penis exhibited the least sensitive end point, based on benchmark dose estimates (OECD 2001). When the overall dose–response curves for agonistic TP were compared, the glans penis was the most sensitive and the seminal vesicle was the least sensitive (OECD 2001). In the present study, it was difficult to select a particularly sensitive organ from among the five tissues examined in the androgen agonistic MT and antagonistic VCZ and *p,p′*-DDE assays. In the Hershberger assay using MT, the CV for the glans penis was smaller than that of the other organs, but the TRT of the ventral prostate was the highest among the values measured in the study. On the other hand, the LAB values of the ventral prostate and Cowper's glands were smaller than the values of the other organs, and the percentage weight change relative to the control value at the highest dose was the greatest in the ventral prostate. These findings demonstrate that the ventral prostate was particularly sensitive based on the TRT, LAB, and increasing percentage of organ weight, whereas the glans penis was sensitive based on the CV values. Similarly, the seminal vesicle was sensitive based on the TRT, LAB, and decreasing percentage of organ weight, whereas the glans penis was sensitive based on the CV values in the assays using antagonistic VCZ and *p,p′*-DDE.

The CV values for the ventral prostate, seminal vesicle, and Cowper's gland were higher than those for the glans penis and BC/LA in the assays for all three chemicals. These organs contain fluid, and the dissection of these organs is technically difficult, compared with that of the glans penis and BC/LA. These technical issues may have influenced the varied CV values obtained for these organs. Furthermore, we did not confirm whether preputial separation had occurred in the rats before castration. Preputial separation has been reported to occur between days 39 and 44 in SD rats (Yamasaki et al. 2001); in this study, the castration was performed between days 40 and 46. Thus, the rats used in this study were likely a mixture of animals with or without preputial separation. The castration times may also have influenced the variation in the CV values for each organ.

In the assay using the androgen antagonistic chemicals, slight differences in the normalized response curves for low doses in the *p,p′*-DDE assay were observed among the laboratories, but the response curves for each organ in the VCZ assay were similar. The fact that the percentages of organ weight relative to the control at high doses in the *p,p′*-DDE assay were lower than those in the VCZ assay suggests that the androgen antagonistic affinity of *p,p′*-DDE is weaker than that of VCZ. On the other hand, the organ weights of the rats given only TP varied among the laboratories. The slight variation in responses among the laboratories for the low dose in the *p,p′*-DDE assay may have been affected by the relationship between the agonistic affinity of TP and the weak antagonistic affinity of *p,p′*-DDE.

In the phase 1 validation study using TP, the OECD reported that no essential differences were observed when the weights of the fresh and fixed organs were compared (OECD 2001). Lab 4 weighed the prostate, seminal vesicle, and Cowper's glands after fixation, whereas the other laboratories measured the weights of fresh organs; the changes in organ weight among the laboratories were essentially similar. Therefore, the difference in the weighing method (fresh vs. fixed organs) did not appear to affect the results of the assay. Although the terminal body weights were different between SD and Wistar rats, the responsiveness of these rats to VCZ and *p,p′*-DDE did not differ in this study. This finding demonstrates that no significant differences exist regarding the use of SD and Wistar rats in the Hershberger assay for the detection of androgen antagonists.

Among the optional organs measured in this study, the weight of the adrenal glands increased significantly in rats given 100 mg/kg/day of VCZ and decreased in rats given 50 mg/kg/day MT. The decrease in adrenal weight may be suppressed by a high dose of

Table 8. Overall mean organ weights, R^2, and CV in rats given 0.2 mg/kg/day TP and *p,p′*-DDE: data and log-transformed data.

	R^2 (%)			*p,p′*-DDE (mg/kg/day)				
Overall means	TRT	LAB	CV (%)	0 (*n* = 30)	3 (*n* = 30)	10 (*n* = 30)	30 (*n* = 30)	100 (*n* = 30)
Overall								
Ventral prostate (mg)	36	32	20	120.6	110.9	117.2	94.3	57.3
Seminal vesicles (mg)	45	31	19	300.8	278.0	296.5	227.0	109.2
BC/LA (mg)	37	42	10	460.0	465.8	442.9	412.3	283.0
Glans penis (mg)	30	37	7	72.8	71.9	71.5	71.1	59.8
Cowper's glands (mg)	33	29	19	27.7	26.4	26.4	24.4	15.6
Log-transformed								
Ventral prostate (mg)	48	23	4.4	2.1	2.0	2.1	2.0	1.7
Seminal vesicles (mg)	59	20	3.7	2.5	2.4	2.5	2.3	2.0
BC/LA (mg)	40	43	1.7	2.7	2.7	2.6	2.6	2.4
Glans penis (mg)	31	35	1.8	1.9	1.9	1.9	1.9	1.8
Cowper's glands (mg)	40	25	6.3	1.4	1.4	1.4	1.4	1.2

Abbreviations: TRT, R^2 values for effects of treatments; LAB, R^2 values for effects among laboratories.

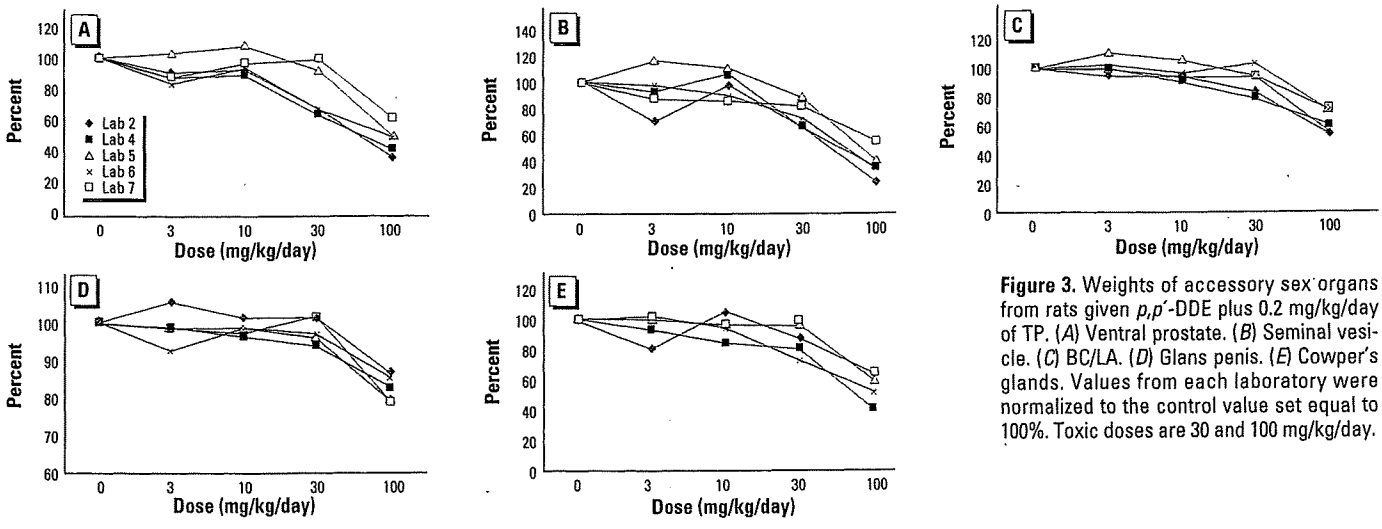

Figure 3. Weights of accessory sex organs from rats given *p,p′*-DDE plus 0.2 mg/kg/day of TP. (*A*) Ventral prostate. (*B*) Seminal vesicle. (*C*) BC/LA. (*D*) Glans penis. (*E*) Cowper's glands. Values from each laboratory were normalized to the control value set equal to 100%. Toxic doses are 30 and 100 mg/kg/day.

androgen in the form of MT, and the adrenal glands may be hypertrophied in response to a high level of antagonist. Increased kidney weights in rats given 50 mg/kg/day of MT and increased liver weights in rats given 30 and 100 mg/kg/day of *p,p′*-DDE suggested toxic effects. On the other hand, a significant decrease or a tendency to decrease of the body weights in the *p,p′*-DDE assay was observed by two out of five laboratories; this response was also considered to be a toxic effect of *p,p′*-DDE.

References

Bülbring E, Burn JH. 1935. The estimation of oestrin and of male hormone in oily solution. J Physiol 85:320–333.

Deanesly R, Parkes AS. 1936. Comparative activities of compounds of the androsterone-testosterone series. Biochem J 30:291–303.

Dingemanse E, Frued J, Laquer E. 1935. Differences between male hormone extracts from urine and from testes. Nature 135:184.

Di Salle E, Briatico G, Giudici D, Ornati G, Panzeri A. 1994. Endocrine properties of the testosterone 5α-reductase inhibitor turosteride (FCE 26073). J Steroid Biochem Mol Biol 48:241–248.

Eisenberg E, Gordan GS. 1950. The levator ani muscle of the rat as an index of mytrophic activity of steroidal hormones. J Pharmacol Exp Therap 99:38–44.

Eisenberg E, Gordan GS, Elliott HW. 1949. Testosterone and tissue respiration of the castrate male rat with a possible test for mytrophic activity. Endocrincology 45:113–119.

Hershberger LG, Shipley EG, Meyer RK. 1953. Myotrophic activity of 19-nortestosterone and other steroids determined by modified levator ani muscle method. Proc Soc Exp Biol Med 83:175–80.

Japanese Association for Laboratory Science. 1992. The Guidelines for Animal Experimentation. Tokyo:Soft Science Inc.

Korenchevsky V. 1932. The assay of testicular hormone preparations. Biochem J 26:413–422.

Korenchevsky V, Dennison M, Kohn-Speyer A. 1933a. Changes produced by testicular hormone in normal and in castrated rats. Biochem J 27:557–579.

———. 1933b. On the assay and the absorption of testicular hormone dissolved in oil. Biochem J 27:778–782.

Korenchevsky V, Dennison M, Schalit R. 1932. The response of castrated male rats to the injection of the testicular hormone. Biochem J 26:1306–1314.

McLachlan JA. 1993. Functional toxicology: a new approach to detect functionally active xenobiotics. Environ Health Perspect 101:386–387.

McLachlan JA, Korach KS. 1995. Symposium on estrogens in the environment, III. Environ Health Perspect 103(suppl 7):3–4.

OECD. 1998. Report of the First Meeting of the OECD Endocrine Disrupter Testing and Assessment (EDTA) Working Group. Paris:Organisation for Economic Co-operation and Development.

———. 2000. The Second Meeting of the OECD Validation Management Group (VMG) for the Screening and Testing of Endocrine Disrupters. Paris:Organisation for Economic Co-operation and Development.

———. 2001. Third Meeting of the OECD Validation Management Group (VMG) for the Screening and Testing of Endocrine Disrupters. Paris:Organisation for Economic Co-operation and Development.

———. 2003. Fourth Meeting of the OECD Validation Management Group (VMG) for the Screening and Testing of Endocrine Disrupters. Paris:Organisation for Economic Co-operation and Development.

Peets EA, Henson MF, Neri R. 1973. On the mechanism of the antiandrogenic action of flutamide (α-α-α-trifluoro-2-methyl-4′-nitro-*m*-propionotoluidide) in the rat. Endocrinology 94:532–540.

Raynaud JP, Bonne C, Moguilewsky M, Lefebvre FA, Bélanger A, Labrie F. 1984. The pure antiandrogen RU 23908 (Anandron), a candidate of choice for the combined antihormonal treatment of prostatic cancer: a review. Prostate 5:299–311.

Raynaud JP, Bouton MM, Moguilewsky M, Ojasoo T, Philibert D, Beck G, et al. 1980. Steroid hormone receptors and pharmacology. J Steroid Biochem 12:143–157.

Sunami O, Kunimatsu T, Yamada T, Yabushita S, Sukata T, Miyata K, et al. 2000. Evaluation of a 5-day Hershberger assay using young mature male rats: methyltestosterone and *p,p′*-DDE, but not fenitrothion, exhibited androgenic or antiandrogenic activity in vivo. Toxicol Sci 25:403–415.

