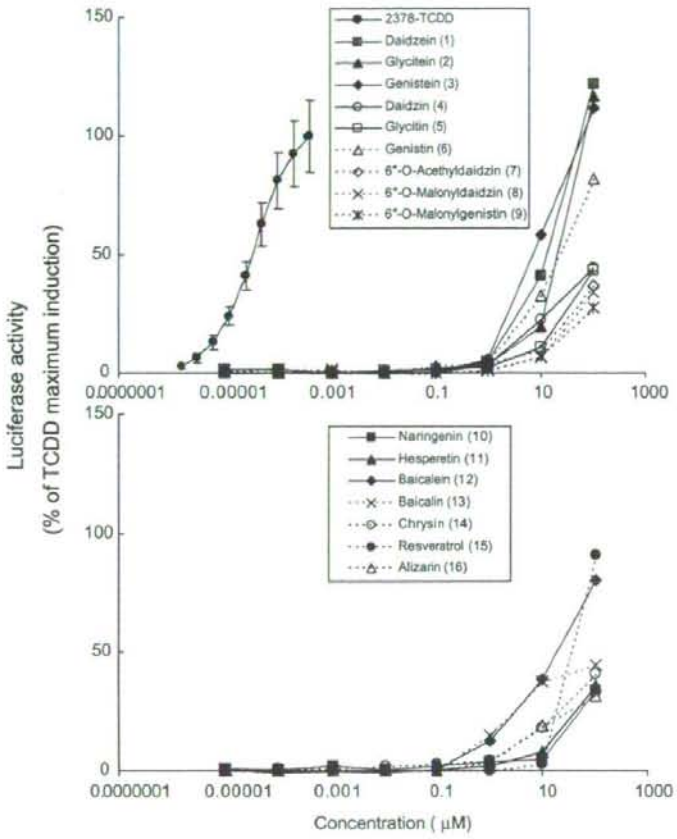

Fig. 3. Concentration–response curves of positive vegetable polyphenols and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for the induction of luciferase activity in the CALUX assay. Each point represents the mean of at least three replicates (40 experiments in the case of TCDD).

on a log scale for some samples are also shown in Fig. 3. Most tested compounds showed no luciferase induction at the concentration level of 100 μM, but some showed luciferase activity at higher concentrations. Of the samples tested, isoflavones produced responses that reached maximal TCDD levels (Fig. 3 and Table 1). EC_{TCDD25} (EC_{TCDD50}) values for daidzein (**1**), glycitein (**2**), and genistein (**3**) were 3.0 (7.9), 4.2 (20.6), and 2.4 (7.0) μM, respectively. Their glycosides showed lower AhR responses than the corresponding aglycones [EC_{TCDD25}: 12.0, 20.0, and 4.2 μM for daidzin (**4**), glycin (**5**), and genistin (**6**), respectively], and their acetylates or malonylates showed much lower induction (EC_{TCDD25}: 32.0, 48.0, and 98.0 μM for 6″-acetyldaidzin (**7**), 6″-malonyldaidzin (**8**), and 6″-malonylgenistin (**9**), respectively).

Among several flavanones, naringenin (**10**) and hesperetin (**11**) elicited agonist-like AhR-mediated activity (EC_{TCDD25}: 53.0 and 38.0 μM). Their glycosides (naringin and hesperidin) and flavanonols including (+)-taxifolin and (+)-fustin did not induce activity at the EC_{TCDD25} level. The tendency for glycosides to weaken these activities was similar to that of isoflavones. Of the flavones, baicalein (**12**) and baicalin (**13**) induced the production of luciferase activity (EC_{TCDD25}: 2.8 and 3.2 μM). Chrysin (**14**) slightly induced the activity at 14.0 μM in EC_{TCDD25}. On the other hand, apigenin (**17**), luteolin (**18**), and others slightly induced the activity at high concentrations in the order of 10–100 μM, but no luciferase induction reached the EC_{TCDD25} level. Also, flavonols, myricetin (**21**), morin (**22**), and others slightly activated luciferase at high concentration on the order of 100 μM.

Based on these findings, the structure–activity correlations in the activation of AhR by flavonoids suggested that the level of activity depends on the molecular size, polarity, and structure of isoflavones and flavanones. Isoflavones such as daidzein (**1**), glycitein (**2**), and genistein (**3**) had similar AhR-inducing potencies, while their glycosides and 6″-*O*-acylates showed lower induction levels. Similarly, flavanone glycosides had weaker activity than their corresponding aglycones such as naringenin (**10**) and hesperetin (**11**), indicating that the increase in the molecule's polarity clearly weakened the activity. Flavanone 3-ols and flavan 3-ols such as (+)-catechin and (−)-epicatechin showed poor induction of luciferase activity. Therefore,

Table 1
Relative responses of the reporter gene system to some polyphenolic constituents (data from Amakura et al., 2003a)

	EC_{TCDD25} (μM)[a]	EC_{TCDD50} (μM)[a]
2,3,7,8-TCDD	1.3×10^{-5} (EC_{25})	3.0×10^{-5} (EC_{50})
Isoflavones		
Daidzein (**1**)	3.0	7.9
Glycitein (**2**)	4.2	20.6
Genistein (**3**)	2.4	7.0
Daidzin (**4**)	12.0	–[b]
Glycitin (**5**)	20.0	–
Genistin (**6**)	4.2	22.6
6″-Acetyldaidzin (**7**)	32.0	–
6″-Malonyldaidzin (**8**)	48.0	–
6″-Malonylgenistin (**9**)	98.0	–
Flavanones		
Naringenin (**10**)	53.0	–
Hesperetin (**11**)	38.0	–
Flavones		
Baicalein (**12**)	2.8	18.8
Baicalin (**13**)	3.2	–
Chrysin (**14**)	14.0	–
Others		
Resveratrol (**15**)	7.3	34.3
Alizarin (**16**)	30.0	–

Each value is the mean of at least three replicates.
[a] Concentration producing luciferase activity equal to 25% (or 50%) of the maximal response to TCDD. Calculated from the slope of the linear portion of each dose–response curve near the origin.
[b] No luciferase induction to the EC_{TCDD50} level observed.

