change in female rats (Quast et al. 1983). In contrast, a tolerable daily intake has been not proposed for 1-CB, and its toxicity would be lower than VDC. In a worst case scenario, if an adult weighing 50 kg ate 100 g of sample number 1 (sausage) containing the maximum levels of VDC (0.020 $\mu g\,g^{-1}$) and 1-CB (0.040 $\mu g\,g^{-1}$) found in this study, the estimated intake would be 0.04 $\mu g\,kg^{-1}$ body weight day^{-1} for VDC and 0.08 $\mu g\,kg^{-1}$ body weight day^{-1} for 1-CB. The VDC intake was 1/1250 of the tolerable daily intake and could be regarded as having no influence on human health from this sample.

Conclusion

An unknown peak detected in the PVDC casing films was identified as 1-CB by GC/MS. In order to determine simultaneously VDC and 1-CB in foods packaged with PVDC casing films, a headspace GC/MS method was successfully developed using 1-chloropropane as the internal standard, and the previous method (Ohno et al. 2005) was modified to determine these compounds in the casing films. In 13 samples tested, VDC was detected at 0.001–0.020 $\mu g\,g^{-1}$ in 11 foodstuffs and at 0.04 $\mu g\,g^{-1}$ in only one casing film. The VDC levels found in the foodstuffs were nearly identical to those by Gilbert et al. (1980). On the other hand, 1-CB was detected in all foodstuffs and casing films, and its presence was first confirmed in this study. 1-CB was detected at 0.004–0.040 $\mu g\,g^{-1}$ in the foodstuffs, while accurate 1-CB levels in the casing films could not be obtained. Further investigation for 1-CB is necessary to clarify the accurate levels and mechanisms of generation in PVDC films. This study has demonstrated that VDC and 1-CB migrated from the casing films into packaged foods and remained there during long storage. The VDC levels in the foodstuffs could be regarded as having no influence on human health; however, no safety evaluation has been undertaken of 1-CB intake from foods packaged with PVDC casing films.

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Migration of dehydroabietic and abietic acids from paper and paperboard food packaging into food-simulating solvents and Tenax TA

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Abstract
An investigation was undertaken to establish the concentration in paper products of dehydroabietic (DHA) and abietic (AA) resin acids, present in rosin, which are major toxicants of pulp- and paper-mill effluent. Their migration was studied from paper and paperboard products into various food-simulating solvents and the substitute fatty food simulant Tenax TA (modified polyphenylene oxide). DHA and AA were detected in five of 10 virgin paper products and in all 10 recycled paperboard products for food-contact use at concentrations of 14–500 and 110–1200 µg/g, respectively. In virgin paper products, the highest migration was into 95% ethanol or heptane, with negligible or no migration into other solvents. In recycled paperboard products, migration was highest into 95% ethanol, but was also observed into 20% ethanol, water and heptane. Migration to Tenax TA was also observed and the migration level increased with time. The maximum migration levels of DHA and AA into food simulants were 0.853 and 3.14 µg/g, respectively. The results suggest that, in the worst case, the daily intake of DHA and AA from paper and paperboard products was 50 times lower than the tolerable daily intake of rosin.

Keywords: *Paper, paperboard, food packaging, migration, dehydroabietic acid, abietic acid, rosin, Tenax TA*

Introduction

Dehydroabietic acid (DHA) and abietic acid (AA), resin acids found in different types of rosin, are major toxicants of pulp- and paper-mill effluent and a primary sources of toxicity in fish (Zanella 1983; Volkman et al. 1993; Wang et al. 1995). DHA increases cell death in human epithelial and fibroblast cells (Söderberg et al. 1996), exerts a toxic effect on human erythrocytes (Toivola and Isomaa 1991) and induces polymorphonuclear leukocytes (Sunzel et al. 1991). AA causes lysis of human alveolar epithelial cells (Ayars et al. 1989), while DHA and AA have been reported to be non-genotoxic in Salmonella/mammalian-microsome assay (Nestmann et al. 1979), but to be genotoxic in *rec*-assay (Ozaki et al. 2005).