U.S. Environmental Protection Agency. 1998. Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) Final Report. EPA/743/R-98/003. Available: http://www.epa.gov/scipoly/oscpendo/history/finalrpt.htm [accessed 27 October 2003].

Wainman P, Shipounoff GC. 1941. The effects of castration and testosterone propionate on the striated perineal musculature in the rat. Endocrinology 29:975–978.

Wakeling A, Furr BJA, Glen AT, Hughes LR. 1981. Receptor binding and biological activity of steroidal and nonsteroidal antiandrogens. J Steroid Biochem 15:355–359.

Yamada T, Sunami O, Kunimatsu T, Kamita Y, Okuno Y, Seki T, et al. 2000. Dissection and weighing of accessory sex glands after formalin fixation, and a 5-day assay using young mature rats are reliable and feasible in the Hershberger assay. Toxicology 162:103–119.

Yamasaki K, Sawaki M, Noda S, Muroi T, Takatsuki M. 2001. Preputial separation and glans penis changes in normal growing Crj:CD(SD) IGS rats. Reprod Toxicol 15:533–536.

Yamasaki K, Sawaki M, Noda S, Takatsuki M. 2002. Uterotrophic and Hershberger assays for *n*-butylbenzene in rats. Arch Toxicol 75:703–706.

Yamasaki K, Takeyoshi M, Yakabe Y, Sawaki M, Imatanaka M, Shinoda K, et al. 2003. Immature rat uterotrophic assay of 18 chemicals and Hershberger assay of 30 chemicals. Toxicology 183:95–115.

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Immature rat uterotrophic assay of 18 chemicals and Hershberger assay of 30 chemicals

Kanji Yamasaki *, Masahiro Takeyoshi, Masakuni Sawaki, Nobuya Imatanaka, Kazutoshi Shinoda, Mineo Takatsuki

Chemicals Evaluation and Research Institute, 3-822, Ishii, Hita, Oita 877-0061, Japan

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Abstract

We performed an immature rat uterotrophic assay of 18 chemicals and Hershberger assay of 30 chemicals to assess the relationship between the results of two assays. The chemicals tested by the uterotopic assay were 4-n-amylphenol, p-dodecyl-phenol, p-(tert-pentyl)phenol, 4-cyclohexylphenol, 4-(1-adamantyl)phenol, 4,4′-thiobis-phenol, diphenyl-p-phenylenediamine, 4-hydroxyazobenzene, 4-(phenylmethyl)phenol, 4,4′-(hexafluoroisopropylidene)diphenol, 2,2-bis(4-hydroxyphenyl)-4-methyl-n-pentane, 4,4′-(octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol, 4,4′-dihydroxybenzophenone, 2,2′,4,4′-tetrahydroxybenzophenone, 4-hydroxybenzophenone, 2,4,4′-trihydroxybenzophenone, testosterone enanthate, and methyltestosterone. The chemicals tested by the Hershberger assay were the 18 chemicals tested in the uterotrophic assay plus the following: 17alpha estradiol, estrone, equilin, norethindrone, norgestrel, ethynyl estradiol, bisphenol A, bisphenol B, bisphenol F, 4-tert-octylphenol, p-cumyl phenol, and nonylphenol. All chemicals examined in this study were positive in a reporter gene assay for ER-alpha. In the immature rat uterotrophic assay, all chemicals induced uterotrophy and p-(tert-pentyl)phenol, 4,4′-thiobis-phenol, 4-(phenylmethyl)phenol, 4,4′-(hexafluoroisopropylidene)diphenol, 2,2-bis(4-hydroxyphenyl)-4-methyl-n-pentane, 4,4′-(octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol, 4,4′-dihydroxybenzophenone, 2,2′,4,4′-tetrahydroxybenzophenone, 4-hydroxybenzophenone, and 2,4,4′-trihydroxybenzophenone exerted both estrogen agonistic effect and reduced the estrogenic effect of ethynylestradiol. In the Hershberger assay, a clear androgen agonistic effect was detected in the androgen derivatives testosterone enanthate and methyltestosterone.

Keywords: Androgenic effect; Endocrine; Estrogenic effect; Hershberger assay; Rat; Uterotrophic assay

1. Introduction

There is concern that certain chemicals may have the potential to interfere with normal sexual differentiation and development in animals and humans (McLachlan, 1993; McLachlan and Korach, 1995), and the Organisation for Economic

* Corresponding author. Tel.: +81-973-24-7211; fax: +81-973-23-9800

E-mail address: yamasaki-kanji@ceri.jp (K. Yamasaki).

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Cooperation and Development (OECD) has proposed the uterotrophic assay and the Hershberger assay as screening tests to detect estrogenic and androgenic properties of potentially endocrine disrupting chemicals (OECD, 2001). In the uterotrophic assay, the test method that chemical compounds are injected subcutaneously to immature female rats for 3 consecutive days is one of option among four standardized protocols, and validation studies were already finished. In the Hershberger assay, on the other hand, chemicals are orally administration to castrated rats for 10 days, and validation studies have been performed. Although there have been reports of estrogenic compounds displaying androgenic effects in toxicological studies (Atanassova et al., 1999; Yamasaki et al., 2002a), the relationship between the agonistic and antagonistic effects of the same estrogenic chemical or between the estrogenic and androgenic effects of the same chemical have never been adequately studied. We, therefore, performed uterotrophic assays and the Hershberger assay of 18 chemicals and Hershberger assay of 12 chemicals identified as having estrogenic properties in our previous study (Yamasaki et al., 2002b).

2. Materials and methods

The studies were performed under Good Laboratory Practice guidelines.

2.1. Uterotrophic assay

2.1.1. Chemicals

The chemicals tested in the uterotrophic assay are listed in Table 1, and chemical structures of test compounds are shown in Fig. 1. All chemicals were dissolved in olive oil (Fujimi Pharmaceutical, Osaka, Japan) before use. All of chemicals tested in the uterotrophic assay in this study were positive in the reporter gene assay for ER-alpha based on their PC10 values. Chemicals tested in this assay were selected based on the list of suspected endocrine disrupters published by the EU (Commission of the European Communities, 2001).

2.1.2. Animals

Crj:CD (SD) rats, dams and their 10-day-old pups, were purchased from Charles River Japan (Shiga, Japan). The dams and pups were housed in polycarbonate pens until weaning. All pups were weaned at 17 days of age and subsequently individually housed in stainless steel wire-mesh cages throughout the study. The immature rats were weighed, weight-ranked, and assigned randomly to each of the experimental and control groups. Body weight and clinical signs were recorded daily throughout the study. Rats were provided with tap water and a commercial diet (CRF-1, Oriental Yeast, Tokyo, Japan) ad libitum before weaning, and with water automatically and a commercial diet (MF, Oriental Yeast) ad libitum after weaning. The animal room was maintained at a temperature of 23 ± 2 °C and a relative humidity of $55 \pm 5\%$, and it was artificially illuminated with fluorescent light on a 12-h light/dark cycle (06:00–18:00 h). All animals were cared for according to the principles outlined in the guide for animal experimentation prepared by *the Japanese Association for Laboratory Animal Science*.

2.1.3. Study design

Each chemical was subcutaneously injected on 3 consecutive days into the back of 19-day-old rats. The doses of each chemical are shown in Table 4. In some rats, ethynyl estradiol (EE, CAS No. 57-63-6, 98% purity, Sigma Chemical) in olive oil was also subcutaneously injected into the back at a dose of 0.6 μg/kg per day on 3 consecutive days after the administration of each chemical at the same doses. A vehicle control group was injected with olive oil alone, and a positive control group was injected with EE after administration of olive oil. A group injected with the estrogen antagonist chemical tamoxifen at a dose of 1 mg/kg per day plus EE was also established to confirm the reliability of this study. Each group consisted of six rats. The doses of each chemical were based on the results of a preliminary study. The volume of the olive oil solution containing the chemical or EE for subcutaneous injections was 2 ml/kg. The animals were killed by bleeding from the abdominal vein under deep ether anesthesia approximately 24 h after the final dose. At necropsy, the

Table 1
Chemicals tested in the uterotrophic assay

Chemicals	CAS no.	Source	Purity (%)
4-n-Amylphenol	14938-35-3	Tokyo Kasei Kogyo, Co.	99.2
p-Dodecyl-phenol	104-43-8	Kanto Chemical Co.	Unknown
p-(Tert-pentyl)phenol	80-46-6	Wako Pure Chemicals	100.0
4-Cyclohexylphenol	1131-60-8	Tokyo Kasei Kogyo Co.	99.7
4-(1-Adamantyl)phenol	29799-07-3	Aldrich Co.	97
4,4′-Thiobis-phenol	2664-63-3	Tokyo Kasei Kogyo Co.	99.8
Diphenyl-p-phenylenediamine	74-31-7	Wako Pure Chemicals	97.2
4-Hydroxyazobenzene	1689-82-3	Wako Pure Chemicals	96
4-(Phenylmethyl)phenol	101-53-1	Tokyo Kasei Kogyo, Co.	99.8
4,4′-(Hexafluoroisopropylidene)diphenol	1478-61-1	Aldrich Co.	98.8
2,2-Bis(4-hydroxyphenyl)-4-methyl-n-pentane	6807-17-6	Wako Pure Chemicals	100.0
4,4′-(Octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol	1943-97-1	Across Organics	99.5
4,4′-Dihydroxybenzophenone	611-99-4	Wako Pure Chemicals	98.5
2,2′,4,4′-Tetrahydroxybenzophenone	131-55-5	Wako Pure Chemicals	98.2
4-Hydroxybenzophenone	1137-42-4	Sigma Chemical Co.	98.0
2,4,4′-Trihydroxybenzophenone	1470-79-7	Aldrich Co.	95
Testosterone enanthate	315-37-7	Wako Pure Chemicals	99.6
Methyltestosterone	58-18-4	Wako Pure Chemicals	100.0

uteri were carefully dissected free of adhering fat and mesentery and weighed.

2.1.4. Statistical analysis

Differences in body weight and organ weight between the vehicle group and each of the chemical groups and between the vehicle-plus-EE group and each of the chemical plus EE groups were assessed for statistical significance by the two-tailed Student's t-test.

2.2. Hershberger assay

2.2.1. Chemicals

The chemicals tested in the Hershberger assay are listed in Table 2, and chemical structures of test compounds are shown in Fig. 1. All chemicals were dissolved in olive oil (Fujimi Pharmaceutical, Osaka, Japan) before use. Chemicals tested in this assay were selected based on the list of suspected endocrine disrupters published by the EU (Commission of the European Communities, 2001).

2.2.2. Animals

Seven-week-old castrated male Brl Han: WIST Jcl (GALAS) rats were purchased from Clea Japan (Shizuoka, Japan) and housed three per cage in stainless steel wire-mesh cages throughout the study. After allowing 14 days to recover from the operation, the rats were weighed, weight-ranked, and assigned randomly to each of the experimental and control groups. Other housing conditions were essentially the same as in the uterotrophic assay.