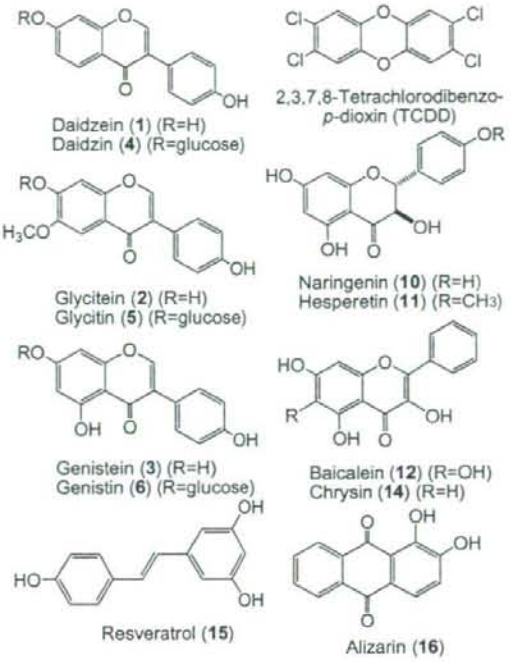

Fig. 4. Structures of TCDD and some tested polyphenols.

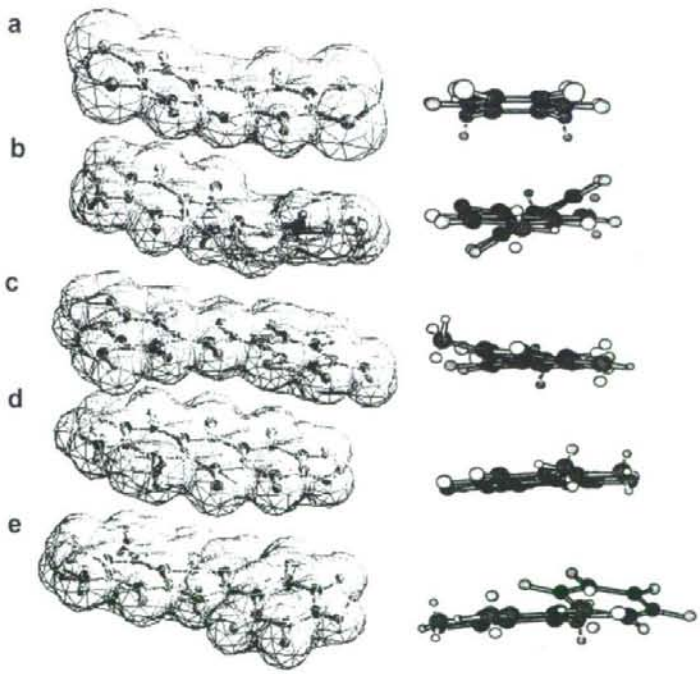

Fig. 5. Molecular models of AhR-activating polyphenols obtained as MM2-minimized structures, (a) TCDD; (b) daidzein (**1**); (c) resveratrol (**15**); (d) alizarin (**10**); (e) baicalein (**12**). Energy-minimized structures were calculated by molecular mechanics using MM2 in CS Chem 3D.

the hydroxyl group at C-3 of the C-ring may also contribute to weakening the activity. Baicalein (**12**), baicalin (**13**), and chrysin (**14**), which induced AhR activation, each possess two or three hydroxyl groups at C-5, -6, and -7 of the A-ring, but none on the B-ring. Other flavones, apigenin (**17**), luteolin (**18**), and vitexin, which weakly induced the activity, each have a hydroxyl group on the B-ring. As for flavones, hydroxyl groups on the B-ring reduced the activation, while the hydroxyl groups on the A-ring had a negligible influence. Similarly, flavonols, which possess hydroxyl group(s) on the B-ring, poorly induced activation, with the exception of myricetin (**21**) and morin (**22**), each of which caused a slight induction at the 100 μM level. Thus, in naturally occurring flavones and flavonols, hydroxyl group(s) on the B-ring and in C-3 of the C-ring may reduce activation.

Among the other compounds tested, resveratrol (**15**), having a *trans*-stilbene structure, showed strong AhR-inducing potency comparable to the maximum induction of TCDD at a high concentration level [7.3 (34.3) μM in EC_{TCDD25} (EC_{TCDD50})], while analogues possessing longer carbon chains [rosmarinic acid, curcumin (**35**)] showed significantly lower AhR inducing capabilities. Previously, the AhR ligand activity of *trans*-stilbene was reported, so the present result further demonstrated that the molecular size in the *trans*-stilbene is important for this activity (Kato et al., 2002). Among the anthraquinones, alizarin (**16**) showed AhR activation (30.0 μM in EC_{TCDD25}), whereas emodin (**32**) and aloe-emodin (**33**) exhibited only weak AhR activity. Although they each possess a structure similar to that of TCDD, substituents (–OH, $–CH_3$, and $–CH_2OH$) in emodin (**32**) and aloe-emodin (**33**) may contribute less than in alizarin (**16**) to the activity. Condensed and hydrolyzable tannins, phenolcarboxylic acids, rosmarinic acid, and curcumin (**35**) induced little production of luciferase at EC_{TCDD25} levels.