Paper and paperboard are widely used in food container and packaging items, such as dishes, cups, kitchen paper and food boxes. Paper products are classified into virgin products, which are made from wood chips, and recycled-products, which are made from recovered fibre. As this material is subjected to extensive treatment involving the use of inks, adhesives, *etc.*, various contaminants are found in recycled products (Sturaro et al. 1994; Castle et al. 1997; Sipiläinen-Malm et al. 1997; Vinggaard et al. 2000; Binderup et al. 2002; Ozaki et al. 2004). Toxicological studies of virgin and recycled paper have shown that recycled paper is more toxic than virgin paper, but without identifying the toxicants (Baba et al. 1998; Fauris et al. 1998; Binderup et al. 2002). We previously examined the genotoxicity of virgin and recycled paper products using *rec*-assay, and identified the unknown genotoxicants as DHA and AA (Ozaki et al. 2005).

The purpose of the present study was to investigate whether DHA and AA (Figure 1) are present in

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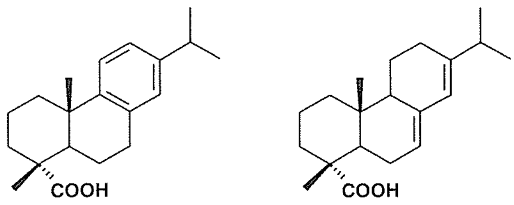

Dehydroabietic acid (DHA, Cas No. 1740-19-8)

Abietic acid (AA, Cas No. 514-10-3)

Figure 1. Chemical structures of DHA and AA.

paper and paperboard products used in contact with food. The migration level of DHA and AA from paper and paperboard products into a number of food-simulating solvents and into the substitute fatty food simulant Tenax TA (modified polyphenylene oxide) was also examined to further elucidate the level of consumer exposure to these compounds. Distilled water, 4% acetic acid and 20% ethanol were used as aqueous simulants, and 95% ethanol and heptane were used as alternative fatty simulants. Ethanol (20%) is used as the alcoholic simulant in Food Sanitation Law in Japan.

Materials and methods

Materials

Solvents and reagents. Dehydroabietic acid (DHA) was purchased from Wako Pure Chemical Industries (Osaka, Japan), and abietic acid (AA) from Tokyo Kasei Kogyo (Tokyo, Japan). Tenax TA (60–80 mesh) was obtained from Supleco (Bellefonte, PA, USA). Ammonium acetate and organic solvents were purchased from Wako Pure Chemical or Kanto Chemical (Tokyo, Japan). All organic solvents were of HPLC or residual pesticide analytical reagent grade.

Samples. Recycled paperboard (recycled fibre >95%) was used to explore experimental conditions for the migration tests. Twenty paper and paperboard products used in contact with food were classified into two groups: 10 virgin paper products and 10 recycled paperboard products. The products were purchased at a supermarket in Japan in 2005.

Methods

Liquid chromatography/mass spectrometry (LC/MS). Chromatographic analysis was performed according to the method of Ozaki et al. (2005) using a Hewlett–Packard 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an API 2000 mass spectrometer fitted with an electrospray ionization interface (ESI) operating in negative-ion mode (Applied Biosystems, Foster City, CA, USA). LC conditions were as follows: column: ZORBAX Eclipse XDB-C_{18} (150 × 2.1 mm I.D., 5 μm particle size; Agilent Technologies); oven temperature held at 30°C; mobile phase: 50 mM ammonium acetate in water/acetonitrile (2 : 8, v/v); flow rate: 0.2 ml/min; injection volume: 10 μl. MS conditions were as follows: ion spray voltage: −3800 V; ion source temperature: 550°C; declustering potential: −66 V; focusing potential: −330 V for DHA, −200 V for AA; entrance potential: −10 V for DHA, −4.5 V for AA. Quantitative analysis was performed using selected ion monitoring of m/z 299.1 for DHA and m/z 301.2 for AA. Measurement was carried out using the respective calibration curves for DHA and AA, ranging from 0.1 to 50 ng.