2.2.3. Study design

Each chemical was orally administered via a stomach tube for 10 consecutive days beginning on postnatal day 56. A vehicle control group given only olive oil was also established. Testosterone propionate (TP, CAS No. 57-63-6, 98% purity, Sigma), 0.2 mg/kg per day, was also administered to some rats by subcutaneous injection into the back after oral administration of each chemical, and a positive control injected with TP was also established. A group given the androgen antagonist chemical flutamide, 10 mg/kg per day, plus TP was established to confirm the reliability of this study. Each group consisted of six rats. The doses of each chemical were selected based on the results of a preliminary study. In the preliminary study, each chemical was orally administered to non-castrated rats for 7 days beginning on postnatal day 56. The volume of the olive oil solution

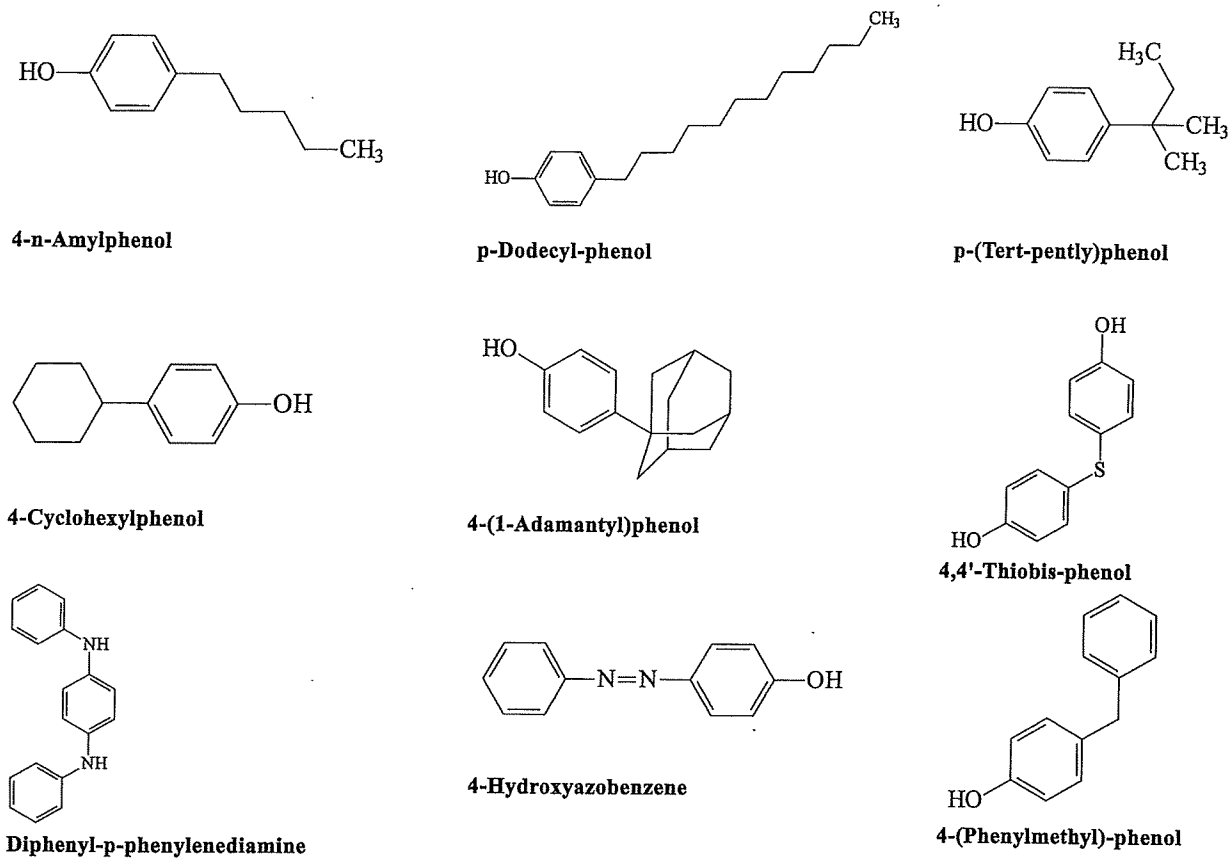


Fig. 1. Chemical structures of test compounds.

containing TP was 1 ml/kg, and the volume of the olive oil solution containing the chemical was 5 ml/kg. The animals were killed by bleeding from the abdominal vein under deep ether anesthesia approximately 24 h after the final dose. The ventral prostate with fluid, seminal vesicle with fluid, bulbocavernosus/levator ani muscle (BC/LA), glans penis, and Cowper's gland were carefully dissected free of adhering fat and weighed.

Because toxic signs, including death and/or decrease in body weight gain, were observed when animals were administered in this study with the high doses of 4-n-amylphenol, p-(tert-pentyl)phenol, 4-cyclohexylphenol, 4-(phenylmethyl)phenol, 4,4′-(hexafluoroisopropylidene)diphenol, 2,2-bis(4-hydroxyphenyl)-4-methyl-n-pentane, 2,2′,4,4′-tetrahydroxybenzophenone, estrone, equilin, and 4-tert-octylphenol, the maximum dose of these compounds were reduced.

2.2.4. Statistical analysis

The analytical method was essentially the same as in study 1.

3. Results

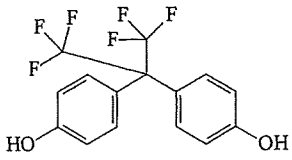
3.1. Uterotrophic assay

3.1.1. Clinical signs and body weight

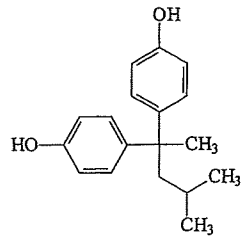
Body weights are shown in Table 4. No clinical abnormalities were observed in any of the groups, and body weight increased normally in all groups.

3.1.2. Uterine weight

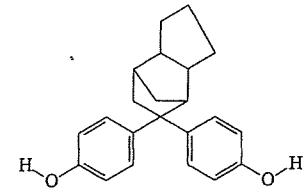
Uterine blotted weights are shown in Table 4. Watery uterine contents were grossly detected in all rats given EE, 800 mg/kg 4-n-amylphenol, 200 mg/kg p-dodecyl-phenol, 40 and 200 mg/kg 4-(1-



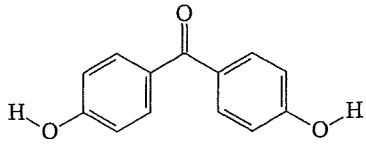
4,4'-(Hexafluoroispropylidene) diphenol



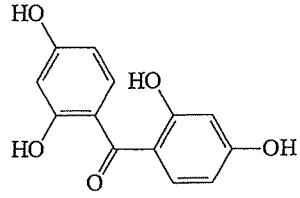
2,2-bis (4-Hydroxyphenyl)-4-methyl-n-pentane



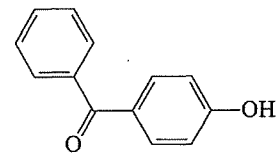
4,4'-(Octahydro-4,7-methano-5H-inden-5-ylidene) bisphenol



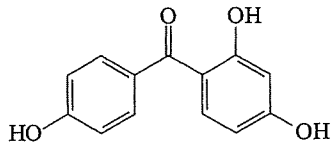
4,4'-Dihydroxybenzophenone



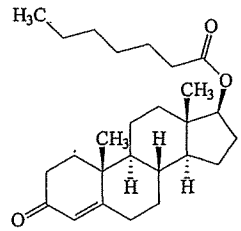
2,2',4,4'-Tetrahydroxybenzophenone



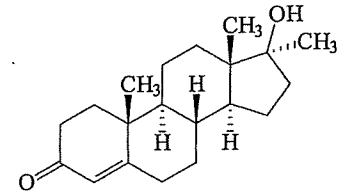
4-Hydroxybenzophenone



2,4,4'-Trihydroxybenzophenone



Testosterone enanthate

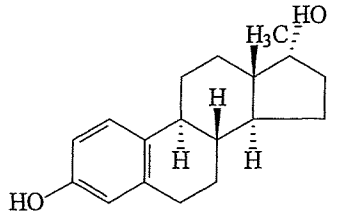


Methyltestosterone

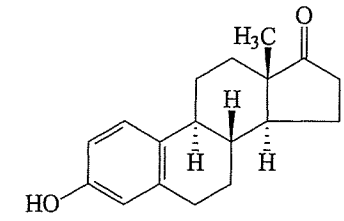
Fig. 1 (*Continued*)

adamantyl)phenol, 100 mg/kg 4,4′-(hexafluoroisopropylidene)diphenol, 10 and 40 mg/kg 2,2-bis (4-hydroxyphenyl)-4-methyl-n-pentane, 10 and 40 mg/kg 4,4′-(octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol, 200 and 800 mg/kg 2,2′,4,4′-tetrahydroxybenzophenone, 40 mg/kg testosterone enanthate, and 40 mg/kg methyltestosterone.

In this study, the uterine weight of rats given EE was higher than in rats given vehicle alone, and the uterine weight of rats given tamoxifen plus EE was lower than in rats given EE, confirming the reliability of this study. Uterine blotted weight increased significantly in rats given 2, 10, and 40 mg/kg 2,2-bis(4-hydroxyphenyl)-4-methyl-n-pentane, 4,4′-(octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol and testosterone enanthate, 8, 40, and 100 mg/kg 4,4′-(hexafluoroisopropylidene)diphenol, 8, 40, and 200 mg/kg 4-(1-adamantyl)phenol, 10 and 40 mg/kg 4,4′-thiobis-phenol and methyltestosterone, 40 and 200 mg/kg p-dodecylphenol, 4-hydroxyazobenzene and 2,4,4′-trihydroxybenzophenone, 100, 400, and 800 mg/kg diphenyl-p-phenylenediamine, 200 and 800 mg/kg 2,2′,4,4′-tetrahydroxybenzophenone and 4-hydroxybenzophenone, 200 mg/kg p-(tert-pentyl)phenol, 4-cyclohexylphenol, 4-(phenylmethyl)phenol and 4,4′-dihydroxybenzophenone, and 800 mg/kg 4-n-amylphenol. Uterine blotted weight decreased significantly in rats given 2 and 10 mg/kg 2,2-bis(4-hydroxyphenyl)-4-methyl-n-pentane plus EE and 4,4′-(octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol plus EE, 10 and 40 mg/kg 4,4′-thiobis-phenol plus EE, 40 and 200 mg/kg 2,2′,4,4′-tetrahydroxybenzophenone plus EE and 2,4,4′-trihydroxybenzophenone plus EE, 40 mg/kg 4,4′-(hexafluoroisopropylidene)diphenol plus EE, and 200 mg/kg p-(tert-pentyl)phenol plus EE, 4-(phenylmethyl)-phenol plus EE, 4,4′-dihydroxybenzophenone plus EE and 4-hydroxybenzophenone plus EE. With these chemicals, the wet absolute and relative weight changes were essentially the same as the blotted weight changes.



17 Alpha estradiol



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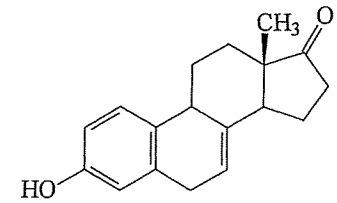
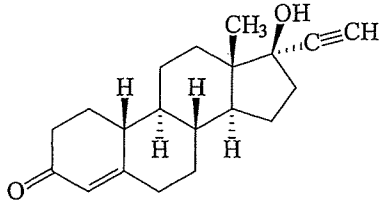
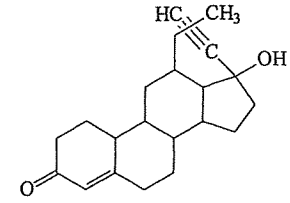


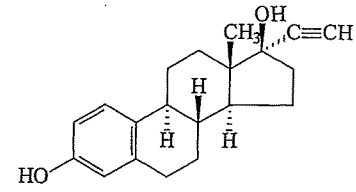
Equilin



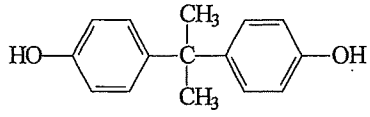
Norethindrone



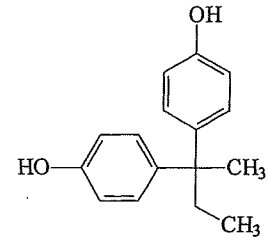
Norgestrel



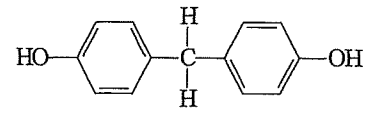
Ethynyl estradiol



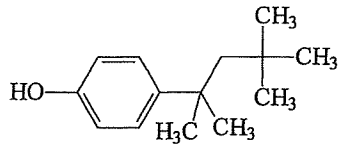
Bisphenol A



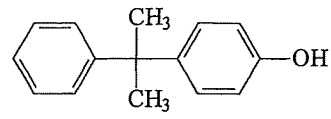
Bisphenol B



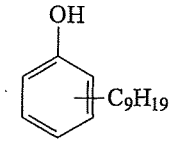
Bisphenol F



4-Tert-octylphenol



p-Cumyl phenol



Nonylphenol

Fig. 1 (*Continued*)