Fig. 5 depicts molecular models of TCDD and several compounds that induced luciferase activity [daidzein (**1**),

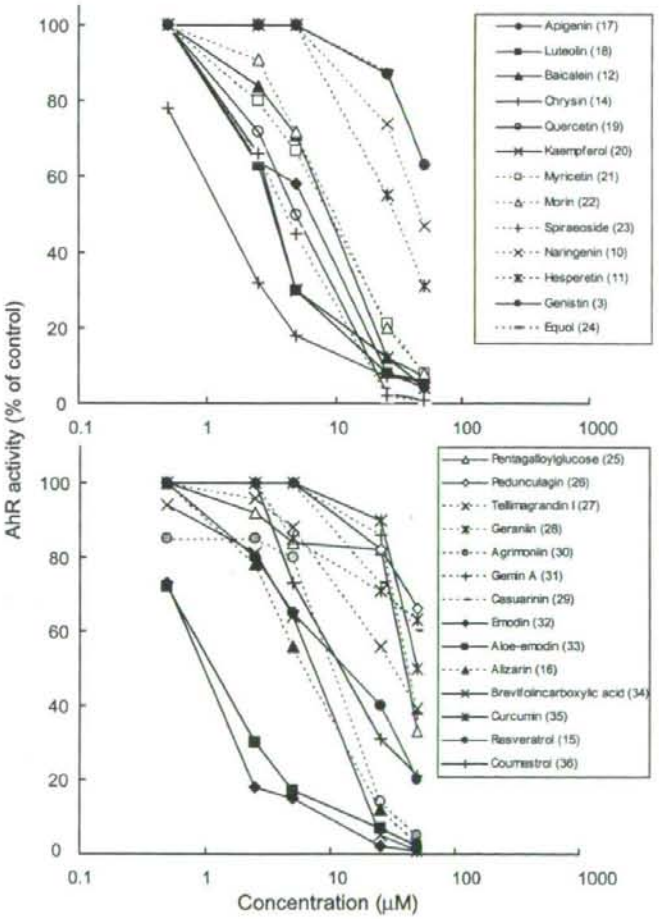

Fig. 6. Dose-dependent inhibitory effect of some vegetable polyphenols on AhR-activation induced by TCDD in Ah-I. Each point represents the mean of two or three replicates.

resveratrol (**15**), alizarin (**16**), and baicalein (**12**)]; these models were each analyzed for their minimum energy conformation. The AhR has been found to favor hydrophobic molecules with quasi-planar structures and to accommodate a ligand-binding pocket (Safe, 1986; Landers and Bunce, 1991; Denison and Nagy, 2003). As shown in Fig. 5, these active compounds have molecular sizes and planar structures similar to those of TCDD.

Isoflavones such as daidzein (**1**), the so-called phytoestrogens, exhibited AhR activation in this *in vitro* experimental system. Southeast Asian and Japanese people often consume soybeans and soybean-derived products, which contain isoflavones in abundance. It is also known that these soy isoflavones may have some health-enhancing properties (Murkies et al., 1998; Setchell and Cassidy, 1999). Therefore, it is considered that a modest AhR-inducer including foods may perform some beneficial regulatory role in the homeostasis, proliferation, and differentiation of cells in animals (Harper et al., 2006).

Isoflavones are known to be mostly metabolized in the body, and their metabolites may not induce AhR because equol, which is regarded as the final metabolite of isoflavones, only slightly induced AhR activity at high concentrations. On the other hand, it has also been reported that two soy isoflavones, daidzein (**1**) and genistein (**2**), appear to be incorporated into tissues after ingestion of baked soybean powder by humans (Watanabe et al., 1998). Since the daily intake and serum excretions of daidzein (**1**) and genistein (**2**) by Japanese has been assessed [daily intake of daidzein (**1**) and genistein (**2**) were *ca.* 20 and 30 mg/day, respectively; serum excretions were *ca.* 120 and 475 nM, respectively] (Yamamoto et al., 2001), it is clear that they are incorporated intact into the body. Considering the bioavailability of isoflavones in these reports, it can be inferred that, in the quantities typically consumed, these isoflavones might not function as agonists to AhR, while a large excess intake of AhR-inducers such as isoflavones may merit attention as a risk factor for health.

3. Inhibitory effects of polyphenol constituents on the AhR-mediated activity induced by TCDD

To evaluate the effect of polyphenol constituents on the AhR pathway induced by TCDD, we used an AhR-based bioassay for dioxins, the Ah-Immunoassay (Ah-I; Paracelsian, USA), which previously proved sensitive as a preliminary experimental model (Amakura et al., 2003b). The Ah-I kit method is a receptor-binding assay using cytosol containing AhR extracted from mammalian liver cells. It immunologically measures the dioxin level by utilizing an antigen-antibody reaction (Fig. 2b). This technique detects the reactivity of AhR with dioxins and dioxin-like compounds on an ELISA plate without using living cells. It is useful for screening the biological toxicity of dioxins (Kobayashi et al., 2002).

Fig. 6 shows the dose–response curves plotted on a log scale for some individual samples (see Figs. 4 and 7 for structures). Most of the compounds inhibited AhR activation by TCDD at high concentrations around the 50 μM level. Some showed marked inhibitory effects at low concentrations of 0.5–2.5 μM. The concentrations showing AhR activity equal to 70% of the maximal response to TCDD in controls were calculated and expressed as EC_{70} values. Table 2 shows the EC_{70} values of the compounds on AhR-based bioassay activation. The inhibitory effects

Table 2
Inhibitory effects of some polyphenolic constituents on TCDD-induced activation of AhR estimated using the AhR-based bioassay (data from Amakura et al. (2003b))

	EC_{70} (μM)[a]		EC_{70} (μM)
Flavones		*Hydrolyzable Tannins*	
Apigenin (**17**)	1.9	Pentagalloylglucose (**25**)	29.6
Luteolin (**18**)	1.8	Pedunculagin (**26**)	42.0
Baicalein (**12**)	5.1	Tellimagrandin I (**27**)	12.4
Chrysin (**14**)	0.7	Geraniin (**28**)	27.3
Flavonols		Casuarinin (**29**)	29.3
Quercetin (**19**)	2.7	Agrimoniin (**30**)	6.4
Kaempferol (**20**)	2.1	Gemin A (**31**)	31.5
Myricetin (**21**)	4.3	*Others*	
Morin (**22**)	5.3	Emodin (**32**)	0.6
Spiraeside (**23**)	2.1	Aloe-emodin (**33**)	0.5
Flavanones		Alizarin (**16**)	3.2
Naringenin (**10**)	27.7	Brevifolincarboxylic acid (**34**)	3.9
Hesperetin (**11**)	14.6	Curcumin (**35**)	35.4
Isoflavones		Resveratrol (**15**)	3.9
Genistein (**3**)	40.8	Coumestrol (**36**)	5.6
Equol (**24**)	41.2		

Each value is the mean of at least three replicates.

