

Estrogenic Activities of 517 Chemicals by Yeast Two-Hybrid Assay

Tsutomu Nishihara,^{*,a} Jun-ichi Nishikawa,^a Tomohiko Kanayama,^a Fumi Dakeyama,^a Koichi Saito,^a Masayoshi Imagawa,^{a,1} Satoshi Takatori,^b Yoko Kitagawa,^b Shinjiro Hori,^b and Hideo Utsumi^c

^aGraduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565–0871, Japn, ^bOsaka Prefectural Institute of Public Health, Higashinari, Osaka 537–0025, Japan, and ^cGraduate School of Pharmaceutical Sciences, Kyushu University, Higashi, Fukuoka 812–8582, Japan

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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-7 cell 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.

MATERIALS AND METHODS

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

*To whom correspondence should be addressed: Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565–0871, Japan. Tel.: +81-6-6879-8240; Fax: +81-6-6879-8244; E-mail: nishihara@phs.osaka-u.ac.jp

¹Present address: Faculty of Pharmaceutical Sciences, Nagoya City University

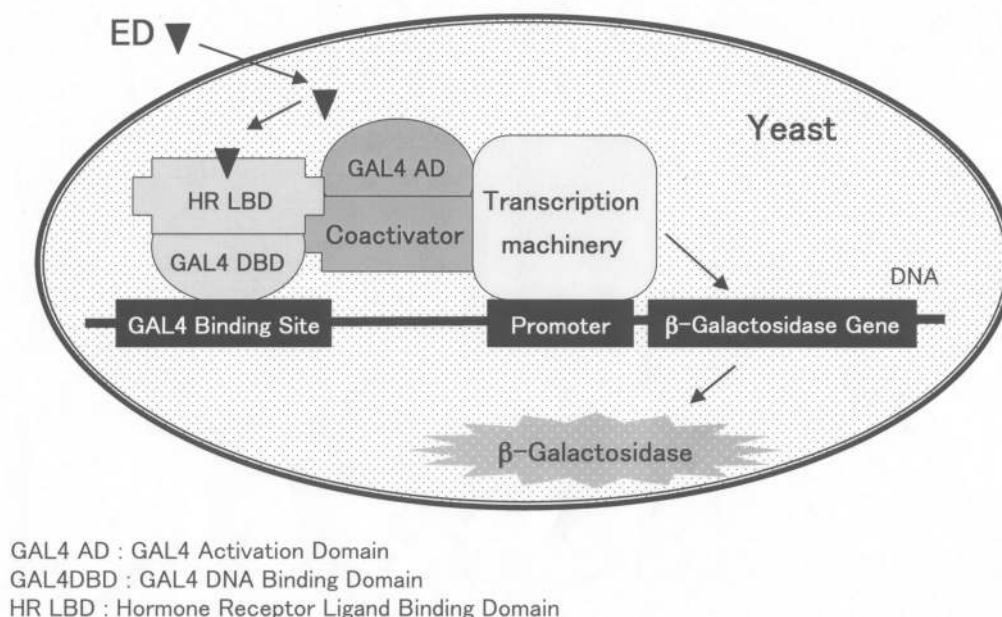


Fig. 1. Outline and Principle of Yeast Two-Hybrid Assay for Estrogenic Activity

In this paper, ER α 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, ER α , 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 pre-incubated overnight at 30°C in SD medium free from tryptophan and leucine. The culture (250 μ l) in a small test tube was then mixed with a DMSO solution (2.5 μ l) 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 μ l) at 30°C for 15 min. The lysate was mixed with 4 mg/ml ONPG (40 μ l) and reacted until development of a yellow color (usu-

ally 30 min) before the reaction was stopped by the addition of 1 M Na₂CO₃ (100 μ l). An aliquot (150 μ l) 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 Compounds by Yeast Two-Hybrid Assay

