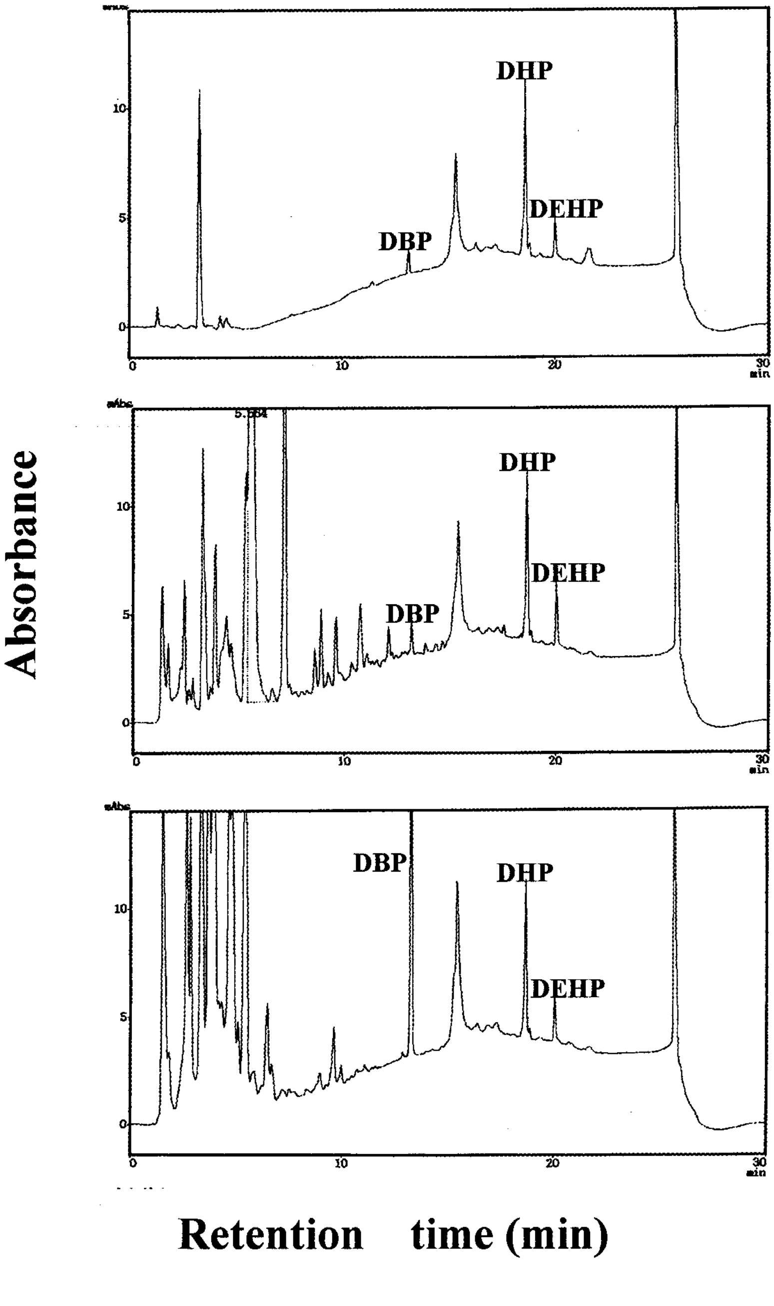

Figure 1. HPLC chromatograms of water (A), Korean red wine (B), and Japanese red wine (C). Diheptylphthalate (DHP) was used as an internal standard (7.5 μ g of DHP in 5 g of a sample).

polluted drinking water, it is important to collect the reliable data on phthalate levels in foodstuffs in the separated regions for evaluating the epidemiological data that might be due to the estrogenic effects of phthalate on humans. Thus, we have investigated the amounts of DBP and DEHP in the same kinds of beverages sold in Japan and Korea.

