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Endocrine-Mediated Effects of a Benzophenone-Related Chemical, 2,3,4,4'-Tetrahydroxybenzophenone, Based on Uterotrophic Assay, Hershberger Assay, and Subacute Oral Toxicity Study

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Abstract

The purpose of this study was to investigate the endocrine-mediated effects of a benzophenone-relative compound, 2,3,4,4'-tetrahydroxybenzophenone, based on the OECD protocols. In the uterotrophic assay, female SD rats were subcutaneously injected with the chemical at doses of 0, 100, 300, and 1,000 mg/kg on each of 3 days from postnatal day 20 to day 22, and the uterine weight of rats given the 300 and 1,000 mg/kg doses of the test chemical increased. In the Hershberger assay, the test chemical was orally administered at doses of 0, 60, 200, and 600 mg/kg/day to castrated male SD rats for 10 consecutive days beginning on postnatal day 56, and no changes were observed. On the other hand, when the test chemical was orally administered at doses 0, 60, 200, and 600 mg/kg/day for at least 28 days, serum T4 values decreased in male rats in the 600 mg/kg group. This suggests a mechanism of action related to the inhibition of thyroid peroxidase. The uterotrophic assay used in this study showed that the chemical has estrogen-agonist properties, however, estrogenic effects were not observed in a 28-day repeated-dose toxicity study. On the other hand, endocrine-mediated effects such as thyroid hormone dysfunction were detected in growing rats based on the results of the OECD test guideline No. 407.

Keywords: 2,3,4,4'-tetrahydroxybenzophenone; Uterotrophic assay; Hershberger assay; Test guideline 407; Rat; Endocrine effects

Introduction

Since it was reported that a considerable number of chemicals may have endocrine-disrupting activity in humans and animals, the Organization for Economic Co-operation and Development (OECD) reviewed the original OECD Test Guideline No. 407 and introduced *in vivo* screening tests in 2008 to detect endocrine-mediated effects. Endocrine-disrupting effects are one of the important parameters used to assess the risk of chemicals in the REACH program.

Benzophenones are widely used as Ultraviolet (UV) light filters and stabilizers in cosmetics, skin creams, and body lotions and as corrosion inhibitors in building materials, automobile components, and automotive antifreeze cooling systems. Benzophenones have been reported to occur in the environment, and some benzophenones have also been reported to possess estrogenic activity based on uterotrophic and various *in vitro* assays [1-5]. Recently, effects on steroidogenesis in testicular leydig cells and a reduction in thyroid peroxidase by 2,2',4,4'-tetrahydroxybenzophenone have been reported [6,7]. On the other hand, 2,3',4,4'-tetrahydroxybenzophenone has been reported to have potent estrogenic activity in human breast cancer cell line MCF-7 and androgenic activity in rat fibroblast cell line NIH3T3, however, a structurally related 2,3,4,4'-tetrahydroxybenzophenone has no estrogenic and androgenic properties [5]. These facts demonstrated that the endocrine-mediated activity differs among hydrated benzophenones. We therefore subjected 2,3,4,4'-tetrahydroxybenzophenone to an uterotrophic assay, the Hershberger assay and TG 407 assay according to the OECD protocols to investigate whether it has endocrine-mediated effects.

Materials and Methods

The study was performed under Good Laboratory Practice guidelines. Animals were cared for according to the principles outlined in the guide for animal experimentation prepared by *The Japanese Association for Laboratory Animal Science*.

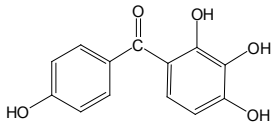

Figure 1: Structure of 2,3,4,4'-tetrahydroxybenzophenone.

Uterotrophic Assay

Chemical: The test chemical, 2,3,4,4'-tetrahydroxybenzophenone (CAS No. 31127-54-5, >101.3% pure, Figure 1), was obtained from the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), and olive oil (Maruishi Parmaceutical, Co., Ltd.) was used as a vehicle.