Extraction of DHA and AA from test samples. To prepare each sample, three examples of the product were purchased and equal amounts of them were weighed and mixed together. The samples were cut into pieces (1 × 1 cm), and 5 g of sample was refluxed with 100 ml ethanol for 2 h. After filtration through funnels with fritted glass discs, the ethanol solution was evaporated and diluted to 10 ml with methanol. A 1-ml sample of the methanol solution was diluted to 10 ml with the LC/MS mobile phase and 1 ml of the resulting solution again diluted to 10 ml with the mobile phase. After filtration, the finally resulting solution was subjected to LC/MS analysis.

Migration tests. To detect possible DHA and AA transfer from paper samples into food, migration tests were carried out. Distilled water, 4% acetic acid and 20% ethanol were used as aqueous simulants and 95% ethanol, heptane and Tenax TA (modified polyphenylene oxide) as alternative fatty simulants. The time and temperature conditions were as follows: for distilled water, 4% acetic acid and 20% ethanol: 30 min at 60 and 95°C, and 1 day at 40°C; for 95% ethanol: 30 min at 60°C; for heptane: 1 h at 25°C; for Tenax TA: 30 min at 60, 100 and 150°C, and 1 and 2 days at 40°C. In one-side tests, samples were cut into circles (50 cm^2 contact area) and the side intended for food contact exposed to the simulants. The ratios of liquid and Tenax TA simulant volume to contact area were 2 ml/cm^2 and 0.04 g/cm^2, respectively. Five ml of the distilled water, 4% acetic acid, and 20 and 95% ethanol migration solutions was diluted to 10 ml with the LC/MS mobile phase and filtered for LC/MS analysis. One ml of the heptane migration solutions was

Table I. Recoveries of DHA and AA.

		Spiked amount (μg)	Recovery ± SD (%) DHA	AA
Extraction test		100	98 ± 7.1	92 ± 5.2
Migration test	Water	1	85 ± 0.3	85 ± 0.7
		10	91 ± 1.2	92 ± 1.2
	4% Acetic acid	1	78 ± 0.3	75 ± 0.2
		10	82 ± 3.6	90 ± 3.0
	20% Ethanol	1	102 ± 0.4	99 ± 0.2
		10	94 ± 0.2	96 ± 0.8
	95% Ethanol	1	102 ± 0.2	93 ± 0.2
		10	98 ± 2.6	103 ± 2.4
	Heptane	0.1	97 ± 0.5	88 ± 0.6
		1	100 ± 3.1	98 ± 2.1
	Tenax TA	0.1	103 ± 2.6	80 ± 0.9
		1	95 ± 1.5	88 ± 1.8

Results are mean ± S.D. of three trials.

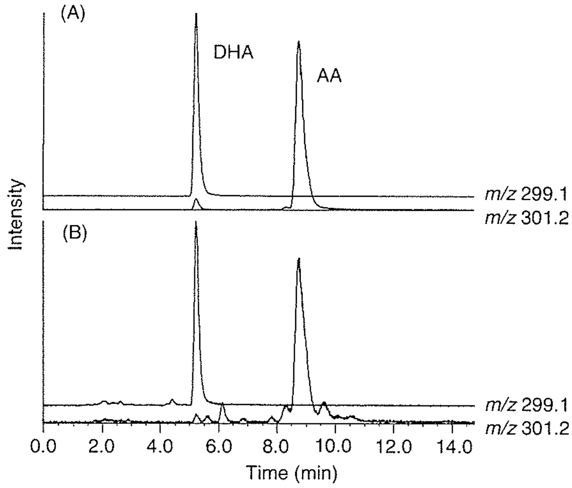

Figure 2. LC/MS chromatograms of DHA and AA in standard solution and paper sample solution. (A) DHA and AA 1 μg/ml, (B) food box-1 in the migration test with 20% ethanol.

dried under a nitrogen stream, dissolved in 1 ml of the mobile phase and filtered for analysis. Tenax TA migration tests were carried out on the basis of European standard EN 14338 (CEN 2003). After the migration test, the simulant (2 g) was extracted twice with 20 ml of acetone. After evaporation, the residue was dissolved in 1 ml of mobile phase and filtered for analysis.