MATERIALS AND METHODS

All solvents, water, sodium chloride, and sodium sulfate used were high purity grade for analysis of phthalates and obtained from Wako Pure Chemical Industries, LTD, (Osaka, Japan). Standard phthalates, DBP, diheptylphthalate (DHP) and DEHP, were also purchased from Wako Pure Chemical Industries, LTD. Bottled waters, beers, fiber drinks, juices, alcoholic drinks (*sho-chu*), nutritive drinks, vinegars, rice punches (*nigori sake*), and red and white wines were purchased from supermarkets or local open markets in Saitama (Japan) and Seoul (Korea) in 1998. The nutritive drinks, fiber drinks and wines used were packed in glass bottles with metal caps, the beers in aluminum cans, the rice punches in stainless steel cans or glass cups with metal caps, the alcoholic drinks and juices in cartons and the others in plastic bottles with plastic caps. Korean samples were sent to the department of chemistry at Saitama Medical School for evaluation of phthalate levels, and kept in a refrigerator until used.

All glassware used for the extraction and aluminum foil were heated at 220℃ overnight before use, and the glassware was wrapped with the aluminum foil prior to the experiment. To 5 g of a sample, were added 500 mg of sodium chloride and 50 μ l of DHP solution (15 μ g/ml of acetonitrile) as an internal standard. The resulting mixture was extracted with 2.5 ml of a mixture of hexane and dichloromethane (10:1) twice. The organic solution separated was dried over 500 mg of sodium sulfate, and the solvents were removed under nitrogen flow. To the dried sample, was added 200 μ l of acetonitrile, and the resulting solution was kept in a refrigerator until analyzed. A Shimadzu LC-10A high-performance liquid chromatograph (HPLC) (Shimadzu, Corp., Kyoto, Japan) was used for analysis of phthalates (Yano *et al.*, 2000). Typical HPLC conditions were as follows: column, TSK-GEL (4.6 mm x 25 cm) (Toyo Soda, Tokyo, Japan); column temperature, 40℃; gradient elution, from acetonitrile-water (50%) to acetonitrile (100%) over 15 min; flow rate, 1.3 ml/min; UV detection, 254 nm. The test solutions (20 μ l, each) were injected into the HPLC column.

RESULTS AND DISCUSSION

We chose retail beverages as the samples for analysis of phthalates in food, because the same kinds of samples that were made in Japan and Korea were easily collected in both countries. The amounts of phthalates in the bottled waters (n=3), beers (n=3), fiber mini (n=3), alcoholic drinks (n=2), nutritive drinks (n=4), vinegars (n=2), juices (n=2), red wine (n=1), white wine (n=1), and rice punches (n=2) were measured by the HPLC method. The typical HPLC chromatograms of water, Korean red wine, and Japanese red wine are shown in Figure 1. To

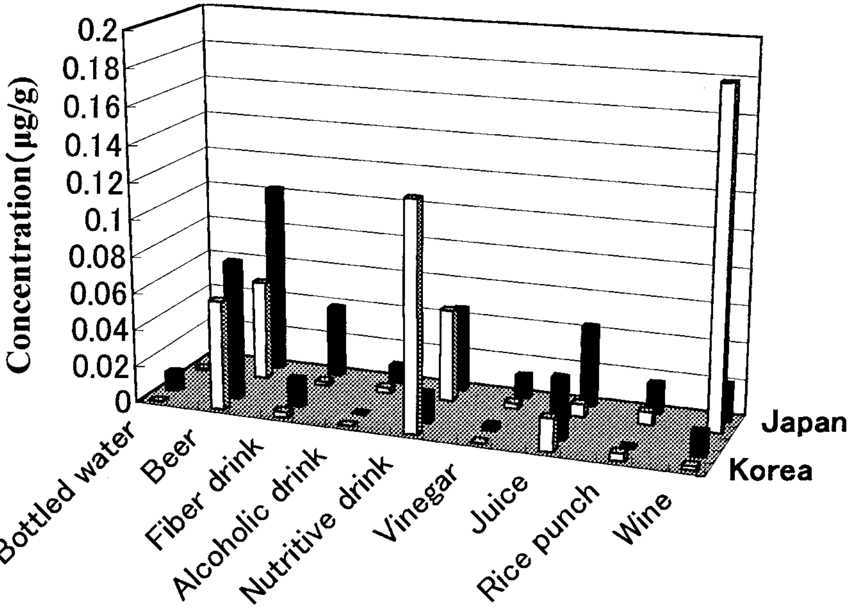

Figure 2. Averages of phthalate levels in each group of samples. The bottled waters (n=3), beers (n=3), fiber mini (n=3), alcoholic drinks (n=2), nutritive drinks (n=4), vinegars (n=2), juices (n=2), red wine (n=1), white wine (n=1), and rice punches (n=2) were used, and each sample was measured twice. The white bars indicate the concentrations of DBP and the black bars indicate the concentrations of DEHP.