| Category | Total Positive compounds | |
|--|--------------------------|--|
| | No. No. | |
| Naturals, and related | 49 | 14 |
| | N1 | 17 β -Estradiol |
| | N2 | 17 α -Estradiol |
| | N3 | Apigenin |
| | N4 | Coumestrin |
| | N5 | Coumestrol |
| | N6 | Daidzein |
| | N7 | Daidzin |
| Medicines, food additives, and related | 20 | 7 |
| | M1 | 17 α -Ethinylestradiol |
| | M2 | β -Estradiol-17-acetate |
| | M3 | Diethylstilbesterol (DES) |
| | M4 | Ethyl 4-hydroxybenzoate |
| Pesticides, and related | 118 | 0 |
| Industrial chemicals | 330 | 43 |
| PCBs, PCDFs, PAHs, and related | 55 | 11 |
| | P1 | 2-Hydroxy benzo[<i>a</i>]pyrene |
| | P2 | 2-Hydroxy fluorene |
| | P3 | 3-Hydroxy benzo[<i>a</i>]pyrene |
| | P4 | 3,8-Dihydroxy-2-chlorodibenzofuran |
| | P5 | 4-Hydroxy-2',4',6'-trichlorobiphenyl |
| | P6 | 7-Hydroxy-3,4-dichlorodibenzofuran |
| Phenols | 78 | 28 |
| | F1 | 2,2-Bis(4-hydroxy-3-methylphenyl)propane |
| | F2 | 2,2-Bis(4-hydroxy-phenyl)butane |
| | F3 | 2,4-Dichlorophenol |
| | F4 | 3,4-Dichlorophenol |
| | F5 | 4,4'-Dihydroxybenzophenone |
| | F6 | 4,4'-Dihydroxybiphenyl |
| | F7 | 4,4'-Thiobiphenyl |
| | F8 | 4-(branched)-Nonylphenol |
| | F9 | 4-Bromophenol |
| | F10 | 4-Chloro-3,5-xyleneol |
| | F11 | 4-Chloro-3-methylphenol |
| | F12 | 4-Chlorophenol |
| | F13 | 4-Ethylphenol |
| | F14 | 4-Hydroxyacetophenone |
| Benzenes and heterocyclics | 80 | 1 |
| | B1 | <i>cis</i> -1,2-Diphenylcyclobutane |
| Phthalates and adipates | 26 | 3 |
| | A1 | Benzylbutyl phthalate (BBP) |
| | A2 | Di- <i>iso</i> -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 ER α -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

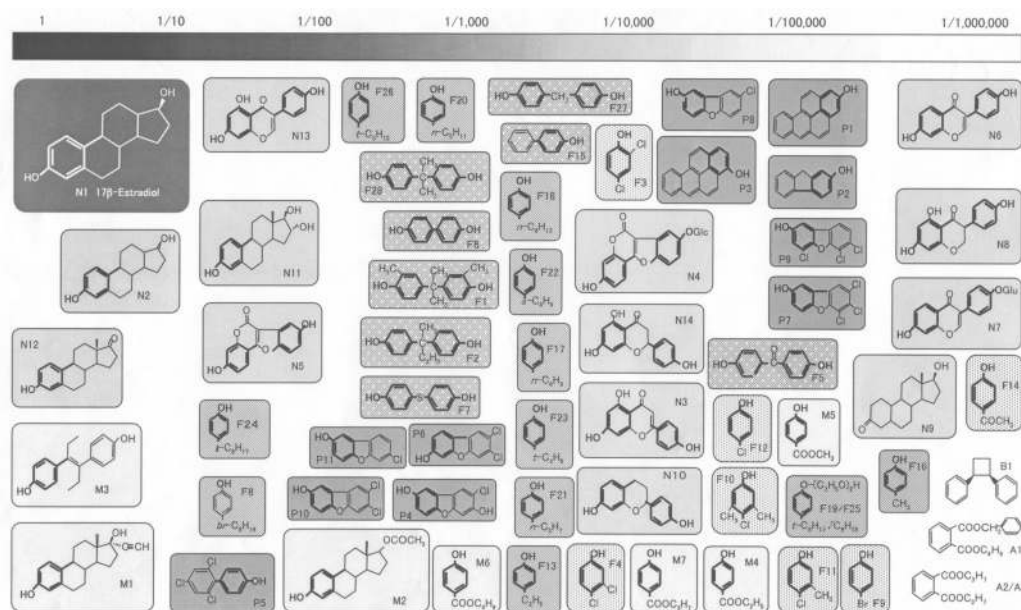


Fig. 2. Chemical Structures of Estrogenic Compounds Determined by Yeast Two-Hybrid Assay