[a] Concentration producing AhR activity equal to 70% of the maximal response to TCDD. Calculated from the slope of the linear portion of each dose–response curve near the origin.

of most samples were weak, less than the EC_{70} level even at high concentrations (inhibitory effect *ca.* 20%).

The flavones and flavonols in Table 2 had strong inhibitory potencies (EC_{70}: 0.7–5.3 μM) against AhR activation induced by TCDD. However, three flavonol 3-*O*-glycosides, quercitrin, rutin, and isoquercitrin, did not inhibit activity to the EC_{70} level. In contrast, spiraeoside (**23**) (a quercetin 4′-*O*-glucoside) showed a strong inhibitory effect, comparable to that of the aglycone [quercetin (**19**)]. This suggested that the inhibitory effects of flavonols might be little influenced by glycosidation of the B-ring. Among the flavanones, hesperetin (**11**) and naringenin (**10**) showed inhibitory effects with EC_{70} values of 14.6 and 27.7 μM, while their 7-*O*-glycosides (naringin, hesperidin, taxifolin, and fustin) did not inhibit activity to the same level. The tendency of glycosides to weaken these activities was similar to those of flavones and flavonols. The isoflavones did not show an inhibitory effect on AhR activation at the EC_{70} level, and they slightly inhibited activity at high concentrations of 25–50 μM (*ca.* 10–20% inhibition), although

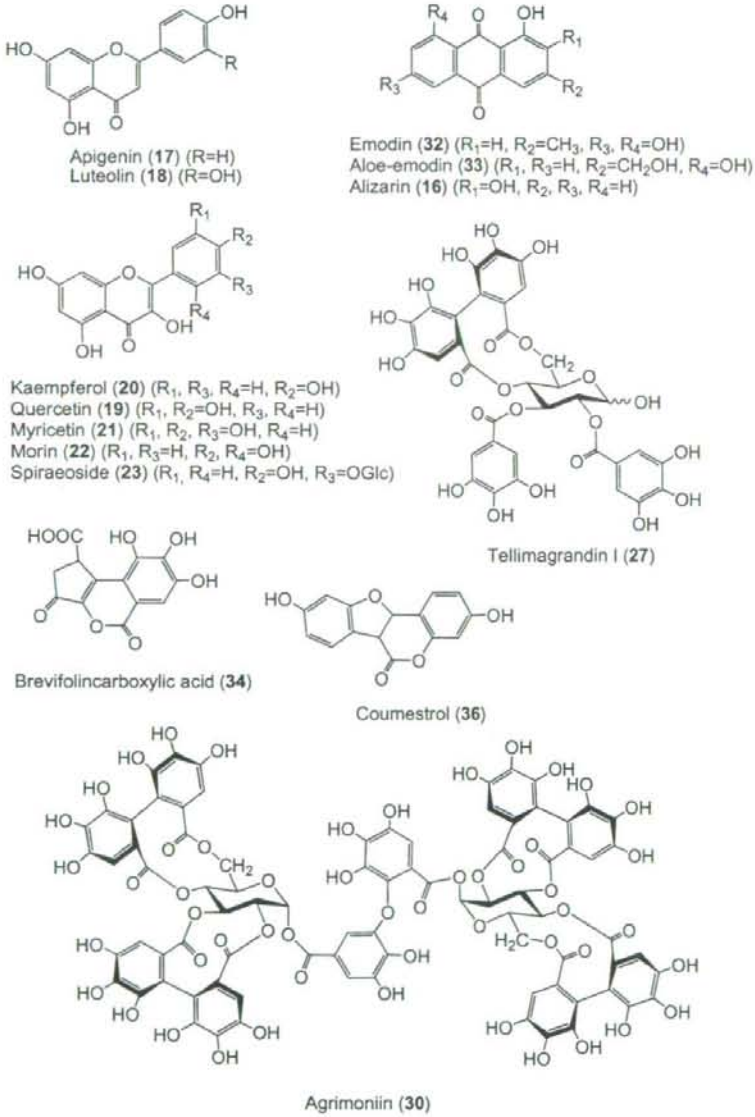

Fig. 7. Structures of some tested polyphenols.

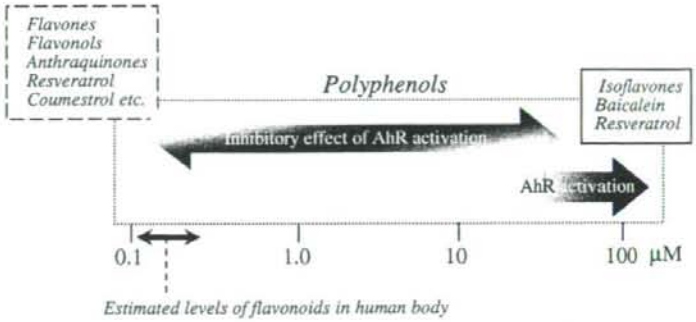

Fig. 8. Estimated ranges of AhR activation and inhibitory effects on AhR activation by TCDD of some polyphenols.

genistein (**3**) had a slight inhibitory effect at 40.8 μM in EC_{70} and equol inhibited activity at 41.2 μM.

Anthraquinones showed remarkable inhibition of AhR activation, comparable to flavones and flavonols [EC_{70} values of aloe-emodin (**33**), emodin (**32**), and alizarin (**16**) were 0.5, 0.6, and 3.2 μM, respectively]. Resveratrol (**15**), brevifolincarboxylic acid (**34**), coumestrol (**36**), and curcumin (**35**) also inhibited activation in this assay (EC_{70} values were 3.9, 3.9, 5.6, and 35.4 μM, respectively). Among these, resveratrol (**15**) and curcumin (**35**) were reported to show antagonist effects on AhR (Ciolino et al., 1998a, b; Casper et al., 1999). As they also showed inhibitory effects in our assay system, they would be promising candidates as prophylactic agents for the prevention of dioxin toxicity.

