Estrogenic Activities of 517 Chemicals by Yeast Two-Hybrid Assay

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One of the urgent tasks in understanding endocrine disruptors (EDs) is to compile a list of suspected substances among the huge number of chemicals by using the screening test method. We developed a simple and rapid screening method using the yeast two-hybrid system based on the ligand-dependent interaction of nuclear hormone receptors with coactivators. To date, we have tested the estrogenic activity of more than 500 chemicals including natural substances, medicines, pesticides, and industrial chemicals. 64 compounds were evaluated as positive, and most of these demonstrated a common structure; phenol with a hydrophobic moiety at the para-position without bulky groups at the ortho-position. These results are expected to facilitate further risk assessment of chemicals.

Key words —— endocrine disruptor, yeast two-hybrid assay, estrogenic activity, 517 chemicals, structure–activity relationship, risk assessment

INTRODUCTION

In their book "Our Stolen Future," Colborn *et al*.[1] pointed out that a number of chemicals exist in the environment that affect the hormonal system and produce an adverse effect on animals and probably on humans. Such chemicals are referred to as endocrine disruptors (EDs), so-called environmental hormones, and have caused great concern all over the world, including Japan. One of the most urgent tasks to aid in the clarification of this matter is to compile a list of suspected EDs among the huge number of chemicals on the market. For this purpose, it is essential to develop simple screening test methods and many have been developed or proposed.[2] Among these, an *in vitro* screening test is potentially a simpler and more useful tool for primary selection of suspected EDs. Such tests include a MCF-7cell proliferation assay (E-Screen), receptor binding assays, reporter gene expression assays using cultured cells and yeast cells (YES), and a yeast two-hybrid assay. We have previously developed the latter method using a yeast two-hybrid system.[3,4] This system is based on the ligand-dependent interaction of two proteins, a hormone receptor and a coactivator, and hormonal activity is detected by β-galactosidase activity (Fig. 1). This assay is a very simple and inexpensive test method with high repeatability, and has been used by more than 30 laboratories and institutes.[5–17] To date, we have tested more than 500 chemicals, including natural substances, medicine, pesticides, and industrial chemicals, for estrogenic activity by our method. Here we report the results and discuss the structure–activity relationship.

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MATERIALS AND METHODS

Chemicals —— Most chemicals were of the highest grade commercially available, and tested without further purification. For example, 17β-estradiol (> 97%; E2), estriol (98%), estrone (98%), and β-sitosterol (99.5%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), while 5α-dihydrotestosterone (100%; DHT) was purchased from Sigma Chemical Co. (St. Louis, MO). Bisphenol A (99%), genistein (> 99%), 4-nonylphenol (technical grade; a mixture of

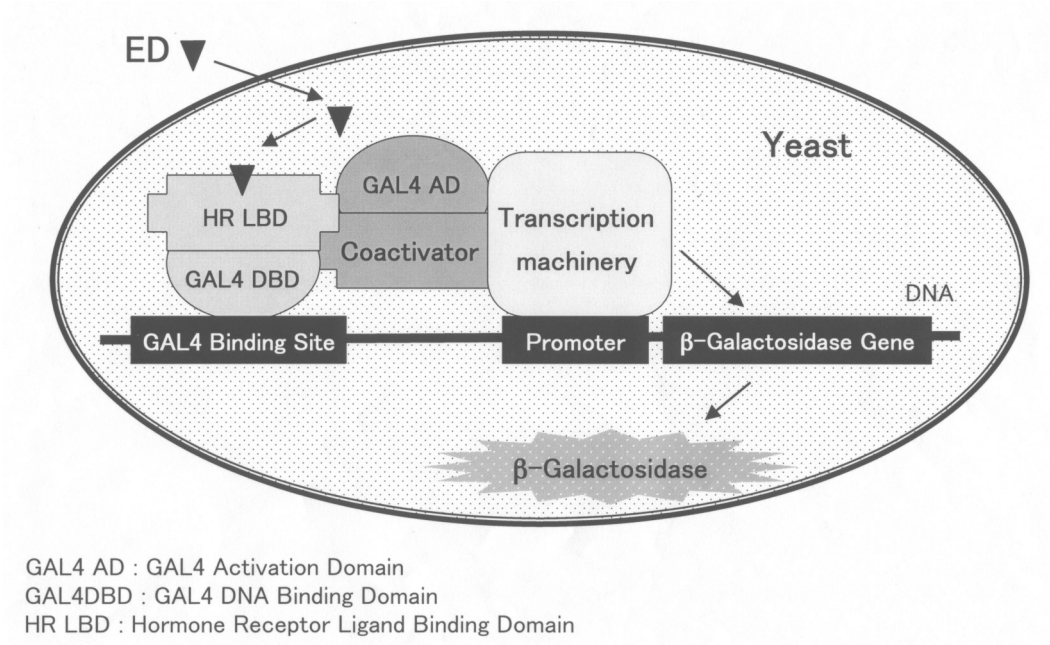

Fig. 1. Outline and Principle of Yeast Two-Hybrid Assay for Estrogenic Activity
In this paper, ERα and TIF2 were used as the receptor and coactivator systems, unless otherwise cited.

nonylphenols with branched alkyl chains) and diethylstilbesterol (99%; DES) were purchased from Nacalai Tesque (Kyoto, Japan). Among these, 255 compounds were prepared as solutions in dimethylsulfoxide or distilled water in the project, "research on the development of the total evaluation technique for hazardous impact of chemical substances on humans and the ecology" supported by Fundamental Research for the Environmental Future from the Environment Agency of Japan.

Yeast Two-Hybrid Assay —— In this study, we used the yeast two-hybrid assay system with the estrogen receptor, ERα, and the coactivator, TIF2, by the method described in our previous paper,[3] after modifying the procedure of cell disruption. Briefly, we introduced two expression plasmids, pGBT9-ERLBD and pGAAD424-TIF-2, into yeast cells (*Saccharomyces cerevisiae* Y190), which carry a β-galactosidase reporter gene and require tryptophan and leucine for growth (Fig. 1). The cells were preincubated overnight at 30°C in SD medium free from tryptophan and leucine. The culture (250 μl) in a small test tube was then mixed with a DMSO solution (2.5 μl) of test chemical and incubated for 4 h at 30°C. After washing by centrifugation, the cells were digested enzymatically by incubation with 1 mg/ml Zymolyase 20T (200 μl) at 30°C for 15 min. The lysate was mixed with 4 mg/ml ONPG (40 μl) and reacted until development of a yellow color (usually 30 min) before the reaction was stopped by the addition of 1 M Na_2CO_3 (100 μl). An aliquot (150 μl) was taken into each of 96 wells of a microplate. Absorbances at 420 and 550 nm were read on a microplate reader to estimate estrogenic activity.

The results were evaluated by relative activity, expressed as REC10 (10% relative effective concentration), that is the concentration of the test chemical showing 10% of the agonist activity of 10^{-7} M E2, which is the optimum concentration for E2. When the activity of the test substance was higher than REC10 within the concentration tested, we judged the chemical as positive. When it was judged to be negative, we indicated the highest dose we tested.

RESULTS AND DISCUSSION

Yeast Two-Hybrid Assay

Because it is expected that the major key target of EDs is the nuclear hormone receptor, which binds specifically to the steroid hormone and regulates its gene expression, the yeast two-hybrid assay has been developed. Unlike YES, another reporter gene assay using yeast cells, our method contains the coactivator, so that the system more closely resembles the mammalian hormonal system. Moreover, it takes less time to test with fairly high sensi-