A symbol nearby the structure in the column, e.g., N2 and F4, corresponds that in Table 1. For example, N2 and F4 are 17 α -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 ER α , 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 ER β , AR, TR, GR, MR, and are planning to test chemicals using these systems with an automatic testing robot. ER α used here is the ligand binding domain from rat ER α , but when compared to the amino acid sequences among mammals, 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 | $> 1 \times 10^{-4}$ | | | |
| Acetylugenol | $> 1 \times 10^{-4}$ | | | |
| Aflatoxin B1 | $> 2 \times 10^{-6}$ | | Toxin | |
| ●Apigenin | 1×10^{-5} | | Phytoestrogen | |
| Aplysiaterpenoid A | $> 10 \text{ mg/l}$ | | | |
| 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 | $> 1 \times 10^{-4}$ | | | |
| Ferulic 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) | $> 2 \text{ mg/l}$ | | Toxin | |
| MelQx | $> 2 \times 10^{-5}$ | | Scorch | |
| Microcystin RR | $> 1.34 \text{ mg/l}$ | | | |
| ●Naringenin | 3×10^{-5} | | Phytoestrogen | |
| Phloretin | $> 1 \times 10^{-4}$ | | | |
| PhIP | $> 2 \times 10^{-5}$ | | 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 | | | | |
| •17 α -Ethinylestradiol | 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 | |
| • <i>n</i> -Butyl 4-hydroxybenzoate | 3×10^{-6} | | Paraben | |
| • <i>n</i> -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 <i>H</i> -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 | |
| Cypermethrin | $> 1 \times 10^{-4}$ | 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 [<i>O</i> -Ethyl- <i>O</i> -(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 | |
| <i>o,p'</i> -DDD | $> 3 \times 10^{-4}$ | 19 | DDT metabolite | |
| <i>o,p'</i> -DDE | $> 3 \times 10^{-4}$ | 19 | DDT metabolite | |
| <i>o,p'</i> -DDT | $> 3 \times 10^{-4}$ | 18 | Insecticide | |
| <i>p,p'</i> -DDD | $> 3 \times 10^{-4}$ | 19 | DDT metabolite | |
| <i>p,p'</i> -DDE | $> 1 \times 10^{-4}$ | 19 | DDT metabolite, antagonist (AR) | |
| <i>p,p'</i> -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 | |
| Xyllylcarb | $> 3 \times 10^{-4}$ | | Insecticide | |
| Zineb | $> 40 \text{ mg/l}$ | 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-Dimethylnaphthalene | $> 1 \times 10^{-4}$ | | 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)phenyl] propane (BisGMA) | $> 1 \times 10^{-3}$ | | Bisphenol | Hashimoto |
