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REPORT OF THE VALIDATION OF THE UTEROTROPHIC BIOASSAY: ADDITIONAL DATA SUPPORTING THE TEST GUIDELINE ON THE UTEROTROPHIC BIOASSAY IN RODENTS

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Environment Directorate

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The Environment, Health and Safety Division publishes free-of-charge documents in ten different series: Testing and Assessment; Good Laboratory Practice and Compliance Monitoring; Pesticides and Biocides; Risk Management; Harmonisation of Regulatory Oversight in Biotechnology; Safety of Novel Foods and Feeds; Chemical Accidents; Pollutant Release and Transfer Registers; Emission Scenario Documents; and the Safety of Manufactured Nanomaterials. More information about the Environment, Health and Safety Programme and EHS publications is available on the OECD's World Wide Web site (http://www.oecd.org/ehs/).

This publication was developed in the IOMC context. The contents do not necessarily reflect the views or stated policies of individual IOMC Participating Organizations.

The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The participating organisations are FAO, ILO, OECD, UNEP, UNIDO, UNITAR and WHO. The World Bank and UNDP are observers. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

FOREWORD

This document is made of two distinct parts "Additional data on the specificity of the Uterotrophic Bioassay" and "Validation of the Uterotrophic Bioassay in mice by bridging data to rats".

The first one, "Additional data on the specificity of the Uterotrophic Bioassay", was developed in response to the recommendations of the Peer Review Panel (PRP) in charge of the review of the validation of the Uterotrophic Bioassay. The PRP expressed the need for additional work to finalize the validation exercise for the purpose of developing the OECD Test Guideline. Thus this section provides, in a retrospective manner, additional information on negative compounds, to fully assess the assay in term of specificity and sensitivity.

The second one, "Validation of the Uterotrophic Bioassay in mice by bridging data to rats", was developed to extend the Test Guideline for the Uterotrophic Bioassay to the use of mice. The results presented in this section are meant to bridge between the extensive validation for the rat Uterotrophic Bioassay and data on mice, so that mice can be used in a corresponding screening procedure. This bridging approach with a limited number of test chemicals, participating laboratories and without coded sample testing has been selected for animal welfare reasons not to use an unnecessary large number of experimental mice.

This document was written by a consultant for the OECD Secretariat. The first draft report was circulated to the Validation Management Group for mammalian testing (VMG-mam) and the Task Force for Endocrine Disrupters Testing and Assessment (EDTA) in October 2006. The report has been modified on the basis of the comments received. The revised report was approved by the VMG-mam, and endorsed by the EDTA Task Force and the Working group of the National Coordinators of the Test Guidelines Programme (WNT) at their respective meetings.

This document is published on the responsibility of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology.

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PART I

ADDITIONAL DATA ON THE SPECIFICITY OF THE UTEROTROPHIC BIOASSAY

Part I of this document includes additional data on the specificity of the Uterotrophic Bioassay. The sections "comparison with results of an enhanced 1-generation study" and "specificity of the Uterotrophic Bioassay defined by in vitro screening data" rely on data of the Japanese MOE and METI. It is gratefully acknowledged that MOE and METI made this information available to the OECD.

Background

1. Under the umbrella of the OECD a preliminary Test Guideline for validation of the rodent Uterotrophic Bioassay was developed. Subsequently, an international validation program was initiated to show the reliability and reproducibility of the bioassay with potent reference estrogens, weak estrogen receptor agonists, a strong estrogen receptor antagonist, and a negative reference chemical. Many laboratories from the private and public sector of various OECD member countries participated in this validation program.

2. The results of the validation program were submitted to a peer review panel of recognized international experts to evaluate whether the data produced substantiated the validity of the Uterotrophic Bioassay. The most important question was whether, on the basis of this validation program, an official OECD Test Guideline could be developed. In this respect, approximately 30 charge questions were given by the OECD secretariat to be answered by the peer review panel.

3. For many of the charged questions there was a common response by the peer review panel, but for some of these there was a split opinion. The major critique centered around the fact that only one negative reference chemical was included in the validation programme. Thus, according to the peer review panel the specificity of the Uterotrophic Bioassay was not adequately demonstrated. This led to a split opinion on the overall validation status: While some members accepted that this in vivo bioassay was sufficiently validated, there was also an extreme position that this international programme could only be considered as a prevalidation study. The general consensus in this respect was that more work would be necessary to define the limits of the test, either by further testing or by substantiating the specificity by additional (literature) data.

4. In the following, additional data not contained within the OECD validation programme are reviewed to substantiate the specificity of the Uterotrophic Bioassay. As a starting point, chemicals that are negative in a Uterotrophic Bioassay, similar to the protocol of the OECD validation program, are selected. Three approaches are used to verify non-estrogenicity of these chemicals:

- Comparison with data of a 2-generation study carried out according to the most recent guidelines;
- Comparison with results of an "enhanced 1-generation test";
- Comparison with screening test results of a lower tier.