Results

Analytical procedure

The results of recovery test are shown in Table I. Each value is taken from the average of three trials ($n = 3$). In the extraction test, recoveries of 92–98% were obtained for a paper sample (5 g) spiked with 100 μg of DHA and AA. In the migration test, recoveries of 75–103% were obtained for food simulant samples (heptane: 1 ml, Tenax TA: 2 g, other simulants: 5 ml) spiked with DHA and AA (heptane and Tenax TA: 0.1 and 1 μg each, other solvents: 1 and 10 μg each) in the migration tests. The limit of quantification ($S/N \geq 10$) for DHA/AA were 0.01 μg/ml standard solution, and the method showed good linearity over the concentration range tested, 0.01–5 μg/ml ($R \geq 0.9999$). The repeatability for DHA and AA standard solution (1 μg/ml, $n = 5$) was 0.72 and 0.75% RSD with peak area evaluation, and 0.12 and 0.08% RSD with migration time evaluation, respectively. The mass chromatograms of the standard and test solutions are shown in Figure 2.

Extraction test

DHA and AA levels in the virgin paper and recycled paperboard products are shown in Table II. In the 10 virgin paper products, both DHA and AA were detected in five samples at respective levels of 14–500 and 110–1200 μg/g. DHA and AA were detected in three of the four dishes, in absorbent paper and in cups, but not in kitchen papers and napkins. In the 11 recycled paperboard products, both DHA and AA were detected in all samples tested at respective levels of 55–230 and 260–880 μg/g.

Migration test of recycled paperboard

Recycled paperboard was tested under various food-simulating conditions to select the appropriate conditions for the migration test of food-contact paper products. The time and temperature conditions were as follows: for distilled water, 4% acetic acid and 20% ethanol: 30 min at 60 and 95°C, and 1 day at 40°C; for 95% ethanol: 30 min at 60°C; for heptane: 1 h at 25°C. Results are shown in Table III. Under the same migration time and temperature conditions (30 min at 60°C), DHA and AA migrated most to 95% ethanol, followed by 20% ethanol and water. In the case of 4% acetic acid, there was no detectable migration under any conditions. Migration levels into heptane (1 h at 25°C) were similar to those into 20% ethanol and water under conditions of 30 min at 60 or 95°C. With the same solvent, the migration level showed a great increase when the temperature was reduced from 60 or 95 to 40°C, but the time was extended from 30 min to 1 day. However, after testing for 1 day at 40°C, paperboard absorbed too much simulant to retain

Table II. Content of DHA and AA in virgin paper and recycled paperboard products.

Virgin paper products			Recycled paperboard products		
	Content (μg/g)			Content (μg/g)	
Sample	DHA	AA	Sample	DHA	AA
Dish-1	500	330	Paperboard	140	370
Dish-2	24	180	Food box-1	230	830
Dish-3	51	460	Food box-2	72	270
Dish-4	ND	ND	Food box-3	55	540
Kitchen paper-1	ND	ND	Food box-4	110	320
Kitchen paper-2	ND	ND	Food box-5	59	260
Napkin-1	ND	ND	Food box-6	120	880
Napkin-2	ND	ND	Food box-7	83	580
Absorbent paper	14	110	Food box-8	130	300
Cup	240	1200	Food box-9	130	490
			Food box-10	70	330

ND <2 μg/g.

Table III. Migration levels of DHA and AA into food-simulating solvents from recycled paperboard under various conditions.

		Migration (μg/ml)	
Solvent	Conditions	DHA	AA
Water	60°C, 30 min	0.05	ND
	95°C, 30 min	0.08	0.07
	40°C, 1 day	0.74	0.40
4% Acetic acid	60°C, 30 min	ND	ND
	95°C, 30 min	ND	ND
	40°C, 1 day	ND	ND
20% Ethanol	60°C, 30 min	0.04	ND
	95°C, 30 min	0.10	0.10
	40°C, 1 day	1.48	1.05
95% Ethanol	60°C, 30 min	0.08	0.12
Heptane	25°C, 1 h	0.05	0.04

Results are mean of two trials. ND <0.02 μg/ml.

Table IV. Migration levels of DHA and AA into Tenax TA from recycled paperboard under various conditions.