| 2,2-bis[4(methacryloxy)]phenyl propane (BisMEPP) | $> 1 \times 10^{-3}$ | | Bisphenol | Hashimoto |
| 2,2-bis[4(methacryloxypropoxy)phenyl] propane (MPEPP) | $> 1 \times 10^{-3}$ | | Bisphenol | Hashimoto |
| 2,2'-Dihydroxybiphenyl | $> 1 \times 10^{-3}$ | | | |
| 2,4,6-Tribromophenol | $> 1 \times 10^{-3}$ | | | |
| 2,4,6-Trichlorophenol | $> 1 \times 10^{-3}$ | | | |
| 2,4-Dibromophenol | $> 1 \times 10^{-3}$ | | | Shiraishi |
| ●2,4-Dichlorophenol | 4×10^{-5} | | | |
| 2,4-Dimethylphenol | $> 1 \times 10^{-5}$ | | | |
| 2,4-Dinitrophenol | $> 1 \times 10^{-3}$ | | | |
| 2,5-Dichlorophenol | $> 1 \times 10^{-3}$ | | | |
| 2,6-Di- <i>tert</i> -butyl-4-methylphenol | $> 1 \times 10^{-4}$ | | | |
| 2-Hydroxybiphenyl (OPP) | $> 1 \times 10^{-3}$ | | | |
| 2-Methacryloyloxyethyl phenyl phosphate (Phenyl-P) | $> 1 \times 10^{-3}$ | | | Hashimoto |
| 2-Methylphenol (<i>o</i> -cresol) | $> 1 \times 10^{-5}$ | | | |
| 2-Nitrophenol | $> 1 \times 10^{-3}$ | | | |
| 2- <i>sec</i> -Butylphenol | $> 1 \times 10^{-3}$ | | | |
| 2- <i>tert</i> -Butylphenol | $> 1 \times 10^{-3}$ | | | |
| ●3,4-Dichlorophenol | 2×10^{-5} | | | Shiraishi |
| 3-Aminophenol | $> 1 \times 10^{-3}$ | | | |
| 3-Nitrophenol | $> 1 \times 10^{-3}$ | | | |
| 3- <i>tert</i> -Butylphenol | $> 1 \times 10^{-3}$ | | | |
| ●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-xyleneol | 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 | $> 3 \times 10^{-3}$ | | | Fujita |
| ●4-Hydroxybiphenyl | 3×10^{-5} | | | |
| 4-iso-Propyl-3-methylphenol | $> 1 \times 10^{-3}$ | | | |
| ●4-Methylphenol (<i>p</i> -cresol) | 3×10^{-4} | | | |
| ●4- <i>n</i> -Butylphenol | 1×10^{-5} | | | |
| 4- <i>n</i> -Heptylphenol | $> 1 \times 10^{-3}$ | | | |
| ●4- <i>n</i> -Hexylphenol | 1×10^{-5} | | | |
| 4- <i>n</i> -Nonylphenol | $> 1 \times 10^{-3}$ | 36 | | |
| 4- <i>n</i> -Octylphenol | $> 1 \times 10^{-3}$ | 36 | | |
| 4-Nonylphenol polyethoxylate (10) | $> 3 \times 10^{-5}$ | | Surfactant | Nakamuro |
| 4-Nonylphenol polyethoxylate (15) | $> 2 \times 10^{-5}$ | | Surfactant | Nakamuro |
| ●4-Nonylphenol polyethoxylate (2) | 1×10^{-3} | | Surfactant | |
| 4-Nonylphenol polyethoxylate (23) | $> 1 \times 10^{-5}$ | | Surfactant | Nakamuro |
| 4-Nonylphenol polyethoxylate (5) | $> 1 \times 10^{-5}$ | | Surfactant | Nakamuro |
| ●4- <i>n</i> -Pentylphenol | 3×10^{-6} | 36 | | |
| ●4- <i>n</i> -Propylphenol | 7×10^{-5} | | | |