Animals: Crl: CD (SD) rats, dams and their 13-day-old pups were purchased from Charles River Japan Inc. (Atugi, Japan). The dams and pups were housed in polycarbonate pens until weaning. All pups were weaned at 19 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

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were recorded daily throughout the study. Rats were given free access to automatically dispensed water and a commercial diet (CRF-1, Oriental Yeast Co., Tokyo, Japan). The animal room was maintained at a temperature of 23 ± 2°C and a relative humidity of 55 ± 15%, and it was artificially illuminated with fluorescent light on a 12 h light / dark cycle (0800-2000 h).

Study design: The chemical was subcutaneously injected at doses of 100, 300, and 1,000 mg/kg/day into the back of 20-day-old rats on 3 consecutive days. The concentration and stability of the test chemical in the vehicle were confirmed. In 6 rats, ethinyl estradiol (EE, CAS No. 57-63-6, Tokyo Chemical Industry Co., Ltd.) in the vehicle was also subcutaneously injected into the back at a dose of 0.6 μg/kg/day on the same 3 consecutive days after injection of the test chemical at the doses above. The test chemical and EE were dissolved in olive oil as the vehicle. A vehicle control group was injected with olive oil alone, and a positive control group was injected with EE at a dose of 0.6 μg/kg/day. A group injected with the estrogen-antagonist chemical tamoxifen (CAS No. 10540-29-1, MP Biochemicals, Inc.) at a dose of 1 mg/kg/day plus EE was also established to confirm the reliability of this study. Each group consisted of 6 rats. The doses were based on the results of a preliminary study. The animals were killed by bleeding from the abdominal aorta under deep ether anesthesia approximately 24 h after the final dose. At necropsy, the uteri were carefully dissected free of adhering fat and mesentery, and weighed.

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.

Hershberger assay

Chemical: The test chemical used in this assay was same in used in the uterotrophic assay.

Animals: Five-week-old Crl: CD (SD) rats were purchased from Charles River Japan, Inc. (Atugi, Japan). The rats were castrated at 49 days of age. After 8 days allow them to recover from the operation, the rats were weighed, weight-ranked, and randomly assigned to each of the experimental and control groups. Other housing conditions were essentially the same as for the uterotrophic assay.

Study design: The chemical was orally administered at doses of 60, 200, and 600 mg/kg/day via a stomach tube on 10 consecutive days beginning on postnatal day 56. The concentration and stability of the test chemical in the vehicle were confirmed. A vehicle control group given olive oil alone was also established. After oral administration of the test chemical, testosterone propionate (TP, CAS No. 57-63-6, Wako Pure Chemical Industries, Ltd.), 0.2 mg/kg/day, was administered to some rats by subcutaneous injection into the back, and a positive control group injected with TP was also established. A group orally injected with the androgen antagonist chemical flutamide (CAS No. 13311-84-7, LKT Laboratories, Inc.), 10 mg/kg/day, plus TP was established to confirm the reliability of this study. Each group consisted of 6 rats. The doses of each chemical were selected based on the results of a preliminary study. The animals were killed by bleeding from the abdominal aorta under deep ether anesthesia approximately 24 h after the final dose. The ventral prostate with fluid, seminal vesicle with fluid, bulbocavernosus/levator ani muscle, glans penis, and Cowper's gland were carefully dissected free of adhering fat, and weighed.

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

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Chemical: The the chemical used in this assay was same in used in the uterotrophic assay.

Animals: Crl: CD (SD) rats were purchased from Charles River Laboratories Japan, Inc. (Atugi, Japan). Animals were weighed, weight-ranked, and randomly assigned to each of the treatment groups and control group before administration, and then housed individually in stainless steel, wire-mesh cages throughout the study. Rats were provided with water automatically and with a commercial diet (CRF-1, Oriental Yeast Co., Tokyo, Japan) ad libitum. Other housing conditions were essentially the same as for the uterotrophic assay.