3.2. Hershberger assay

3.2.1. Clinical signs and body weights

Summary of general toxic signs in rats given chemicals and body weights are shown in Tables 3 and 5, respectively. Decreased spontaneous locomotion was seen in rats given 600 mg/kg 4-n-amylphenol, 200 and 600 mg/kg p-(tert-pentyl)phenol, 600 mg/kg 4-cyclohexylphenol, 200 and 600 mg/kg 4,4′-(hexafluoroisopropylidene)diphenol, 200 mg/kg 2,2-bis(4-hydroxyphenyl)-4-methyl-n-pentane, 600 mg/kg 2,2′,4,4′-tetrahydroxybenzophenone, 600 mg/kg 4-hydroxybenzophenone, 600 mg/kg bisphenol B, 1000 mg/kg bisphenol F, 200 and 600 mg/kg 4-tert-octylphenol, 200 and 600 mg/kg p-cumyl phenol, and 200 mg/kg nonylphenol. This sign was also detected in rats given the above chemicals plus TP. In addition, one rat given 600 mg/kg 4-n-amylphenol plus TP, two rats given 600 mg/kg 4,4′-(hexafluoroisopropylidene)diphenol plus TP, one rat given 600 mg/kg 2,2′,4,4′-tetrahydroxybenzophenone, and all rats given 600 mg/kg 4-tert-octylphenol and 600 mg/kg 4-tert-octylphenol plus TP died during the administration period. Significant decrease in body weight gain was observed in rats given 600

Table 2
Chemicals tested in the Hershberger assay

Chemicals	CAS no.	Source	Purity (%)
4-n-Amylphenol	14938-35-3	Tokyo Kasei Kogyo, Co.	99.2
p-Dodecyl-phenol	104-43-8	Kanto Chemical Co.	Unknown
p-(Tert-pentyl)phenol	80-46-6	Wako Pure Chemicals	100.0
4-Cyclohexylphenol	1131-60-8	Tokyo Kasei Kogyo Co.	99.7
4-(1-Adamantyl)phenol	29799-07-3	Aldrich Co.	97
4,4′-Thiobis-phenol	2664-63-3	Tokyo Kasei Kogyo Co.	99.8
diphenyl-p-phenylenediamine	74-31-7	Wako Pure Chemicals	97.2
4-Hydroxyazobenzene	1689-82-3	Wako Pure Chemicals	96
4-(Phenylmethyl)phenol	101-53-1	Tokyo Kasei Kogyo, Co.	99.8
4,4′-(Hexafluoroisopropylidene)diphenol	1478-61-1	Aldrich Co.	98.8
2,2-bis(4-Hydroxyphenyl)-4-methyl-n-pentane	6807-17-6	Wako Pure Chemicals	100.0
4,4′-(Octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol	1943-97-1	Across Organics	99.5
4,4′-Dihydroxybenzophenone	611-99-4	Wako Pure Chemicals	98.5
2,2′,4,4′-Tetrahydroxybenzophenone	131-55-5	Wako Pure Chemicals	98.2
4-Hydroxybenzophenone	1137-42-4	Sigma Chemical Co.	98.0
2,4,4′-Trihydroxybenzophenone	1470-79-7	Aldrich Co.	95
Testosterone enanthate	315-37-7	Wako Pure Chemicals	99.6
Methyltestosterone	58-18-4	Wako Pure Chemicals	100.0
17 Alpha estradiol	57-91-0	Wako Pure Chemicals	100
Estrone	53-16-7	Wako Pure Chemicals	100
Equilin	474-86-2	Sigma Chemical Co.	99.5
Norethindrone	68-22-4	Wako Pure Chemicals	100.7
Norgestrel	797-63-7	Sigma Chemical Co.	99
Ethynyl estradiol	57-63-6	Sigma Chemical Co.	98
Bisphenol A	80-05-7	Kanto Chemical Co.	99.9
Bisphenol B	77-40-7	Tokyo Kasei Kogyo, Co.	99.8
Bisphenol F	620-92-8	Kanto Chemical Co.	99.9
4-Tert-octylphenol	140-66-9	Wako Pure Chemicals	98.5
p-Cumyl phenol	599-64-4	Wako Pure Chemicals	99.9
Nonylphenol	25154-52-3	Kanto Chemical Co.	96.7

mg/kg 4-n-amylphenol and 4-n-amylphenol plus TP, 600 mg/kg 4-cyclohexylphenol, 200 mg/kg 4-(1-adamantyl)phenol and 4-(1-adamantyl)phenol plus TP, 200 mg/kg 4,4′-thiobis-phenol, 600 mg/kg 4-(phenylmethyl)-phenol, 200 and 600 mg/kg 4,4′-(hexafluoroisopropylidene)diphenol and 4,4′-(hexafluoroisopropylidene)diphenol plus TP, 50 and 200 mg/kg 2,2-bis(4-hydroxyphenyl)-4-methyl-n-pentane and 200 mg/kg 2,2-bis(4-hydroxyphenyl)-4-methyl-n-pentane plus TP, 10 and 50 mg/kg 4,4′-(octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol plus TP, 600 mg/kg 4,4′-dihydroxybenzophenone plus TP, 200 and 600 mg/kg 2,2′,4,4′-tetrahydroxybenzophenone and 600 mg/kg 2,2′,4,4′-tetrahydroxybenzophenone plus TP, 600 mg/kg 2,4,4′-trihydroxybenzophenone, 2 and 10 mg/kg 17alpha-estradiol plus TP, 2 and 10 mg/kg estrone and 10 mg/kg estroen plus TP, in all rats given equilin and equilin plus TP, in rats given 10 mg/kg norethindrone and norethindrone plus TP, 100 mg/kg norgestrel and norgestrel plus TP, 50 and 200 μg/kg EE and 200 μg/kg EE plus TP, 600 mg/kg bisphenol B and bisphenol B plus TP, 1000 mg/kg bisphenol F and 200 and 1000 mg/kg bisphenol F plus TP, 200 mg/day 4-tert-octylphenol, 600 mg/kg p-cumyl phenol and p-cumyl phenol plus TP, and 200 mg/kg nonylphenol.

3.2.2. Organ weights

Relative accessory sex organ weights are shown in Table 5. The accessory sex organ weights of rats given TP were higher than in rats given the vehicle alone, and the organ weights of rats given flutamide plus TP were lower than those of rats given

Table 3
General toxic signs in rats given each chemical without testosterone propionate in the Hershberger assay

Chemicals	Toxic signs
4-n-Amylphenol	Decreased body weight gain and decreased spontaneous locomotion in 600 mg/kg group
p-Dodecyl-phenol	No abnormalies detected
p-(Tert-pentyl)phenol	Decreased spontaneous locomotion in 200 and 600 mg/kg groups
4-Cyclohexylphenol	Decreased body weight gain and decreased spontaneous locomotion in 600 mg/kg group
4-(1-Adamantyl)phenol	Decreased body weight gain in 200 mg/kg group
4,4′-Thiobis-phenol	Decreased body weight gain in 200 mg/kg group
Diphenyl-p-phenylenediamine	No abnormalies detected
4-Hydroxyazobenzene	No abnormalies detected
4-(Phenylmethyl)phenol	Decreased body weight gain in 600 mg/kg group
4,4′-(Hexafluoroisopropylidene)diphenol	Decreased body weight gain and decreased spontaneous locomotion in 200 and 600 mg/kg groups
2,2-Bis(4-hydroxyphenyl)-4-methyl-n-pentane	Decreased body weight gain in 50 and 200 mg/kg groups and decreased spontaneous locomotion in 200 mg/kg group
4,4′-(Octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol	No abnormalies detected
4,4′-Dihydroxybenzophenone	No abnormalies detected
2,2′,4,4′-Tetrahydroxybenzophenone	Decreased body weight gain in 200 and 600 mg/kg groups, and decreased spontaneous locomotion and a dead animal in 600 mg/kg group
4-Hydroxybenzophenone	Decreased spontaneous locomotion in 600 mg/kg group
2,4,4′-Trihydroxybenzophenone	Decreased body weight gain in 600 mg/kg group
Testosterone enanthate	No abnormalies detected
Methyltestosterone	No abnormalies detected
17 Alpha estradiol	No abnormalies detected
Estrone	Decreased body weight gain in 2 and 10 mg/kg groups
Equilin	Decreased body weight gain in 0.5, 2 and 10 mg/kg groups
Norethindrone	Decreased body weight gain in 10 mg/kg group
Norgestrel	Decreased body weight gain in 100 mg/kg group
Ethynyl estradiol	Decreased body weight gain in 50 and 200 mg/kg groups
Bisphenol A	No abnormalies detected
Bisphenol B	Decreased body weight gain and decreased spontaneous locomotion in 600 mg/kg group
Bisphenol F	Decreased body weight gain and decreased spontaneous locomotion in 1000 mg/kg group
4-Tert-octylphenol	Decreased body weight gain in 200 mg/kg group, decreased spontaneous locomotion in 200 and 600 mg/kg groups and dead animals in 600 mg/kg group
p-Cumyl phenol	Decreased body weight gain in 600 mg/kg group and decreased spontaneous locomotion in 200 and 600 mg/kg groups
Nonylphenol	Decreased body weight gain and decreased spontaneous locomotion in 200 mg/kg group

TP confirming the reliability of this study. Since body weight gain decreased in the high or middle and high dose groups of many chemicals in this study, we mainly used the relative organ weight changes to assess the effect of each chemical.

Relative accessory sex organ weights increased significantly in rats given testosterone enanthate and methyltestosterone. Relative ventral prostate, seminal vesicle, and glans penis weights increased significantly in rats given 2 and 10 mg/kg equilin. Relative ventral and seminal vesicle weights increased in rats given 30 and 100 mg/kg norgestrel, and relative glans penis weights increased in rats given 100 mg/kg norgestrel. The absolute weight changes with these chemicals were essentially the same, and some organ weights also increased in rats given each chemical plus TP. Relative ventral prostate weights increased significantly in rats given 10 mg/kg estrone and 50 μg/kg EE. In addition, relative ventral prostate weights decreased significantly in rats given 50 and 600 mg/kg 4-hydroxybenzophenone, and relative seminal vesicle weights increased significantly in rats given 800 mg/kg diphenyl-p-phenylenediamine, 10 mg/kg estrone, 50 μg/kg EE and 50 mg/kg 4-tert-

octylphenol. Relative BC/LA weights decreased in rats given 100 mg/kg p-dodecyl-phenol, 30 mg/kg 4-hydroxyazobenzene, 200 mg/kg 4,4′-(hexafluoroisopropylidene)diphenol, 200 mg/kg 4,4′-dihydroxybenzophenone and 200 and 600 mg/kg bisphenol B. Relative glans penis weight increased significantly in rats given 50 mg/kg diphenyl-p-phenylenediamine, 50 and 200 mg/kg 4-(phenylmethyl)phenol, 600 mg/kg 4,4′-(hexafluoroisopropylidene)diphenol, 50 and 200 μg/kg EE and 600 mg/kg bisphenol A. On the other hand, the relative ventral prostate weight increased significantly in rats given 10 and 200 mg/kg 4,4′-thiobis-phenol plus TP, 50 and 800 mg/kg diphenyl-p-phenylenediamine plus TP, 10 and 200 mg/kg 2,2-bis(4-hydroxyphenyl)-4-methyl-n-pentane plus TP and 200 and 600 mg/kg bisphenol B plus TP, whereas its weight decreased in rats given 200 mg/kg 4,4′-dihydroxybenzophenone plus TP. Relative seminal vesicle weight increased significantly in rats given 50 mg/kg p-(tert-pentyl)phenol plus TP, 200 mg/kg 4-(1-adamantyl)phenol plus TP, 10 and 200 mg/kg 4,4′-thiobis-phenol plus TP, 50 mg/kg 4-(phenylmethyl)phenol plus TP, 50 and 600 mg/kg 4,4′-(hexafluoroisopropylidene)diphenol plus TP, in all rats given estrone plus TP, in rats given 50 and 200 μg/kg EE plus TP, and 600 mg/kg bisphenol B plus TP, but its weight decreased in rats given 200 mg/kg nonylphenol plus TP. Relative BC/LA weight increased significantly in rats given 600 mg/kg bisphenol B plus TP, but it decreased in rats given 600 mg/kg 4-n-amylphenol plus TP and 200 mg/kg 4,4′-dihydroxybenzophenone plus TP. Relative glans penis weight increased significantly in rats given 10 mg/kg 4-hydroxyazobenzene plus TP, 50 and 600 mg/kg 4,4′-(hexafluoroisopropylidene)diphenol plus TP, 10 and 50 mg/kg 4,4′-(octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol plus TP, 600 mg/kg 4,4′-dihydroxybenzophenone plus TP, 2 and 10 mg/kg estrone plus TP, 10 mg/kg norethindrone plus TP, 200 μg/kg EE plus TP, and 600 mg/kg bisphenol B plus TP. Relative Cowper's gland increased significantly in rats given 200 mg/kg 4-(1-adamantyl)phenol plus TP, 600 mg/kg 4,4′-(hexafluoroisopropylidene)diphenol plus TP, 600 mg/kg bisphenol B plus TP, 200 mg/kg bisphenol F plus TP, and 200 mg/kg 4-tert-octylphenol plus TP.