Comparison with data of a 2-generation study (Styrene)

5. A negative Uterotrophic Bioassay was reported by Date et al. (2002) based on the protocols of US EPA (1998), Odum et al. (1997), and Laws et al. (2000). Three groups of pubertal rats, 21 days old, were given subcutaneous injections of styrene in corn oil daily over 3 days at dose levels of 0, 20, and 200 mg/kg body weight (dosing volume 5ml/kg). Animals were sacrificed 24 h after the last dose and wet uterine weights were determined. Styrene did not show an effect on uterus weight. In contrast, estradiol-17β (40µg/kg body weight) led to a massive increase in uterine weight of about 400 - 600% (estimated by a graph) and p-nonylphenol (200mg/kg body weight) to a statistically significant uterine weight increase of 230 %. Thereby, styrene proved to be negative in a test series that was positive for a potent as well as for a weak estrogen agonist.

6. A 2-generation reproduction study (Cruzan et al., 2005a) in combination with a developmental neurotoxicity study (Cruzan et al., 2005b) has recently been published. In this study 25 rats per sex per dose group were exposed by inhalation to 0, 50, 150, and 500 ppm styrene daily for six hours per day. The inhalation exposure was carried out in the P-generation for at least 70 consecutive days prior to mating. Inhalation exposure for the F0 and F1 females continued throughout mating and gestation until gestation day 20. During lactational days 1-4 styrene was given to the dams by gavage at dose levels of 0,66, 117 and 300 mg/kg body weight/day in olive oil. Oral dosing was selected to achieve approximately the same internal exposure as after inhalation. Offsprings were weaned on postnatal day 21 and exposed to inhalation beginning with the next day. The F1 pups that were not used for breeding of the F2 generation were sacrificed on postnatal day 21 and the F2 pups that were not used for the developmental neurotoxicity part were sacrificed on postnatal day 28.

7. The following parameters were determined in the 2-generation reproduction study (Cruzan et al., 2005): Clinical symptomatology, body weight, food consumption, reproductive performance (including vaginal smears for estrous cycle), spermatogenic endpoints, selective organ weights and histopathology of F0 and F1 animals, selective organ weights without histopathology of F1 and F2 pups sacrificed on postnatal day 21 or 28.

8. In the 2-generation reproduction study the following findings were noted: The MTD was clearly achieved (reduced body weight gain in F0 and F1 animals at 500 ppm and partly also at 150 ppm; relative liver weights were increased in F0 and F1 males at 500 and 150 ppm; olfactory degeneration was found by histopathology at all dose levels. In addition, female F2 animals showed a decrease of the absolute (but not relative) weights of pituitary, thymus and uterus, but this effect was attributed to growth retardation. In the F2 male animals the absolute and relative pituitary weights were reduced at 500 ppm. No effects were noted on gestational parameters of F0 and F1 dams, on ovarian follicle and corpora lutea counts in F1 females, on the reproductive performance of males and females (e.g. estrous cycle time to coitus, spermatogenic endpoints), and on the number and survival of offspring.

9. In the developmental neurotoxicity part (Cruzan et al., 2005 b) the F2 offsprings of the 2-generation study (Cruzan et al., 2005a) were weaned on PND 21 and not directly exposed to styrene. Neurobehavioral (FOB, grip strength, locomotor activity etc) and neuropathological evaluations were conducted through PND 72 on one F2 pup/sex/litter. Maturation was assessed on selected F1 and F2 pups by monitoring developmental landmarks, among others pinna detachment, incisor eruption, eye opening, preputial separation, vaginal patency.

10. In F2 males and females there were subtle indications of a delay in the acquisition of some developmental landmarks, which accompanied the decreased bodyweights. In general, the mean ages of acquisition were not statistically significantly increased, but higher level exposed animals acquired the landmarks later than the controls. This was the case among others for preputial separation, but this was

only a numerical but not a statistically significant finding. Most importantly, there was no effect on the age of vaginal opening that can be regarded as one of the most sensitive parameter for an estrogenic effect.

11. In conclusion, the 2-generation reproduction study conducted at doses up to the MTD (Cruzan et al., 2005a) in combination with a developmental neurotoxicity study (Cruzan et al., 2005b) did not show effects on gonadal function, reproductive performance, offspring survival, or developmental effects that may be related to an estrogenic action of styrene (e.g. especially vaginal opening). This study supported negative findings in previous studies like a 3-generation study (Belides et al,. 1985), and several subchronic studies that did not show any effects on the gonads as referenced by Cruzan et al. (2005a). The results clearly disagreed with a former gavage study of Srivastava et al. (1989) who reported at 400 mg/kg alterations of marker enzymes for testicular function, testicular pathology, and decreased sperm counts.