Study design: Rats were orally gavaged with 0, 60, 200 and 600 mg/kg/day of test chemical. These doses were selected on the basis of a preliminary test in which rats were orally gavaged with 0, 30, 100, 300 and 1,000 mg/kg/day of test chemical for 14 days, with result that some toxic effects such as reduced body weight gains, reduced hemoglobin values, increased platelet counts, increased alanine aminotransferase values and reduced thymus weights were detected in the 1,000 mg/kg group. A vehicle control group was gavaged with olive oil (using 5 ml/kg of olive oil containing the test chemical) and the concentration and stability of the test chemical in the vehicle were confirmed before use. Each group consisted of 10 males and 10 females. Animals were killed by exsanguinations under ether anesthesia, and blood samples were obtained from the abdominal aorta and examined for hematological parameters, clinical biochemistry and hormonal parameters. In addition to the requirements of the current OECD Test Guideline 407, we also adopted hormone analysis, estrous cycling and spermatology as optional endpoints.

General observations: Clinical signs were recorded daily. Once before the first dose and once a week thereafter, detailed clinical observations of all animals were made outside the home cage. The signs for which the animals were examined included changes in skin, fur, eyes, and mucous membranes, frequency of urine and feces, and autonomic activity (e.g. lacrimation, piloerection, pupil size, respiratory pattern). Changes in gait, posture, response to handling, the occurrence of clonic or tonic movements, stereotypes (e.g. excessive grooming, circling), or bizarre behavior (e.g. self-mutilation, walking backwards), were also recorded. In the 4th week, a functional observation battery (FOB) that tested sensory reactivity to stimuli of different types (e.g. auditory, visual, and proprioceptive), assessed grip strength, and assessed motor activity, was also conducted.

Body weight and food consumption: Individual body weight was recorded twice weekly and immediately before necropsy. Food consumption was measured weekly.

Hematology: The following were examined in the hematology examinations: red blood cell count, white blood cell count, hemoglobin concentration, hematocrit value, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, reticulocyte count, prothrombin time, activated partial thromboplastin time, and differential leukocyte count.

Clinical biochemistry: Serum levels of the following were measured in the clinical biochemistry examination: aspirate aminotransferase,

Dosages (mg/kg/d)	Body weight (g)	Uterine wet weight		Uterine blotted weight	
		Absolute (mg)	Relative (mg/100g)	Absolute (mg)	Relative (mg/100g)
0	66.2 ± 3.3	48.5 ± 3.4	73.3 ± 4.6	45.2 ± 3.2	68.4 ± 3.5
100	65.2 ± 2.7	58.7 ± 11.1	90.2 ± 18.4	54.5 ± 10.6	83.8 ± 17.3
300	66.2 ± 3.9	74.5 ± 14.9**	112.6 ± 21.5**	70.4 ± 13.6**	106.4 ± 19.7**
1,000	61.9 ± 2.4*	170.3 ± 25.2**	276.7 ± 50.7**	129.6 ± 11.5**	210.1 ± 24.7**
EE	64.8 ± 2.4	176.2 ± 30.1	272.2 ± 46.9	141.0 ± 14.6	217.8 ± 21.9
100+EE	64.4 ± 4.2	161.3 ± 36.5	252.4 ± 66.6	131.0 ± 13.3	203.9 ± 20.3
300+EE	65.0 ± 2.5	144.6 ± 31.6	222.6 ± 49.6	112.6 ± 17.0#	173.2 ± 26.0##
1,000+EE	62.3 ± 3.2	190.8 ± 40.4	305.9 ± 62.3	140.0 ± 19.8	224.6 ± 30.9
TMX+EE	63.2 ± 3.6	110.4 ± 11.8##	174.5 ± 12.5##	103.5 ± 10.4##	163.6 ± 11.4##

EE; ethinyl estradiol, TMX; tamoxifen.
* Significantly different from control at $P<0.05$.
** Significantly different from control at $P<0.01$.
# Significantly different from EE at $P<0.05$.
Significantly different from EE at $P<0.01$.

Table 1: Body weights and uterine weights (mean ± SD) in the uterotrophic assay.

alanine aminotransferase, alkaline phosphatase, cholinesterase, γ-glutamyl transpeptidase, total cholesterol, triglyceride, glucose, total protein, albumin, blood urea nitrogen, creatinine, total bilirubin, calcium, inorganic phosphorus, sodium, potassium and chlorine, and the albumin-globulin ratio was calculated.