4. Discussion

The OECD proposed the rat uterotrophic assay and the Hershberger assay as screening methods to detect the estrogenic and androgenic properties of endocrine disrupting chemicals, and these assays have been reported to be useful in this regard (OECD, 2001). In the present study, we performed uterotrophic assays and Hershberger assays of 18 chemicals and Hershberger assays alone on 12 chemicals that showed an estrogen agonistic effect in our previous study (Yamasaki et al., 2002b).

All of the chemicals tested were positive in the uterotrophic assay, and thus they have estrogen agonistic properties. Androgen derivatives, such as testosterone enanthate and methyltestosterone, also tested positive in the uterotrophic assay. These androgen chemicals may be aromatized to estradiol. A decrease in uterine weight was observed in the high-dose group receiving p-(tert-pentyl)phenol plus EE, 4-(phenylmethyl)phenol plus EE, and 4,4′-dihydroxybenzophenone plus EE and also in the middle- and high-dose groups receiving 4,4′-thiobis-phenol plus EE and 2,4,4′-trihydroxybenzophenone plus EE. These findings clearly demonstrate that the above chemicals have partial agonistic properties and that they also reduce the agonistic effect of EE. In addition, a decrease in uterine weight was detected in the middle-dose groups receiving 4,4′-(hexafluoroisopropylidene)diphenol plus EE and 4-hydroxybenzophenone plus EE and also in the low- and middle-dose groups receiving 2,2-bis(4-hydroxyphenyl)-4-methyl-n-pentane plus EE, 4,4′-(octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol and 2,2′,4,4′-tetrahydroxybenzophenone plus EE. These chemicals are also thought to reduce the agonistic effect of EE as well as the agonistic effects of the high dose. Interestingly, ten of the 18 chemicals examined in this study exhibited both estrogen agonistic properties and reduced the agonistic effect of EE. All of the (di and tri) phenyl methanes and benzophenone derivatives displayed both agonistic properties and reduced the agonistic effect of EE. In the benzophenone derivatives, the position of hydroxyl groups may be related to the uterotrophic affinity. Moreover, the observation that the agonistic effect of 4-(1-

Table 4
Body weight and uterine blotted weight in the uterotrophic assays of 18 chemicals with and without ethynyl estradiol (EE)

Chemicals	PC10 values for ER alpha	Groups (mg/kg per day)	Body weight (g)	Uterus blotted weight	
				Absolute (mg)	Relative (mg/100 g)
Para-alkylphenols					
4-n-Amylphenol	177 639	Vehicle control	57.8±4.8	29.7±3.8	51.2±4.7
		100	56.5±2.7	25.8±2.3	45.8±3.8
		400	56.4±2.6	37.2±9.5	65.9±16.1
		800	51.2±3.9	64.5±9.9**	126.1±18.0**
		Vehicle+EE	57.1±2.0	102.2±7.5	179.2±13.8
		100+EE	57.4±3.0	83.2±25.1	143.7±37.1
		400+EE	56.8±2.7	93.1±19.4	163.4±30.0
		800+EE	54.7±3.5	100.3±17.7	182.6±24.1
		Tamoxifen+EE	55.7±2.5	83.2±6.1**	149.3±7.0**
p-Dodecyl-phenol	23 645	Vehicle control	59.0±2.6	27.4±3.0	46.3±3.2
		8	57.7±1.7	28.9±2.9	50.0±4.9
		40	58.4±3.6	37.6±7.2*	64.5±11.8*
		200	56.7±3.4	97.3±15.1**	171.5±23.8**
		Vehicle+EE	56.3±2.6	100.3±25.6	177.1±37.9
		8+EE	56.0±2.8	99.6±10.8	177.7±15.4
		40+EE	57.2±1.6	100.6±11.5	175.6±17.6
		200+EE	58.2±3.3	116.1±11.7	199.5±15.9
		Tamoxifen+EE	55.6±2.6	76.0±4.7	137.1±12.7*
p-(Tert-pentyl)phenol	401 969	Vehicle control	57.4±3.1	29.4±3.6	51.2±4.9
		8	57.3±2.7	27.8±2.1	48.6±3.5
		40	57.9±1.9	33.8±7.8	58.4±13.6
		200	53.6±2.1	72.2±7.1**	134.5±9.7**
		Vehicle+EE	58.3±1.9	110.8±6.2	190.4±13.8
		8+EE	57.2±2.9	103.2±17.4	180.8±31.1
		40+EE	58.0±2.1	108.2±12.6	186.5±20.4
		200+EE	56.6±3.9	91.2±9.0**	161.0±10.9**
		Tamoxifen+EE	56.4±0.9	85.1±2.6**	151.1±4.9**
Phenol derivatives					
4-Cyclohexylphenol	64 256	Vehicle	56.6±2.4	27.9±1.1	49.5±2.6
		8	55.2±2.2	27.9±2.9	50.6±5.2
		40	55.6±1.5	29.7±4.5	53.4±8.6
		200	55.2±2.5	60.2±7.9**	108.9±11.8**
		Vehicle+EE	56.1±1.3	96.8±5.9	172.7±11.5
		8+EE	54.4±3.3	100.4±9.6	184.8±17.4
		40+EE	54.9±1.9	103.3±18.3	188.3±34.1
		200+EE	55.2±3.0	92.1±12.6	166.8±19.9
		Tamoxifen+EE	54.6±3.1	81.2±4.2**	149.4±14.1*
4-(1-Adamantyl)phenol	1248	Vehicle	59.0±3.7	31.4±3.3	53.1±4.0
		8	57.4±3.1	52.0±14.0*	90.5±23.7*
		40	57.5±2.9	102.3±15.5**	178.1±27.8**
		200	54.9±4.2	138.4±7.9**	252.8±17.0**
		Vehicle+EE	59.3±3.2	112.9±18.6	191.0±33.0
		8+EE	58.6±3.6	119.6±18.0	204.3±27.1
		40+EE	58.6±3.5	112.7±20.7	192.0±28.0
		200+EE	58.0±5.1	132.7±7.7*	230.6±26.7*
		Tamoxifen+EE	57.8±3.2	85.5±10.2*	148.4±19.4*
4,4′-Thiobis-phenol	20 087	Vehicle	55.2±2.0	28.6±1.5	51.8±2.9
		2	55.8±3.7	33.7±4.9	60.2±6.7*
		10	55.5±2.2	36.5±3.2**	66.0±6.6**

Table 4 (*Continued*)

Chemicals	PC10 values for ER alpha	Groups (mg/kg per day)	Body weight (g)	Uterus blotted weight	
				Absolute (mg)	Relative (mg/100 g)
		40	55.0±2.9	42.1±2.6**	76.5±4.7**
		Vehicle+EE	56.4±3.0	111.0±7.4	197.0±12.1
		2+EE	55.2±1.9	112.1±11.3	203.5±23.7
		10+EE	56.8±3.6	100.0±8.8*	176.3±17.9*
		40+EE	56.1±2.8	77.1±15.8**	136.8±23.8**
		Tamoxifen+EE	54.9±2.1	79.8±6.5**	145.4±11.5**
Non-condensed polycyclic compounds					
Diphenyl-p-phenylenediamine	2 300 407	Vehicle	52.8±3.4	24.6±2.8	46.6±5.5
		100	55.2±4.6	29.8±4.9*	53.9±7.6
		400	52.8±1.3	33.4±4.7**	63.4±9.3**
		800	52.0±2.9	48.9±6.9**	94.2±12.9**
		Vehicle+EE	54.2±3.0	98.2±7.5	181.8±18.4
		100+EE	54.3±1.3	113.6±13.7*	209.5±27.4
		400+EE	53.9±2.4	104.2±14.6	194.1±31.7
		800+EE	50.8±7.2	106.1±17.5	209.8±25.6
		Tamoxifen+EE	53.2±2.4	82.2±6.7**	154.5±12.7*
4-Hydroxyazobenzene	164 424	Vehicle	57.8±3.7	27.5±2.6	47.9±6.9
		8	57.0±4.0	29.5±4.2	51.8±7.0
		40	56.8±2.7	43.2±6.9**	76.1±11.9**
		200	56.4±2.4	56.2±6.0**	99.6±9.6**
		Vehicle+EE	57.8±2.6	104.2±13.5	180.8±26.7
		8+EE	58.0±3.1	112.5±19.1	194.4±32.8
		40+EE	57.6±3.5	108.8±4.3	189.3±7.2
		200+EE	57.0±2.1	93.4±3.7	164.4±11.8
		Tamoxifen+EE	56.1±4.3	81.1±7.1**	145.5±19.9*
(Di or tri) phenyl methanes					
4-(Phenylmethyl) phenol	1 198 024	Vehicle	61.1±4.8	33.2±8.1	54.1±10.7
		8	62.5±4.2	32.9±5.3	52.4±6.3
		40	60.9±3.2	36.0±4.9	59.1±7.9
		200	60.4±4.5	58.3±5.6**	96.8±9.2**
		Vehicle+EE	62.9±2.9	119.4±11.8	190.0±17.6
		8+EE	62.1±3.3	130.7±8.7	210.6±12.6*
		40+EE	62.8±3.5	120.6±9.1	192.3±13.6
		200+EE	62.3±3.3	101.2±14.0*	163.6±28.4*
		Tamoxifen+EE	60.3±3.3	81.6±8.0**	135.5±11.9**
(Di or tri) phenyl methanes					
4,4′-(Hexafluoro isopropylidene) diphenol	6906	Vehicle	56.1±4.3	28.6±4.9	50.9±7.4
		8	55.0±4.5	47.2±9.9**	85.1±11.9**
		40	56.6±4.0	65.9±9.8**	116.0±11.7**
		100	54.7±4.2	96.4±9.0**	177.2±22.2
		Vehicle+EE	56.5±3.8	110.3±15.7	195.4±25.0
		8+EE	56.7±3.8	95.1±6.1	168.0±9.4*
		40+EE	55.5±2.1	74.5±9.1**	134.2±15.4**
		100+EE	55.9±3.2	99.3±11.9	177.8±18.9
		Tamoxifen+EE	54.7±3.4	79.5±5.2**	145.9±14.4**
2,2-Bis (4-hydroxyphenyl)-4-methyl-n-pentane	1892	Vehicle	58.3±2.8	28.8±2.6	49.4±3.0
		2	58.0±2.7	58.4±8.8**	100.5±14.2**
		10	59.2±3.3	84.0±11.9**	141.6±15.3**

Table 4 (*Continued*)