12. Further in vitro studies support the conclusion that styrene does not exhibit an estrogen agonist activity. Date et al. (2002) did not find an estrogen receptor binding activity up to a concentration of 10^{-5} mol/l and Soto et al. (1995) reported styrene to be negative in the E-SCREEN assay. In addition Ohno et al., (2001) reported styrene to be negative in the estrogen receptor binding assay, the luciferase reporter gene assay, and the MCF-7 proliferation assay. In all of these tests estradiol-17ß, DES, bisphenol A, and p-nonylphenol gave positive responses.

Comparison with results of a "enhanced 1-generation study"

13. The Ministry of the Environment (MoE) in Japan developed an "enhanced 1-generation test" with the following basic test design: female F0 animals were orally exposed to the test chemicals throughout gestation and lactation. Thereby the F1-offspring were indirectly treated via the milk. Afterwards the F1-offspring were kept without treatment until sexual maturation. The following endpoints were examined in the F1-offspring: anogenital distance, sexual development, weights of sex organs, sperm analyses, necropsy, histopathology, and expression of mRNA (ERs, AR, early responsive genes).

14. The data obtained by this test method (Annex 2) may give an indication whether or not the test chemical exhibits estrogenic activity. But there are two general problems to be considered in this respect:

- This bioassay was specifically designed to identify possible "low dose" effects. Therefore, there were generally 4 exposure groups with dose levels 3 – 5 orders of magnitude lower than doses used in "routine" toxicity testing. Only 1 – 2 groups received such higher dose levels that could be compared to doses in the Uterotrophic Bioassay. Therefore, generally no dose-response relationships are available for the high dose range.

- A multiplicity of parameters was investigated, that could be taken as an indication for endocrine effects. Therefore, with most test chemicals there were some findings possibly related to some hormonal interaction, but not specifically to estrogenicity. But due to lack of further mechanistic data a clear proof for non-estrogenicity of such effects may in some cases not be possible.

15. To circumvent the above mentioned problems only the following effects in the F1-generation should be taken as a clear indication for an estrogenic effect:

- increase in uterine weight
- acceleration of vaginal opening

Both of these effects are highly sensitive endpoints for chemicals with an estrogenic mode of action. If in the "enhanced 1-generation study" there was no change in uterine weight or time to vaginal opening, the test chemical was assumed not to exhibit an estrogenic mode of action.

16. In total, 35 chemicals were tested in the "enhanced 1-generation test" and of these the following were negative in the Uterotrophic Bioassay of the CERI/METI test series (Annex 1):

- Di-(2-ethylhexyl)phthalate
- Amitrole
- Di-(2-ethylhexyl)adipate
- Diethylphthalate
- Dihexylphthalate
- Dipentylphthalate
- Dipropylphthalate
- Pentachlorophenol

17. In the following the findings in the "enhanced 1-generation test" are listed for the above mentioned chemicals that might have some relationship to an hormonal interaction without specifically indicating to an estrogenic mode of action:

a) Di-(2-ethylhexyl)phthalate: no effects indicating to endocrine activity

b) Amitrole: effects on pituitary in F0 dams, F1 males and females. Delay of preputial separation, changes in testes weight in F1 males. Delay of vaginal opening, low ovary weight in F1 females.

c) Di-(2-ethylhexyl)adipate: high number of stillborns and low weaning rate in F1 pups. Low serum testosterone in F1 males. Low ERα-mRNA expression in ovaries of F1 females.

d) Diethylphthalate: low pituitary weight in F0 dams. Low viability and number of live offspring in F1 pups. Low sperm motility, delay of preputial separation, changes in serum FSH, low weight and histopathological changes of testes in F1 males. Increase in anogenital distance in F1 females.

e) Dihexylphthalate: low number of pups. Decrease in anogenital distance in F1 males.

f) Dipentylphthalate: low fertility rate and number of pups for F0 dams.

g) Dipropylphthalate: low viability of F1 pups. Delay of vaginal opening on F1 females.

h) Pentachlorophenol: high male sex ratio, low number of delivered / viable F1 pups. High testicular sperm count, delay of preputial separation, decrease of testis and prostate weights in F1 males.

18. In summary, for all of these chemicals being negative in the Uterotrophic Bioassay, none of these findings in the "enhanced 1-generation test" can be taken as an evidence for estrogenic activity. Specifically there was no increase in uterine weight or acceleration of vaginal opening in the F1 females, effects that would be assume to be most sensitive for an estrogenic mode of action. Therefore, as the "enhanced 1-generation test" design should be considered highly predictive for endocrine mediated effects, the results thereby obtained strongly support the negative Uterotrophic Bioassay data.

Comparison of the Uterotrophic Bioassay with in vitro screening data

19. CERI/METI tested 65 chemicals in the Uterotrophic Bioassay, in the hER receptor binding assay, and in the hER α-reporter gene assay for agonist activity (Annex 1; Tables 1 and 2). In the Uterotrophic Bioassay 20 days old immature rats were given the test material subcutaneously on three consecutive days. The highest dose level represented the MTD as calculated “from preliminary tests for detecting estrogenic

activity". Three dose groups and a solvent control group were used. Some further details on the study design are given in the publication of Yamasaki et al. (2002). The data of CERI/METI presented to the OECD are given in the annex.

