Phthalates, Adipates, Citrate and Some of the Other Plasticizers Detected in Japanese Retail Foods: a Survey

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Plasticizers in Japanese retail foods were determined by gas chromatography/mass spectrometry (GC/MS) (SIM). The plasticizers tested were as follows: dibutyl phthalate, butylbenzyl phthalate, di(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate, di(2-ethylhexyl) adipate, diisononyl adipate (DINA), dialkyl adipate, dibutyl sebacate, *O*-acetyl tributyl citrate (ATBC) and diacetyllauroyl glycerol (DALG). A total of 93 samples were analyzed. For the analysis, each sample was extracted by a method suitable to its nature and cleaned using Florisil® and Bondesil PSA® dual layer columns. The recovery of plasticizers from fortified food samples was 62.0–131.0%, except in the case of DINA. The limit of detection (LOD) was different for each sample species and plasticizers. For example, the LOD for plasticizers in retort-pouched baby food was 0.0004–0.037 μg/g. A retort-pouched baby food sample was found to be contaminated by DEHP at the Japanese tolerable daily intake (TDI) level, 40 μg/kg/day. The source of contamination was presumed to be disposable gloves because the baby food was produced before the prohibition of DEHP-containing poly vinyl chloride (PVC) gloves by the Japanese government. After that prohibition, products generally contained much lower levels of DEHP. A higher level of DALG was detected in the other baby food samples, although it became clear that DALG did not originate as contamination from plastics but was added as a food additive. ATBC was detected in bottled *sake* samples at levels of around 3–7 μg/g, having migrated from the gasket of the bottle cap. ATBC and DALG levels in the above foods were quite low compared with their no observed adverse effect level (NOAEL) or guideline levels as food additives.

Key words —— plasticizer, food, survey, phthalate, adipate

INTRODUCTION

A wide range of plastic materials are used as containers or wrappings in such a way that they contact foods directly. It is known in the field of food safety that additives or monomers in plastics may migrate into foods. Poly vinyl chloride (PVC) and poly vinylyden chloride (PVDC) contain plasticizers in larger amounts than other additives, such as anti-oxidants or stabilizers; therefore their degree of migration would be larger.[1)]

The authors conducted a study to determine the Japanese daily intake of phthalates, a common and important group of plasticizers, by means of a duplicate diet study based on meals obtained from hospitals and packed lunches from convenience stores in 1999. All of the packed lunches and parts of meals from hospitals were found to contain high levels of di(2-ethylhexyl) phthalate (DEHP).[2,3)] The authors ascertained the source of the contamination to be disposable PVC gloves used in cooking.[3)] DEHP causes liver or testicular damage in rats and mice, and the "no observed adverse effect level" (NOAEL) has been shown to be 3.7 or 14 mg/kg/day.[4,5)] In June 2000, the Japanese government set the tolerable daily intake (TDI) level for DEHP to be 40–140 μg/kg/day, and prohibited the use of DEHP-containing gloves for food contact purposes.[6)] The authors investigated the DEHP levels in retail packed lunches after the prohibition of PVC gloves and found them to be about 4% of the levels before prohibition.[7)]

In a previous study, a high DEHP content of 6 μg/g was also found in retort-pouched baby food, with the source being PVC tubing used in production.[2)] No survey of plasticizers in Japanese foods had been done adequately at that time, so that unexpected high DEHP contamination was successively encountered. Widespread use of PVC for gloves, tubing or some

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Table 1. List of Plasticizers Tested

No.	Compound	Abbreviation	CAS No.	Ions for quantification (m/e)	Ions for confirmation (m/e)	Internal standard	Relative retention time (RRT)[a]
1	Di-*n*-butyl phthalate	DBP	84-74-2	149	104, 150	DBP-d_4[b]	0.832
2	*n*-Butylbenzyl phthalate	BBP	85-68-7	149	206	BBP-d_4[b]	1.000
3	Di(2-ethylhexyl) phthalate	DEHP	117-81-7	149	167, 279	DEHP-d_4[b]	1.078
4	Diisononyl phthalate	DINP	28553-12-0	149	293	DNP-d_4[b]	1.259[d]
5	Di-*n*-alkyl adipate (C=6,8,10)	DAA		129	213	[f]	0.917[d]
6	Di(2-ethylhexyl) adipate	DEHA	103-23-1	129	147	DEHA-d_8[c]	1.013
7	Diisononyl adipate	DINA	33703-08-1	129	255	[f]	1.140[d]
8	Dibutyl sebacate	DBS	109-43-3	185	241	[f]	0.924
9	*O*-Acetyl tributyl citrate	ATBC	77-90-7	185	129	[f]	0.956
10	Diacetyllauroyl glycerol[e]	DALG	30899-62-8	159	183	[f]	0.966[d]

a) Relative retention time compared to BBP. Retention time of BBP was 22.92 min. *b*) Quantification ion was 153 for deuterated isomer. *c*) Quantification ion was 137 for deuterated isomer. *d*) RRT of the highest peak is shown because the standard material was a mixture of isomers. *e*) Produced by acetylation of monolauroyl glycerol from vegetable oil. *f*) Internal standard was not used.

of the other purposes can distort the estimation of daily intake. The estimated daily intake data on duplicated diet samples in 1999 has lost its reliability because the intake of DEHP should have been lowered by the prohibition of PVC gloves.