Chemicals	PC10 values for ER alpha	Groups (mg/kg per day)	Body weight (g)	Uterus blotted weight	
				Absolute (mg)	Relative (mg/100 g)
		40	57.4±3.2	115.1±16.6**	200.3±25.3**
		Vehicle+EE	59.7±2.7	107.6±7.1	180.4±11.8
		2+EE	58.0±3.8	96.2±10.3*	165.7±13.3
		10+EE	59.0±3.0	92.5±7.9**	157.3±17.1*
		40+EE	57.9±3.1	112.3±15.9	193.8±23.9
		Tamoxifen+EE	58.1±2.4	87.5±5.5**	151.0±14.1**
4,4′-(Octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol	37 162	Vehicle	54.9±3.2	28.3±4.4	51.6±7.1
		2	56.2±3.1	63.4±5.2**	112.9±7.8**
		10	53.2±3.8	86.2±6.5**	162.5±13.7**
		40	54.6±1.7	92.5±7.1**	169.3±10.3**
		Vehicle+EE	55.7±3.0	97.4±8.3	174.9±12.6
		2+EE	54.8±2.6	78.7±8.4**	143.9±16.7**
		10+EE	55.9±2.3	82.3±9.4*	147.4±15.3**
		40+EE	53.0±4.2	93.5±8.3	176.8±16.6
		Tamoxifen+EE	52.4±2.6	79.7±3.6**	152.2±4.7**
Benzophenone derivatives					
4,4′-Dihydroxybenzophenone	124 213	Vehicle	56.3±4.2	31.0±6.6	55.0±9.7
		8	54.3±6.1	28.9±3.9	53.8±8.9
		40	53.7±4.2	28.3±4.5	54.5±6.8
		200	56.1±3.9	45.0±5.0**	80.4±7.5**
		Vehicle+EE	54.9±2.9	95.0±5.4	173.1±9.2
		8+EE	55.8±5.2	110.1±17.4	197.8±30.1
		40+EE	55.7±5.1	106.9±17.8	192.3±29.0
		200+EE	54.4±4.4	69.8±14.8**	128.3±23.5**
		Tamoxifen+EE	54.2±4.5	84.7±7.8*	157.0±17.0
2,2′,4,4′-Tetrahydroxybenzophenone	106 427	Vehicle	58.1±3.3	34.2±4.2	58.9±6.8
		40	57.9±3.3	37.6±2.6	65.1±5.7
		200	57.4±1.5	77.2±10.4**	134.2±15.3**
		800	54.8±3.9	119.7±12.1**	218.9±20.2**
		Vehicle+EE	57.6±3.5	117.8±11.3	205.3±25.0
		40+EE	57.4±2.9	00.5±15.3*	175.1±27.6
		200+EE	56.8±3.4	67.0±10.3**	134.2±15.3**
		800+EE	53.0±2.4	124.3±9.2	218.9±20.2**
		Tamoxifen+EE	56.5±4.4	87.8±4.6**	156.1±12.6**
4-Hydroxybenzophenone	1 096 217	Vehicle	59.6±3.5	34.9±6.1	58.7±10.1
		40	57.6±2.8	39.6±3.7	68.8±5.3
		200	55.9±9.1	48.3±6.3**	88.7±19.2**
		800	55.9±2.5	86.1±12.7**	154.1±22.3**
		Vehicle+EE	57.5±2.5	108.3±11.5	188.4±19.6
		40+EE	59.5±2.4	115.1±12.2	194.2±26.9
		200+EE	57.2±2.9	84.7±15.1*	148.7±29.2*
		800+EE	57.4±2.2	95.5±12.1	166.4±20.0
		Tamoxifen+EE	57.7±3.3	85.2±8.9**	142.9±12.2**
Benzophenone derivatives					
2,4,4′-Trihydroxybenzophenone	43 765	Vehicle	59.2±4.5	34.9±2.1	59.1±4.5
		8	59.9±3.7	37.8±3.5	63.1±3.4
		40	58.2±4.3	45.1±4.2**	77.8±9.0**
		200	58.2±5.9	72.8±10.2**	125.1±12.0**

Table 4 (*Continued*)

Chemicals	PC10 values for ER alpha	Groups (mg/kg per day)	Body weight (g)	Uterus blotted weight	
				Absolute (mg)	Relative (mg/100 g)
		Vehicle+EE	58.2±3.5	116.5±12.6	199.7±12.1
		8+EE	58.2±3.2	109.5±15.0	187.5±17.8
		40+EE	58.1±2.8	94.5±10.4**	163.1±20.0**
		200+EE	55.2±3.3	67.7±11.6**	123.4±25.0**
		Tamoxifen+EE	58.8±3.3	84.2±3.8**	143.4±8.7**
Androgen derivatives					
Testosterone enanthate	17140	Vehicle	59.7±3.0	36.6±3.0	61.3±4.2
		2	61.1±2.9	30.8±3.2**	50.5±6.3**
		10	61.9±2.3	60.5±9.6**	98.1±17.3**
		40	60.5±4.0	89.0±6.7**	147.3±10.0**
		Vehicle+EE	60.0±5.2	121.4±18.2	201.6±16.7
		2+EE	59.7±3.3	108.8±6.3	182.5±12.4*
		10+EE	60.9±3.0	121.7±12.6	199.9±19.0
		40+EE	61.0±2.4	129.6±16.8	211.9±20.4
		Tamoxifen+EE	58.1±2.8	86.7±6.3**	149.3±11.8**
Methyltestosterone	173235	Vehicle	62.7±2.5	34.9±7.0	55.7±10.9
		2	63.1±4.3	35.5±3.1	56.4±4.4
		10	62.7±3.9	60.9±9.7**	97.6±18.0**
		40	63.3±3.2	96.1±17.4**	151.8±26.4**
		Vehicle+EE	61.9±2.2	115.8±16.1	187.2±25.8
		2+EE	63.6±3.4	103.3±7.7	162.4±7.0
		10+EE	64.4±2.9	118.3±7.1	184.0±11.8
		40+EE	62.2±1.1	129.6±7.8	208.5±13.3
		Tamoxifen+EE	61.6±3.3	86.8±9.6**	140.8±11.5**

* Significantly different from vehicle control or vehicle control plus EE at $P < 0.05$.
** Significantly different from vehicle control or vehicle control plus EE at $P < 0.01$.

adamantyl)phenol was enhanced by EE was quite interesting.

In the previous study, we performed a reporter gene assay for ER alpha-mediated transcriptional activation and an immature rat uterotrophic assay of 23 chemicals (Yamasaki et al., 2002b). In the reporter gene assay, the transcriptional activity of each chemical was tested over concentrations ranging from 10 pM to 10 μM. The EC50, PC50, and PC10 values were then calculated, and the results showed that the PC10 values were superior to the EC50 and PC50 values for predicting the estrogenic activity of chemicals. The PC50 and PC10 values defined as the test chemical concentrations estimated to show 50 and 10%, respectively, of the transcriptional activity of positive control wells treated with natural ligands (1 nM of 17β-estradiol) were calculated in our own made software. We selected all of the chemicals for the uterotrophic assay in this study based on their PC10 values in the reporter gene assay and found that all had estrogen agonistic effects. This demonstrates that the PC10 value is superior as a parameter for predicting estrogen agonistic activity of chemicals with a wide range of estrogenic potency and that the reporter gene assay is a potentially useful method for prioritizing chemicals to be tested in subsequent screening tests.

In the Hershberger assay, a clear androgen agonistic effect was detected in two androgen derivatives: testosterone enanthate and methyltestosterone. The weights of some accessory sex organs also increased in rats given estrogen equilin, norgestrel or estrone. However, whether these chemicals have an androgenic effect remains uncertain because these accessory sex organs may

Table 5
Body weight and relative accessory sex organ weight in rats given 30 chemicals with and without testosterone propionate (TP) for 10 days

Chemicals	Doses (mg/kg per day)	Body weight (g)	Ventral prostate (mg/100 g bw)	Seminal vesicle (mg/100 g bw)	BC/LA (mg/100 g bw)	Glans penis (mg/100 g bw)	Cowper's gland (mg/100 g bw)
Para-alkylphenols							
4-n-Amylphenol	Vehicle control	269.9±15.2	6.3±2.6	13.9±2.8	46.0±4.5	12.3±1.3	1.6±0.6
	50	266.0±13.0	5.5±0.5	13.3±2.1	50.3±8.4	12.6±1.9	1.2±0.4
	200	268.7±19.4	6.9±2.6	14.8±1.9	48.8±7.1	13.1±1.4	1.8±0.4
	600(400)[a]	248.8±15.5*	5.5±2.8	15.3±6.0	47.4±11.0	12.8±3.1	1.7±0.7
	Vehicle+TP	271.8±12.3	33.9±2.6	79.8±14.7	119.3±10.6	25.5±3.2	7.2±1.4
	50+TP	273.1±17.4	35.1±4.3	80.0±26.7	113.5±15.0	25.1±2.1	6.8±1.1
	200+TP	280.2±11.9	33.4±4.3	69.4±12.2	109.0±12.5	22.8±2.4	5.9±2.3
	600(400)+TP	254.2±11.9*	34.9±7.9	74.4±33.3	96.2±13.8*	25.6±0.6	7.0±1.2
	Flutamide+TP	276.0±12.9	5.8±1.2**	13.1±2.6**	52.8±8.2**	13.5±2.3**	1.6±0.6**
p-Dodecyl-phenol	Vehicle control	273.9±19.0	6.8±1.9	13.7±3.7	53.6±6.4	12.2±2.4	1.5±0.4
	10	264.6±21.4	6.7±2.9	16.2±6.1	48.0±8.0	12.5±3.7	1.5±0.4
	30	260.4±18.2	7.3±4.5	14.9±2.5	52.7±6.9	12.6±2.5	1.5±0.4
	100	249.7±21.3	6.6±2.0	16.5±1.9	46.1±3.8*	13.7±2.2	2.0±0.8
	Vehicle+TP	275.3±14.5	35.7±2.8	83.8±16.1	112.3±13.3	24.1±2.3	7.1±0.9
	10+TP	273.0±11.3	34.5±7.6	84.1±29.9	111.2±5.8	23.6±1.8	6.9±1.2
	30+TP	271.3±18.8	37.2±3.5	98.6±22.9	121.3±5.8	24.8±1.6	8.0±0.8
	100+TP	262.2±17.0	36.8±5.6	104.1±19.7	119.0±13.0	25.1±1.1	6.9±2.2
	Flutamide+TP	271.8±19.6	6.4±0.8**	14.0±3.6**	51.9±8.4**	13.2±1.8**	1.9±0.5**
p-(Tert-pentyl) phenol	Vehicle control	274.9±13.8	6.2±0.6	13.0±2.2	50.2±3.5	13.5±2.6	1.2±0.4
	50	267.6±6.9	6.6±1.2	15.0±2.5	53.2±7.0	14.0±2.0	1.7±0.7
	200	271.6±10.2	6.9±1.0	12.9±1.9	54.1±9.4	12.9±2.2	1.2±0.2
	600(400)	270.2±11.4	6.4±1.2	10.8±1.7	46.5±4.3	10.8±1.7	1.3±0.4
	Vehicle+TP	280.6±8.7	34.6±6.1	69.8±12.6	103.5±8.9	23.0±1.4	6.8±1.0
	50+TP	279.7±10.7	31.6±7.6	85.9±9.7*	107.0±21.6	23.7±2.1	6.0±1.4
	200+TP	279.9±13.4	39.2±3.2	79.8±13.9	108.6±15.5	22.7±2.8	7.7±1.6
	600(400)+TP	265.5±15.8	35.0±7.0	65.5±9.6	96.4±9.8	22.5±2.0	6.3±2.3
	Flutamide+TP	261.3±24.8	7.2±2.1**	13.7±3.1**	56.1±9.6**	12.2±2.3**	1.5±0.3**
Phenol derivatives							
4-Cyclohexylphenol	Vehicle control	280.7±12.3	5.7±0.6	13.1±1.4	50.4±8.3	12.5±2.5	1.5±0.3
	50	282.0±14.3	6.1±1.6	14.5±4.1	44.3±3.5	13.8±2.9	1.7±0.5
	200	281.7±10.4	6.4±0.7	14.3±3.8	50.9±9.7	12.2±2.9	1.7±0.4
	600(400)[a]	266.6±6.6*	6.3±0.7	12.8±1.2	42.1±8.7	12.2±1.8	1.8±0.6
	Vehicle+TP	283.2±10.3	38.9±6.6	99.8±34.1	110.9±12.3	23.1±3.7	7.9±2.2
	50+TP	287.3±16.4	39.5±11.0	79.8±26.2	110.4±12.1	23.1±2.5	7.1±0.8
	200+TP	287.4±12.0	39.7±7.6	76.5±25.5	113.7±12.4	22.6±1.3	7.4±1.0
	600(400)+TP	270.6±14.4	35.4±6.2	84.6±18.8	104.3±15.4	24.1±1.9	8.0±1.6
	Flutamide+TP	287.5±7.1	6.0±0.7**	11.5±2.3**	51.7±7.7**	11.1±2.3**	1.6±0.2**
4-(1-Adamantyl) phenol	Vehicle control	276.9±16.6	5.0±1.1	10.8±3.8	53.7±11.3	9.8±3.8	1.5±0.5