quantitatively evaluate the amounts of phthalates, the correlation coefficients were calculated from the calibration lines of DEHP/DHP (w/w) vs. DEHP/DHP (A/A) and DBP/DHP (w/w) vs. DBP/DHP(A/A), giving 0.92 and 1.43, respectively. Using these values, we determined the amounts of DBP and DEHP in the beverages and the solvents used for extraction, and then subtracted the solvent value from each sample value. The detection limits were 0.004 μ g/g. Since the values within each sample in the same group were scattering, the averages in each group were calculated for a comparison of phthalate levels in both countries. The results are summarized in Figure 2.

DBP level in the Japanese red wine was extremely high when compared with that in the Korean one. To confirm this high DBP level, a similar type of another Japanese red wine (180ml in a glass bottle with a metal cap) produced by the other factory was analyzed in the same condition, giving a very similar result (data not shown here). On the other hand, out of four Korean nutritive drinks, two drinks contained a large amount of various ingredients as well as quite a large amount of DBP. These findings suggest that accumulation of DBP might have occurred through the ingredients that have some affinity with DBP or through the various processes of production.

We directly compared the phthalate levels in all the samples examined here using the average values. The DBP level (μ g/g) in the Japanese beverages (0.034 ± 0.059) was about 1.5 times higher than that in the Korean beverages (0.023 ± 0.042), but it was not statistically significant. On the other hand, the DEHP level (μ g/g) in the Japanese beverages (0.032 ± 0.028) was 1.8 times higher than that in the Korean beverages (0.018 ± 0.22) ($p<0.01$). Further the DBP level in Japanese beverages was found to be rather higher than the DEHP level in agreement with the result published by Tomita *et al.*(1977). The high level of DBP in the living environment in Japan was also supported by our finding that the DBP level in the blood of breeding monkeys is much higher than that of the wild monkeys (Asaoka, *et al.*, 2000). Furthermore, Tokyo Sanitary Bureau (2000) reported that the DBP level in the indoor air in Japan is about 10 times higher than that in the outdoor air and that the DBP level in the indoor is about 3 times higher than the DEHP level. These results indicate that Japan seems to be more polluted by these phthalates than Korea and that everywhere in the town sites in Japan seems to be highly polluted by DBP.

To get some information on the factors to affect phthalate levels in each country, the amounts of plastic production and consumption were compared in Japan and Korea in 1998 when the samples were collected. The former were 13,909,000t (Japan) and 8,940,000t (Korea) and the latter were 10,938,000t (Japan) and 3,270,000t (Korea), respectively (Japan Plastics Industrial Association 2000). These data indicate that Japan produced 1.6 times and consumed 3.3 times larger amounts of plastics than Korea did. These ratios suggest that the phthalate pollution of beverages observed here is somehow related to the processes of plastic production and/or the consumption of plastics since the beverages are contaminated with phthalates by contacting containers, processing equipment, and various types of tubing, which are made of plastic or plastic parts

.

The highest values examined here are 0.275 μ g/g of DBP and 0.127 μ g/g of DEHP in Japanese red wine and beer, respectively, corresponding to 0.275 mg/kg and 0.127 mg/kg, which are well below the specific migration limits of DBP (6 mg/kg) and DEHP (3 mg/kg) laid down by the EU Scientific Committee for Food (1999). Thus, these phthalate levels may not provide serious problems for adult people to take these beverages. It may be better, however, for women in pregnancy to avoid taking the beverages containing the high level of phthalates since phthalates easily pass through the placenta (Tomita *et al.* 1977), because organs of the developing embryo and fetus are especially sensitive to low concentrations of endocrine disruptors (Bigsby *et al.*, 1999).

Faced with gradually increasing number of children with abnormal behavior such as ADHD in the industrialized countries (Robinson *et al.*,1999; Krimsky, 2000;

Rice, 2000), it may be considered that the behavioral and neurophysiological effects induced by endocrine disruptors are more important rather than their reproductive effects. At present, we don't know whether phthalates themselves or their cocktails with other chemicals may exert profound effects on the normal brain development or not. Ema *et al.* (1994), however, reported that some phthalates have teratogenic effects when pregnant rats are treated with them, suggesting that the perceived increase in ADHD in the population may be in part the result of early exposure to the phthalates. Therefore, it is urgent to get the reliable data on the amounts of phthalates not only in food but also in our living environments to correctly understand their biological effects on humans (Page *et al.*, 1995; Colón *et al.*, 2000).