Table 1. Estrogenic Componds by Yeast Two-Hybrid Assay

Category	Total No.	Positive compounds No.				
Naturals, and related	49	14	N1	17β-Estradiol	N8	Dihydrogenistein
			N2	17α-Estradiol	N9	Dihydrotestosterone (DHT)
			N3	Apigenin	N10	Equol
			N4	Coumestrin	N11	Estriol
			N5	Coumestrol	N12	Estrone
			N6	Daidzein	N13	Genistein
			N7	Daidzin	N14	Naringenin
Medicines, food additives, and related	20	7	M1	17α-Ethynylestradiol	M5	Methyl 4-hydroxybenzoate
			M2	β-Estradiol-17-acetate	M6	*n*-Butyl 4-hydroxybenzoate
			M3	Diethylstilbesterol (DES)	M7	*n*-Propyl 4-hydroxybenzoate
			M4	Ethyl 4-hydroxybenzoate		
Pesticides, and related	118	0				
Industrial chemicals	330	43				
PCBs, PCDFs, PAHs, and related	55	11	P1	2-Hydroxy benzo[*a*]pyrene	P7	8-Hydroxy-2,3,4-trichlorodibenzofuran
			P2	2-Hydroxy fluorene	P8	8-Hydroxy-2-monochlorodibenzofuran
			P3	3-Hydroxy benzo[*a*]pyrene	P9	8-Hydroxy-3,4,6-trichlorodibenzofuran
			P4	3,8-Dihydroxy-2-chlorodibenzofuran	P10	8-Hydroxy-3,4-dichlorodibenzofuran
			P5	4-Hydroxy-2′,4′,6′-trichlorobiphenyl	P11	8-Hydroxy-3-monochlorodibenzofuran
			P6	7-Hydroxy-3,4-dichlorodibenzofuran		
Phenols	78	28	F1	2,2-Bis(4-hydroxy-3-methylphenyl)propane	F15	4-Hydroxy biphenyl
			F2	2,2-Bis(4-hydroxy-phenyl)butane	F16	4-Methylphenol(*p*-cresol)
			F3	2,4-Dichlorophenol	F17	4-*n*-Butylphenol
			F4	3,4-Dichlorophenol	F18	4-*n*-Hexylphenol
			F5	4,4′-Dihydroxybenzophenone	F19	4-Nonylphenol polyethoxylate (2)
			F6	4,4′-Dihydroxybiphenyl	F20	4-*n*-Pentylphenol
			F7	4,4′-Thiobiphenyl	F21	4-*n*-Propylphenol
			F8	4-(branched)-Nonylphenol	F22	4-*sec*-Butylphenol
			F9	4-Bromophenol	F23	4-*tert*-Butylphenol
			F10	4-Chloro-3,5-xylenol	F24	4-*tert*-Octylphenol
			F11	4-Chloro-3-methylphenol	F25	4-*tert*-Octylphenol polyethoxylate (2)
			F12	4-Chlorophenol	F26	4-*tert*-Pentylphenol
			F13	4-Ethylphenol	F27	Bis(4-hydroxyphenyl)methane
			F14	4-Hydroxyacetophenone	F28	Bisphenol A
Benzenes and heterocyclics	80	1	B1	*cis*-1,2-Diphenylcyclobutane		
Phthalates and adipates	26	3	A1	Benzylbutyl phthalate (BBP)	A3	Di-*n*-propyl phthalate
			A2	Di-*iso*-propyl phthalate		
Aliphatics	75	0				
Inorganics and organometals	16	0				
Total	517	64				

tivity (detectable level for E2 is 3×10^{-10} M; REC10 for E2) and high repeatability. Accordingly, more than 30 laboratories in Japan have been using this method. Some data in this paper were collected from laboratories other than our own.

Test Chemicals

We tested the estrogenic activity of 517 compounds by our method using the ERα-TIF2 system, including 94 chemicals in the list of suspected EDs published by the Japan Environment Agency.[18] Table 1 shows a summary of the test compounds with the names of 64 positive compounds, the chemical structures of which are seen in Fig. 2. Tested chemicals consisted of natural substances (metabolites, oxidation products, *etc*.), medicines, food additives, pesticides, and industrial chemicals (PCBs, PCDFs, PAHs, phenols, benzenes, phthalates and adipates, and others). The details are shown in Table 2, where positive compounds are marked by closed circles

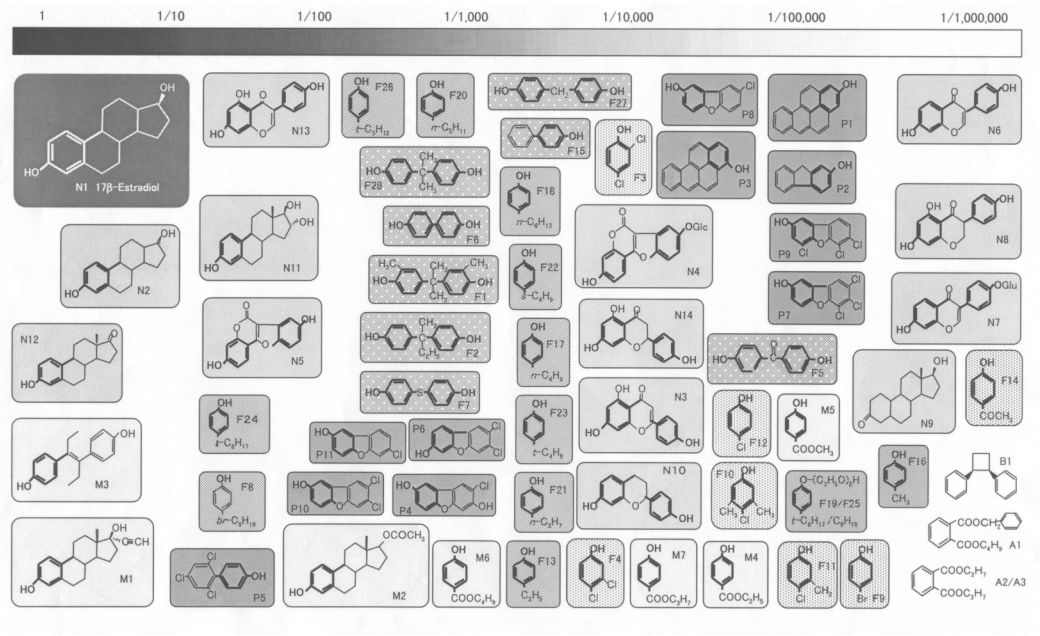

Fig. 2. Chemical Structures of Estrogenic Compounds Determined by Yeast Two-Hybrid Assay

A symbol nearby the structure in the column, *e.g.,* N2 and F4, corresponds that in Table 1. For example, N2 and F4 are 17α-estradiol and 3,4-dichlorophenol, respectively.

with REC10 values.

When the activity of the test substance was more than 10% of the activity of 10^{-7} M E2, the chemical was considered positive. No positive compounds were detected among pesticides, benzenes (besides phenols), heterocyclics, aliphatics, inorganics, and organometals. The tested compounds included 94 chemicals of the suspected EDs listed by the Japan Environmental Agency,[18] but only 15 (6/67 items) were positive. That is, our test may give false negative results for many of the listed compounds. The reasons for this are considered as follows: 1) some act *via* receptors other than ERα, 2) some involve a pathway other than *via* receptor-mediated gene expression, 3) some act as antagonists, 4) some act after being metabolized by animal cells, 5) some have inhibitory activity against the galactosidase assay and biocidal activity against the yeast cell, and 6) some cannot be transported into the cell, resulting in cellular concentrations below the sensitivity level.

Regarding 1), few chemicals with such a future have been reported. We have already created systems using other receptors, such as ERβ, AR, TR, GR, MR, and are planning to test chemicals using these systems with an automatic testing robot. ERα used here is the ligand binding domain from rat ERα, but when compared to the amino acid sequences among mammalians, it is more than 90% homologous,[19] indicating a low possibility of large errors. Indeed, most of the positive compounds have been reported to be positive by other methods or are expected to be positive from findings with analogous compounds.[19–22]

Regarding 2), there are some reports indicating that certain chemicals act in a different manner. For example, it is reported that tributyltin inhibits aromatase which catalyzes the transformation of testosterone to E2,[23] PCDDs interfere with the function of estrogen *via* the Ah receptor,[24] and so forth. However, this type of case is expected to involve only a small number of chemicals, although we do not understand yet the full array of possible mechanisms of endocrine disruption.

In the case of 3) and 4), a considerably large number of chemicals are likely to be involved. In this paper, we cited only the results for estrogenic compounds, that is estrogen agonists. In our system, antagonists and metabolites were detectable when samples were tested in the presence of the original ligand such as E2 and DHT, and were pretreated with S9mix, respectively.[14,25] Of course, we can detect activity when we have metabolites as test chemicals. For example, some hydroxylated PCDFs were positive, and the free acid of 4-hydroxybenzoate was negative.