	Migration (μg/g)	
Conditions	DHA	AA
60°C, 30 min	0.006	0.009
100°C, 30 min	0.062	0.066
150°C, 30 min	0.17	0.30
40°C, 1 day	0.13	0.15
40°C, 2 days	0.24	0.38

Results are mean of two trials.

sufficient operating strength and the findings were concluded to be inconsistent with reality. Migration level increased only slightly when the temperature was raised from 60 to 95°C, and 60°C was selected to compare the migration level under the same temperature from food-contact paper and paperboard products to water, 4% acetic acid, 20% ethanol and 95% ethanol. From these results, the conditions chosen for the migration test of food-contact paper and paperboard products were 30 min at 60°C for water, 4% acetic acid, and 20 and 95% ethanol, and 1 h at 25°C for heptane.

Next, time and temperature conditions for Tenax TA were explored. The conditions tested were as follows: 30 min at 60, 100 and 150°C, and 1 and 2 days at 40°C. Results are shown in Table IV. Migration levels increased around 30-fold when temperature was raised from 60 to 150°C. However, even when the temperature was kept relatively low (40°C), migration levels after 2 days at 40°C were higher than after 30 min at 150°C. Since paper products are rarely used under conditions of high temperature, such as heating in the microwave oven, conditions of 1 and 2 days at 40°C and 30 min at 60°C were chosen for the migration test of food-contact paper and paperboard products.

Migration test of food-contact paper packaging

Results of the tests of DHA and AA migration into food-simulating solvents are shown in Table V. Three kinds of dish (virgin paper product) and three kinds of food box (recycled paperboard product) containing DHA and AA were tested. The DHA and AA contained in the dishes showed the highest migration into heptane or 95% ethanol, with negligible or no migration into other solvents. The DHA and AA contained in food boxes, on the other hand, showed the highest migration into 95% ethanol and also migrated into 20% ethanol, water and heptane. The highest migration levels for

Table V. Migration levels of DHA and AA into food-simulating solvents from paper/paperboard products (µg/ml).

		Dish-1		Dish-2		Dish-3		Food box-1		Food box-2		Food box-3	
Solvent	Condition	DHA	AA	DHA	AA	DHA	AA	DHA	AA	DHA	AA	DHA	AA
Water	60°C, 30 min	0.02	ND	ND	ND	ND	0.02	0.23	0.31	0.07	0.12	0.04	0.19
4% Acetic acid	60°C, 30 min	0.07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20% Ethanol	60°C, 30 min	0.09	0.03	ND	ND	ND	0.11	0.17	0.28	0.06	0.13	0.03	0.17
95% Ethanol	60°C, 30 min	0.31	0.19	0.04	0.24	0.05	0.68	0.37	2.52	0.10	0.36	0.05	0.41
Heptane	25°C, 1h	0.79	0.45	0.02	0.14	ND	0.09	0.06	0.26	0.029	0.11	ND	0.11

Results are mean of two trials. ND: water, 4% acetic acid, 20% ethanol and 95% ethanol: <0.02 µg/g; heptane <0.01 µg/g.

Table VI. Migration levels of DHA and AA into Tenax TA from paper/paperboard products (µg/g).

	Dish-1		Dish-2		Dish-3		Food box-1		Food box-2		Food box-3	
Conditions	DHA	AA	DHA	AA	DHA	AA	DHA	AA	DHA	AA	DHA	AA
60°C, 30 min	0.057	0.034	ND	0.017	ND	0.031	ND	0.007	ND	0.008	ND	0.009
40°C, 1 day	0.725	0.396	0.063	0.228	0.027	0.157	0.047	0.135	0.022	0.092	0.016	0.164
40°C, 2 days	0.853	0.504	0.069	0.286	0.045	0.256	0.063	0.254	0.030	0.133	0.022	0.209

Results are mean of two trials. ND <0.005 µg/g.

DHA and AA, respectively, were observed from dish-1 into heptane (0.79 µg/ml) and food box-1 into 95% ethanol (2.52 µg/ml); these two packaging items contained the highest levels of DHA (500 µg/g) and AA (830 µg/g).