20. The hER receptor binding activity is given in terms of the RBA (relative binding activity). A test was judged to be positive if a numerical IC50 value could be calculated by the data. In some cases an exact calculation of the IC_{50} was not possible for the hER receptor binding assay. To define positives/negatives in this assay the following assumptions were made: The test result was taken negative, if the maximum concentration that could be used led to a detachment of < 20%; the test was taken questionable positive, if the maximum concentration led to a detachment of > 20%. The results of the hER α-reporter gene agonist assay are reported as PC10*. This is defined as the chemical test concentration estimated to show 10% of the transcriptional activity of the positive control substance (Estradiol-17β) (Yamasaki et al., 2002).

21. The data were analyzed qualitatively and semi- quantitatively. For the qualitative approach, the number of chemicals being positive or negative in the Uterotrophic Bioassay and in the in vitro screening tests were compared. For the semi-quantitative approach, cut-off levels for the RBA (hER receptor binding assay) and the PC10 (hERα-reporter gene assay) were arbitrarily selected and a comparison was made with the positive/negative Uterotrophic Bioassay data.

22. In total 65 chemicals were tested in the Uterotrophic Bioassay and in both of the in vitro screening tests. Of these, 31 were positive in the Uterotrophic Bioassay and 34 were negative.

23. For the qualitative comparison the following results were obtained: of the 34 chemicals negative in the Uterotrophic Bioassay, 8 were negative in both in vitro assays, 4 were positive in the reporter gene assay only, 12 were positive or questionable positive (2) in the receptor binding assay only, and 10 were positive in both in vitro assays (thereof 1 questionable positive in the receptor binding assay). In contrast, of the 31 chemicals being positive in the uterotrophic assay 26 were positive in both in vitro assays, 1 was negative and 1 questionable positive in the receptor binding assay only, 2 were negative in the reporter gene assay only, and 1 chemical was negative in both in vitro assays. In conclusion, there was a clear tendency for chemicals positive in the Uterotrophic Bioassay, to also test positive in the in vitro assays and vice versa.

24. The data base of those chemicals being positive in the Uterotrophic Bioassay but negative in one or both of the screening tests is discussed in more detail:

a) 4-Diethylaminobenzaldehyde was negative in both of the in vitro tests. The structure by itself does not give any indication whether this chemical may or may not exhibit estrogenic activity. The mean blotted uterus relative weights (mg/100 g) were 52.2, 56.2, 70.9 ($p < 0.05$), and 64.9 ($p < 0.05$) for the vehicle control group and the dose groups treated with 60, 200, and 600 mg/kg/d. Thus, there is only a small increase in uterus weight and a clear dose response relationship is missing. In addition, the mean body weights of the test and control animals at the beginning was quite high with 65.2 g (calculated from the body weights given in Table 2). The total test series comprised approx. 580 test and control groups for estrogenicity and antiestrogenicity. Only eleven of these groups had an initial mean body weight of > 65 g (cf. Table 2).

* Note: A recalculation of the PC10 values is under consideration. This may lead to a shift of the absolute values, but will not have much of an influence on the relationship of the values to each other. Thus, this recalculation may lead to a different cut-off level but will not have a substantial influence on the chemicals above and below this level.

In conclusion, the positive response of 4-diethylaminobenzaldehyde in the Uterotrophic Bioassay is at best very week and may even be considered as questionable taking into account the unclear dose response relationship and the high initial body weight of the immature animals. (Initial age and body weight of the immature animals is one of the most critical variables to be controlled in the Uterotrophic Bioassay.)

b) 17α-Methyltestosterone was positive in the reporter gene assay but only questionably positive in the receptor binding assay. It was clearly positive in the Uterotrophic Bioassay with the following mean blotted relative uterus weights (mg/100 g): 55.7; 56.4; 97.6 ($p < 0.01$); 151.8 ($p < 0.01$) for the vehicle control group and the test groups dosed with 2; 10; and 40 mg/kg/d.

It is well known, that androgens may elicit a positive response in the Uterotrophic Bioassay either by direct action or via metabolic aromatisation to estrogens. This test compound is therefore expected to give a positive response in the Uterotrophic Bioassay. Thus, the positive Uterotrophic Bioassay supersedes the questionable result of the receptor binding assay.

c) Testosterone enanthate was positive in the reporter gene assay and was considered negative in the receptor binding assay. Similar to 17α-methyltestosterone the Uterotrophic Bioassay was clearly positive with mean blotted relative uterine weights (mg/100 g) of 61.3; 50.5; 98.1 ($p < 0.01$); and 147.3 ($p < 0.01$) for the vehicle control and the dose groups of 2; 10; and 40 mg/kg/d.