When the dose-response pattern showed a decrease in activity at a higher concentration, we doubted the biocidal effect in case 5), and carefully

Table 2. Estrogenic Activity of 517 Chemicals by Yeast Two-Hybrid Assay

Compound[a]	REC10 (M)[b]	No.[c]	Remarks	Data source[d]
•17β-Estradiol (E2)	3×10^{-10}		Estrogen	
Natural products and related				
•17α-Estradiol	4×10^{-9}		E2 metabolite	Shiraishi
4-Aminobenzoic acid	$> 1 \times 10^{-4}$			
4-Aminobenzoic acid diglucoside	$> 1 \times 10^{-4}$			
Acetyleugenol	$> 1 \times 10^{-4}$			
Aflatoxin B1	$> 2 \times 10^{-6}$		Toxin	
•Apigenin	1×10^{-5}		Phytoestrogen	
Aplysiaterpenoid A	> 10 mg/l			
Biochanin A	$> 1 \times 10^{-4}$			
β-Sitosterol	$> 1 \times 10^{-4}$			
Caravacrol	$> 1 \times 10^{-4}$			
•Coumestrin	1×10^{-5}		Phytoestrogen	
•Coumestrol	3×10^{-7}		Phytoestrogen	
Cucumechinoside D	$> 5 \times 10^{-8}$		Toxin	
Cyclosporin A	$> 1 \times 10^{-5}$		Toxin	
•Daidzein	1×10^{-4}		Phytoestrogen	
•Daidzin	3×10^{-3}			Hayakawa
•Dihydrogenistein	1×10^{-4}			Hayakawa
Dihydroglycitein	$> 1 \times 10^{-3}$			Hayakawa
•Dihydrotestosterone (DHT)	5×10^{-5}		Androgen	
•Equol	1×10^{-5}			Hayakawa
•Estriol	3×10^{-7}		E2 metabolite	
•Estrone	3×10^{-10}		E2 metabolite	
Eugenol	$> 1 \times 10^{-4}$			
Feruic acid	$> 1 \times 10^{-4}$			
Flavone	$> 1 \times 10^{-4}$			
•Genistein	1×10^{-7}		Phytoestrogen	
Genistin	$> 1 \times 10^{-2}$			Hayakawa
Glycitein	$> 1 \times 10^{-3}$			Hayakawa
Glycitin	$> 1 \times 10^{-3}$			Hayakawa
Glycyrrhizic acid, 2K	$> 3 \times 10^{-4}$			
Hinokitiol	$> 1 \times 10^{-4}$			
Hinokitiol acetylglucoside	$> 1 \times 10^{-4}$			
Hinokitiol glucoside	$> 1 \times 10^{-4}$			
Isoeugenol	$> 1 \times 10^{-4}$			
Kaempferol	$> 1 \times 10^{-4}$			
Kojic acid	$> 1 \times 10^{-4}$			
Marthasteroside A1 (20S-Acanthaglycoside E)	> 2 mg/l		Toxin	
MelQx	$> 2 \times 10^{-5}$		Scorch	
Microcystin RR	>1.34 mg/l			
•Naringenin	3×10^{-5}		Phytoestrogen	
Phloretin	$> 1 \times 10^{-4}$			
PhlP	$> 2 \times 10^{-5}$		Scorch	
Quercetin	$> 1 \times 10^{-4}$			
Testosterone	$> 1 \times 10^{-3}$			
Thymol	$> 1 \times 10^{-4}$			
Trp-P-2	$> 2 \times 10^{-5}$		Scorch	
Tyramine	$> 3 \times 10^{-4}$		Tyrosine metabolite	
Tyrosine	$> 3 \times 10^{-4}$			

Table 2. Continued

Compound[a]	REC10 (M)[b]	No.[c]	Remarks	Data source[d]
Medicines, food additives, and related				
•17α-Ethynylestradiol	2×10^{-10}			
4-Hydroxybenzoic acid	$> 1 \times 10^{-3}$		Paraben	
4-Hydroxy-tamoxifen	$> 1 \times 10^{-4}$		Antagonist (ER)	
Benzoic acid	$> 1 \times 10^{-3}$		Benzene	
•β-Estradiol-17-acetate	5×10^{-7}			
Butylated hydroxyanisole (BHA)	$> 1 \times 10^{-3}$		Antioxidant; phenol	
Butylated hydroxytoluene (BHT)	$> 1 \times 10^{-3}$		Antioxidant; phenol	
Dexamethasone	$> 1 \times 10^{-5}$		Antagonist (AR)	
•Diethylstilbesterol (DES)	2×10^{-10}			
Dodecylol polyethoxylate (15)	$> 3 \times 10^{-5}$		Surfactant; aliphatic	Nakamuro
Dodecylol polyethoxylate (3)	$> 1 \times 10^{-5}$		Surfactant; aliphatic	Nakamuro
Dodecylol polyethoxylate (5)	$> 3 \times 10^{-5}$		Surfactant; aliphatic	Nakamuro
Dodecylol polyethoxylate (9)	$> 2 \times 10^{-5}$		Surfactant; aliphatic	Nakamuro
•Ethyl 4-hydroxybenzoate	1×10^{-4}		Paraben	
Ethylenediaminetetraacetic acid, 2Na (EDTA)	$> 1 \times 10^{-3}$		Aliphatic	
Hydroxy-flutamide	$> 1 \times 10^{-4}$		Antagonist (AR)	
•Methyl 4-hydroxybenzoate	4×10^{-4}		Paraben	
•*n*-Butyl 4-hydroxybenzoate	3×10^{-6}		Paraben	
•*n*-Propyl 4-hydroxybenzoate	1×10^{-5}		Paraben	
Tamoxifen	$> 1 \times 10^{-2}$			Hayakawa
Pesticides and related				
1,2-Dibromo-3-chloropropane	$> 1 \times 10^{-3}$	17	Nematocide	
1,4-Dichlorobenzene	$> 1 \times 10^{-4}$		Insecticide	
2,4,5-Trichlorophenol	$> 1 \times 10^{-3}$		Bactericide, herbicide	
2,4,5-Trichlorophenoxyacetic acid	$> 1 \times 10^{-4}$	6	Bactericide, herbicide	
2,4-Dichlorophenoxyacetic acid (2,4-D)	$> 1 \times 10^{-3}$	7	Herbicide	
2-Mercaptobenzothiazole	$> 1 \times 10^{-4}$		Fungicide	
Acephate	$> 1 \times 10^{-4}$		Insecticide	
α-Hexachlorocyclohexane (HCH, BHC)	$> 3 \times 10^{-4}$	12	Insecticide	
Alachlor	$> 1 \times 10^{-4}$	10	Herbicide	
Aldicarb	$> 3 \times 10^{-4}$	49	Acaricide, insecticide	
Aldrin	$> 3 \times 10^{-4}$	21	Insecticide	
Amitrole (3-Amino-1*H*-1,2,4-triazole)	$> 1 \times 10^{-3}$	8	Herbicide	
Atrazine	$> 1 \times 10^{-4}$	9	Herbicide	Shiraishi
Benzoepinesulfate	$> 3 \times 10^{-4}$		Acaricide, insecticide	Kawagoshi
β-Hexachlorocyclohexane (HCH, BHC)	$> 3 \times 10^{-4}$	12	Insecticide	
Bifenox	$> 5 \times 10^{-5}$		Herbicide	
BPMC	$> 1 \times 10^{-4}$		Insecticide	
Bromobutide	$> 1 \times 10^{-5}$		Herbicide	Shiraishi
Captans	$> 1 \times 10^{-4}$		Fungicide	
Carbaryl (NAC)	$> 1 \times 10^{-4}$	13	Insecticide	
Carbendazim	$> 3 \times 10^{-4}$		Fungicide	
Carbofuran	$> 1 \times 10^{-4}$		Acaricide, insecticide, nematocide	Shiraishi
Chinomethionat	$> 3 \times 10^{-4}$		Acaricide, fungicide, insecticide	
Chlornitrofen (CNP)	$> 1 \times 10^{-5}$		Herbicide	
Chlorobenside	$> 3 \times 10^{-4}$		Acaricide	
Chlorobenzilate	$> 3 \times 10^{-4}$		Acaricide, insecticide	
Chloropropham	$> 3 \times 10^{-4}$		Herbicide	
Chlorothalonil (TPN)	$> 1 \times 10^{-4}$		Fungicide	