Results of the tests of DHA and AA migration into Tenax TA are shown in Table VI. Migration levels of DHA and AA in 30 min at 60°C were from not detected (ND) to 0.057 µg/g and 0.007–0.034 µg/g, respectively, but were 5–19-fold greater under conditions of 1 day at 40°C and 8–36-fold greater under conditions of 2 days at 40°C. The highest levels of DHA and AA migration were observed from dish-1, which contained 500 and 330 µg/g, respectively, of the two substances.

Discussion

DHA and AA are major resin acids of the abietic type found in different types of rosin. Rosin has a wide variety of applications and is commonly used in the manufacture of soap, paints, adhesives, printing inks, coatings and paper-sizing. In the present study, DHA and AA were detected in all recycled paperboard food boxes. There are a number of possible origins. Wood-pulp type and pulping method may correlate with content of DHA and AA, as the two substances are major components of conifer oleoresin found in pine, spruce and fir trees. In chemical pulping, most of the rosin components are separated from the pulp, leaving tall oil rosin as a by-product. In pulp from mechanical pulping, however, which is mainly used for newspaper, most of the wood's original components are retained and rosin components are present. As various types of post-consumer paper, such as newspaper, magazines and corrugated boxboard, are used as material for recycled paperboard, the pulp is of mixed type and DHA and AA were found in all recycled paperboard products. The type of printing ink and adhesive used in post-consumer paper may also be relevant. In the present study, DHA and AA were detected in half of the virgin paper products. There was some tendency for products used to hold water or oil, such as dishes and cups, to contain DHA and AA. As paper-sizing agent gives the paper resistance to moisture and controls ink penetration, the DHA and AA contained in dishes and cups may originate from rosin used as sizing agent. The DHA and AA detected in cup material, which was the only product coated with polyethylene film, may also originate from adhesives used between the paper and the film.

Rates of migration from paper products to food-simulating solvents were calculated from the results of extraction and migration tests. As shown in Figure 3, migration rates into 95% ethanol were the highest (7–10%), with rates for dishes and food boxes almost the same. Migration rates to water, 20% ethanol and heptane, on the other hand, differed between dishes and food boxes. For both DHA and AA, the migration rates from food boxes into water and 20% ethanol were in the respective ranges of 2–5 and 2–4%, much higher than from dishes (0.1 and 0.4–0.8%, respectively). Although the rate of DHA and AA migration from

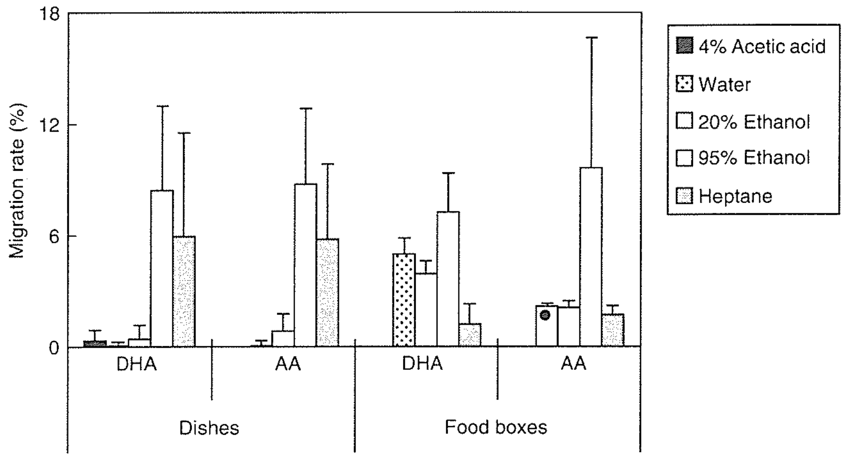

Figure 3. Migration rates of DHA and AA from dishes and food boxes into food-simulating solvents. Migration conditions: heptane 25°C/1 h, other solvents 60°C/30 min.