Hormone analysis: The serum concentrations of the following hormones were measured at the end of the test period: Thyroid-Stimulating Hormone (TSH), Triiodothyronin (T3), and Thyroxin (T4), and Follicle Stimulating Hormone (FSH). Testosterone in males and estradiol in females were also measured.

Estrous cycling: The estrous cycles of all females were assessed daily from day 22 until the day of sacrifice by examining vaginal smears stained with Giemsa stain.

Spermatology: Sperm morphology (200 smeared spermatozoa) and the sperm count were determined by examining by using specimens obtained from the right epididymis.

Necropsy: Males were necropsied on day 29. Females were necropsied after having been dosed for at least 29 days and sacrificed on days 29-33 to allow them to be sacrificed in the diestrous stage.

Organ weight: The following organs were weighed after necropsy: testes, epididymides, seminal vesicles, prostate (ventral and dorsolateral lobes), ovaries, uterus, adrenals, liver, spleen, kidneys, heart, brain, and thymus, as fresh organs, and the ventral prostate, dorsolateral prostate, thyroid and pituitary gland, after organ fixation. The ovaries, uterus and pituitary were weighed optionally in addition to the current OECD Test Guideline 407.

Histopathology: The following organs were fixed in 10% neutral buffered formalin and examined: prostate, seminal vesicles, ovaries, uterus, vagina, mammary gland, brain, thyroid, adrenals, liver, spleen, kidneys, pancreas, thymus, parathyroids, heart and pituitary gland. The epididymides and testes were fixed in Bouin's solution before examining them. In the present study, we did not examine the following organs listed in the current OECD Test Guideline 407 because we focused on the changes in endocrine-related organs: stomach, intestine, urinary bladder, eye ball, Harderian gland, sciatic nerve and spinal cord.

Statistical analysis: Bartlett's variance test was performed for the parametric data (Body weight, food consumption, hematological data, clinical biochemical data, hormonal data, organ weight, grip strength and locomotor activity). Bartlett's test revealed a homogeneous variance, so one-way analysis of variance was conducted and if the result of the one-way analysis was significant, Dunnett's test was performed to compare the comparison between the treated and the control groups. Data with an inhomogeneous variance shown by Bartlett's test, or non-parametric data (FOB numerical data: the number of stools, the number of urinary pools and incidence rate of abnormal spermatozoa) was subjected to Kruskal-Wallis' rank test, and if a significant difference was observed, Dunnett's approach was carried out. Incidence rate of abnormal estrous cycles and histopathological changes were analyzed by the Fisher's exact probability test and Chi-square test, respectively. In the evaluation of the results, when a difference from the control was found at a significance level of 1% or 5%, it was regarded as a significant change.

Results

Uterotrophic assay

Clinical signs and body weight: No abnormal clinical findings were detected in the rats given the test chemical. A decrease in body weight gains was found in the 1,000 mg/kg group (Table 1).

Uterine weight: Uterine wet weight, blotted weight, and relative weight increased in the 300 and 1,000 mg/kg groups (Table 1). The wet, blotted, and relative weights of the uteri of rats given EE increased compared to the rats given vehicle alone, and the uterine weights of rats given tamoxifen plus EE decreased compared to the rats given EE alone.

Hershberger assay

Clinical signs and body weight: No abnormal clinical findings or body weight changes were detected in the rats given the test chemical (Table 2).

Organ weight: No dose-dependent changes were detected in any of the accessory sex organs (Table 2). The organ weight of all accessory sex organs of the rats given TP increased compared to the rats given vehicle alone, and the organ weights of the rats given flutamide plus TP were decreased compared to the rats given TP alone.

TG 407

Body weight and food consumption: The changes in body weight are shown in Table 3. A decrease in body weight gains was found in the 600 mg/kg group of both sexes, accompanied by decreased food consumption.