Table 2. Continued

| Compound ^{a)} | REC10(M) ^{b)} | No. ^{c)} | Remarks | Data source ^{d)} |
|---|------------------------|-------------------|------------|---------------------------|
| ●4- <i>sec</i> -Butylphenol | 1×10^{-5} | 36 | | Nishimura |
| ●4- <i>tert</i> -Butylphenol | 3×10^{-5} | | | |
| ●4- <i>tert</i> -Octylphenol | 2×10^{-7} | | | |
| 4- <i>tert</i> -Octylphenol polyethoxylate (10) | $> 2 \times 10^{-5}$ | | Surfactant | Nakamuro |
| 4- <i>tert</i> -Octylphenol polyethoxylate (15) | $> 2 \times 10^{-5}$ | | Surfactant | Nakamuro |
| ●4- <i>tert</i> -Octylphenol polyethoxylate (2) | 3×10^{-4} | | Surfactant | |
| 4- <i>tert</i> -Octylphenol polyethoxylate (23) | $> 1 \times 10^{-5}$ | | Surfactant | Nakamuro |
| 4- <i>tert</i> -Octylphenol polyethoxylate (5) | $> 1 \times 10^{-5}$ | | Surfactant | Nakamuro |
| ●4- <i>tert</i> -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 | $> 10 \text{ mg/l}$ | | | |
| 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 (<i>o</i> -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- <i>N</i> -oxide | $> 1 \times 10^{-4}$ | | Heterocyclic | |
| 4-Nitrotoluene | $> 1 \times 10^{-3}$ | 47 | | |
| 4- <i>tert</i> -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 | $> 100 \text{ mg/l}$ | | | Eguchi |
| Benzophenone | $> 1 \times 10^{-3}$ | 46 | | |
| Benzylalcohol | $> 1 \times 10^{-3}$ | | | |
| Biphenyl | $> 1 \times 10^{-3}$ | | | |
| Chlorhexidine gluconate | $> 1 \times 10^{-4}$ | | | |
| Chlorobenzene | $> 1 \times 10^{-3}$ | | | |
| <i>cis</i> -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 | |
| <i>N,N</i> -Dimethylaniline | $> 1 \times 10^{-3}$ | | | |
| <i>n</i> -Butylbenzene | $> 1 \times 10^{-3}$ | 67 | | |
| Neophentylglycol dimethacrylate | $> 1 \times 10^{-3}$ | | | Hashimoto |
| <i>N</i> -Ethylaniline | $> 1 \times 10^{-3}$ | | | |
| Nitrobenzene | $> 1 \times 10^{-4}$ | | | |
| <i>N</i> -Methylaniline | $> 1 \times 10^{-3}$ | | | |
| <i>N</i> -Nitrosodiphenylamine | $> 1 \times 10^{-4}$ | | | |
| <i>N</i> -Phenyl-1-naphthylamine | $> 1 \times 10^{-3}$ | | | |
| <i>N</i> -Phenyl-2-naphthylamine | $> 1 \times 10^{-4}$ | | | |
| <i>o</i> -Tolidine (3,3'-dimethylbenzidine) | $> 1 \times 10^{-3}$ | | | |
| Phenylhydrazine | $> 1 \times 10^{-3}$ | | | |
| Quinoline | $> 1 \times 10^{-3}$ | | Heterocyclic | |
| • <i>cis</i> -1,2-Diphenylcyclobutane | 3×10^{-4} | 66 | Styrene dimer | |
| <i>trans</i> -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 | |
| Terephthalic acid | $> 1 \times 10^{-3}$ | | | |
| Toluene | $> 1 \times 10^{-3}$ | | | |
| <i>trans</i> -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- <i>iso</i> -butyl adipate | $> 3 \times 10^{-5}$ | | Adipate | |
| Di- <i>iso</i> -butyl phthalate (DIBP) | $> 1 \times 10^{-4}$ | | Phthalate | |
| Di- <i>iso</i> -decyl phthalate | $> 3 \times 10^{-4}$ | | Phthalate | |
| Di- <i>iso</i> -nonyl phthalate | $> 2 \times 10^{-3}$ | | Phthalate | Kawagoshi |
| Di- <i>iso</i> -octyl phthalate | $> 2 \times 10^{-3}$ | | Phthalate | Kawagoshi |
| Di- <i>iso</i> -propyl adipate | $> 3 \times 10^{-4}$ | | Adipate | |
| •Di- <i>iso</i> -propyl phthalate | 2×10^{-3} | | Phthalate | Kawagoshi |
| Dimethyl adipate | $> 3 \times 10^{-5}$ | | Adipate | |
| Dimethyl phthalate | $> 1 \times 10^{-3}$ | | Phthalate | |
| Di- <i>n</i> -butyl phthalate (DBuP) | $> 1 \times 10^{-3}$ | 40 | Phthalate | |
| Di- <i>n</i> -hexyl phthalate | $> 5 \times 10^{-3}$ | | Phthalate | Kawagoshi |
| Di- <i>n</i> -pentyl phthalate | $> 2 \times 10^{-3}$ | | Phthalate | Kawagoshi |
| •Di- <i>n</i> -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 trimeric 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 | $> 1 \times 10^{-3}$ | | | |
| <i>N,N</i> -Dimethylformamide | $> 1 \times 10^{-4}$ | | Solvent | |
| <i>n</i> -Butyl acrylate | $> 1 \times 10^{-3}$ | | | |
| <i>n</i> -Decyl alcohol | $> 1 \times 10^{-3}$ | | | |
| Nitrilotriacetic acid | $> 1 \times 10^{-4}$ | | | |
| <i>N</i> -Nitrosodiethylamine | $> 1 \times 10^{-4}$ | | | |
| <i>N</i> -Nitrosodimethylamine | $> 1 \times 10^{-4}$ | | | |
| Nonoxynol iodide | $> 100 \text{ mg/l}$ | | | Eguchi |
| Polyalkylpolyaminoethylglycine HCl | $> 100 \text{ mg/l}$ | | | Eguchi |
| Sodium lauryl sulfate (SDS) | $> 1 \times 10^{-3}$ | | | |
| Tetrachloroethylene | $> 1 \times 10^{-3}$ | | | |
| Tetraethylenepentamine | $> 0.01 \text{ ml/l}$ | | | |
| 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)} |
|--|------------------------|-------------------|---------------|---------------------------|
| Triethoxylethylene glycol 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 17 β -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