Table 2. Continued

Compound[a)]	REC10 (M)[b)]	No.[c)]	Remarks	Data source[d)]
Chlorpyrifos-methyl	$> 3 \times 10^{-4}$		Insecticide	
Cyanazin	$> 1 \times 10^{-4}$		Herbicide	Shiraishi
Cyfluthrin	$> 3 \times 10^{-4}$		Insecticide	
Cyhalothrin	$> 3 \times 10^{-4}$		Insecticide	
Cypermetrin	$> 1 \times 10^{-4}$	56	Insecticide	
Diazinon	$> 1 \times 10^{-4}$		Acaricide, insecticide	
Dichlorvos (DDVP)	$> 2 \times 10^{-4}$		Insecticide	
Dicloran	$> 3 \times 10^{-4}$		Fungicide	
Dieldrin	$> 3 \times 10^{-4}$	23	Insecticide	
Diflubenzuron	$> 3 \times 10^{-4}$		Insecticide	
Dimepiperate	$> 5 \times 10^{-5}$		Herbicide	Shiraishi
Dimethoate	$> 1 \times 10^{-4}$		Acaricide, insecticide	
Edifenfos (EDDP)	$> 1 \times 10^{-4}$		Fungicide	
Endosulfan (α-Benzoepin)	$> 3 \times 10^{-4}$	24	Acaricide, insecticide	Kawagoshi
Endosulfan (β-Benzoepin)	$> 3 \times 10^{-4}$	24	Acaricide, insecticide	Kawagoshi
Endosulfan (Benzoepin)	$> 1 \times 10^{-3}$	24	Acaricide, insecticide	
Endrin	$> 3 \times 10^{-4}$	22	Insecticide, rodenticide	
EPN [*O*-Ethyl-*O*-(4-nitrophenyl) phenyl-phosphono thioate]	$> 1 \times 10^{-4}$		Acaricide, insecticide	
Esprocarb	$> 2 \times 10^{-5}$		Herbicide	Shiraishi
Ethyl parathion	$> 3 \times 10^{-4}$	12	Acaricide, insecticide	
Fenbutatin oxide	$> 3 \times 10^{-4}$		Acaricide, insecticide	
Fenitrothion (MEP)	$> 1 \times 10^{-4}$		Insecticide	
Fenobcarb	$> 3 \times 10^{-4}$		Insecticide	
Fenoprop	$> 3 \times 10^{-4}$		Herbicide, plant growth regulator	
Fensulfothion	$> 3 \times 10^{-4}$		Insecticide, nematocide	
Fenthion (MPP)	$> 1 \times 10^{-4}$		Insecticide	
Fenvalerate	$> 1 \times 10^{-3}$	58	Insecticide	
Fluazifop-butyl	$> 3 \times 10^{-4}$		Herbicide	
Flucythrinate	$> 3 \times 10^{-4}$		Insecticide	
Fluvalinate	$> 3 \times 10^{-4}$		Insecticide	
γ-Hexachlorocyclohexane (HCH, BHC)	$> 1 \times 10^{-3}$	12	Insecticide	
Heptachlor	$> 3 \times 10^{-4}$	25	Insecticide	
Heptachlor epoxide	$> 3 \times 10^{-4}$	26	Heptachlor metabolite	
IBP	$> 1 \times 10^{-4}$		Fungicide	
Iprodione	$> 3 \times 10^{-4}$		Fungicide	
Isoprothiolane	$> 1 \times 10^{-4}$		Fungicide, insecticide	Shiraishi
Isoxathion	$> 2 \times 10^{-5}$		Insecticide	Shiraishi
Kelthane (Dicofol)	$> 1 \times 10^{-4}$	20	Acaricide	
Linuron	$> 3 \times 10^{-4}$		Herbicide	
Malaoxon	$> 1 \times 10^{-4}$		Insecticide	Shiraishi
Malathion	$> 1 \times 10^{-4}$	27	Acaricide, insecticide	
Maneb	$> 1 \times 10^{-5}$	53	Fungicide	
Manzeb (Mancozeb)	$> 1 \times 10^{-5}$	52	Fungicide	
Mefenacet	$> 1 \times 10^{-4}$		Herbicide	Shiraishi
Metamitron	$> 3 \times 10^{-4}$		Herbicide	
Methidathion	$> 3 \times 10^{-4}$		Insecticide	
Methomyl	$> 2 \times 10^{-4}$	28	Insecticide	
Methoxychlor	$> 1 \times 10^{-4}$	29	Insecticide	
Metribuzin	$> 3 \times 10^{-4}$	55	Herbicide	
Molinate	$> 1 \times 10^{-4}$		Herbicide	

Table 2. Continued

Compound[a)]	REC10 (M)[b)]	No.[c)]	Remarks	Data source[d)]
Nebron	$> 3 \times 10^{-4}$		Herbicide	
Nitrofen (NIP)	$> 1 \times 10^{-4}$	31	Herbicide	
o,*p*′-DDD	$> 3 \times 10^{-4}$	19	DDT metabolite	
o,*p*′-DDE	$> 3 \times 10^{-4}$	19	DDT metabolite	
o,*p*′-DDT	$> 3 \times 10^{-4}$	18	Insecticide	
p,*p*′-DDD	$> 3 \times 10^{-4}$	19	DDT metabolite	
p,*p*′-DDE	$> 1 \times 10^{-4}$	19	DDT metabolite, antagonist (AR)	
p,*p*′-DDT	$> 3 \times 10^{-4}$	18	Insecticide	
Paraquat	$> 1 \times 10^{-4}$		Herbicide	
Pendimethalin	$> 3 \times 10^{-4}$		Herbicide	
Pentachloronitrobenzene (PCNB)	$> 1 \times 10^{-4}$		Fungicide	
Pentachlorophenol	$> 1 \times 10^{-5}$		Acaricide, fungicide, herbicide	Shiraishi
Permethrin	$> 1 \times 10^{-4}$	59	Insecticide	
Phenthoate (PAP)	$> 1 \times 10^{-4}$		Acaricide, insecticide	
Phosalone	$> 3 \times 10^{-4}$		Acaricide, insecticide	
Pretilachlor	$> 1 \times 10^{-4}$		Herbicide	Shiraishi
Primiphos-methyl	$> 3 \times 10^{-4}$		Insecticide	
Procymidone	$> 3 \times 10^{-4}$		Fungicide	
Propanil (DCPA)	$> 1 \times 10^{-4}$		Herbicide	
Propazin	$> 5 \times 10^{-5}$		Herbicide	Shiraishi
Propoxur	$> 1 \times 10^{-4}$		Insecticide	Shiraishi
Propyzamide	$> 3 \times 10^{-4}$		Fungicide, herbicide	
Simazine (CAT)	$> 1 \times 10^{-4}$	11	Herbicide	
Simetryne	$> 2 \times 10^{-4}$		Herbicide	
Tefluthrin	$> 3 \times 10^{-4}$		Herbicide	
Tetrachlorofthalide	$> 1 \times 10^{-4}$		Insecticide	
Tetrachlorovinphos	$> 3 \times 10^{-4}$		Insecticide	
Thiabendazole	$> 3 \times 10^{-4}$		Fungicide, nematocide	
Thiobencarb	$> 5 \times 10^{-4}$		Herbicide	
Thiophanate-methyl	$> 1 \times 10^{-4}$		Fungicide	
Thiram	$> 1 \times 10^{-4}$		Fungicide, rodenticide	
Toxaphene (camphechlor)	$> 5 \times 10^{-5}$	32	Insecticide	Kawagoshi
Triadimefon	$> 3 \times 10^{-4}$		Fungicide	
Triadimenol	$> 3 \times 10^{-4}$		Fungicide	
Trifluralin	$> 1 \times 10^{-4}$	35	Herbicide	
Triforine	$> 3 \times 10^{-4}$		Fungicide	
Vinclozolin	$> 1 \times 10^{-4}$	60	Fungicide	
Xylylcarb	$> 3 \times 10^{-4}$		Insecticide	
Zineb	$>$ 40 mg/l	61	Fungicide	
Ziram	$> 1 \times 10^{-4}$	62	Fungicide	
Industrial chemicals				
PCBs, PCDFs,PAHs, and related				
1,2-Dimethylnaphthalene	$> 1 \times 10^{-4}$		PAH	
1,6-Dinitropyrene	$> 1 \times 10^{-6}$		PAH	
1,8-Dimethylnaphthalene	$> 1 \times 10^{-4}$		PAH	
1,8-Dinitropyrene	$> 1 \times 10^{-6}$		PAH	
10-Hydroxy benzo[*a*]pyrene	$> 1 \times 10^{-4}$		PAH metabolite	Hayakawa
11-Hydroxy benzo[*a*]pyrene	$> 1 \times 10^{-4}$		PAH metabolite	Hayakawa
12-Hydroxy benzo[*a*]pyrene	$> 1 \times 10^{-4}$		PAH metabolite	Hayakawa
1-Hydroxy pyrene	$> 1 \times 10^{-3}$		PAH metabolite	Hayakawa