General observations: Grip strength changes and locomotor

Dosages (mg/kg/d)	Body weight (g)	Ventral prostate (mg/100g)	Seminal Vesicle (mg/100g)	BC/LA (mg/100g)	Glans penis (mg/100g)	Cowper's gland (mg/100g)
0	299 ± 18	7.3 ± 0.8	14.4 ± 4.2	65.2 ± 13.5	18.7 ± 3.4	2.6 ± 0.4
60	305 ± 22	8.6 ± 0.9*	12.9 ± 1.8	60.1 ± 1.7	18.5 ± 1.6	2.2 ± 0.3
200	307 ± 15	9.6 ± 1.7*	14.2 ± 1.4	59.9 ± 5.2	19.7 ± 2.5	2.8 ± 0.7
600	283 ± 20	8.4 ± 1.2	17.2 ± 1.8	56.7 ± 9.2	20.8 ± 2.0	2.8 ± 1.0
TP	337 ± 21	48.7 ± 5.8	130.9 ± 11.2	155.2 ± 10.6	28.5 ± 2.8	9.3 ± 0.7
60+TP	341 ± 21	45.9 ± 6.6	140.1 ± 27.2	157.2 ± 14.2	29.0 ± 1.3	10.1 ± 2.3
200+TP	332 ± 21	47.9 ± 7.3	144.0 ± 23.0	179.0 ± 12.8##	30.1 ± 1.5	10.4 ± 2.4
600+TP	313 ± 19	43.8 ± 7.7	141.8 ± 17.0	159.2 ± 24.1	29.8 ± 3.2	11.7 ± 2.0
FT + TP	329 ± 24	8.4 ± 1.3##	15.0 ± 2.3##	63.2 ± 7.8##	18.6 ± 3.8##	3.0 ± 0.7##

TP; testosterone propionate, FT; flutamide, BC/LA; Bulbocavernosus and levator ani muscles.
* Significantly different from control at $P<0.05$.
Significantly different from TP at $P<0.01$.

Table 2: Body weights and relative accessory sex organ weights (mean ± SD) in the Hershberger assay.

Male	Control	60mg/kg/day	200mg/kg/day	600mg/kg/day
Terminal body weights (g)	419 ± 25	421 ± 30	412 ± 25	371 ± 22**
Kidneys (g)	2.81 ± 0.23	2.86 ± 0.25	2.79 ± 0.16	2.87 ± 0.17
(%)	0.66 ± 0.03	0.67 ± 0.04	0.67 ± 0.03	0.77 ± 0.04**
Brain (g)	2.09 ± 0.06	2.07 ± 0.08	2.03 ± 0.07	2.07 ± 0.10
(%)	0.49 ± 0.03	0.49 ± 0.04	0.49 ± 0.03	0.55 ± 0.03**
Female	Control	60mg/kg/day	200mg/kg/day	600mg/kg/day
Terminal body weights (g)	276 ± 19	275 ± 26	272 ± 19	252 ± 17*
Brain (g)	1.92 ± 0.05	1.90 ± 0.09	1.94 ± 0.10	1.94 ± 0.07
(%)	0.67 ± 0.04	0.67 ± 0.06	0.69 ± 0.04	0.76 ± 0.05**

* Significantly different from control at $P<0.05$.
** Significantly different from control at $P<0.01$.
%: organ weight as a percentage of body weight.

Table 3: Terminal body weights and abnormal organ weights (mean ± SD) in the TG 407.

