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Uptake of Phthalate Esters, Di(n-butyl)phthalate and Di(2-ethylhexyl)phthalate, as Environmental Chemicals in Monkeys in Japan

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Some phthalate esters are known to have the toxicity, mutagenicity, teratogenicity, and carcinogenicity (Keith 1997). In human environments, usage of phthalate esters is increasing because of their addition to many plastic products as plasticizers and to disposable devices used for foods and medicines to enhance flexibility. Among them, di(n-butyl)phthalate (DBP) and di(2-ethylhexyl)phthalate (DEHP) which are the main plasticizers occupy about 2.9% and 53.1%, respectively of all plasticizers in Japan (Kasozai Kogyo Kai 1998).

Although estrogenic effects of these compounds are uncertain on humans (Gangolli 1982; Editorial 1995; Poster 1997), there is some evidence for their effects on animals (Keith 1997; Jobling et al. 1995). Both humans and animals are exposed mainly to these phthalates via their diets due to food contamination and polluted drinking water (Page and Lacroix 1995). To obtain some information on our environmental pollution by phthalate esters, we studied the levels of DBP and DEHP in the blood samples obtained from monkeys living in two different environments, breeding and wild, in Japan. A part of this study has been reported in a proceeding form (Asaoka et al. 1998).

MATERIALS AND METHODS

Blood samples (about 1 ml each) were collected from Japanese monkeys living in two different places. Thirty three blood samples were obtained from the breeding monkeys (13 males and 20 females) fed with artificial foods at Primate Research Institute, Kyoto University, Inuyama, Aichi,

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Japan. Ten blood samples were obtained from the wild monkeys (9 males and 1 female) captured by the animal control program of Chiba prefecture permission in Chiba area. The ages of the breeding monkeys were recorded and those of the wild monkeys were estimated by the observation of their dental conditions. The collected samples were sealed and kept in a freezer at -80°C until used. The standard experimental procedure was as follows: To 0.5 ml of each blood sample, were added 0.25 ml of di(heptyl)phthalate ester (DHP) (15 μ g / 1 ml of acetonitrile) as an internal standard, 1.5 ml of water, 0.1 ml of methanol, 1.5 ml of acetonitrile, and 2.0 ml of hexane (Karle et al. 1997). The resulting solution was mixed well with a vortex and centrifuged for 5 min at 2,000 rpm. The separated upper layer was dried under nitrogen flow. To the dried residue, 500 μ l of acetonitrile was added and a portion of this, each 20 μ l, was used for HPLC analysis (Yano et al. 1998). The analysis conditions were as follows: TSK ODS-column (4.0 mm x 260 mm), column temperature; 40°C, gradient elution; acetonitrile-water (50%) to acetonitrile (100%) for 15 min, flow rate; 1.3 ml / min, UV detection; at 254 nm. The amount of DBP or DEHP was calculated by using each regression line obtained from the ratio of DBP/DHP (absorbance) vs. DBP/DHP (weight) or DEHP/DHP (absorbance) vs. DEHP/DHP (weight).

RESULTS AND DISCUSSION

The typical HPLC chromatograms of the blood samples of the wild and breeding monkeys were shown in Figure 1, in which DBP, DEHP and DHP were completely separated. In the breeding monkeys, DBP was detected in all blood samples of 33 monkeys and DEHP was detected in 58% of the monkeys. In the wild monkeys, six out of ten monkeys showed uptake of DBP and nine showed uptake of DEHP. Correlation between the uptake amounts of the phthalate esters and the monkey ages was not shown significantly.