Table 2. Continued

Compound[a]	REC10 (M)[b]	No.[c]	Remarks	Data source[d]
1-Methylnaphthalene	$> 1 \times 10^{-3}$		PAH	
1-Nitropyrene	$> 1 \times 10^{-5}$		PAH	
2,6-Dimethylnapthalene	$> 1 \times 10^{-4}$		PAH	
2-Aminoanthracene	$> 1 \times 10^{-4}$		PAH	
2-Aminoanthraquinone	$> 1 \times 10^{-3}$		PAH	
•2-Hydroxy benzo[*a*]pyrene	5×10^{-5}		PAH metabolite	Hayakawa
•2-Hydroxy fluorene	1×10^{-4}		PAH metabolite	Hayakawa
2-Hydroxydibenzofuran	$> 1 \times 10^{-5}$	1	PCDF metabolite	Kuroki
2-Nitrofluorene	$> 1 \times 10^{-3}$		PAH	
3,3′,4,4′,5-Pentachlorobiphenyl (PCB 126)	$> 1 \times 10^{-5}$	2	PCB	
•3,8-Dihydroxy-2-chlorodibenzofuran	8×10^{-7}	1	PCDF metabolite	Kuroki
•3-Hydroxy benzo[*a*]pyrene	1×10^{-5}		PAH metabolite	Hayakawa
3-Hydroxy-2,8-dichlorodibenzofuran	$> 1 \times 10^{-5}$	1	PCDF metabolite	Kuroki
3-Methylcholanthrene (MC)	$> 2 \times 10^{-5}$		PAH	
3-Nitrofluoranthene	$> 1 \times 10^{-4}$		PAH	
4-Hydroxy benzo[*a*]pyrene	$> 1 \times 10^{-4}$		PAH metabolite	Hayakawa
4-Hydroxy-2′,3,5,5′-tetrachlorobiphenyl	$> 1 \times 10^{-5}$	2	PCB metabolite	Kuroki
•4-Hydroxy-2′,4′,6′-trichlorobiphenyl	4×10^{-7}	2	PCB metabolite	Kuroki
4-Hydroxy-4′-monochlorobiphenyl	$> 1 \times 10^{-5}$	2	PCB metabolite	Kuroki
5-Hydroxy benzo[*a*]pyrene	$> 1 \times 10^{-4}$		PAH metabolite	Hayakawa
6-Hydroxy benzo[*a*]pyrene	$> 1 \times 10^{-4}$		PAH metabolite	Hayakawa
6-Hydroxy-3,4-dichlorodibenzofuran	$> 1 \times 10^{-5}$	1	PCDF metabolite	Kuroki
7-Hydroxy benzo[*a*]pyrene	$> 1 \times 10^{-4}$		PAH metabolite	Hayakawa
7-Hydroxy-1,2,3,6,8-pentachlorodibenzofuran	$> 1 \times 10^{-5}$	1	PCDF metabolite	Kuroki
•7-Hydroxy-3,4-dichlorodibenzofuran	6×10^{-6}	1	PCDF metabolite	Kuroki
8-Hydroxy benzo[*a*]pyrene	$> 1 \times 10^{-4}$		PAH metabolite	Hayakawa
•8-Hydroxy-2,3,4-trichlorodibenzofuran	1×10^{-5}	1	PCDF metabolite	Kuroki
•8-Hydroxy-2-monochlorodibenzofuran	1×10^{-5}	1	PCDF metabolite	Kuroki
•8-Hydroxy-3,4,6-trichlorodibenzofuran	1×10^{-5}	1	PCDF metabolite	Kuroki
•8-Hydroxy-3,4-dichlorodibenzofuran	5×10^{-6}	1	PCDF metabolite	Kuroki
•8-Hydroxy-3-monochlorodibenzofuran	7×10^{-6}	1	PCDF metabolite	Kuroki
9-Hydroxy benzo[*a*]pyrene	$> 1 \times 10^{-4}$		PAH metabolite	Hayakawa
9-Hydroxy fluorene	$> 1 \times 10^{-3}$		PAH metabolite	Hayakawa
9-Hydroxy-2,6-dichlorodibenzofuran	$> 1 \times 10^{-5}$	1	PCDF metabolite	Kuroki
9-Hydroxy-3,4-dichlorodibenzofuran	$> 1 \times 10^{-5}$	1	PCDF metabolite	Kuroki
Anthracene	$> 1 \times 10^{-4}$		PAH	
Benz[*a*]anthracene	$> 2 \times 10^{-4}$		PAH	
Benzo[*a*]pyrene	$> 1 \times 10^{-4}$	43	PAH	
Benzo[*b*]fluoranthene	$> 5 \times 10^{-5}$		PAH	
Benzo[*e*]pyrene	$> 2 \times 10^{-5}$		PAH	
Benzo[*g*,*h*,*i*]perylene	$> 2 \times 10^{-5}$		PAH	
Benzo[*k*]fluoranthene	$> 2 \times 10^{-6}$		PAH	
Dibenz[*a*,*h*]anthracene	$> 1 \times 10^{-4}$		PAH	
Hydroxy-tetrachlorobiphenyl	$> 1 \times 10^{-5}$	2	PCB metabolite	Kuroki
Hydroxy-trichlorobiphenyl	$> 1 \times 10^{-5}$	2	PCB metabolite	Kuroki
Naphthalene	$> 1 \times 10^{-3}$		PAH	
Pyrene	$> 1 \times 10^{-4}$		PAH	
Phenols				
2,2-Bis(3,5-dibromo-4-hydroxyphenyl) propane	$> 1 \times 10^{-3}$		Bisphenol	
•2,2-Bis(4-hydroxy-3-methylphenyl)propane	3×10^{-6}	Bisphenol	Fujita	

Table 2. Continued

Compound[a)]	REC10(M)[b)]	No.[c)]	Remarks	Data source[d)]
•2,2-Bis(4-hydroxy-phenyl)butane	3×10^{-6}		Bisphenol	Fujita
2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy) pheny] propane (BisGMA)	$>1\times10^{-3}$		Bisphenol	Hashimoto
2,2-bis[4(methacryloxy)]pheny propane (BisMEPP)	$>1\times10^{-3}$		Bisphenol	Hashimoto
2,2-bis[4(methacryloxypolyethoxy)pheny] propane (MPEPP)	$>1\times10^{-3}$		Bisphenol	Hashimoto
2,2′-Dihydroxybiphenyl	$>1\times10^{-3}$			
2,4,6-Tribromophenol	$>1\times10^{-3}$			
2,4,6-Trichlorophenol	$>1\times10^{-3}$			
2,4-Dibromophenol	$>1\times10^{-3}$			Shiraishi
•2,4-Dichlorophenol	4×10^{-5}			
2,4-Dimethylphenol	$>1\times10^{-5}$			
2,4-Dinitrophenol	$>1\times10^{-3}$			
2,5-Dichlorophenol	$>1\times10^{-3}$			
2,6-Di-*tert*-butyl-4-methylphenol	$>1\times10^{-4}$			
2-Hydroxybiphenyl (OPP)	$>1\times10^{-3}$			
2-Methacryloyloxyethyl phenyl phosphate (Phenyl-P)	$>1\times10^{-3}$			Hashimoto
2-Methylphenol (*o*-cresol)	$>1\times10^{-5}$			
2-Nitrophenol	$>1\times10^{-3}$			
2-*sec*-Butylphenol	$>1\times10^{-3}$			
2-*tert*-Butylphenol	$>1\times10^{-3}$			
•3,4-Dichlorophenol	2×10^{-5}			Shiraishi
3-Aminophenol	$>1\times10^{-3}$			
3-Nitrophenol	$>1\times10^{-3}$			
3-*tert*-Butylphenol	$>1\times10^{-3}$			
•4,4′-Dihydroxybenzophenone	3×10^{-4}			Fujita
•4,4′-Dihydroxybiphenyl	5×10^{-6}			
•4,4′-Thiobiphenyl	3×10^{-6}			Fujita
•4-(branched)-Nonylphenol	2×10^{-7}	36		
•4-Bromophenol	8×10^{-5}			
•4-Chloro-3,5-xylenol	1×10^{-4}			
•4-Chloro-3-methylphenol	5×10^{-5}			
•4-Chlorophenol	2×10^{-4}			
•4-Ethylphenol	3×10^{-5}			
•4-Hydroxyacetophenone	2×10^{-4}			Fujita
4-Hydroxybenzaldehyde	$>3\times10^{-3}$			Fujita
•4-Hydroxybiphenyl	3×10^{-5}			
4-iso-Propyl-3-methylphenol	$>1\times10^{-3}$			
•4-Methylphenol (*p*-cresol)	3×10^{-4}			
•4-*n*-Butylphenol	1×10^{-5}			
4-*n*-Heptylphenol	$>1\times10^{-3}$			
•4-*n*-Hexylphenol	1×10^{-5}			
4-*n*-Nonylphenol	$>1\times10^{-3}$	36		
4-*n*-Octylphenol	$>1\times10^{-3}$	36		
4-Nonylphenol polyethoxylate (10)	$>3\times10^{-5}$		Surfactant	Nakamuro
4-Nonylphenol polyethoxylate (15)	$>2\times10^{-5}$		Surfactant	Nakamuro
•4-Nonylphenol polyethoxylate (2)	1×10^{-3}		Surfactant	
4-Nonylphenol polyethoxylate (23)	$>1\times10^{-5}$		Surfactant	Nakamuro
4-Nonylphenol polyethoxylate (5)	$>1\times10^{-5}$		Surfactant	Nakamuro
•4-*n*-Pentylphenol	3×10^{-6}	36		
•4-*n*-Propylphenol	7×10^{-5}			