Items	Male				Female			
	Control	60mg/kg/day	200mg/kg/day	600mg/kg/day	Control	60mg/kg/day	200mg/kg/day	600mg/kg/day
Grip strength (g)								
Forelimb	454 ± 64	375 ± 109	332 ± 98**	292 ± 55**	298 ± 55	265 ± 65	239 ± 48	255 ± 52
Hindlimb	235 ± 28	232 ± 46	200 ± 51	195 ± 40	171 ± 52	149 ± 26	164 ± 35	162 ± 46
Motor activity								
Interval count (min)								
0-10	2327 ± 1173	2400 ± 983	3286 ± 1367	2648 ± 856	4496 ± 1899	3557 ± 1553	3790 ± 1164	4493 ± 1293
10-20	1133 ± 768	1483 ± 1205	1826 ± 858	1354 ± 1037	3360 ± 1474	2311 ± 1338	2985 ± 916	3583 ± 1557
20-30	1123 ± 959	914 ± 1040	1612 ± 1148	776 ± 699	2541 ± 1666	1568 ± 1552	2289 ± 1124	2609 ± 1601
30-40	853 ± 1274	1155 ± 958	792 ± 565	688 ± 496	2057 ± 1535	1790 ± 1208	1857 ± 1790	2378 ± 1690
40-50	355 ± 672	689 ± 920	794 ± 885	474 ± 550	1920 ± 1678	1498 ± 885	1363 ± 1339	2057 ± 1710
50-60	897 ± 743	404 ± 267	755 ± 952	614 ± 626	1264 ± 1080	1452 ± 1424	1526 ± 1167	1994 ± 1563
Total count (60 min)	6688 ± 3884	7044 ± 3951	9066 ± 4633	6565 ± 1919	15639 ± 7808	12176 ± 6811	13810 ± 5569	17113 ± 8196

** Significantly different from control at $P<0.01$.

Table 4: Grip strengths and locomotor activities (mean ± SD) in the TG 407.

activities are shown in Table 4. Forelimb grip strength was reduced in male rats in the 200 and 600 mg/kg groups, but no abnormal locomotor activities were observed in any male groups. No significant abnormalities were observed in any of the female rat groups.

Hematological and clinical biochemical findings: Abnormal hematological and clinical biochemical findings are shown in Table 5. In male rats, triglyceride values decreased, and alanine aminotransferase values and A/G ratio increased in the 600 mg/kg group. In female rats, hemoglobin, hematocrit, glucose, total cholesterol, triglyceride, and phospholipid values decreased in the 200 and/or 600 mg/kg groups, and total bilirubin values and A/G ratio increased in the 600 mg/kg group.

Hormonal findings: Hormonal findings are shown in Table 6. Serum T4 values decreased significantly in male rats in the 600 mg/kg group. TSH values increased in male rats in the 200 mg/kg group, but no changes were observed in the 600 mg/kg group.

Organ weights: Changes in organ weights are shown in Table 3. In male rats, the relative weights of the kidney and brain increased in the 600 mg/kg group. In female rats, the relative weights of the brain increased in the 600 mg/kg group.

Estrous cycling, Sperm analysis, and Gross morphological and Histopathological findings

No abnormalities were detected in any of the groups.

	Male				Female			
Items	Control	60mg/kg/day	200mg/kg/day	600mg/kg/day	Control	60mg/kg/day	200mg/kg/day	600mg/kg/day
Hemoglobin (g/dl)	15.8 ± 0.4	15.8 ± 0.4	15.6 ± 0.5	15.6 ± 0.5	14.7 ± 0.4	14.6 ± 0.7	14.2 ± 0.5	14.0 ± 0.4*
Hematocrit (%)	41.6 ± 1.1	41.8 ± 1.5	41.0 ± 1.3	41.3 ± 1.2	38.6 ± 1.3	38.6 ± 2.0	37.1 ± 1.2	37.0 ± 1.2*
T-Bil (mg/dl)	0.12 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.13 ± 0.01*
Glucose (mg/dl)	185 ± 10	182 ± 16	186 ± 10	180 ± 10	198 ± 15	189 ± 12	194 ± 10	181 ± 14*
T-Cho (mg/dl)	66 ± 11	69 ± 12	63 ± 9	63 ± 7	68 ± 9	64 ± 12	60 ± 8	52 ± 10**
Triglyceride (mg/dl)	91 ± 42	99 ± 45	75 ± 34	40 ± 14**	77 ± 41	80 ± 36	45 ± 17	36 ± 12**
Phospholipid (mg/dl)	129 ± 9	137 ± 19	125 ± 10	121 ± 12	150 ± 20	143 ± 19	131 ± 12*	119 ± 16**
ALT (IU/l)	33 ± 4	34 ± 5	32 ± 5	38 ± 5*	30 ± 5	29 ± 5	27 ± 3	32 ± 3
A/G ratio	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1*	1.6 ± 0.2	1.7 ± 0.2	1.8 ± 0.2	2.0 ± 0.2**

T-Bil; total bilirubin, T-Cho; total cholesterol, ALT; alanine aminotransferase.
* Significantly different from control at $P<0.05$.
** Significantly different from control at $P<0.01$.