The same considerations apply as for 17α-methyltestosterone. A positive response is to be expected in the Uterotrophic Bioassay and it outweighs the negative response of the receptor binding assay.

d) 4-tert-Butylcatechol was positive in the receptor binding assay and negative in the reporter gene assay. The Uterotrophic Bioassay led to a clear positive response. The mean blotted relative uterine weights (mg/100 g) were 59.7; 63.9; 71.7; and 111.1 ($p < 0.01$) for the control and the test groups with 100; 300; and 1,000 mg/kg/d.

The positive response in the Uterotrophic Bioassay is plausible: the test chemicals listed in the annex included many phenolic compounds and most of them were positive in the Uterotrophic Bioassay as well as in both of the screening assays. Therefore, the positive in vivo Uterotrophic Bioassay might outweigh the negative reporter gene assay.

e) 2,3,4-Trihydroxybenzophenone was negative in the receptor gene assay and positive in the receptor binding assay. Again, the Uterotrophic Bioassay is clearly positive with mean blotted relative uterine weights of 57.1; 55.3; 82.3 ($p < 0.01$); and 106.8 ($p < 0.01$) (mg/100 g) for the control and test groups with 100; 300; and 1,000 mg/kg/d.

This chemical also has a phenolic structure and therefore the positive Uterotrophic Bioassay might outweigh the negative reporter gene assay.

In summary, the positive Uterotrophic Bioassay for 4-diethylaminobenzaldehyde with both a negative receptor binding and reporter gene assay is considered questionable while for the other 4 chemicals the positive Uterotrophic Bioassay is more relevant in comparison to the conflicting in vitro test results (for each of these 4 chemicals one in vitro test was positive and the other one was negative).

25. For the semi-quantitative comparison the following arbitrary cut-off levels were selected:

- for the hER receptor binding assay a RBA of 0.01

- for the hER α-reporter gene agonist assay a PC10 of 10^{-6}

On the basis of these arbitrary cut-off limits, the distribution of chemicals being positive and negative in the Uterotrophic Bioassay was as follows:

- 6/34 uterotrophic negatives but 23/31 uterotrophic positives had a RBA >0.01 in the hER receptor binding assay;
- 3/34 of the uterotrophic negatives but 21/31 of the uterotrophic positives had a PC10 $<10^{-6}$ in the hER α-reporter gene agonist assay.

This is consistent with a similar analysis performed by CERI in Annex 3 comparing the ER binding assay and uterotrophic assay. A total of 74 chemicals were subjected to comparison. The lowest Relative Binding Affinities (RBA) in ER binders, which showed uterotrophic activity was 0.0023%. When this cut-off (0.002%) was applied to the comparison between ER-binding and Uterotrophic assay, the concordance was improved, from 65% to 81%. This analysis also shows that the ER binding assay is more sensitive than the uterotrophic assay and a weak response (RBA =<0.002%) may not be translated into a biologically meaningful signal in the uterotrophic and would be negative in that assay.

In summary, in this semi-quantitative comparison, the Uterotrophic Bioassay differentiated well between chemicals showing weak/strong activities in these two screening assays.

Conclusion

26. The database presented for styrene shows that this chemical is negative in a recent Uterotrophic Bioassay and is without estrogenic activity in a 2-generation study carried out according to today's standard. The specificity of the Uterotrophic Bioassay is further supported by some former in vivo and in vitro studies.

27. The specificity of the Uterotrophic Bioassay would have been even more substantiated, if for some chemicals with positive results in the Uterotrophic Bioassay "enhanced 1-generation tests" had also been carried out for comparison purposes. And such substances should then show clear signs of estrogenicity in the "enhanced 1-generation test". Unfortunately, such examples are not available in these test series. But if one allows for reading across similar chemical structures an additional good indication for the specificity can be obtained for such substances being positive in the Uterotrophic Bioassay: 4-Dodecylphenol, 4-tert.-amylpenol, 4-n-amylphenol, and 4-(1-adamantyl)phenol all tested positive in the Uterotrophic Bioassay. 4-Nonylphenol (branched) and 4-tert-octylphenol - being very similar by their chemical structure - showed an acceleration of vaginal opening in the F1 females, a clear indication for estrogenic activity. This concordance for positive effects in both test systems gives additional support to the specificity of the Uterotrophic bioassay.

28. Chemicals that were negative in the Uterotrophic Bioassay did not show an indication for an estrogenic mode of action in the "enhanced 1-generation test" developed by the MoE in Japan. This new test design may be considered to be very sensitive to endocrine mediated effects in the F1-offsprings.

29. The qualitative and semi-quantitative comparison of Uterotrophic Bioassay data with those from two screening assays demonstrate, that the Uterotrophic Bioassay can well differentiate between chemicals with strong/weak estrogen receptor binding and agonist activity but that ER binding assay is more sensitive than the uterotrophic assay for weak responses (RBA<0.002%). Thereby it has to be taken into account that the in vitro tests belong to a lower level of the "OECD Conceptual Framework" and more weight has to be given to the results of the in vivo Uterotrophic Bioassay.