The purpose of this study is to ascertain the presence or absence of food contamination due to plasticizers by conducting a wide survey. This study is a necessary step in the accurate estimation of intake. Ten plasticizers were analyzed and are abbreviated in Table 1. These include 4 phthalates [di-*n*-butylphthalate (DBP), *n*-butylbenzyl phthalate (BBP), DEHP and diisononyl phthalate (DINP)], 3 adipates [di-*n*-alkyl adipate (DAA), di(2-ethylhexyl) adipate (DEHA), diisononyl adipate (DINA)] and 3 other plasticizers [dibutyl sebacate (DBS), *O*-acetyl tributyl citrate (ATBC) and diacetyllauroyl glycerol (DALG)]. These plasticizers were either frequently detected in foods in our previous study[2,3] or were often added to the wraps or containers for food consumed in Japan.[1] The isotope dilution technique was partly used for quantification, and the surrogates are also specified in Table 1.

MATERIALS AND METHODS

Food Samples —— Retail food samples were purchased from stores in the Kinki area of Japan from September 2000 to February 2001. The manufacturers of these foods were distributed throughout Japan. Number of samples/type of samples are mentioned in Table 2. Three baby food samples in which high DEHP or DALG was detected, were purchased and tested repeatedly.

Chemicals —— Plasticizers and the deuterated isomers were purchased from Wako Pure Chemicals (Osaka, Japan), Hayashi Pure Chemicals (Osaka, Japan), Tokyo Kasei Kogyo (Tokyo, Japan) and Kanto Kagaku (Tokyo, Japan). Each compound was dissolved in *n*-hexane (stock solution) and diluted by *n*-hexane for calibration curves or acetonitrile for recovery tests (working solutions). A Florisil + Bondesil PSA column was prepared by packing 2 g Florisil PR® (60–100 mesh, Floridin Co., New York, U.S.A.) as a slurry in a glass syringe (1.5 cm i.d. × 11 cm) with a cotton plug, and 0.5 g Bondesil PSA® (acid-washed silica bonded by ethylenediamine-*N*-propyl, 40 μm, Varian Inc., CA, U.S.A.) and 1 g anhydrous sodium sulfate (Na_2SO_4) were placed on this slurry using *n*-hexane. The system was then passed through 10 ml acetone and 10 ml *n*-hexane before use. Extrelut 20® was purchased from Merck (Darmstadt, Germany). Acetone, *n*-hexane, water and Na_2SO_4 were of a special grade for phthalates purchased from Kanto Kagaku and Wako Pure Chemicals. Acetonitrile and NaCl were of pesticide analysis grade (Wako Pure Chemicals). Florisil and NaCl were heated at 200ºC for 2 hr and cooled before use.

Apparatus —— The homogenizer used was a Dancing Agitator, Hirosawa Ironworks (Tokyo, Japan). To mix a homogenized sample with acetonitrile, Ultra-Turrax, Janke & Kunkel GmbH & Co (Staufen, Germany) was used. The rotary evaporator was an

Table 2. Plasticizers in Food Samples; Minimum, Maximum and Median Levels Detected[a)]