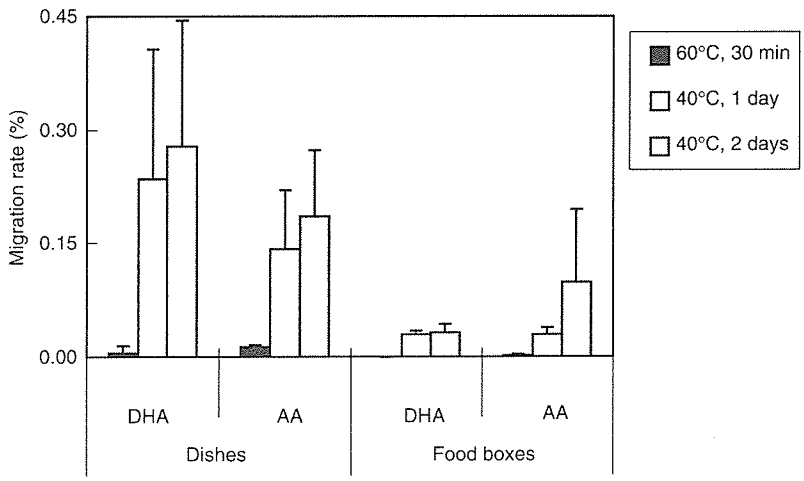

Figure 4. Migration rates of DHA and AA from dishes and food boxes into Tenax TA.

dishes into heptane (6%) was much higher than from food boxes, this was mostly attributable to dish-1, which showed an especially high level of migration into heptane (Table V). Migration rates from paper products into Tenax TA, calculated from the results of extraction and migration tests, are shown in Figure 4. Migration rates were no higher than 0.46%. Under all conditions, migration rates from dishes were higher than from food boxes. The differences between dishes and food boxes also indicate differing origins of DHA and AA in the products. DHA and AA in dishes are presumed to be added to the surface as sizing agent, and were easy to migrate even to heptane, which has relatively small affinity with the paper. On the other hand, DHA and AA in food boxes are presumed to originate from recycled fibres, so exist not only on the surface but also inside the paperboard. Therefore, water and 20% ethanol, which have enough affinity to soak into the paperboard, resulted in high migration rates. Moreover, migration rates to Tenax TA might were higher in the dishes, where DHA and AA exist on the surface. However, there are many other factors which may affect migration rates, such as initial content and diffusion coefficient of DHA and AA in paper/paperboard. Further study is required for clarification.

From migration data, we roughly estimated the daily worst-case intake of DHA and AA, and compared it with the tolerable daily intake (TDI) of rosin. TDI was used because few genotoxicological studies have been published for

DHA and AA, and several other *in vitro* and *in vivo* tests are required to confirm their genotoxicity. Rosin TDI, 1 mg/kg body weight/day, is established by the EU Scientific Committee on Food as the sum of resin acid, fatty acid tall oil, *etc.* (SCF 1978, 1987). We have determined the content of DHA and AA in one rosin sample and percentage compositions were 8.5 and 26.2%, respectively (data not shown).

Daily worst-case intake of DHA and AA was estimated using the following equation: [Worst-case migration (μg/g) × consumption factor (CF) × consumption of food per day (g)]/body weight (kg). CF is the consumption factor of paper products, which represents the percentage of the diet expected to have been in contact with articles fabricated from paper products. A value of 10% was used for this (US FDA). Worst-case migration data for this calculation were selected from Tables V and VI. The highest migration values were 0.853 μg/g from dish-1 into Tenax TA for DHA and 2.52 μg/ml (3.14 μg/g) from food box-1 into 95% ethanol for AA. Consumption of food per day and body weight was set at 3 and 60 kg, respectively (US FDA). From these factors, daily intake of DHA and AA in the worst-case scenario was as follows: $[(0.853 + 3.14) \times 0.1 \times 3000]/60 = 20.0\,\mu g/kg$ body weight/day or 0.020 mg/kg body weight/day. The calculated data was 50 times lower than TDI for rosin. Moreover, migration to actual food will be less than above because migrations to food simulants are usually higher than to actual foods. Consequently, the value calculated for the worst case was 50 times lower than the TDI, indicating that there is no safety concern relating to DHA and AA in the tested virgin and recycled paper products.

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