Table 5 (*Continued*)

Chemicals	Doses (mg/kg per day)	Body weight (g)	Ventral prostate (mg/100 g bw)	Seminal vesicle (mg/100 g bw)	BC/LA (mg/100 g bw)	Glans penis (mg/100 g bw)	Cowper's gland (mg/100 g bw)
	10	266.2±13.9	5.3±0.8	12.6±2.0	45.3±6.3	11.5±2.3	1.9±0.3
	50	266.0±12.8	5.7±1.2	12.5±3.4	48.0±5.9	11.5±2.7	1.8±0.7
	200	250.5±12.3*	6.1±1.3	13.7±2.4	53.6±6.1	12.9±2.8	2.0±0.9
	Vehicle+TP	282.1±15.8	38.2±5.5	78.1±14.7	123.3±12.5	23.9±1.3	7.4±0.8
	10+TP	278.7±8.7	37.6±5.7	75.2±9.8	110.3±10.7	22.3±1.5	7.4±1.7
	50+TP	279.4±14.2	35.1±4.9	73.8±17.4	118.6±6.6	22.9±1.3	6.8±1.6
	200+TP	259.3±13.5*	41.1±3.5	101.9±11.7*	126.5±17.6	26.8±3.0	9.2±1.1**
	Flutamide+TP	279.8±17.5	6.1±1.2**	11.4±2.4**	57.8±5.5**	11.3±3.3**	1.7±0.4**
4,4′-Thiobis-phenol	Vehicle control	269.6±9.7	5.6±0.7	12.3±2.1	45.5±11.2	11.3±1.5	1.5±0.4
	10	270.0±9.1	5.6±0.9	13.3±1.5	53.3±6.8	12.6±2.1	1.6±0.3
	50	271.8±17.6	5.5±0.8	12.0±1.9	51.1±7.1	10.7±2.3	1.7±0.4
	200	252.9±11.4*	5.6±0.5	13.0±1.6	47.4±6.8	13.1±1.3	1.4±0.4
	Vehicle+TP	277.7±10.8	31.9±4.3	79.3±8.8	117.6±6.0	23.2±2.7	7.4±0.9
	10+TP	277.8±17.6	43.7±6.8**	103.8±13.9**	119.3±10.8	23.7±1.4	8.0±1.1
	50+TP	274.8±13.9	37.9±6.5	95.3±35.2	125.3±21.3	25.0±1.8	7.3±2.5
	200+TP	267.2±15.2	38.5±5.3*	99.8±4.0**	119.4±13.5	24.7±1.4	7.5±0.9
	Flutamide+TP	271.1±15.5	7.0±1.0**	14.6±3.1**	53.0±4.8**	13.1±2.0**	1.4±0.3**
Non-condensed polycyclic compounds							
Diphenyl-p-phenylenediamine	Vehicle control	273.6±10.6	5.2±1.0	9.4±2.0	48.2±8.5	11.0±1.7	1.9±1.2
	50	259.1±16.4	5.6±1.1	11.9±2.7	50.8±5.8	13.8±1.1**	1.6±0.4
	200	258.1±13.5	5.9±0.7	10.6±2.6	51.8±7.0	10.4±1.3	1.4±0.4
	800	255.7±18.7	5.5±1.4	13.6±1.0**	51.8±5.4	12.7±2.8	1.5±0.5
	Vehicle+TP	269.0±15.8	27.8±3.6	63.1±14.0	111.0±11.5	21.3±4.5	6.4±1.8
	50+TP	275.1±17.6	33.4±4.8*	71.0±13.5	116.4±11.6	22.3±2.6	6.7±1.1
	200+TP	274.7±11.5	31.0±4.0	68.7±15.1	111.3±11.2	21.2±1.8	7.1±1.7
	800+TP	260.6±13.5	33.0±3.8*	68.9±7.9	110.4±14.9	23.5±2.6	6.8±1.3
	Flutamide+TP	268.3±16.5	6.5±0.8**	13.4±2.0**	48.7±6.3**	12.2±2.8**	1.5±0.3**
4-Hydroxy azobenzene	Vehicle control	280.2±18.7	5.9±0.8	13.8±0.8	53.0±6.2	11.9±1.7	2.0±0.5
	10	277.2±14.2	5.5±1.1	12.8±1.9	49.2±8.0	11.8±2.0	1.9±0.8
	30	275.5±11.1	5.7±2.1	12.5±2.5	43.6±5.4*	10.6±2.8	1.3±0.7
	100	271.5±12.4	6.3±1.1	12.5±1.9	49.4±7.7	12.4±2.1	2.0±0.9
	Vehicle+TP	282.4±11.6	33.0±5.9	70.6±14.4	105.1±10.6	21.2±1.7	6.8±1.2
	10+TP	283.0±17.7	34.4±2.8	74.1±12.4	104.3±12.4	24.1±1.6*	7.3±0.6
	30+TP	287.7±11.7	32.4±1.3	65.8±5.9	114.5±10.0	21.1±1.6	6.8±1.5
	100+TP	276.3±18.7	30.9±6.2	70.8±9.3	100.9±9.8	22.5±1.9	7.9±1.7
	Flutamide+TP	278.6±20.6	6.6±1.2**	13.4±3.6**	48.0±5.3**	11.2±2.6**	1.4±0.4**
(Di or Tri) phenyl methanes							
4-(Phenylmethyl) phenol	Vehicle control	279.4±14.3	5.7±0.6	12.8±2.1	51.1±4.1	10.0±2.0	1.7±0.4
	50	274.1±15.0	5.8±1.8	17.0±4.7	49.9±4.2	12.9±1.7*	1.9±0.4
	200	269.8±10.0	5.9±0.7	14.6±4.9	47.9±11.0	12.2±0.9*	1.5±0.4

Table 5 (*Continued*)

Chemicals	Doses (mg/kg per day)	Body weight (g)	Ventral prostate (mg/100 g bw)	Seminal vesicle (mg/100 g bw)	BC/LA (mg/100 g bw)	Glans penis (mg/100 g bw)	Cowper's gland (mg/100 g bw)
	600(400)[a]	261.1±13.4*	5.4±0.7	13.9±4.6	48.1±5.6	11.7±2.5	1.7±0.7
	Vehicle+TP	280.1±9.5	37.4±3.6	75.1±12.1	108.9±5.1	23.4±1.7	7.8±1.6
	50+TP	283.1±13.7	37.7±3.9	98.6±13.8*	114.9±13.0	23.5±2.4	7.7±1.6
	200+TP	282.8±8.8	35.4±3.5	78.3±15.6	111.6±8.7	22.9±1.5	7.4±0.9
	600(400)+TP	269.3±14.4	35.2±7.2	85.9±12.0	111.5±7.7	25.1±2.6	8.3±2.0
	Flutamide+TP	278.8±14.0	6.1±0.7**	12.5±1.2**	49.5±11.5**	11.4±2.7**	2.0±0.2**
(Di or tri) phenyl methanes							
4,4′-(Hexafluoroisoproylidene)diphenol	Vehicle control	275.1±9.7	5.8±0.9	11:3±2.1	53.2±6.3	12.6±1.0	1.5±0.5
	50	257.0±18.2	5.5±1.5	12.0±1.8	55.9±8.4	12.8±1.1	1.7±0.5
	200	259.9±12.8*	5.7±1.4	13.2±2.3	44.2±4.7*	12.2±2.0	1.3±0.4
	600(400)[a]	219.6±30.9*	6.2±1.6	13.6±1.2	48.9±3.5	15.1±1.8*	1.7±0.3
	Vehicle+TP	283.3±9.2	34.5±7.3	73.0±11.6	119.6±7.6	22.8±1.5	6.8±1.1
	50+TP	277.0±14.4	42.4±6.1	99.4±19.7*	120.2±13.4	25.5±1.7*	6.0±3.0
	200+TP	260.6±14.9*	39.3±10.2	109.0±37.8	118.7±20.1	25.3±2.6	8.4±1.6
	600(400)+TP	209.4±33.1*	37.1±2.5	126.7±27.7*	115.3±17.6	30.6±5.0*	8.5±0.8*
	Flutamide+TP	275.3±12.8	6.1±0.5**	13.2±1.0**	51.9±4.2**	13.0±1.4**	1.3±0.3**
2,2-Bis(4-hydroxyphenyl)-4-methyl-n-pentane	Vehicle control	275.7±12.7	5.8±1.2	12.9±2.7	54.0±5.7	13.1±1.3	1.7±0.2
	10	278.4±12.0	5.7±0.9	13.7±3.3	48.4±7.7	10.8±2.2	1.4±0.6
	50	254.7±10.6*	6.4±0.5	14.2±1.8	53.0±4.8	13.2±0.9	1.8±0.2
	200(100)	243.5±9.2**	6.1±0.3	13.8±1.7	54.5±11.0	13.8±1.7	2.0±1.0
	Vehicle+TP	275.6±11.1	32.2±6.2	78.9±25.3	108.7±14.9	22.2±3.7	7.0±1.2
	10+TP	280.9±19.8	39.5±4.8*	103.9±23.0	121.7±5.2	24.8±1.5	7.7±2.2
	50+TP	269.9±12.4	35.0±4.3	92.0±21.9	123.5±10.4	24.7±2.8	8.1±1.6
	200(100)+TP	253.6±16.3*	40.7±6.5*	101.5±20.3	122.8±11.5	26.2±2.6	8.8±2.3
	Flutamide+TP	276.7±11.1	7.1±1.0**	15.3±2.8**	53.5±7.2**	12.2±2.0**	1.5±0.6**
4,4′-(Octahydro-4,7-methano-5H-inden-5-ylidene)bisphenol	Vehicle control	276.2±13.9	5.5±0.9	12.5±3.3	52.7±9.9	13.6±1.0	1.6±0.2
	2	276.6±10.5	5.3±0.5	10.8±2.6	53.7±8.9	12.4±2.2	1.5±0.5
	10	267.5±11.4	5.5±0.6	12.8±1.0	56.8±8.0	12.2±1.5	1.6±0.3
	50	254.8±21.7	6.0±0.4	12.2±1.6	46.3±6.0	12.3±2.4	1.8±0.4
	Vehicle+TP	282.3±8.7	34.7±6.7	83.3±14.0	117.9±13.3	22.6±0.5	6.8±1.1
	2+TP	277.7±21.5	38.0±9.2	95.6±21.2	113.3±11.9	24.6±2.3	7.1±0.8
	10+TP	271.7±5.5*	36.3±3.8	92.5±20.4	119.9±10.8	25.2±1.3**	7.3±1.1
	50+TP	260.9±12.3**	36.7±5.5	94.3±12.5	115.9±17.7	25.8±2.1*	7.4±0.6
	Flutamide+TP	284.1±14.2	6.4±1.0**	11.5±1.7**	54.0±4.2**	12.3±2.0**	1.6±0.3**
Benzophenone derivatives							
4,4′-Dihydroxy benzophenone	Vehicle control	277.9±14.5	5.0±0.9	15.0±3.3	57.0±6.7	13.2±1.3	2.2±0.7
	50	280.0±12.8	6.4±0.7*	13.2±1.7	51.0±10.3	11.5±2.0	1.6±0.6
	200	264.4±15.5	6.3±1.0	14.2±2.9	43.7±5.7**	13.3±2.2	1.9±0.5