Food species	Number of samples	Concentrations (μg/g) minimum - maximum (median)					
		DBP	BBP	DEHP	DINP	DEHA	ATBC
Beverages							
Sake	5	ND[b)]–0.006 (tr)	ND	ND–0.014 (ND)	ND	ND–Tr[c)] (ND)	ND–7.30 (3.14)
Wine	3	Tr–0.66 (0.007)	ND–0.002 (0.001)	ND	ND	ND	ND–0.009 (ND)
Beer	3	ND–Tr (ND)	ND–Tr (ND)	ND–0.027 (ND)	ND	ND–Tr (ND)	ND
Fat and oil							
Butter	3	ND	ND–0.056 (ND)	1.02–2.83 (1.61)	ND	0.79–2.78 (1.40)	Tr–0.56 (0.064)
Margarine	3	ND	ND	ND	ND	ND	ND
Fat spread	3	ND	ND–0.13 (ND)	ND	ND	ND	ND
vegetable oil	8	ND–2.40 (ND)	ND–0.62 (0.037)	ND–1.75 (0.45)	ND	ND–0.49 (0.040)	ND
Dairy products							
Cheese	3	ND	Tr–0.008 (Tr)	0.33–0.57 (0.35)	ND	0.036–0.046 (0.044)	0.052–0.22 (0.080)
Milk	3	ND	ND	0.063–0.10 (0.064)	ND	0.040–0.081 (0.049)	0.013–0.11 (0.013)
Ice cream	3	ND	ND	0.17–0.39 (0.17)	ND	0.097–0.15 (0.097)	0.016–0.026 (0.024)
Refreshments							
Cookies	3	ND–0.070 (0.027)	ND	0.10–0.68	ND	ND–0.12 (0.010)	ND–0.028 (ND)
Chocolate	3	ND–0.027 (ND)	ND–Tr (ND)	0.077–0.21 (0.080)	ND	0.009–0.023 (0.015)	ND
Solty pastry	3	ND	ND–Tr (ND)	Tr–0.15 (0.071)	ND	ND–0.012 (ND)	ND–Tr (ND)
Fast foods							
Hamburger set	3	ND	ND–Tr (ND)	ND–0.039 (ND)	ND	ND	ND
Gyu-don	3	ND	ND	ND	ND	ND	ND
Pizza	3	ND	ND–0.002 (Tr)	0.096–0.40 (0.23)	ND	ND–0.015 (ND)	ND
Instant food							
retort-pouched food	11	ND	ND–0.010 (Tr)	ND–0.44 (0.067)	ND	ND–0.015 (ND)	ND–0.003 (ND)
dry noodles	3	ND–0.051 (Tr)	ND	ND–0.42 (0.092)	ND	ND–0.017 (ND)	ND–Tr (ND)
Baby food							
retort-pouched baby food	13	ND–0.011 (ND)	ND–0.003 (Tr)	ND–4.25 (0.081)	ND–0.064 (ND)	ND–0.44 (0.006)	ND–0.016 (ND)
baby snack	5	ND–Tr (ND)	ND–Tr (ND)	0.12–0.45 (0.32)	ND–1.83 (0.17)	ND–0.018 (0.010)	ND–0.012 (ND)
Infant formula	6	0.013–0.25 (0.14)	ND–0.003 (ND)	0.028–0.28 (0.13)	ND	Tr–0.041 (0.010)	ND
Total	93	ND–2.40	ND–0.62	ND–4.25	ND–1.83	ND–2.78	ND–3.14

a) DINA, DBS and DAA were not detected in any sample. DALG was detected only in two samples of retort-pouched baby food at levels of 5.47 and 4.76 μg/g. *b*) ND: not detected; ND $<$ LOD. *c*) Tr: trace level; LOD $\leq$ Tr $<$ LOQ.

Table 3. Limit of Detection (LOD) for Plasticizers in Food Samples[a)]

Food species	Method of analysis[b)]	Sampling weight (g)	Number of blank test	Limit of detection (μg/g)[c)]									
				DBP	BBP	DEHP	DINP	DAA	DEHA	DINA	DBS	ATBC	DALG
Retort-pouched baby food	A	50	6	0.003	0.0004	0.037	0.006	0.003	0.0009	0.008	0.002	0.0006	0.003
Hamburger, retort-pouched food	A	50	9	0.099	0.0004	0.019	0.006	0.003	0.004	0.014	0.002	0.0006	0.003
Gyu-don,[d)] pizza	A	25	9	0.20	0.0008	0.037	0.01	0.006	0.007	0.027	0.003	0.001	0.006
Sake, wine	B	100	5	0.001	0.0002	0.004	0.003	0.002	0.0003	0.004	0.0008	0.0004	0.002
Beer	C	20	3	0.066	0.001	0.004	0.02	0.008	0.0005	0.02	0.004	0.002	0.008
Butter, margarine, fat spread	D	2	4	0.28	0.01	0.19	0.2	0.08	0.021	0.2	0.04	0.02	0.08
Vegetable oil	E	2	4	0.051	0.01	0.053	0.2	0.08	0.017	0.2	0.04	0.02	0.08
Refreshments, cheese, dry noodles	F	5	8	0.015	0.004	0.029	0.06	0.03	0.005	0.08	0.02	0.006	0.03
Infant formula	F	20	3	0.002	0.001	0.013	0.02	0.008	0.001	0.02	0.004	0.002	0.008
Milk	G	10	4	0.010	0.002	0.025	0.03	0.02	0.010	0.04	0.008	0.003	0.02
Ice cream	G	5	4	0.020	0.004	0.049	0.06	0.03	0.020	0.08	0.02	0.006	0.03

a) Limit of quantification (LOQ) was set at a level twice that of LOD. *b*) The details of each method are described in the text. *c*) Limit of detection is calculated as three times the standard deviation of the plasticizer levels detected in the blank test, or as the mechanical detection limit of GC/MS. *d*) Boiled rice with spiced beef and onion.