Figure 2 showed the average amounts (μ g / ml) with standard deviation of DBP and DEHP in the blood samples from both wild and breeding monkeys. In the breeding monkeys, the total average value of DBP was 0.0821 ± 0.0303. The average value of DBP in the male monkeys (0.0880 ± 0.0194) was slightly higher than that in the female monkeys (0.0782 ± 0.0356) but this difference was not significant. With DEHP, total average amount was 0.1939 ± 0.1050. An average amount in the male monkeys (0.2348 ± 0.0955) was also slightly higher than that in the female monkeys, (0.1642 ± 0.1057). In the wild

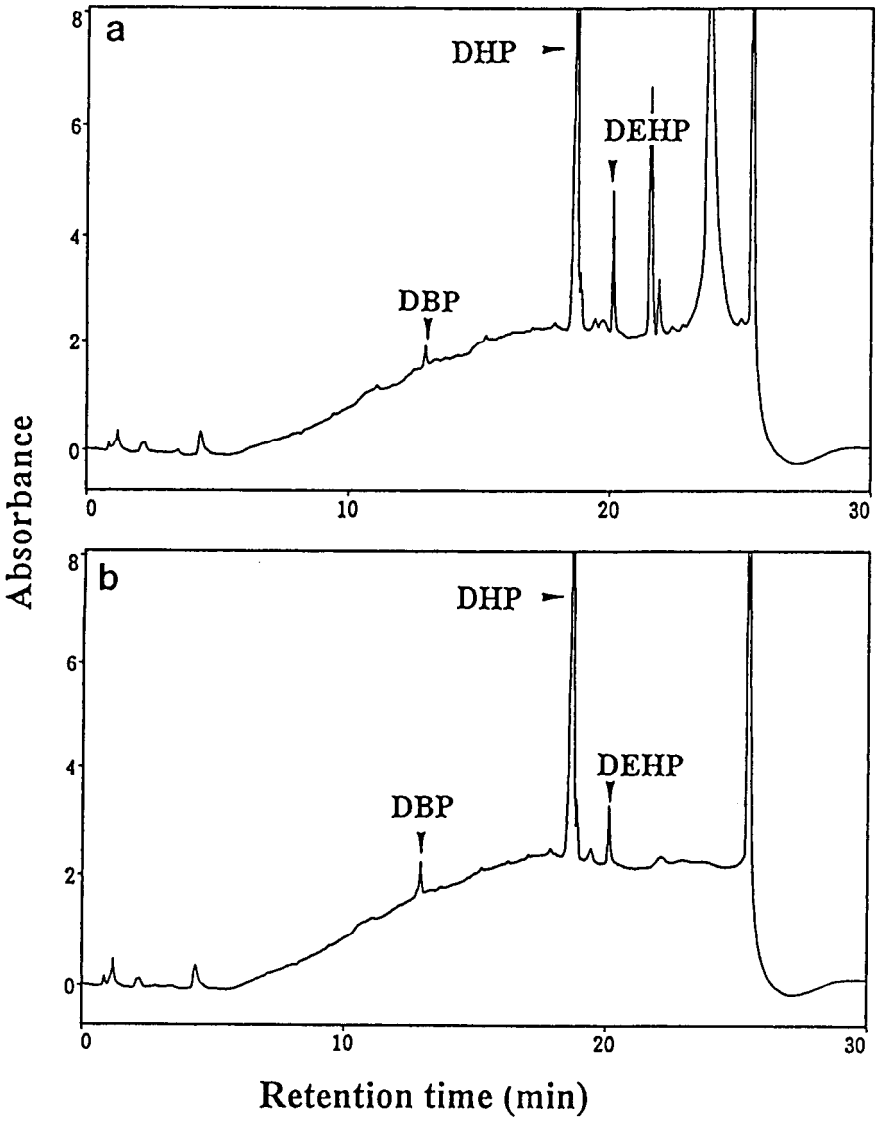

Figure 1. The HPLC chromatograms of DBP, DEHP and DHP in the monkey blood. a: wild living monkey. b: breeding monkey. The HPLC conditions were described in Materials and Methods.