On the other hand, some hydrolyzable tannins in Table 2, which are large molecules with molecular weights of 1000–2000 higher than the phenolics mentioned above, inhibited AhR activation with EC_{70} of 6.4–42.0 μM, while condensed tannins showed no inhibitory effects. As tannins are known to form complexes with various proteins, to function as enzyme inhibitors, and so on (Haslam, 1989), their inhibitory effects in this assay system may partly be ascribed to a non-specific binding to AhR. Further study will be required to elucidate these effects.

In Section 2, we described the AhR-mediated activity of vegetable polyphenolics using the CALUX assay. The active compounds were classified into the so-called phytoestrogens, and their structural characteristics were similar to those of TCDD. Flavones such as chrysin (**14**) showed remarkable activation in the AhR-based assay as well as strong inhibitory effects on AhR-mediated activity induced by TCDD at the EC_{70} level, suggesting that they may be both agonists and antagonists of AhR, depending on the amount. On the other hand, flavones such as apigenin (**17**) and flavonols were regarded as strong antagonists of AhR because they showed little AhR activation. Most isoflavones had slight inhibitory effects, less than the EC_{70} level, whereas genistein (**3**) and equol (**24**) showed inhibition at the EC_{70} level. Anthraquinones, brevifolincarboxylic acid (**34**), and coumestrol (**36**), each of which slightly activated AhR at high concentrations, also produced inhibitory effects on AhR activity at the EC_{70} level.

Some reports have measured the amounts of flavonoid ingestion and absorption in the human body. For example, the daily consumption of flavonols was recently estimated at only 20–35 mg/day (Manach et al., 2005). Another paper reported that the ingestion of 8, 20, and 50 mg quercetin (**19**) resulted in concentrations of 0.14, 0.22, and 0.29 μM quercetin (**19**) in plasma, respectively (Yamamoto et al., 2001). Another study, performed on ten healthy volunteers, showed that ingestion of 68 ± 13 mg quercetin equivalents from onion resulted in a maximum quercetin (**19**) concentration in plasma of 0.74 ± 0.15 μM (Hollman et al., 1997). Taking into account the average intake (*ca.* 20–35 mg/day) and the absorption amount (*ca.* 0.1–0.3 μM) of flavonoids in general, it can be suggested that flavonoids might act as antagonists of AhR at usual intake levels.

Fig. 8 summarizes the estimated levels and inhibitory effects of AhR activation by TCDD of some low-molecular-weight polyphenols. Thus, some vegetable polyphenolics with low molecular weights and planar structures exhibited the properties of agonistic and/or antagonistic effects of AhR in the *in vitro* bioassays, and it can be inferred that they may have an antagonistic function in our usual dietary intake.

4. Interactions between some plant food extracts and AhR determined by *in vitro* bioassay

The influences of aqueous alcohol extracts of some plant foods on the AhR-signaling pathway were also investigated (Amakura et al., 2002, 2004, 2005). The *in vitro* AhR-inducing potencies of 39 plant food extracts including vegetables, fruits, herbs, and teas were determined by the CALUX assay. The induction of luciferase by each extract is shown in Fig. 9. Among the vegetables, shungiku (*Glebionis coronaria*) and spinach extracts induced production of luciferase activity of about 50% relative to TCDD maximum induction at concentrations of 1 mg/ml (plant extract dissolved in DMSO), while the other extracts did not

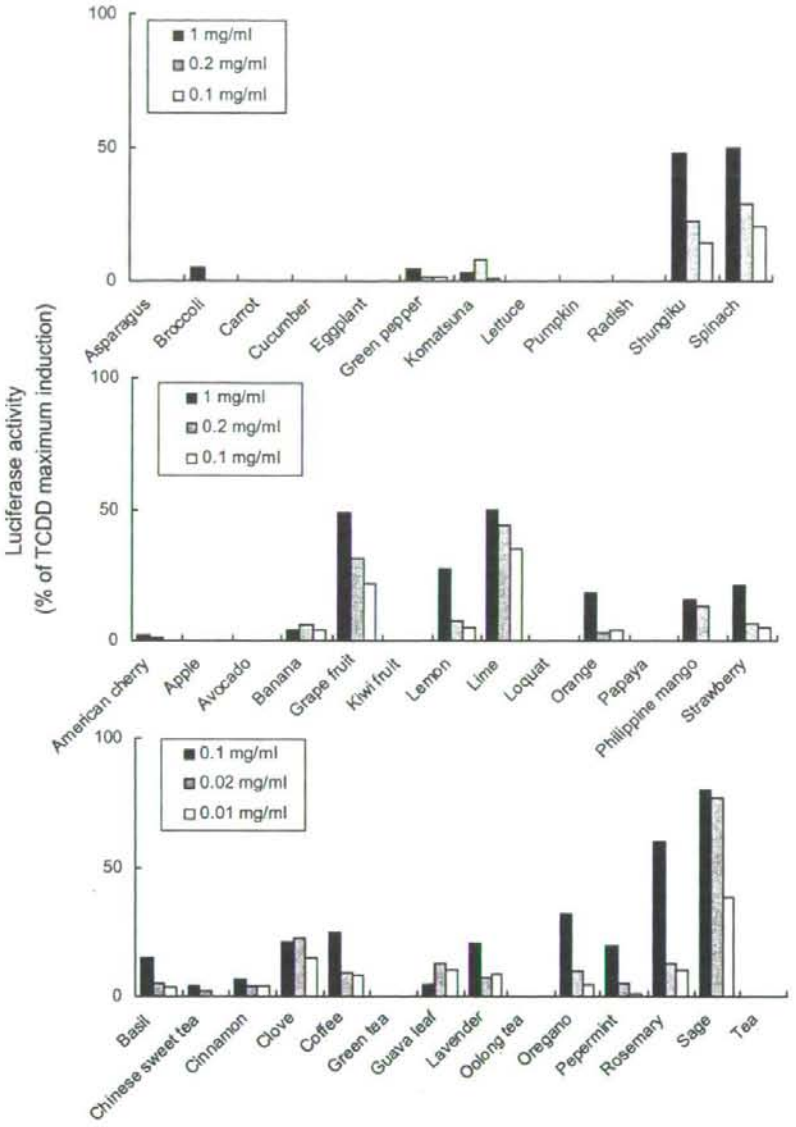

Fig. 9. Vegetable, fruit, and herb extracts tested for the induction of luciferase activity in the CALUX assay. The final sample concentrations added were 0.1, 0.2, and 1 mg/ml (or 0.01, 0.02, and 0.1 mg/ml for dried herbs) as extracts in three steps. Values represent the mean of triplicate determinations and are expressed relative to the activity obtained with TCDD.