RE 111 Rotavapor (Buchi, Shibata Kagaku Kikai Kogyo, Tokyo, Japan) equipped with a water bath and vacuum pump (Model JS-75A, Advantec, Tokyo, Japan). The water bath was maintained at 40ºC. All glassware and stainless materials were heated at 200ºC for 2 hr, cooled, and rinsed with hexane before use.

Surrogate Mixture —— Four deuterated isomers of phthalates and one isomer of DEHA were diluted with *n*-hexane, and a portion of each solution was mixed. The concentration of each was 2000 ng/ml.

Sample Extraction and Cleanup —— Food samples were extracted by suitable methods depending on their nature as described below. At the start of extraction 1 ml of a surrogates mixture solution was added to the sample. The sampling weights for each food species are listed in Table 3.

Method A: Retort-pouched foods, pizza and *gyu-don*. A homogenized sample was mixed with 100 ml acetonitrile for 1 min, then centrifugated at 3000 rpm for 5 min. The acetonitrile layer was separated and transferred to a separatory funnel, and residual homogenate was mixed again with 100 ml acetonitrile and centrifuged. This layer was gathered, treated with 7 g NaCl, and vigorously shaken for 5 min. The aqueous layer was removed. To the organic layer, 40 ml hexane saturated with acetonitrile was added, and the solution was shaken. The acetonitrile layer was evaporated to remove the acetonitrile completely. To the residue 5 ml hexane was added, and the extract was transferred to a test tube. After shaking the capped test tube for 30 sec, the upper layer was charged on a conditioned Florisil and Bondesil PSA dual layer column, and the eluent was discarded. Another 5 ml *n*-hexane was added to the test tube and shaken, the *n*-hexane layer was charged on to the column, and the eluent was discarded. Plasticizers were eluted by 20 ml 5% (v/v) acetone in *n*-hexane. The eluate was evaporated and reconstituted in 2 ml *n*-hexane for gas chromatography/mass spectrometry (GC/MS) analysis.

Method B: *Sake* and wine. To the sample 15 g NaCl and 100 ml *n*-hexane were added, and the mixture was vigorously shaken for 5 min. The organic layer was taken, dried over Na_2SO_4, evaporated and cleaned using Florisil and Bondesil PSA dual layer column as in Method A.

Method C: Beer. Degassed sample was charged on an Extrelut 20® and let stand for 10 min. One hundred ml of ethyl acetate was passed through the Extrelut 20®, and the eluent was evaporated to dryness. The residue was cleaned as in Method A.

Method D: Butter, margarine, fat spread. Sample was melted on a water bath then extracted with 100 ml acetone 2 times. The acetone mixture was passed through a glass filter and evaporated to dryness. To the residue 50 ml of 10% NaCl solution was added, and plasticizers were extracted by 50 ml ethyl acetate and *n*-hexane mixture (1 : 2) 2 times. The organic layer was gathered and dried over Na_2SO_4 followed by evaporation. To the residue 10 ml *n*-hexane was added, and the plasticizers were extracted with 20 ml acetonitrile 3 times. The lower layer was gathered, evaporated and cleaned as in Method A.

Method E: Vegetable oil. The operation performed was very similar to that used in the study by Kawamura *et al*.[1)] The sample was dissolved in 20 ml

hexane and extracted with 30 ml acetonitrile 3 times. To the acetonitrile layer 250 ml of 10% NaCl solution was added, and the plasticizers were extracted with 100 ml *n*-hexane 2 times. The organic layer was dried over Na_2SO_4 then evaporated and cleaned as in Method A.

Method F: Infant formula, chocolate, cookies, cheese, snacks, and dry noodles. Sample was extracted with 100 ml acetonitrile 2 times. The acetonitrile layer was treated with 40 ml hexane to remove fat or oil then evaporated and cleaned as in Method A.

Method G: Fresh milk and ice cream. The AOAC method for pesticides[8] was modified for plasticizers. To the sample 40 ml water, 50 ml ethanol, 0.5 g sodium oxalate and 50 ml ether and *n*-hexane mixture (1 : 1) were added, and the mixture was shaken for 5 min. The organic layer was separated, and the aqueous layer was extracted 2 more times with 25 ml of an ether and *n*-hexane mixture. The organic layer was gathered and dried over 20 g Na_2SO_4 then evaporated. The residue was dissolved in 100 ml acetonitrile, and fat or oil was removed by extraction in 20 ml *n*-hexane. The acetonitrile layer was evaporated and cleaned as in Method A.