30. In addition to the negative result obtained in the international validation program with dibutylphthalate (the negative reference chemical tested) these data give strong evidence for a good specificity of the Uterotrophic Bioassay which is an in vivo level 3 screening assay in the "OECD Conceptual Framework for Testing Endocrine Disrupting Chemicals"**.**

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ANNEX 1: CERI/METI DATA

Immature Rat Uterotrophic Assay

The uterotrophic assay was conducted by subcutaneously administering the test substances to immature rats (20 days old) for 3 days, following Protocol B, as proposed by the OECD. The test animals were divided into a high-dose group (which was administered the maximum tolerated dose calculated on the basis of the results from preliminary tests for detecting estrogenic activity), a medium-dose group, a low-dose group, a solvent control group, antiestrogenicity detection groups (which were administered EE in combination with the above-mentioned doses for detecting antiestrogenicity), and a positive control group for antiestrogenicity which was co-administered tamoxifen (TMX) and EE. All test substances were subcutaneously administered for 3 days.

Table 1. The Results of Uterotrophic Assay, Human ERα Binding Assay and Reporter Gene Assay

TestSub No.	Chemical_Name	Cas_No	Uterotrophic assay _estrogenicity	Uterotrophic assay _antiestrogenicity	hER receptor binding assay _binding activity RBA	hERα reporter gene assay _agonist activity (stable) PC10(M)	hERα reporter gene assay _antagonist activity (stable) IC30(M)
1-011	Bis(2-ethylhexyl)adipate (= Di(2-ethylhexyl) adipate)	103-23-1	−	−	N.B.	−	N.E.
1-020	Disulfiram	97-77-8	−	+	0.0461	−	4.30E-06
2-043	17alpha-Methyltestosterone	58-18-4	+	−	N.D.	2.08E-07	−
2-049	Testosterone enanthate	315-37-7	+	−	N.B.	1.65E-08	−
3-005	4-n-Amylphenol	14938-35-3	+	−	0.0032	2.84E-07	−
3-009	4-Dodecyl-phenol	104-43-8	+	−	0.238	4.65E-08	−
3-013	p-(tert-Butyl)phenol	98-54-4	+	+	0.00233	1.18E-06	−
3-014	p-(tert-Amyl) phenol	80-46-6	+	+	0.0173	3.65E-07	−
3-019	4-Cyclohexylphenol	1131-60-8	+	−	0.0396	1.01E-07	−
3-020	4-(1-Adamantyl)phenol	29799-07-3	+	−	1.7	1.05E-09	−
3-030	2,4-Di-tert-butylphenol	96-76-4	−	−	0.00155	6.59E-06	N.E.
3-041	p-Hydroxybenzoic acid	99-96-7	−	−	N.B.	−	N.E.
3-042	Ethyl-p-hydroxybenzoate	120-47-8	−	−	N.D.	4.70E-06	−
3-046	2-Ethylhexyl-4-hydroxybenzoate	5153-25-3	+	+	0.0519	4.40E-08	−
3-062	4-tert-Butylcatechol	98-29-3	+	+	0.0189	−	−
3-103	p-Dichlorobenzene	106-46-7	−	−	N.B.	1.24E-06	−
3-136	Pentachloro-phenol	87-86-5	−	−	N.B.	−	N.E.
3-159	Diethyl phthalate	84-66-2	−	−	N.B.	−	N.E.
3-160	Di-n-propylphthalate	131-16-8	−	−	N.D.	−	N.E.
3-162	Di-n-pentyl phthalate	131-18-0	−	−	0.00165	1.02E-07	−
3-163	Di-n-hexyl phthalate	84-75-3	−	−	0.000918	−	N.E.
3-164	Diheptyl phthalate	3648-21-3	−	−	0.00113	−	N.E.
3-173	Diisononyl phthalate	28553-12-0	−	−	0.000325	2.47E-06	N.E.
3-174	1,2-Benzenedicarboxylic acid diisodecyl ester	26761-40-0	−	−	0.000343	5.85E-06	N.E.
3-175	Di-sec-octyl phthalate (= Di(2-ethylhexyl) phthalate)	117-81-7	−	−	0.071	−	−
3-193	Diallyl terephthlate	1026-92-2	−	−	N.B.	1.31E-06	−
3-212	Flutamide	13311-84-7	−	−	N.B.	−	N.E.
3-345	4-Diethylaminobenzaldehyde	120-21-8	+	−	N.B.	−	N.E.
4-011	4,4-Dihydroxydiphenyl	92-88-6	+	+	0.0883	2.13E-07	−
4-018	4'-Hydroxy-4-biphenylcarbonitrile	19812-93-2	−	−	0.00144	1.19E-06	−
4-019	3,3',5,5'-Tetramethyl-(1,1'-biphenyl)-4,4'-diol	2417-04-1	−	−	0.00412	1.65E-06	−
4-023	4-(Phenylmethyl)-phenol	101-53-1	+	+	0.0223	1.14E-06	−
4-024	4,4'-Dihydroxydiphenylmethane	620-92-8	+	+	0.0719	1.68E-07	−
4-029	2,2-Bis(4-hydroxyphenyl)-4-	6807-17-6	+	+	2.79	2.51E-09	−