Table 2. Continued

Compound[a]	REC10(M)[b]	No.[c]	Remarks	Data source[d]
•4-*sec*-Butylphenol	1×10^{-5}			
•4-*tert*-Butylphenol	3×10^{-5}			Nishimura
•4-*tert*-Octylphenol	2×10^{-7}	36		
4-*tert*-Octylphenol polyethoxylate (10)	$> 2 \times 10^{-5}$		Surfactant	Nakamuro
4-*tert*-Octylphenol polyethoxylate (15)	$> 2 \times 10^{-5}$		Surfactant	Nakamuro
•4-*tert*-Octylphenol polyethoxylate (2)	3×10^{-4}		Surfactant	
4-*tert*-Octylphenol polyethoxylate (23)	$> 1 \times 10^{-5}$		Surfactant	Nakamuro
4-*tert*-Octylphenol polyethoxylate (5)	$> 1 \times 10^{-5}$		Surfactant	Nakamuro
•4-*tert*-Pentylphenol	1×10^{-6}	36		
•Bis(4-hydroxyphenyl)methane	2×10^{-5}		Bisphenol	Hashimoto
Bis(4-hydroxyphenyl)sulfone	$> 1 \times 10^{-3}$		Bisphenol	Hashimoto
•Bisphenol A	3×10^{-6}	37	Bisphenol	
Bisphenol-A-bischloroformate (BPA-GF)	$> 1 \times 10^{-3}$		Bisphenol	Hashimoto
Bisphenol-A-diglycidyl ether (BPA-GE)	$> 1 \times 10^{-3}$		Bisphenol	Hashimoto
Bisphenol-A-dimethacrylate (BisDMA)	$> 1 \times 10^{-3}$		Bisphenol	Hashimoto
Bisphenol-A-ethoxylate (BPA-E)	$> 1 \times 10^{-3}$		Bisphenol	Hashimoto
Bisphenol-A-ethoxylate diacrylate (BPA-ED)	$> 1 \times 10^{-3}$		Bisphenol	Hashimoto
Bisphenol-A-proxylate (BPA-P)	$> 1 \times 10^{-3}$		Bisphenol	Hashimoto
Catechol	$> 1 \times 10^{-4}$			
Clomiphene	$> 1 \times 10^{-3}$			
Hexachlorophene	> 10 mg/l			
Hydroquinone	$> 1 \times 10^{-3}$			
Pentachlorophenol (PCP)	$> 1 \times 10^{-3}$	5		
Phenol	$> 1 \times 10^{-3}$			
Resorcinol	$> 1 \times 10^{-3}$			
Tetrabromobisphenol A	$> 1 \times 10^{-5}$			Shiraishi
Tetrachlorobisphenol A	$> 5 \times 10^{-5}$			Shiraishi
Benzenes and heterocyclics				
1,2,3-Trichlorobenzene	$> 1 \times 10^{-3}$			
1,2,3-Trimethylbenzene	$> 4 \times 10^{-3}$			Kawagoshi
1,2,4,5-Tetramethylbenzene	$> 4 \times 10^{-3}$			Kawagoshi
1,2,4-Trichlorobenzene	$> 1 \times 10^{-3}$			
1,2,4-Trimethylbenzene	$> 4 \times 10^{-3}$			Kawagoshi
1,2-Dichlorobenzene	$> 1 \times 10^{-3}$			
1,2-Diethylbenzene	$> 4 \times 10^{-3}$			Kawagoshi
1,2-Dinitrobenzene	$> 1 \times 10^{-3}$			
1,2-Epoxyethylbenzene	$> 1 \times 10^{-3}$			
1,3,5-Triethyltoluene	$> 4 \times 10^{-3}$			Kawagoshi
1,3,5-Trimethylbenzene	$> 4 \times 10^{-3}$			Kawagoshi
1,4-Dichlorobenzene	$> 3 \times 10^{-4}$			
1,4-Diethylbenzene	$> 4 \times 10^{-3}$			Kawagoshi
1-Chloro-2,4-dinitrobenzene	$> 1 \times 10^{-3}$			
1-Chloro-2-nitrobenzene	$> 1 \times 10^{-3}$			
2,2′-Dihydroxybiphenyl	$> 1 \times 10^{-3}$			
2,4-Diaminotoluene	$> 1 \times 10^{-3}$			
2,4-Dichloroaniline	$> 1 \times 10^{-3}$			
2,4-Dinitroaniline	$> 1 \times 10^{-3}$			
2,5-Dichloroaniline	$> 1 \times 10^{-3}$			
2-Aminotoluene (*o*-toluidine)	$> 1 \times 10^{-4}$			
2-Ethyltoluene	$> 4 \times 10^{-3}$			Kawagoshi

Table 2. Continued

Compound[a)]	REC10(M)[b)]	No.[c)]	Remarks	Data source[d)]
2-Mercaptoimidazoline	$> 1 \times 10^{-4}$		Heterocyclic	
2-Methylpyridine	$> 1 \times 10^{-3}$		Heterocyclic	
2-Phenylene diamine	$> 1 \times 10^{-4}$			
3-Ethyltoluene	$> 4 \times 10^{-3}$			Kawagoshi
4-Amino butylbenzoate	$> 1 \times 10^{-3}$			
4-Chloroaniline	$> 1 \times 10^{-3}$			
4-Chloronitrobenzene	$> 1 \times 10^{-4}$			
4-Chlorotoluene	$> 1 \times 10^{-4}$			
4-Nitroquinoline-*N*-oxide	$> 1 \times 10^{-4}$		Heterocyclic	
4-Nitrotoluene	$> 1 \times 10^{-3}$	47		
4-*tert*-Butylbenzoic acid	$> 1 \times 10^{-3}$			
4-Toluenesulfonamide	$> 1 \times 10^{-3}$			
α-Methylstyrene	$> 1 \times 10^{-3}$			
Aniline	$> 1 \times 10^{-5}$			
Benzaldehyde	$> 1 \times 10^{-3}$			
Benzalkonium chloride	> 100 mg/l			Eguchi
Benzophenone	$> 1 \times 10^{-3}$	46		
Benzylalcohol	$> 1 \times 10^{-3}$			
Biphenyl	$> 1 \times 10^{-3}$			
Chlorhexidine gluconate	$> 1 \times 10^{-4}$			
Chlorobenzene	$> 1 \times 10^{-3}$			
cis-Stilbene	$> 1 \times 10^{-5}$			Shiraishi
Cumene	$> 1 \times 10^{-3}$			
Dibenzyl ether	$> 1 \times 10^{-3}$			
Diethylbenzene, mixture	$> 1 \times 10^{-3}$			
Diphenylamine	$> 1 \times 10^{-4}$			
Diphenylmethane	$> 1 \times 10^{-3}$			
Ethyl benzene	$> 1 \times 10^{-3}$			
Hippuric acid, Na	$> 3 \times 10^{-4}$		Toluene metabolite	
Melamine	$> 1 \times 10^{-3}$		Heterocyclic	
Menadione	$> 1 \times 10^{-3}$		Oxidant	
N,*N*-Dimethylaniline	$> 1 \times 10^{-3}$			
n-Butylbenzene	$> 1 \times 10^{-3}$	67		
Neophenthylglycol dimethacrylate	$> 1 \times 10^{-3}$			Hashimoto
N-Ethylaniline	$> 1 \times 10^{-3}$			
Nitrobenzene	$> 1 \times 10^{-4}$			
N-Methylaniline	$> 1 \times 10^{-3}$			
N-Nitrosodiphenylamine	$> 1 \times 10^{-4}$			
N-Phenyl-1-naphthylamine	$> 1 \times 10^{-3}$			
N-Phenyl-2-naphthylamine	$> 1 \times 10^{-4}$			
o-Tolidine (3,3′-dimethylbenzidine)	$> 1 \times 10^{-3}$			
Phenylhydrazine	$> 1 \times 10^{-3}$			
Quinoline	$> 1 \times 10^{-3}$		Heterocyclic	
•*cis*-1,2-Diphenylcyclobutane	3×10^{-4}	66	Styrene dimer	
trans-1,2-Diphenylcyclobutane	$> 1 \times 10^{-3}$	66	Styrene dimer	
1,3-Diphenylpropane	$> 1 \times 10^{-3}$	66	Styrene dimer	
2,4-Diphenyl-1-butene	$> 1 \times 10^{-3}$	66	Styrene dimer	
Styrene	$> 1 \times 10^{-3}$		Styrene monomer	
2,4,6-Triphenyl-1-hexene	$> 1 \times 10^{-3}$	66	Styrene trimer	
1,3,5-Triphenylcyclohexane	$> 1 \times 10^{-3}$	66	Styrene trimer	