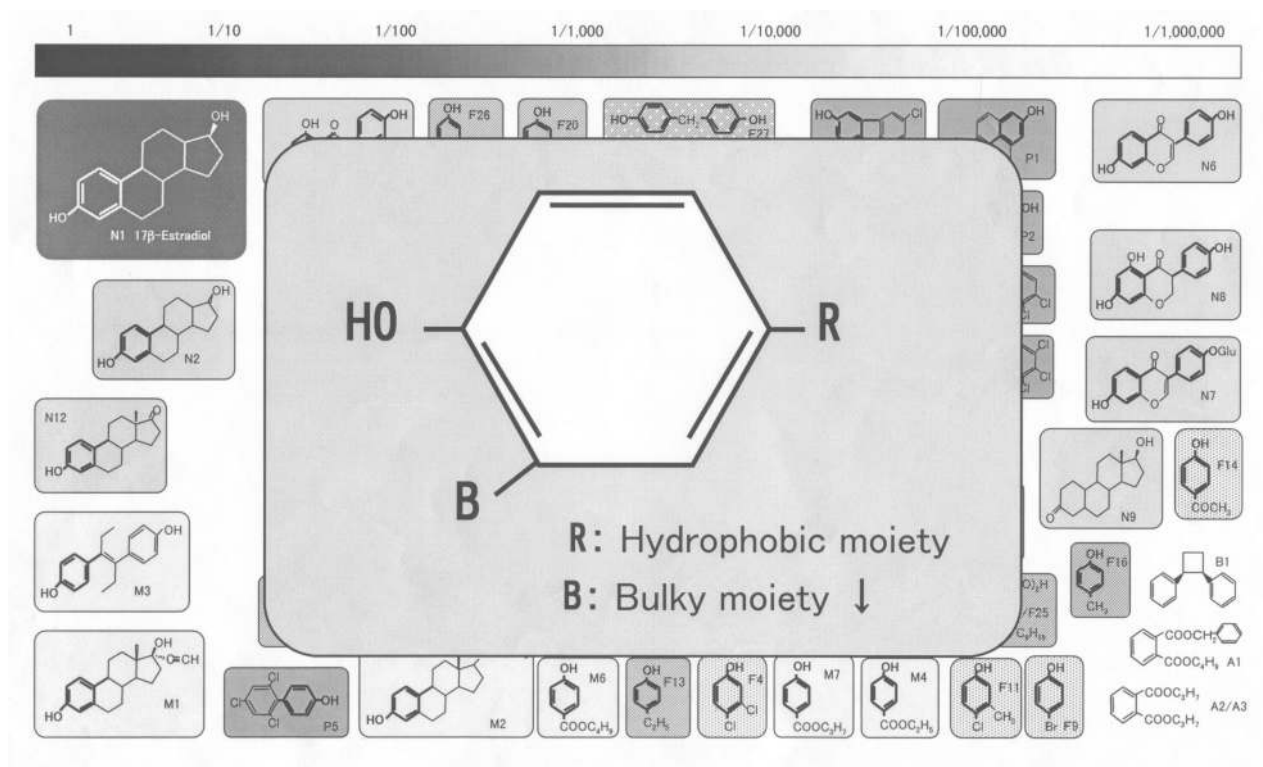


Fig. 3. Common Structure in Estrogenic Compounds Determined by the Yeast Two-Hybrid Assay

additive), pentachlorophenol and 2,4,5-trichlorophenol were negative. When we investigated *n*-alkylphenols, the activity was $C5 > C6 = C4 = C3 = C2 = C1 \gg C7 = C8 = C9$. However, the last 3 compounds showed binding activity to ER α , 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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