induce luciferase activity (below 10%). Of the fruits, citrus fruits such as grapefruit and lime induced luciferase activity. Among the dried herbs and teas, sage and rosemary induced luciferase activity about 80% and 70%, respectively, at 0.1 mg/ml. On the other hand, in the assay using Ah-I to measure the inhibitory effects of these extracts on AhR activation by TCDD, green leafy vegetables such as spinach and komatsuna, citrus such as orange and grapefruit, and herbs such as sage and peppermint showed marked inhibitory effects (Fig. 10).

The agonistic and antagonistic effects of green leafy vegetables, citrus, and sage, as indicated by the induction of luciferase activity and the inhibitory effect on AhR-induced activation by TCDD, respectively, could be due to the flavonoids and/or related ingredients in these extracts.

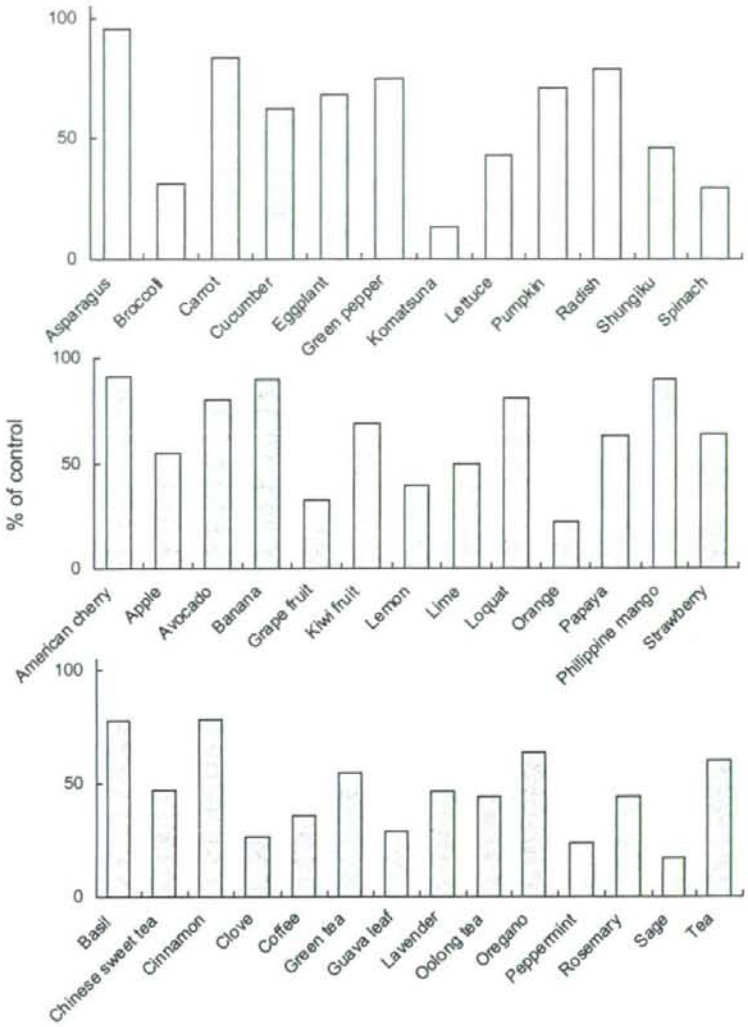

Fig. 10. Inhibitory effect of vegetable, fruit, and herb extracts on AhR induction by TCDD. Sample extracts at final concentrations of 500 μh/ml were used. The values indicate AhR binding activity. The value obtained without the addition of the sample solution was considered 100% of the control.

5. Concluding remarks

As part of a study on food function and safety, various food polyphenols were assessed for their effects on AhR in relation to toxic dioxins, using *in vitro* assays of AhR antagonistic and agonistic effects. In the CALUX assay, isoflavones, resveratrol (**15**), and some flavonoids activated AhR (agonistic effect). Then, a screening of the inhibitory effects of food polyphenols on TCDD-induced AhR activation was conducted using Ah-I, demonstrating that flavones, flavonols, anthraquinones, coumestrol (**36**), brevifolincarboxylic acid (**34**), and resveratrol (**15**) remarkably inhibited activation (antagonistic effect). In addition, aqueous alcohol extracts of consumables such as some green leafy vegetables, citrus fruits, and herbs that are rich in flavonoids were shown to interact with the AhR-signaling pathway in *in vitro* bioassays (CALUX assay and Ah-I) (agonistic effect at high concentrations and antagonistic effect at low concentrations).

To discuss the utility of food polyphenols for humans, it is necessary to understand not only their functional effects but also the influences of polyphenols on health and safety. Some vegetable polyphenolics with low molecular weights and planar structures exhibited properties of agonistic and/or antagonistic effects of AhR in the *in vitro* bioassays. However, in light of the bioavailability of such polyphenols, it can be inferred that they may have an antagonistic function in our usual dietary intake. The

AhR for polyphenols in usual intake might function biodefensively to protect the incorporation of foreign chemical compounds such as dioxin. On the other hand, the large excessive intake of foods that contain AhR-activators may be conducive to dioxin-like toxicity, therefore it may be necessary to pay attention to how much of these foods people eat. The results suggest that a well-balanced meal is also important in preventing dioxin-like toxicity.