	methyl-n-pentane						
4-033	4,4'-(Hexafluoroisopropylidene)diphenol	1478-61-1	+	+	0.774	9.15E-09	–
4-034	4,4'-Cyclohexylidenebisphenol	843-55-0	+	+	0.212	1.14E-07	–
4-035	4,4'-(Octahydro-4,7-methano-5H-inden-5-ylidene) bisphenol	1943-97-1	+	+	2.21	4.68E-08	2.44E-07
4-041	4,4'-(1,3-Phenylenediisopropylidene)bisphenol	13595-25-0	+	+	0.175	4.31E-07	–
4-044	4,4'-Sulfonyldiphenol	80-09-1	+	+	0.0055	6.73E-07	–
4-054	1,1,3-Tris(2-methyl-4-hydroxy-5-tert-butylphenyl)butane	1843-03-4	–	–	0.0216	–	–
4-061	4,4'-Dimethoxytriphenylmethane	7500-76-7	–	–	N.D.	–	N.E.
4-103	4-Hydroxybenzophenone	1137-42-4	+	+	0.0108	1.12E-06	–
4-106	4,4'-Dihydroxybenzophenone	611-99-4	+	+	0.017	3.07E-07	–
4-107	2,4-Dihydroxybenzophenone	131-56-6	+	+	0.0139	1.06E-06	–
4-108	2,4,4'-Trihydroxybenzophenone	1470-79-7	+	+	0.0738	1.22E-07	–
4-111	4,4'-Dimethoxybenzophenone	90-96-0	–	–	N.B.	3.24E-06	N.E.
4-112	2,2',4,4'-Tetrahydroxybenzophenone	131-55-5	+	+	0.0925	1.36E-07	–
4-114	4-Fluoro-4'-hydroxybenzophenone	25913-05-7	+	–	0.00314	1.08E-06	–
4-115	2,3,4-Trihydroxybenzophenone	1143-72-2	+	+	0.00883	–	5.04E-06
4-137	4-Hydroxyazobenzene	1689-82-3	+	–	0.0747	1.70E-07	–
4-144	3,3,3',3'-Tetramethyl-1,1'-spirobisindane-5,5',6,6'-tetrol	77-08-7	–	+	0.0999	1.40E-07	–
4-147	4,4'-Thiobis-phenol	2664-63-3	+	+	0.243	3.62E-08	–
4-150	Diphenyl-p-phenylenediamine	74-31-7	+	–	0.0136	2.27E-06	N.E.
4-172	Clomiphene citrate (cis and trans mixture)	50-41-9	+	+	37	3.61E-08	8.30E-08
4-408	3,3'-Dichlorobenzidine dihydrochloride	612-83-9	–	–	0.000441	1.79E-07	N.E.
5-021	2-Naphthol	135-19-3	–	–	0.00105	1.93E-06	N.E.
5-136	Benzoanthrone	82-05-3	–	+	N.B.	1.27E-06	N.E.
6-008	Atrazine	1912-24-9	–	+	N.B.	–	N.E.
6-026	Amitrol	61-82-5	–	–	N.B.	–	N.E.
6-032	Benomyl	17804-35-2	–	–	N.B.	–	N.E.
6-067	Captafol (ISO) (= 1,2,3,6-Tetrahydro-N-(1,1,2,2-tetrachloroethylthio)phthalimide)	2425-06-1	–	–	0.0452	–	–
6-071	N-Cyclohexyl-2-	95-33-0	–	–	0.00464	–	N.E.

	benzothiazolesulfenamide						
6-072	2,2'-Dithiobis[benzothiazole]	120-78-5	−	−	0.0128	−	N.E.
6-074	2-Benzothiazolethiol	149-30-4	−	−	0.00165	−	N.E.
6-087	Hexachlorocyclopentadiene	77-47-4	−	−	0.0108	−	−
—	Triphenyltin chloride	639-58-7	−	+	N.E.	N.E.	N.E.

+: positive, -: negative

N.D.: a test substance whose IC50 value could not be calculated from the tested concentration range but whose maximum tested concentration resulted in detachment of 20% or more of the standard ligand from the receptor.

N.B.: a test substance whose IC50 value could not be calculated from the tested concentration range but whose maximum tested concentration resulted in detachment of less than 20% of the standard ligand from the receptor.