Table 5: Abnormal hematological and clinical biochemical values (mean ± SD) in the TG 407.

	Male				Female			
Items	Control	60mg/kg/day	200mg/kg/day	600mg/kg/day	Control	60mg/kg/day	200mg/kg/day	600mg/kg/day
T3 (ng/dl)	2.4 ± 0.3	2.5 ± 0.3	2.2 ± 0.3	2.2 ± 0.3	2.4 ± 0.3	2.5 ± 0.2	2.4 ± 0.2	2.5 ± 0.3
T4 (ng/ml)	89.6 ± 16.0	99.2 ± 20.4	86.4 ± 13.1	71.8 ± 11.0*	67.7 ± 10.9	73.8 ± 9.3	71.5 ± 8.2	62.3 ± 7.4
TSH (ng/ml)	1.8 ± 0.8	2.6 ± 1.8	3.8 ± 2.2*	1.4 ± 0.7	0.7 ± 0.3	1.3 ± 0.6	0.9 ± 0.3	0.8 ± 0.2
FSH (ng/ml)	3.3 ± 1.0	3.2 ± 1.2	3.6 ± 1.0	3.7 ± 0.7	0.7 ± 0.2	0.8 ± 0.3	1.2 ± 1.0	0.9 ± 0.3
Testosterone(ng/ml)	1.7 ± 1.4	3.3 ± 2.3	3.0 ± 3.4	2.4 ± 2.0	NE	NE	NE	NE
Estradiol (pg/ml)	NE	NE	NE	NE	23.6 ± 4.4	24.1 ± 3.6	23.0 ± 3.1	22.0 ± 3.5

NE; not examined.
* Significantly different from control at $P<0.05$.

Table 6: Hormonal values (mean ± SD) in the TG 407.

Discussion

This study was conducted to investigate the endocrine-mediated effects of 2,3,4,4'-tetrahydroxybenzophenone in accordance with OECD Test Guidelines.

In the uterotrophic assay the uterine weight of rats given EE was higher than in rats given the vehicle alone, and the organ weights of the rats given tamoxifen plus EE were lower than in the rats given EE alone, and in the Hershberger assay the weights of the accessory sex organ 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 in rats given TP alone, thereby confirming the reliability of the uterotrophic and Hershberger assays used in this study. The uterine weight of the rats given the 300 and 1,000 mg/kg doses of the test chemical was higher than in the control group, indicating that the test chemical has estrogen-agonist properties. On the other hand, this chemical is not considered to have androgen-agonist or -antagonist properties, because no changes in the weight of accessory sex organs were detected in the Hershberger assay used in this study.

In the TG 407 assay used in this study, serum T4 values decreased in male rats in the 600 mg/kg group. A study on propylthiouracil based on the protocol in the OECD Test Guideline No. 407 found that serum T3 and T4 values decreased [8,9]. The OECD Test Guideline No. 407 also states that thyroxin and propylthiouracil have a similar effect on serum T3 and T4 values, and that these changes are accompanied by changes in TSH and histopathological changes in the thyroid [10,11]. Furthermore, the hypothyroidism induced in rats by various thyroid hormone modulators, such as barbital and methimazole, has also been characterized by increased serum TSH and/or decreased T3 and T4 values [12,13]. Changes in serum T4 values in the present study were not accompanied by changes in serum T3 or TSH values, and histopathological abnormalities were not detected in the thyroid. In a study using ovariectomized rats [14], it was reported that 2,2',4,4'-tetrahydroxybenzophenone was associated with decreased T4 values and increased TSH values without changes in T3 values, and it has been suggested that this compound and benzophenone affect thyroid hormone homeostasis by inhibiting or inactivating thyroid peroxidase [7,14]. The changes in serum T4 values seen in this study appear to be due to the effects of thyroid peroxidase as well as other benzophenone-related compounds. The fact that serum TSH values did not change may be related to the degree to which T4 values decreased.