Acknowledgements

This work was supported by a Health Sciences Research Grant from Ministry of Health, Labour and Welfare of Japan.

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Dioxin Concentrations in Commercial Health Tea Materials in Japan

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(Received October 27, 2008; Accepted February 4, 2009)

This study determined the concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (dioxin-like PCBs) in five selected plant materials [dokudami (from houttuynia herb), rose hip (from rosa fruit), ebisugusa (from cassia seed), rooibos, and tochu (from eucommia leaf)] used as health teas in Japan. The toxic equivalent (TEQ) levels for dioxins in the samples ranged from < 0.001 to 0.27 pg-TEQ/g weight, when undetectable and trace amounts were taken as zero. The mean of total TEQ level in commercial tea materials was estimated as 0.08 pg-TEQ/g (n = 5). The total TEQ in these samples was mainly dominated by the levels of PCDD/Fs (representing *ca.* 80% of the total TEQ).

Key words —— dioxin, polychlorinated dibenzo-*p*-dioxin, polychlorinated dibenzofuran, dioxin-like polychlorinated biphenyl, health tea, food

INTRODUCTION

In Japan, foods have been generally recognized as the main route of human intake of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs), which are referred to collectively as dioxins, contributing to more than 95% of the daily intake of these compounds.[1] Thus, it is very important to survey the levels of dioxins in various kinds of foodstuffs in order to evaluate the risk to humans. For food safety and security, we have been regularly performing daily intake surveys of dioxins by total diet studys and dioxin-pollution surveys of individual foods in Japan. From previous results, fishery products have been regarded as the main source of dioxins in the Japanese diet.[2,3] In addition, the levels of dioxins in retail fish and shellfish including dioxin contamination in the edible parts of Japanese common squid and saury were elucidated.[4,5]

In recent years, the demand for functional foods, including health teas, has expanded significantly.[6] Functional foods tend to be concentrates of selected foods; therefore, they may have a risk of excessive intake of harmful chemical contaminants in the foods even if those chemicals in individual food are at such a small level that they have no influence on health routinely. Previously, the levels of dioxin contaminants in fish oil supplements on the Japanese market were reported.[7] As a part of these ongoing studies on the actual situation of dioxin contamination in functional foods in Japan, it is reported herein on the dioxin levels in health teas, which have not been described previously with the exception of studies on green tea leaf.[8,9]

MATERIALS AND METHODS

Samples and Reagents —— Five tea materials [sample 1: dokudami (from houttuynia herb; aerial part of *Houttuynia cordata*), sample 2: ebisugusa (from cassia seed; seed of *Cassia obtusifolia* or *Cassia tora*), sample 3: rooibos (from the leaves of *Aspalatus linearis*), sample 4: rose hip (from the fruit of *Rosa* spp.) and sample 5: tochu (from the leaves of *Eucommia ulmoides*)] were obtained from stores in Japan (in 2006). Reagents used in this study were the same as described in the previous paper.[10]

Extraction and Cleanup Procedure —— The method of extraction and cleanup followed that of the tentative guideline for the analysis of dioxins in foods in Japan.[11] Briefly, 50 g of homogenized tea material was spiked with a mixture of ^{13}C-labeled internal quantitative standards [seventeen PCDD/Fs and twelve dioxin-like PCBs that have toxic equivalency factor (TEF) values proposed by the World Health Organization (WHO)], then extracted twice

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by shaking with acetone-*n*-hexane (1 : 1, total 200–360 ml) for 1 hr at room temperature, and filtered (0.8 μm filter, GPF filter paper of Kiriyama, Tokyo, Japan). After extraction, sulfuric acid treatment and column chromatography (CC) procedures (silver nitrate-silica gel CC, alumina CC, and activated carbon mixed silica gel CC) were carried out for cleanup, and each fraction obtained was spiked with ^{13}C-labeled recovery standards before analysis by high-resolution gas chromatography (HRGC)/high-resolution mass spectrometry (HRMS). The details were described in the previous paper.[10]

Dioxin Analysis —— Dioxin analysis was performed under HRGC/HRMS conditions using an Agilent 6890 plus gas chromatograph (Agilent Technologies, Santa Clara, CA, U.S.A.) coupled to a Micromass Autospec mass spectorometer (Micromass, Manchester, U.K.). The approximate limits of quantification in this study were; 0.01 pg/g for Tetra (Te)CDDs, Penta (Pe)CDDs, Tetra (Te)CDFs, and Penta (Pe)CDFs; 0.02 pg/g for Hexa (Hx)CDDs, Hepta (Hp)CDDs, Hexa (Hx)CDFs, and Hepta (Hp)CDFs; 0.05 pg/g for Octa (O)CDD and Octa (O)CDF; 0.1 pg/g for non-*ortho* PCBs; and 1 pg/g for mono-*ortho* PCBs. The toxic equivalent (TEQ) values for congeners were calculated using the WHO-TEFs.[12] The total TEQ in a sample was calculated based on the assumption that all isomer concentrations lower than the limits of quantification were equal to zero.

RESULTS AND DISCUSSION

Table 1 shows the concentrations of the sums for PCDDs, PCDFs, and dioxin-like PCBs, as well as the total TEQ, for five plant materials used as health teas. The recoveries calculated for the spiked compounds were always in the range defined as sufficient recovery in the tentative guideline.[11] On the whole, the samples had low dioxin levels around the limit of quantification or below. The highest total concentration in pg/g product weight was 37.15 pg/g in sample 5 (tochu). Subsequently samples 1 (dokudami) and 4 (rose hip) were found to have contamination levels of 17.46 and 11.42 pg/g, respectively. The dioxin concentrations of samples 2 (ebisugusa) and 3 (rooibos) were low, 2.46 and 0.66 pg/g, respectively. The mean of dioxin concentrations of commercial tea materials was 13.83 pg/g (n = 5, 3.18 pg/g for PCDDs, 0.79 pg/g for PCDFs, and 9.86 pg/g for dioxin-like PCBs, respectively). The maximum TEQ values corresponded to sample 5 (0.27 pg-TEQ/g) followed by sample 1 (0.13 pg-TEQ/g), and the mean of total TEQ level in commercial tea materials was 0.08 pg-TEQ/g (n = 5).