Table 5 (*Continued*)

Chemicals	Doses (mg/kg per day)	Body weight (g)	Ventral prostate (mg/100 g bw)	Seminal vesicle (mg/100 g bw)	BC/LA (mg/100 g bw)	Glans penis (mg/100 g bw)	Cowper's gland (mg/100 g bw)
	600	265.3±12.7	5.5±0.8	13.6±3.1	49.5±9.9	12.1±2.6	1.6±0.7
	Vehicle+TP	287.8±13.5	35.8±2.1	74.0±9.6	113.5±7.2	22.6±1.0	6.1±2.0
	50+TP	284.6±19.3	38.1±6.3	68.4±6.8	117.1±6.1	22.5±1.7	7.4±0.8
	200+TP	276.0±10.1	27.8±3.1**	72.7±16.9	98.3±10.3*	21.8±2.1	6.1±1.3
	600+TP	268.6±14.2*	32.2±4.8	77.7±18.3	116.3±12.6	24.7±0.6**	6.9±1.7
	Flutamide+TP	281.2±16.3	6.7±1.4**	13.4±1.9**	55.4±4.7**	12.0±1.6**	1.8±0.4**
2,2′,4,4′-Tetrahydroxy benzophenone	Vehicle control	283.5±15.0	5.2±0.9	15.0±1.5	55.2±10.6	10.9±2.5	1.7±0.2
	50	281.6±11.9	6.1±0.6	15.0±1.8	56.9±4.7	12.5±1.6	1.4±0.4
	200	255.7±19.2*	5.9±1.1	16.8±2.3	54.3±7.6	13.6±2.3	2.1±0.5
	600(400)[a]	258.6±6.4**	5.2±0.7	14.4±3.9	50.9±3.1	11.9±3.8	1.6±0.2
	Vehicle+TP	290.8±10.4	36.5±5.3	76.6±12.9	119.9±14.9	22.9±2.1	7.5±2.3
	50+TP	289.9±13.2	40.8±4.5	81.0±7.2	127.1±13.7	23.5±2.1	8.4±1.2
	200+TP	286.1±13.5	40.2±4.4	77.7±14.0	108.5±12.5	22.8±1.9	7.7±1.1
	600(400)+TP	272.7±11.9*	36.3±5.8	81.4±9.9	112.5±9.5	24.4±1.5	7.4±0.6
	Flutamide+TP	290.6±11.8	6.1±0.8**	15.1±1.8**	54.6±6.5**	11.8±1.8**	1.7±0.3**
4-Hydroxy benzophenone	Vehicle control	280.7±11.7	6.1±0.4	10.3±2.7	53.9±11.9	9.5±2.8	1.2±0.3
	50	280.5±15.0	5.3±0.8*	11.0±3.1	50.9±10.7	10.5±2.0	1.1±0.7
	200	278.6±13.9	6.1±0.9	13.1±3.4	51.6±5.7	11.6±1.4	1.4±0.4
	600	275.1±9.4	5.2±0.5**	12.3±2.8	45.4±6.8	10.3±2.3	1.6±0.8
	Vehicle+TP	281.7±15.2	31.8±5.9	70.7±13.0	116.9±14.3	22.4±1.4	7.0±1.3
	50+TP	288.7±9.8	33.9±4.6	75.9±9.0	112.6±11.8	22.1±1.9	6.1±1.2
	200+TP	284.5±16.1	34.1±4.6	77.0±17.0	117.1±11.9	23.1±1.6	6.1±1.5
	600+TP	282.3±13.7	36.1±7.3	77.5±22.4	117.2±7.5	23.3±0.6	8.0±2.2
	Flutamide+TP	282.1±12.2	6.7±0.7**	13.4±2.9**	52.1±10.6**	13.0±2.0**	1.1±0.4**
Benzophenone derivatives							
2,4,4′-Trihydroxy benzophenone	Vehicle control	279.2±8.1	5.9±0.8	12.2±1.9	52.3±6.7	12.6±1.1	1.4±0.3
	50	282.0±14.6	6.0±0.8	11.5±1.6	49.7±6.3	11.4±1.6	1.4±0.4
	200	264.4±17.5	5.8±0.8	12.0±2.7	51.0±6.4	12.9±1.3	1.5±0.4
	600	256.1±15.7**	6.0±0.9	13.7±2.4	48.5±7.5	13.2±2.0	1.5±0.6
	Vehicle+TP	288.5±21.1	34.5±6.0	84.4±16.7	119.0±15.0	23.6±1.8	7.3±1.6
	50+TP	284.3±11.3	35.6±5.7	84.9±10.9	116.1±10.6	22.9±2.5	6.6±1.4
	200+TP	274.7±13.0	36.3±5.5	90.7±19.6	121.1±13.3	24.6±1.9	7.0±0.8
	600+TP	273.7±11.1	32.0±6.8	75.2±18.0	119.0±12.8	23.0±1.7	7.0±1.6
	Flutamide+TP	287.1±16.2	6.4±0.8**	11.3±1.7**	51.8±10.2**	10.2±2.4**	1.5±0.6**
Androgen derivatives							
Testosterone enanthate	Vehicle control	275.7±7.4	6.4±1.1	13.9±3.0	49.2±6.4	12.0±1.1	1.6±0.3
	50	279.6±14.8	14.5±2.5**	23.9±6.7**	78.8±9.0**	15.5±2.6*	3.2±1.2*
	200	278.8±9.1	27.6±6.3**	48.3±7.8**	124.9±24.0**	22.6±2.8**	7.1±2.0**
	600	270.4±9.4	39.0±4.8**	131.9±34.9**	143.5±10.5**	25.5±1.2**	10.5±1.8**
	Vehicle+TP	285.6±14.7	35.2±4.0	73.9±10.0	111.1±19.5	23.4±1.3	6.6±1.4

Table 5 (*Continued*)

Chemicals	Doses (mg/kg per day)	Body weight (g)	Ventral prostate (mg/100 g bw)	Seminal vesicle (mg/100 g bw)	BC/LA (mg/100 g bw)	Glans penis (mg/100 g bw)	Cowper's gland (mg/100 g bw)
	50+TP	285.6±9.6	42.4±6.5*	98.2±15.7**	130.0±19.9	24.4±1.9	8.1±1.2
	200+TP	282.3±11.5	48.2±7.9**	125.3±24.4**	139.9±8.1*	24.6±1.9	9.4±1.8*
	600+TP	273.9±14.1	53.9±8.3**	176.3±35.1**	154.2±18.0**	27.9±1.4**	10.9±1.5**
	Flutamide+TP	274.5±6.0	6.5±0.9**	14.0±2.8**	50.9±6.7**	12.6±2.1**	2.2±0.7**
Methyltestosterone	Vehicle control	287.3±12.5	6.0±0.5	11.9±2.2	46.1±9.5	10.3±2.6	1.0±0.2
	0.5	283.2±7.4	8.0±0.9**	14.4±1.7*	53.9±5.1	11.8±2.3	2.0±0.2**
	5	284.4±7.9	15.7±3.1**	19.0±3.2**	54.4±7.3	13.8±1.8*	1.6±0.4**
	50	278.5±15.4	48.5±7.6**	100.0±25.4**	135.6±5.9**	25.3±2.2**	8.6±1.1**
	Vehicle+TP	294.1±9.0	37.0±4.8	78.7±5.1	111.0±13.6	23.5±1.5	7.7±0.5
	0.5+TP	293.7±12.0	38.9±3.5	86.2±16.9	117.3±9.0	24.0±1.8	6.9±1.5
	5+TP	292.3±13.0	42.8±6.0	100.5±23.2	125.4±15.0	24.4±2.7	9.4±2.0
	50+TP	287.8±9.5	59.7±10.3**	173.7±64.9*	150.3±14.4**	27.0±1.7**	11.4±1.4**
	Flutamide+TP	283.8±16.7	7.2±1.9**	13.1±2.1**	48.7±3.2**	11.9±0.6**	1.6±0.7**
Steroids							
17Alpha estradiol	Vehicle control	272.1±10.2	5.9±0.7	11.0±2.3	54.4±8.0	12.7±1.2	1.5±0.5
	0.5	265.1±18.1	5.7±1.0	11.1±1.5	56.0±4.3	13.1±2.2	1.6±0.6
	2	270.6±10.9	6.7±1.0	11.5±1.9	54.9±6.9	11.9±0.7	1.6±0.9
	10	254.0±28.3	5.7±0.7	11.1±2.4	52.3±5.3	12.5±3.4	1.4±0.6
	Vehicle+TP	286.2±11.7	38.9±5.2	77.6±8.9	126.4±5.7	24.5±2.5	7.6±1.4
	0.5+TP	287.8±21.2	40.6±5.0	78.9±20.8	125.4±16.9	23.6±2.8	7.7±1.0
	2+TP	267.7±11.0*	43.0±6.0	93.5±21.5	129.1±12.0	25.3±1.7	9.0±1.3
	10+TP	266.6±12.7*	38.7±3.8	90.0±14.7	123.4±10.0	25.3±1.2	8.1±1.3
	Flutamide+TP	280.6±20.3	7.1±1.3**	10.3±1.1**	56.4±6.4**	12.3±2.0**	1.3±0.4**
Estrone	Vehicle control	283.8±12.3	5.9±0.6	12.2±0.6	49.1±4.6	12.1±1.3	1.6±0.3
	0.5	271.4±13.2	6.7±1.0	13.3±2.1	55.8±10.6	13.2±1.9	1.8±0.7
	2	261.8±19.8*	6.0±0.8	13.6±1.9	50.5±2.1	13.0±1.5	1.5±0.6
	10(6)[a]	242.9±10.0**	6.6±0.4*	16.8±2.7**	48.5±7.3	13.6±3.6	2.1±0.6
	Vehicle+TP	285.7±15.3	30.0±5.9	62.0±9.2	109.0±14.7	22.1±1.5	6.3±1.3
	0.5+TP	283.1±9.3	33.3±5.3	76.0±9.7*	115.1±9.5	22.4±1.8	6.4±0.7
	2+TP	270.4±16.8	32.4±4.4	95.7±11.9**	121.6±3.2	25.1±1.3**	7.9±1.7
	10(6)+TP	243.4±13.3**	34.0±3.5	108.0±12.2**	113.7±7.5	26.8±2.6**	7.0±0.8
	Flutamide+TP	284.2±12.0	6.7±1.2**	12.0±2.4**	51.0±7.7**	12.4±2.6**	1.3±0.2**
Equilin	Vehicle control	286.2±12.3	5.4±0.8	12.1±1.6	51.6±9.8	11.8±1.7	1.4±0.4
	0.5	258.4±18.3*	5.8±0.5	13.5±2.5	51.5±7.0	13.6±1.8	1.3±0.5
	2	241.3±8.5**	6.6±0.8*	17.1±1.2**	50.9±3.3	14.2±1.2*	1.7±0.7
	10(6)	225.0±11.0*	6.5±0.5*	19.2±2.9**	50.3±7.8	14.6±0.6**	1.6±0.5
	Vehicle+TP	294.0±19.6	35.0±7.7	72.4±12.0	112.7±17.0	22.9±1.3	6.4±0.7
	0.5+TP	260.7±13.6**	33.6±8.1	96.5±20.1*	123.2±15.2	25.1±2.1	7.9±1.4*
	2+TP	251.4±9.5**	36.4±3.4	98.8±19.7*	123.7±14.6	24.8±2.3	8.2±0.9**
	10(6)+TP	237.8±12.4**	32.2±6.5	109.2±19.6**	117.8±8.7	26.5±2.0**	7.8±0.9*