The estrogenic properties of ethinyl estradiol were detected at a dose of 2–3 μg/kg in the uterotrophic assay [1,15,16], and estrogenic effects were observed starting at 10–50 μg/kg, under OECD test guideline No. 407 [1,17]. In contract, in the uterotrophic assay, bisphenol A's estrogenic properties were detected at a dose of 20 mg/kg and abnormal estrous stages were only observed at a dose of 600 mg/kg in OECD test guideline No. 407 [18]. On the other hand, the uterotrophic properties of genistein and nonylphenol were detected at doses of 20–60 mg/kg and 20–75 mg/kg, respectively [16], and the estrogenic effects were not apparent in rats given 1,000 mg/kg of genistein or 300 mg/kg of nonylphenol in OECD test guideline No. 407 [10,11]. These findings demonstrated the absence of any estrogenic effects of some chemicals under OECD test guideline No. 407, when uterotrophic assay did not indicate a high level of estrogenic activity. Our previous studies conducted in accordance with the OECD Test Guideline No. 407 using bisphenol A-related compounds revealed that estrogenic effects such as abnormal estrous cycles, reduced male accessory sex organ weights and histopathological abnormalities in the sex and accessory sex organs appeared when high estrogenic potency was confirmed by uterotrophic assay [19,20]. In the present study, the estrogenic effects of 2,3,4,4'-tetrahydroxybenzophenone were not detected at a dose of 600 mg/kg under OECD test guideline No. 407, and its estrogenic properties were not apparent in rats given 100 mg/kg in the uterotrophic assay. We

believe that estrogenic effects of the present chemical were not detected because its estrogenic properties, as assessed in the uterotrophic assay, are weak.

Reduced body weight gains, hematological effects, and hepatic and renal abnormalities have been reported in 28-day and 14-week repeated-dose toxicity studies of benzophenone [21]. In this study, reduced body weight gains were detected in the 600 mg/kg group of both sexes, and this change was probably an adverse effect caused by this compound. Slightly reduced hemoglobin and hematocrit values, and increased alanine aminotransferase and total bilirubin values were detected in the 600 mg/kg group of male and/or female rats. In the preliminary test, reduced hemoglobin values, increased platelet counts and increased alanine aminotransferase values were also detected in the 1,000 mg/kg group. Therefore, this compound was considered to have the potential to cause hematological and hepatic disorders. Other abnormal parameters found in the clinical biochemical examination and organ weight changes could be related to reduced body weight gains. On the other hand, reduced forelimb grip strength was observed in male rats in the 200 and 600 mg/kg groups. Unfortunately, histopathological examination of the peripheral nerves and muscles around the forelimbs and spinal cord in the thoracic region was not performed in this study. However, no locomotor abnormalities were detected in these groups and similar reduced grip strengths have not been reported in the repeated-dose toxicity studies using benzophenone and related compounds [2,3,5,21,22]. In addition, it has been reported that changes in grip strength are associated with locomotor abnormalities in rats given a number of neurotoxic compounds [23-28]. The mechanisms underlying reduced grip strength in this study were unclear and we could not determine if this change was an adverse effect. The no observed adverse effect level (NOAEL) for this chemical was estimated to be 200 mg/kg/day.

Conclusion

We performed a uterotrophic assay, Hershberger assay, and TG 407 assay of 2,3,4,4'-tetrahydroxybenzophenone according to the OECD protocols in order to investigate its endocrine-mediated effects. Estrogen-agonist properties were detected in the uterotrophic assay, and serum T4 values decreased in the TG 407 assay. On the other hand, the overall NOAEL for repeated dose toxicity is considered to be 200 mg/kg/day.

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