GC/MS Conditions —— Plasticizers were determined using GC/MS. No specific injection system was used. GC was equipped with a normal septum. The conditions were as follows: GC-17A and GCMS-QP5050 (Shimadzu, Kyoto, Japan) equipped with a methyl silicone-coated fused-silica capillary column DB-5MS (0.25 mm i.d. × 30 m, 0.25 μm film thickness, J&W Scientific, Folsom, CA, U.S.A.) were used. The oven temperature was maintained at 50ºC for the first 1 min, then ramped up at 10ºC/min to 270ºC where it was held for 27 min. The injection port and interface were kept at 260ºC. Helium (100 kPa) served as the carrier gas. The injection mode was splitless, the sampling time was 3 min, and the injection volume was 1 μl. An auto injector AOC-20i (Shimadzu) was used. The MS conditions were the following: ionization mode, electron impact, detection voltage, 1.30 kV in usual cases, and 1.00 kV at higher concentration.

Quantification and Confirmation —— The plasticizers were determined by comparing their peak areas with those of standards in GC/MS. A blank version of the test performed on the food samples was conducted every day using 50 ml water. All of the blank values were averaged, and the average value was subtracted from the detected plasticizer values. For the plasticizers detected in blank tests, the limit of detection (LOD) was determined to be three times the standard deviation of the blank values. For the other plasticizers, the mechanical limit of GC/MS was considered to be the LOD. The limit of quantification (LOQ) was set at twice the LOD. DINP was determined by the highest five peaks, DALG was determined by the main two peaks, and DINA was determined by all peaks on the chromatogram.

Quality Assurance —— The recovery of plasticizers from various food samples was assessed. One ml of plasticizer solution (in acetonitrile) was added to a food sample 30 min before extraction. Fortification levels were 0.005 to 4 μg/g.

Quantification of ATBC in the Cap Liner —— The gasket of the cap was scratched off using a knife, and 0.1 g of the gasket was extracted with 100 ml *n*-hexane for 16 hr at 37ºC. ATBC in the extract was quantified by GC/MS.

RESULTS AND DISCUSSION

Analytical Method

A total of 10 plasticizers were well determined under the GC/MS conditions described in the experimental section. DINP standard was provided as a mixture of isomers and gave a set of peaks similar to what has been previously reported.[9] Chromatograms of DAA, DINA, DBS, ATBC and DALG are shown in Fig. 1. Plasticizers that gave more than one peak were quantified by the sum of the area of the peaks. Stable isomers of DBP, BBP, DEHP and DEHA were available, and they were used as surrogates for the isotope dilution technique. Di-*n*-nonyl phthalate (DNP-d_4) was used as a surrogate of DINP because the deuterated isomer of DINP was not available. DAA, DINA, DBS, ATBC and DALG were directly quantified, and no internal standard was used.

Table 3 shows the LOD for each kind of food sample. DBP and DEHP exist so ubiquitously that all blank tests indicated their presence at some level. BBP and DEHA also were sometimes detected. The blank values reflect the contamination levels present in the laboratory, reagents and apparatus. They were averaged and subtracted from the detected values of the plasticizers. The LOD was set as 3 times the standard deviation of the blank test values. For plasticizers not found in the blank tests, the mechanical detection limit in GC/MS was set as the LOD. The