Table 2. Continued

Compound[a]	REC10(M)[b]	No.[c]	Remarks	Data source[d]
1a-Phenyl-4e-(1′-phenylethyl)tetralin	$> 1 \times 10^{-3}$	66	Styrene trimer	
1a-Phenyl-4a-(1′-phenylethyl)tetralin	$> 1 \times 10^{-3}$	66	Styrene trimer	
1e-Phenyl-4a-(1′-phenylethyl)tetralin	$> 1 \times 10^{-3}$	66	Styrene trimer	
1e-Phenyl-4e-(1′-phenylethyl)tetralin	$> 1 \times 10^{-3}$	66	Styrene trimer	
Terephtalic acid	$> 1 \times 10^{-3}$			
Toluene	$> 1 \times 10^{-3}$			
trans-Stilbene	$> 1 \times 10^{-5}$			Shiraishi
Phthalates and adipates				
2-Butoxyethyl phthalate	$> 4 \times 10^{-3}$		Phthalate	Kawagoshi
Adipic acid	$> 1 \times 10^{-3}$		Adipate	
•Benzylbutyl phthalate (BBP)	5×10^{-4}	39	Phthalate	
Di-2-ethylhexyl adipate	$> 1 \times 10^{-3}$	45	Adipate	
Di-2-ethylhexyl phthalate	$> 2 \times 10^{-3}$	38	Phthalate	
Dibutyl adipate	$> 3 \times 10^{-5}$		Adipate	
Dibutyl phthalate	$> 3 \times 10^{-4}$		Phthalate	
Dicyclohexyl phthalate	$> 3 \times 10^{-4}$	41	Phthalate	
Diethyl adipate	$> 3 \times 10^{-4}$		Adipate	
Diethyl phthalate	$> 1 \times 10^{-3}$	42	Phthalate	
Diheptyl phthalate	$> 3 \times 10^{-4}$		Phthalate	
Di-*iso*-butyl adipate	$> 3 \times 10^{-5}$		Adipate	
Di-*iso*-butyl phathalate (DIBP)	$> 1 \times 10^{-4}$		Phthalate	
Di-*iso*-decyl phthalate	$> 3 \times 10^{-4}$		Phthalate	
Di-*iso*-nonyl phthalate	$> 2 \times 10^{-3}$		Phthalate	Kawagoshi
Di-*iso*-octyl phthalate	$> 2 \times 10^{-3}$		Phthalate	Kawagoshi
Di-*iso*-propyl adipate	$> 3 \times 10^{-4}$		Adipate	
•Di-*iso*-propyl phthalate	2×10^{-3}		Phthalate	Kawagoshi
Dimethyl adipate	$> 3 \times 10^{-5}$		Adipate	
Dimethyl phthalate	$> 1 \times 10^{-3}$		Phthalate	
Di-*n*-butyl phthalate (DBuP)	$> 1 \times 10^{-3}$	40	Phthalate	
Di-*n*-hexyl phthalate	$> 5 \times 10^{-3}$		Phthalate	Kawagoshi
Di-*n*-pentyl phthalate	$> 2 \times 10^{-3}$		Phthalate	Kawagoshi
•Di-*n*-propyl phthalate	1×10^{-3}		Phthalate	Kawagoshi
Dipropyl phthalate	$> 3 \times 10^{-4}$	65	Phthalate	
Aliphatics				
1,1,1,2-Tetrachloroethane	$> 1 \times 10^{-3}$			
1,2,3-Trichloropropane	$> 1 \times 10^{-3}$			
1,2-Dibromoethane	$> 1 \times 10^{-3}$			
1,3-Dichloro-2-propanol	$> 1 \times 10^{-3}$			
1,3-Dichloropropene, mixture	$> 1 \times 10^{-3}$			
1,4-Dioxane	$> 1 \times 10^{-5}$			
1-Butanol	$> 1 \times 10^{-3}$			
1-Nonanol	$> 1 \times 10^{-3}$			
1-Tridecanol	$> 3 \times 10^{-4}$			
2,2′,2″-Nitrilotriethanol	$> 1 \times 10^{-3}$			
2-Aminoethanol	$> 1 \times 10^{-3}$			
2-Chloro-1,1,2-trifluoroethyl ethyl ether	$> 1 \times 10^{-4}$			
2-Ethoxyethanol	$> 1 \times 10^{-4}$			
2-Hydroxyethyl methacrylate (HEMA)	$> 1 \times 10^{-3}$			Hashimoto
2-Methyl-1-propanol	$> 1 \times 10^{-3}$			
4-Acryloyloxyethyl trimeritic acid (4-AETA)	$> 1 \times 10^{-3}$			Hashimoto

Table 2. Continued

Compound[a)]	REC10(M)[b)]	No.[c)]	Remarks	Data source[d)]
4-Acryloyloxyethyl trimeritic acid anhydrate (4-AET)	$> 1 \times 10^{-3}$			Hashimoto
4-Methacryloyloxyethyl trimeritic acid (4-META)	$> 1 \times 10^{-3}$			Hashimoto
4-Methacryloyloxyethyl trimeritic acid anhydrate (4-MET)	$> 1 \times 10^{-3}$			Hashimoto
Acetaldehyde	$> 1 \times 10^{-4}$			
Acetamide	$> 1 \times 10^{-3}$			
Acrylamide	$> 1 \times 10^{-4}$			
Bis(2-chloroethyl) ether	$> 1 \times 10^{-3}$			
Bromodichloromethane	$> 1 \times 10^{-3}$			
Bromoform	$> 1 \times 10^{-3}$			
Camphorquinone	$> 1 \times 10^{-3}$			Hashimoto
Chlorodibromomethane	$> 1 \times 10^{-3}$			
Coumaric acid	$> 1 \times 10^{-4}$			
Cyclohexanol	$> 1 \times 10^{-3}$			
Cyclohexanone	$> 1 \times 10^{-3}$			
Cyclohexylamine	$> 1 \times 10^{-3}$			
Dicyclohexylamine	$> 1 \times 10^{-4}$		Solvent	
Dicyclopentadiene	$> 1 \times 10^{-4}$			
Didecyldimethylammonium chloride	$> 3 \times 10^{-4}$			
Diethoxyleneglycol dimethacrylate	$> 1 \times 10^{-3}$			Hashimoto
Diethyl sulfate	$> 1 \times 10^{-4}$			
Diethylene glycol	$> 1 \times 10^{-3}$			
Diphenyl carbonate	$> 3 \times 10^{-4}$		Polycarbonate material	
Epichlorohydrin	$> 1 \times 10^{-4}$			
Ethanolamine	$> 1 \times 10^{-3}$			
Ethylcarbamate	$> 1 \times 10^{-3}$			
Ethylene glycol	$> 1 \times 10^{-3}$			
Ethylene glycol monoethyl ether	$> 1 \times 10^{-3}$			
Formaldehyde	$> 2 \times 10^{-3}$			
Glutaraldehyde	$> 1 \times 10^{-3}$			
Glyoxal	$> 5 \times 10^{-4}$			
Hexachloro-1,3-butadiene	$> 1 \times 10^{-4}$			
Hydroxylamine sulfate	$> 1 \times 10^{-3}$			
Isophorone	$> 1 \times 10^{-4}$			
Methyl methacrylate (MMA)	$> 1 \times 10^{-3}$			Hashimoto
Monochloroacetic acid	$> 1 \times 10^{-4}$			
Monoethoxyleneglycol dimethacrylate	$> 1 \times 10^{-3}$			Hashimoto
Morpholine	$> 1 \times 10^{-3}$		Solvent	
N,*N*-Dimethylformamide	$> 1 \times 10^{-4}$			
n-Butyl acrylate	$> 1 \times 10^{-3}$			
n-Decyl alcohol	$> 1 \times 10^{-3}$			
Nitrilotriacetic acid	$> 1 \times 10^{-4}$			
N-Nitrosodiethylamine	$> 1 \times 10^{-4}$			
N-Nitrosodimethylamine	$> 1 \times 10^{-4}$			
Nonoxynol iodide	> 100 mg/l			Eguchi
Polyalkylpolyaminoethylglycine HCl	> 100 mg/l			Eguchi
Sodium lauryl sulfate (SDS)	$> 1 \times 10^{-3}$			
Tetrachloroethylene	$> 1 \times 10^{-3}$			
Tetraethylenepentamine	> 0.01 ml/l			
Thiourea	$> 1 \times 10^{-4}$			
Tributyl phosphate	$> 1 \times 10^{-4}$			