Acknowledgments. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, Culture, Science and Technology of Japan (No.13027240), and by a Grant for the Oversea Special Research Program of the Primate Research Institute of Kyoto University in 2000.

REFERENCES

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

Bigsby R, Chapin RE, Daston GP, Davis BJ, Gorski J, Gray LE, Howdeshell KL, Zoeller RT, vom Saal FS (1999) Evaluating the effects of Endocrine Disruptors on Endocrine Function during Development. Environ Hlth Perspect (Suppl. 4) 107: 613-618

Colón I, Caro D, Bourdony CJ, Rosario O (2000) Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. Environ Hlth Perspect 108: 895-900

Ema M, Amano H, Ogawa Y (1994) Characterization of the development toxicity of di-n-butyl phthalate in rats. Toxicology 86: 163-174

EU Commission (1999) Synoptic document-draft of provisional list of monomers and additives used in the manufacture of plastics and coatings intended to come into contact with foodstuffs, Scientific Committee for Food. European Commission, Rue de la Loi 200, B-1049 Brussels, Belgium.

Fredricsson B, Möller L, Pousette Å, 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 RC, 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

Japan Environmental Agency (1998) Strategic Programs on Environmental Endocrine Disruptors' 98, May (in Japanese)
Japan Plastics Industrial Association (2000) Japan Plastics, 51(6), 30 (in Japanese).
Kasozai Kogyo Kai (1999) Kasozai INFORMATION, No.11, 12 (in Japanese)
Krimsky S (2000) Hormonal Chaos. The Scientific and Social Origins of the Environmental Endocrine Hypothesis. (The Johns Hopkins University Press, Baltimore and London), pp. 45-51
Moller H (1996) Change in male: female ratio among newborn infants in Denmark. Lancet 348: 828-829
Murature DA, Tang SY, Steinhardt G, Dougherty RC (1987) Phthalate esters and semen quality parameters. Biomed Environ Mass Spectrosc 14: 473-477
Needham LL, Blount B, Rogers HS, Brock JW, Barr D (2000) Levels of selected nonpersistent endocrine disruptors in Humans. Analysis of Environmental Endocrine Disruptors. Edited by L.H.Keith, T.L. Jones-Lepp, and L.L. Needham (USA; American Chemical Society), pp 147-157
Page BD, Lacroix GM (1995) The occurrence of phthalate ester and di-2-ethylhexyl adipate plasticizers in Canadian packaging and food sampled in 1985-1989: a survey. Food Add Contam 12: 129-151
Rice DC (2000) Parallels between attention deficit hyperactivity disorder and behavioral deficits produced by neurotoxic exposure in monkeys. Environ Hlth Perspect (Suppl.3) 108: 405-408
Robinson LM, Sclar DA, Skaer TL, Galin RS (1999) National trends in the prevalence of attention-deficit/hyperactivity disorder and prescribing of methylphenidate among school-age children. Clin Pediatrics 38: 209-217
Tokyo Sanitary Bureau, Living Environmental Division (2000) Analysis Report August, 2 (in Japanese)
Tomita I, Nakamura Y, Yagi Y (1977) Phthalic acid esters in various foodstuffs and biological materials. Ecotoxicol Environ Saf 1: 275-287
Tyler CR, Jobling S, Sumpter JP (1998) Endocrine disruption in wildlife: A critical review of the evidence. Crit Rev Toxicol 28: 319-361
Vartiainen T, Kartovaara L, Tuomisto J (1999) Environmental chemicals and changes in sex ratio: Analysis over 250 years in Finland. Environ Hlth Perspect 107: 813-815
Yano K, Asaoka K, Moriguchi T, Skamoto Y, Katayama H (2000) Environmental distribution of suspect endocrine disruptors, di(n-butyl)phthalate and di(2-ethylhexyl)phthalate, in Japan. Book of Abstracts, American Chemical Society, 220th National Meeting (USA: Washington), 2, ENVR-261