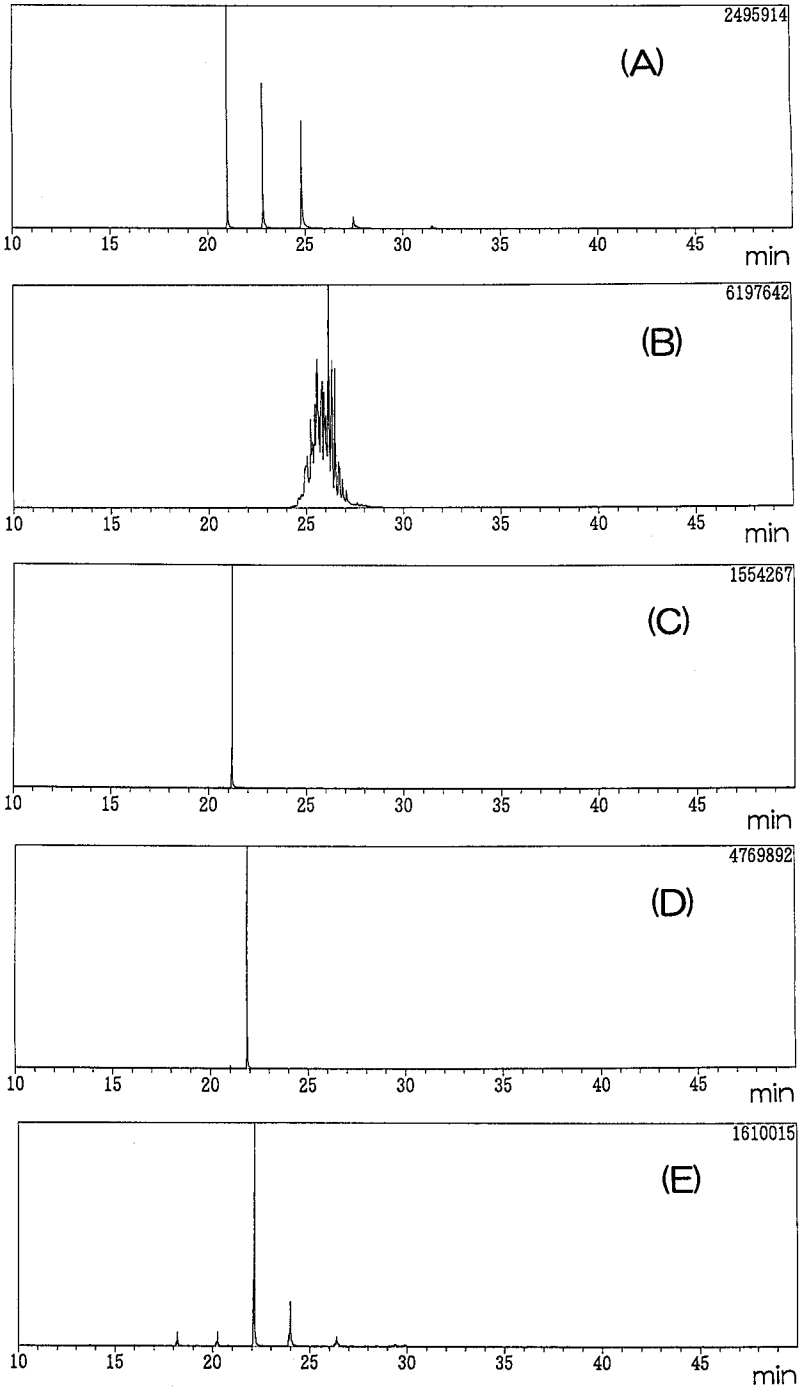

Fig. 1. GC/MS Chromatograms for Plasticizers

(A) DAA; (B) DINA; (C) DBS; (D) ATBC; (E) DALG. GC/MS conditions: instruments: GC-17A and GCMS-QP5050 (Shimadzu); column: DB-5MS (0.25 mm i.d. × 30 m, 0.25 μm film thickness, JandW Scientific; oven temperature: 50ºC (1 min) – (10ºC/min) – 270ºC (27 min); injection port and interface temperature: 260ºC; carrier gas: helium (100 kPa); injection mode: splitless; sampling time: 3 min; ionization mode: electron impact; detection voltage: 1.00 kV; data acquisition mode: scanning; injection volume: 1 μl; concentration of each plasticizer: 1 mg/ml.

blank value and LOD depend on the sample weight. They increase as the sample weight decreases. Sampling weight is different for each food species and the method of analysis. Dry or fatty foods were sampled in small amount because they give much interference in GC/MS chromatogram, whereas more aqueous foods were sampled in larger amount. For example, 50 g of retort-pouched baby foods were sampled, and the LODs for DBP and DEHP were 0.003 and 0.037 μg/g respectively, while 2 g of but-

Table 4. Recovery Rates (%) of Plasticizers from Food Samples ($n = 3$)

Sample	Method of analysis[b]	sampling weight	Plasticizer (Added amount: μg[a])									
			DBP[c] (4)	BBP[c] (0.5)	DEHP[c] (4)	DINP[c] (8)	DAA (4)	DEHA[c] (0.5)	DINA (4)	DBS (4)	ATBC (4)	DALG (4)
Retort-pouched baby food	A	50	96.3	98.4	101.6	124.6	70.4	82.3	32.0	77.1	106.3	75.4
Sake	B	100	99.7	98.1	98.4	103.7	87.9	99.7	89.0	86.2	—[d]	70.3
Wine	B	100	93.4	92.3	91.8	101.1	88.7	91.4	82.2	88.4	75.9	67.4
Beer	C	20	82.2	94.7	100.3	100.9	98.6	93.6	73.6	91.5	65.4	67.6
Butter	D	2	96.0	94.3	92.4	117.9	88.4	92.7	62.4	89.0	99.7	90.4
Vegetable oil	E	2	101.0	101.3	100.6	131.0	80.9	105.4	36.0	82.5	96.6	84.5
Chocolate	F	5	99.1	101.6	106.3	110.1	90.9	101.0	96.9	98.8	115.0	90.3
Infant formula	F	20	98.7	99.1	102.0	105.4	76.1	108.5	53.1	63.4	72.6	62.0
Milk	G	10	98.2	99.4	103.2	124.9	83.1	98.2	39.3	85.1	112.7	113.5
Ice cream	G	5	100.9	102.8	—[d]	115.4	81.2	95.9	71.6	75.2	90.9	91.3