Table 2. Continued

Compound[a)]	REC10(M)[b)]	No.[c)]	Remarks	Data source[d)]
Triethoxyleneglycol dimethacrylate	$> 1 \times 10^{-3}$			Hashimoto
Triethylamine	$> 1 \times 10^{-3}$			
Triethylenetetramine	$> 1 \times 10^{-4}$			
Trimethylpropane triacrylate	$> 1 \times 10^{-3}$			Hashimoto
Trimethylpropane trimethacrylate	$> 1 \times 10^{-3}$			Hashimoto
Tris(2-chloroethyl) phosphate	$> 1 \times 10^{-4}$			
Tris(butoxyethyl) phosphate	$> 1 \times 10^{-4}$			
Urethane dimethacrylate	$> 1 \times 10^{-3}$			Hashimoto
Vinylacetic acid	$> 1 \times 10^{-4}$			
Inorganics and organometals				
Antimony(III) chloride	$> 1 \times 10^{-4}$		Inorganic	
Boric acid	$> 1 \times 10^{-4}$		Inorganic	
Cadmium chloride	$> 1 \times 10^{-4}$		Inorganic	
Copper(II) sulfate	$> 1 \times 10^{-3}$		Inorganic	
Lead nitrate	$> 2 \times 10^{-3}$		Inorganic	
Mercury(II) chloride	$> 1 \times 10^{-3}$		Inorganic	
Methylmercury chloride	$> 1 \times 10^{-4}$		Organic metal	
Nickel(II) chloride	$> 1 \times 10^{-4}$		Inorganic	
Potassium cyanide	$> 1 \times 10^{-3}$		Inorganic	
Potassium dichromate (VI)	$> 1 \times 10^{-4}$		Inorganic	
Sodium arsenite	$> 1 \times 10^{-4}$		Inorganic	
Sodium molybdate	$> 1 \times 10^{-3}$		Inorganic	
Sodium selenate	$> 1 \times 10^{-5}$		Inorganic	
Thallium(I) chloride	$> 1 \times 10^{-5}$		Inorganic	
Tributyltin(IV) chloride (TBT)	$> 1 \times 10^{-4}$	33	Organometal	
Triphenyltin(IV) chloride (TPT)	$> 1 \times 10^{-3}$	34	Organometal	

a) The compound with • is positive. *b*) REC10: The concentration showing 10% activity of 10^{-7} M 17β-estradiol (relative activity). *c*) No. : Number on the list of suspected EDs published by the Japan Environmental Agency. *d*) Name of person who provided data.

checked the optical density of the suspension of yeast cells before and after exposure to test chemicals. The interference effect on the galactosidase assay was neglected, since we removed the chemicals by washing before the enzyme assay. This point must be carefully considered when the method is modified by omitting the washing procedure for simplification.[26,27)]

With regards to 6), this is the largest disadvantage of our method because yeast is a plant, with a cell wall, suggesting a difference in membrane transport of some chemicals from that in animal cells. Fortunately, there are no reports to date that some chemicals are highly positive in tests using animals or animal cells, while giving negative results when tested using yeast cells. The best resolution for this is to develop a modified method with higher sensitivity. We have succeeded to achieve about 10 times higher sensitivity using a chemoluminescence system.[27)] In this study, although we used the original method, this new modified method may be helpful not only in the case 5) and 6) but also in the case of water insoluble samples.

Structure–Activity Relationship

When considering the chemical structure and estrogenic activity, DHT, benzylbutyl phthalate *n*- and iso-propylphthalate, and *cis*-1,2-diphenylbutane (styrene dimer) were the only positive compounds without a phenol ring. All but DHT showed a higher REC10 (mM level), so we may need further investigations for evaluation of these chemicals, including the impurities in these samples, and metabolism in yeast cells. In addition, all of these positive substances had the hydrophobic moiety at the *para*-position. For example, phenol and *ortho*-substitutes such as 2-hydroxybiphenyl (OPP; food additive), and 2,2'-dihydroxybiphenyl. 2,4-D and 2,4,5-T (Pesticides) were negative, and 4-hydroxybenzoic esters (parabens) were positive whereas free acid was negative. Ortho-substitution with a bulky moiety showed reduced activity; dibutylhydroxytoluene (BHT; food

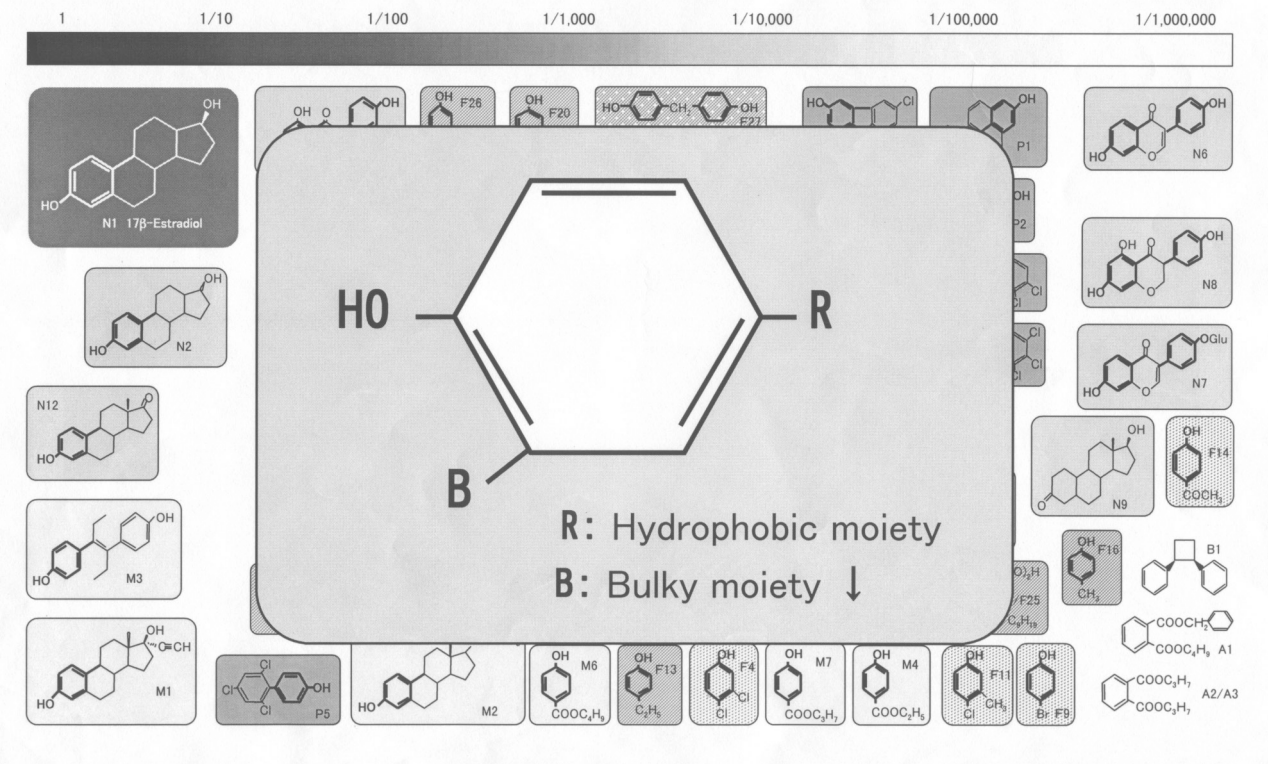

Fig. 3. Common Structure in Estrogenic Compounds Determined by the Yeast Two-Hybrid Assay

additive), pentachlorophenol and 2,4,5-trichlorophenol were negative. When we investigated *n*-alkylphenols, the activity was C5 > C6 = C4 = C3 = C2 = C1 >> C7 = C8 = C9. However, the last 3 compounds showed binding activity to ERα, biocidal activity against the yeast cell and antagonistic activity as great as C6, suggesting that the lower activity might due to water-insolubility and/or impermeability of the cell to these compounds.[28] It is deduced from the above findings that most of the positive compounds have a phenol ring with a moiety of appropriate hydrophobicity at the *para*-position, and that substitution of a bulky moiety at the *ortho*-position reduces activity (Fig. 3).

The greatest advantage of this assay is that it is not necessary to consider endogenous estrogens and receptors because these are absent from yeast cells, Furthermore, this method is less time-consuming and easy to perform. In contrast, the largest disadvantage is the potential for false negative results due to a difference in membrane transport of chemicals because the yeast cell is not an animal cell but a plant cell.

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