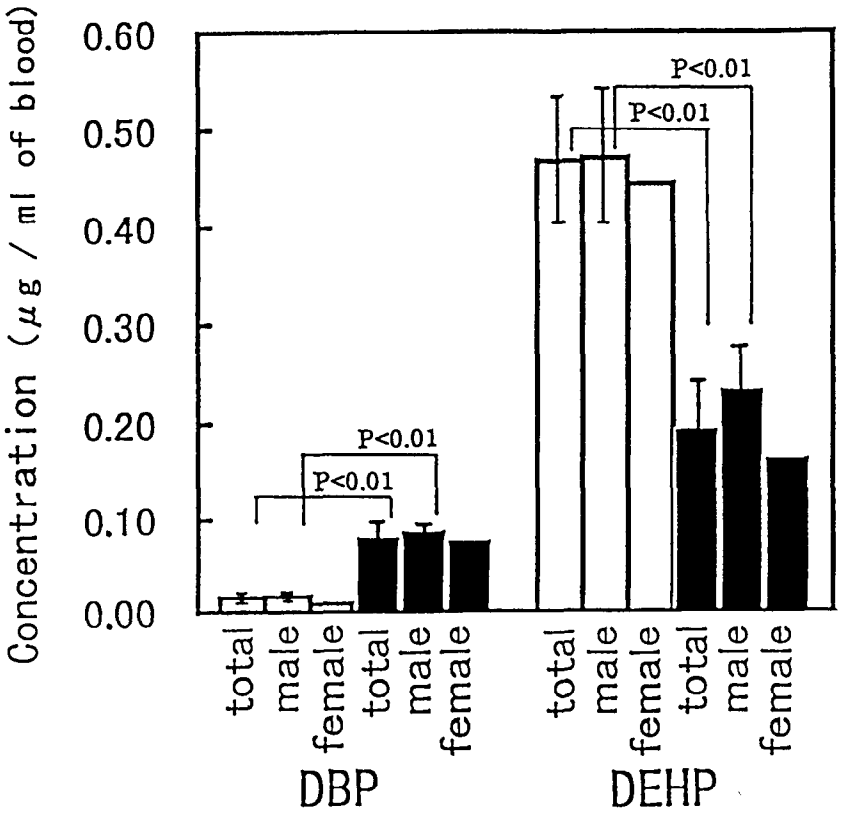

Figure 2. The average concentrations of DBP and DEHP in the blood from monkeys living in different places. White and black blocks show the wild and breeding monkeys, respectively.

monkeys, the total average amounts of DBP and DEHP were 0.0146 ± 0.0107 and 0.4674 ± 0.1346, respectively. With DBP and DEHP, an averages amounts in the male (0.0160 ± 0.0114 and 0.4703 ± 0.1436) were also slightly higher than those in the female (0.0079 and 0.4443), respectively.

A comparison of the phthalate ester levels in both groups suggests that the uptake of DBP is significantly higher in the breeding monkeys than that in the wild monkeys ($P<0.01$). The uptake of DEHP, however, is significantly lower in the breeding monkeys than that in the wild monkeys ($P<0.01$). This may reflect their different diets from contaminated food and polluted drinking water caused by different distribution of plastic products between the town and rural sites.

On the other hand, the amounts of DBP and DEHP in the river water were reported to be 0.0002-0.0014 and 0.0043-0.0068 (μ g / ml), respectively in 1996 (Japan Environment Agency 1996). We take their medium values (0.0008 and 0.0056, respectively) as each standard and compare these values with their concentrations found in the monkey blood. The DBP levels in the wild and breeding monkeys are found to be 18 and 102 fold higher than that in water, respectively, and the DEHP levels in the wild and breeding are about 83 and 35 fold higher than that in water, respectively. Although these phthalate esters are metabolically cleared from their bodies as observed in human (Dirven et al. 1993) some of them may be accumulated in the fatty tissues (Mes et al. 1974) because of their lipophilic nature. Furthermore, our finding that the DEHP uptake occurs in both groups of monkeys, especially higher in the wild monkeys, suggests that plasticizers such as DEHP are spreading over many fields in Japan.

Tomita et al. (1977) reported the levels of DBP and DEHP in Japanese blood after meals as 0.03-0.11 ppm (average 0.097, n=3) and 0.06-0.28 ppm (average 0.17, n=3), respectively. These average values are in good agreement with those with the breeding monkey (0.082 and 0.194, respectively), suggesting that the monkey environments are closely related to human environments. The present results demonstrate that investigation of the pollution in monkey life environments could provide very useful information on our environmental pollution. Metabolic degradations of these phthalate esters in monkey are under investigation in our laboratory.

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