a) Fortification level depends on the sampling weight. It is calculated by dividing the added amount by the sampling weight. *b*) Detail of each method is described in the text. *c*) The recovery value was obtained by the isotope dilution technique. *d*) Correct recovery data could not be gained because the level of originally contained plasticizer was so high.

ter was sampled and the LODs for the two phthalates were 0.28 and 0.19 μg/g respectively. The LOQ was set at a level of twice the LOD.

A total of 10 kinds of food samples were fortified with plasticizers for the recovery test, and the results are shown in Table 4. The fortification levels were different for each food and plasticizer. Phthalates and DEHA were determined by the isotope dilution technique and were recovered at 82.2–131.0%. The recovery of DINP in the fortification test was over 100% as previously reported,[2] possibly due to the lower recovery of DNP-d_4 (40–70%) as its surrogate. Deuterated isomer of DINP was not available, so DNP-d_4 was used instead. The recovery of DINA was lower than 60% for retort-pouched baby foods, butter, vegetable oil, infant formula and fresh milk. The present method is not suitable for DINA quantification, but played a role in monitoring DINA residue. The recovery rates of ATBC and DALG added to chocolate and fresh milk were more than 110%. This result may be due to an enhanced response in the chromatography due to the 'matrix effect.'[10]

Plasticizer Levels in Foods and Possible Sources of Contamination

Table 2 shows the plasticizer levels in 93 samples of retail foods. DBP, BBP, DEHP, DINP, DEHA, ATBC and DALG were detected whereas DAA, DINA and DBS were not detected. Some sources of higher plasticizer contamination in foods have been reported previously, and included not only PVC gloves, but also cling-film,[11–14] milk tubing,[15] alumi laminate,[16] gaskets for glass bottles,[16,17] and casings of sausages.[18] The possible sources of the plasticizers detected in the present study are discussed below.

DEHP was detected in a retort-pouched baby food sample at a level of 4.25 μg/g. This product (Baby Food A) was rice gratin with meat, produced in May 2000. Three samples of Baby Food A produced in September to December 2000 contained less than 0.1 μg DEHP/g as shown in Table 5. The authors inquired about the cooking conditions, and the manufacturer reported that DEHP-containing PVC gloves were formerly used in the production of this food, mixing chopped vegetables and butter, but their use was discontinued when the Japanese government prohibited them in June 2000. Thus the high content of DEHP in Baby Food A was presumed to have originated in gloves that came in contact with foodstuffs. This conclusion is supported by the fact that Baby Food A also contained 0.44 μg DEHA /g. PVC gloves often contain a low level of DEHA in addition to the main plasticizer DEHP.[1,3]

In a previous study conducted in 1999, the authors found 5.99 μg DEHP/g in another retort-pouched baby food. The origin of DEHP was sought and found to be a PVC tube used in production. When fatty and hot foodstuffs passed through the tube, high DEHP elution occurred. The manufacturer then changed to a stainless steel tube. Retort-pouched foods are permitted to be produced using PVC tubing. One sample of retort-pouched baby food pro-

Table 5. Plasticizers in Baby Food A Repeatedly Purchased and Tested[a)]

Sample No.	Production date	Concentrations (μg/g)					
		DBP	BBP	DEHP	DINP	DEHA	ATBC
1	16 May 2000	ND[b)]	0.003	4.25	ND	0.44	Tr[c)]
2	28 Sep 2000	ND	0.001	0.086	ND	0.003	ND
3	5 Dec 2000	ND	0.002	0.099	ND	0.004	ND
4	26 Dec 2000	ND	Tr	Tr	ND	0.006	ND

a) DAA, DINA, DBS and DALG were not detected in any sample. *b*) ND: not detected. *c*) Tr: Trace.

duced by another manufacturer later than June 2000 contained 1.57 μg DEHP/g. Such a higher level of DEHP may be due to the use of PVC tubing in production.