Figure 1 graphically illustrates the TEQ con-

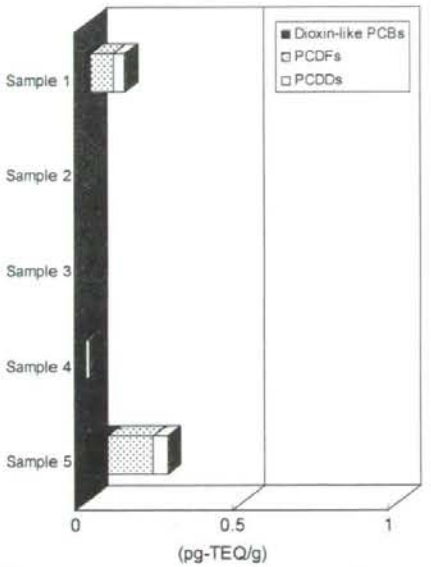

Fig. 1. TEQ Concentrations in Five Commercial Health Tea Materials
Name of samples: see Materials and Methods.

Table 1. Concentrations of PCDD/Fs and Dioxin-like PCBs in Commercial Health Tea Materials in Japan

Tea Materials	Concentration (pg/g prepared weight)				Total TEQ concentration (pg-TEQ/g prepared weight)
	PCDDs	PCDFs	Dioxin-like PCBs	Total	
Sample 1	3.35	1.01	13.1	17.46	0.13
Sample 2	0.16	nd	2.3	2.46	< 0.001
Sample 3	0.06	nd	0.6	0.66	< 0.001
Sample 4	8.50	0.42	2.5	11.42	0.014
Sample 5	3.83	2.52	30.8	37.15	0.2
Average (n = 5)	3.18	0.79	9.86	13.83	0.08

nd: not detected. TEQ was calculated as WHO-TEFs. Values below the limit of detection were takes as zero.

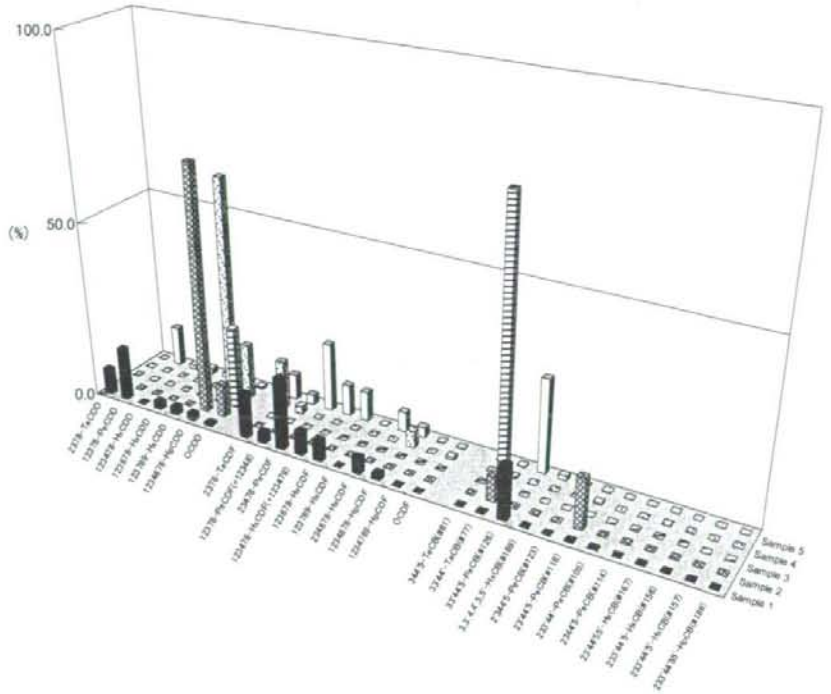

Fig. 2. Percentage Contributions of Congeners Using WHO-TEFs to Total Contamination in Five Commercial Health Tea Materials
Name of samples: see Materials and Methods.

centrations and their components in each health-tea sample. As can be seen, PCDD/Fs were the predominant congeners detected in the samples.

Figure 2 shows the percentage contribution of isomers to the total TEQ levels of tea materials. Total TEQ levels were dominated by 1,2,3,7,8-PeCDD, 2,3,7,8-TeCDF, 2,3,4,7,8-PeCDF, and 3,3′,4,4′,5-PeCB (#126) in samples 1 and 5; 1,2,3,4,6,7,8-HpCDD, OCDD and 2,3,7,8-TeCDF in sample 4; 1,2,3,4,6,7,8-HpCDD and 2,3′,4,4′,5-PeCB (#118) in sample 2; and OCDD and 3,3′,4,4′-TeCB (#77) in sample 3. It was reported previously that the dioxin concentrations in vegetables reflect in the contamination source.[13] For example, dioxin analysis of leafy vegetables indicated that 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, and 3,3′,4,4′-5-PeCB (#126), which are indicator isomers of total TEQ in environmental samples (exhaust gas, ash, and ambient air), are the dominant isomers. In the present study, the dominant isomers contaminating samples 1 and 5 were similar to those found in leafy vegetables. Thus, it is suggested that the dioxin contamination in the teas was caused by absorption or adhesion from the atmosphere to the surface of the plants.

The previous report indicated that the dioxin levels of green tea leaf were also in the range of 0.053–0.856 pg-TEQ/g.[8,9] The present TEQ levels for dioxins in health tea materials were < 0.001–0.27 pg-TEQ/g with the roughly same values as those of green tea. Generally, the plant materials for health teas are decocted in boiling water for drinking. As dioxins are insoluble in water, they are considered not to be ingested from health teas.

In conclusion, the levels of dioxins in health tea materials were surveyed for the first time in Japan. The samples analyzed had low dioxin levels similar to those of leafy vegetables.

Acknowledgements This work was supported by a Health Sciences Research Grant from the Ministry of Health, Labour and Welfare of Japan.

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