In *sake* bottled in glass cups and sealed with caps, ATBC was found in rather high concentrations, 2.61 to 7.30 μg/g. ATBC was detected in the gasket of the cap at levels of 30–48%. It is likely that the ATBC in the *sake* migrated from the gasket of the cap. ATBC is now expected to replace phthalates as the PVC plasticizer,[19)] but the previous report did not mention the use of ATBC in cap sealing.[17)] *Sake* contains 13–19% ethanol, which could be one reason for the high migration of ATBC. The authors found that DEHP migration occurred from PVC gloves enhanced by ethanol.[3)]

DEHP, DEHA and ATBC were frequently found in dairy products. The fat content of the dairy foods is as follows: butter > cheese > ice cream > milk.[20)] The levels of plasticizers in these foods have positive relationship with the fat contents. Regardless of the fact that the foods were produced by various companies and that the original milk was taken from a variety of locations in Japan. Fresh milk may be ubiquitously contaminated with the 3 plasticizers, and the contamination levels increase as the fat of the milk is concentrated. The source of the contamination, however, is not clear.

DALG was found in 2 baby food samples, Baby Foods B and C, at levels of 5.47 and 4.76 μg/g respectively (Table 2). These foods were products of the same manufacturer, and both contained *tofu* hamburg. Each of 2 replicated samples produced on different dates all contained 3.79 to 6.71 μg DALG/g. The authors obtained the plastic containers or bags that came in contact with the products and foodstuffs and analyzed them for plasticizers, but no DALG or any of the other plasticizers were detected. Thus the DALG in the product was assumed to have originated in the food additive 'glycerol ester of fatty acids,' which was added to *tofu* as an anti-bubble agent. The baby food maker speculated that this food additive was used, and the manufacturer of the food additive recognized the identity of this compound and DALG.

The levels of DEHP and DBP in vegetable oils were rather high, to 1.75 and 2.40 μg/g respectively. Fatty foods are often reported to contain plasticizers or the other environmental pollutants.

One-day or One-time Intake of Plasticizers

Table 6 shows the one-time intake of plasticizers from more highly contaminated foods. The intake is given as a ratio to the body weight of the consumer. The one-time intake of DEHP from one of the retort-pouched baby food samples was found to be 39.5 μg/kg bw, which is very similar to the Japanese TDI of 40 μg/kg bw. This high DEHP may have been due to the use of PVC gloves, and is no longer detected in baby foods as mentioned above. As TDI's are derived on the basis of *lifetime* exposure, there would be no problem if babies were fed with the baby foods containing DEHP of TDI levels for a certain period.

ATBC intake from the *sake* was 26.3 μg/kg bw, which is 3800 times smaller than the NOAEL of the EU, 100 mg/kg bw/day.[19)] DALG intake from the baby food was 62.4 μg/kg bw. It was not a plasticizer but rather a food additive as described above, and it is regarded to be a safe compound for which no guidelines for the level that may be added to foods are specified in Japan.

For the other plasticizers, a total of more than 2 μg/kg bw per one-time intake was not observed in this study. Fat and oil are not generally consumed in high one-time amounts, and so the intake of the contaminants in them are not likely to cause a problem in spite of their higher contamination levels. DBP, BBP, DEHP, DEHA and ATBC that were detected in foods in the present study were not higher than the levels detected in previous research, except for the DEHP detected in Baby Food A. For DINP and

Table 6. Intake of Plasticizers from the Food Samples — Highest Levels Detected

Sample	Plasticizer	Concentration (μg/g)	One-time food intake (g)	Intake of plasticizer (μg)	Body weight of consumer (kg)	Intake per body weight (μg/kg)
Grape seed oil	DBP	2.40	20	48.0	50[a)]	0.96
Olive oil	BBP	0.62	20	12.4	50[a)]	0.25
Retort-pouched baby food A	DEHP	4.25	80	340.0	8.6[b)]	39.53
Baby snack	DINP	1.83	3.3	6.0	7.8[c)]	0.77
Butter	DEHA	2.78	20	55.6	50[a)]	1.11
Sake	ATBC	7.30	180	1314.0	50[a)]	26.28
Retort-pouched baby food B	DALG (food additive)	6.71	80	536.8	8.6[b)]	62.42

a) Body weight of an adult. *b*) Body weight of 9-month-old baby. *c*) Body weight of 6-month-old baby.

DALG, no comparable data exist.

In conclusions, plasticizers in Japanese retail foods were determined by GC/MS. A survey of 93 samples showed a retort-pouched baby food sample to be contaminated by DEHP at the Japanese TDI level. The source of contamination was supposed to be PVC gloves, because the baby food was produced before the prohibition of PVC gloves by the Japanese government. After this prohibition, the baby food product contained a much lower level of DEHP. DEHP and the other plasticizers in the other foods under study were quite low compared to their TDI or NOAEL.

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