N.E. Not examined

Table 2. List of Results of Uterotrophic Assay on 66 Substances

TestSub No.	Chemicals (CAS.No)	Groups (mg/kg/day)	Body weight (g)	Absolute Organ weight (mg)		Relative Organ weight (mg/100g)		Test _Year
				Uterus wet wt.	Uterus blotted wt.	Uterus wet wt.	Uterus blotted wt.	
1-011	Bis(2-ethylhexyl)adipate (= Di(2-ethylhexyl) adipate) (103-23-1)	V.C.	60.3 ± 3.0	35.3 ± 4.2	34.6 ± 4.0	58.6 ± 7.4	57.4 ± 7.1	FY2001
		40	61.0 ± 3.3	34.8 ± 5.8	34.0 ± 5.6	56.8 ± 7.6	55.6 ± 7.3	
		200	60.9 ± 2.7	32.5 ± 3.6	32.1 ± 3.7	53.3 ± 4.8	52.7 ± 5.1	
		1000	61.3 ± 2.6	36.0 ± 5.1	35.5 ± 5.0	58.5 ± 6.7	57.7 ± 6.6	
		V + EE	61.4 ± 3.1	127.2 ± 19.9**	110.9 ± 12.6**	207.7 ± 34.4**	180.8 ± 20.6**	
		40 + EE	61.0 ± 3.3	131.5 ± 19.5	109.0 ± 11.3	214.6 ± 21.2	178.2 ± 9.4	
		200 + EE	59.4 ± 3.2	131.5 ± 41.8	108.8 ± 15.7	224.3 ± 82.8	184.2 ± 33.6	
		1000 + EE	61.0 ± 4.6	125.6 ± 16.7	110.9 ± 11.3	206.8 ± 29.8	182.2 ± 16.7	
		TMX+EE	58.3 ± 2.7	90.0 ± 7.9##	86.7 ± 7.5##	154.3 ± 10.2#	148.7 ± 8.8#	
1-020	Disulfiram (97-77-8)	V.C.	64.2 ± 3.3	32.2 ± 4.2	31.2 ± 4.1	50.2 ± 7.2	48.8 ± 7.1	FY2004
		100	62.6 ± 4.5	25.4 ± 3.8 *	24.5 ± 3.7 *	40.7 ± 5.9 *	39.1 ± 5.4 *	
		300	60.5 ± 3.3	26.9 ± 3.3 *	26.1 ± 3.1 *	443 ± 4.0	43.1 ± 3.8	
		1,000	61.3 ± 1.0	25.8 ± 1.8 *	25.1 ± 1.4 *	42.2 ± 2.9 *	40.9 ± 2.5 *	
		V + EE	62.1 ± 2.0	129.0 ± 16.8 **	114.0 ± 7.5	207.2 ± 20.3 **	183.3 ± 7.3 **	
		100 + EE	61.1 ± 2.6	113.6 ± 12.6	99.8 ± 8.1 #	185.8 ± 16.6	163.4 ± 12.1 ##	
		300 + EE	56.4 ± 6.5	106.3 ± 24.7	92.5 ± 14.2 ##	190.2 ± 43.4	165.7 ± 29.3	
		1,000 + EE	57.8 ± 4.3	93.6 ± 24.0 #	86.7 ± 17.1 ##	161.1 ± 35.5 #	149.4 ± 23.5 #	
		TMX+EE	60.3 ± 2.8	81.8 ± 8.4 ##	79.9 ± 8.3 ##	135.7 ± 12.3 ##	132.5 ± 12.0 ##	
2-043	17alpha-Methyltestosterone (58-18-4)	V.C.	62.7 ± 2.5	36.3 ± 7.6	34.9 ± 7.0	57.8 ± 11.7	55.7 ± 10.9	FY2001
		2	63.1 ± 4.3	36.9 ± 3.4	35.5 ± 3.1	58.5 ± 4.8	56.4 ± 4.4	
		10	62.7 ± 3.9	62.9 ± 10.0**	60.9 ± 9.7**	100.9 ± 18.5**	97.6 ± 18.0**	
		40	63.3 ± 3.2	100.1 ± 18.6**	96.1 ± 17.4**	158.1 ± 28.4**	151.8 ± 26.4**	
		V + EE	61.9 ± 2.2	137.6 ± 27.2**	115.8 ± 16.1**	222.4 ± 44.2**	187.2 ± 25.8**	
		2 + EE	63.6 ± 3.4	107.3 ± 8.0#	103.3 ± 7.7	168.6 ± 7.0#	162.4 ± 7.0	
		10 + EE	64.4 ± 2.9	122.9 ± 7.7	118.3 ± 7.1	191.1 ± 11.8	184.0 ± 11.8	
		40 + EE	62.2 ± 1.1	134.7 ± 8.2	129.6 ± 7.8	216.6 ± 13.8	208.5 ± 13.3	
		TMX+EE	61.6 ± 3.3	89.5 ± 10.0##	86.8 ± 9.6##	145.2 ± 12.1##	140.8 ± 11.5##	

2-049	Testosterone enanthate (315-37-7)	V.C.	59.7	±	3.0	37.6	±	3.2	36.6	±	3.0	63.1	±	4.7	61.3	±	4.2	FY2001
		2	61.1	±	2.9	31.8	±	3.3*	30.8	±	3.2**	52.3	±	6.5**	50.5	±	6.3**	
		10	61.9	±	2.3	62.9	±	10.1**	60.5	±	9.6**	101.9	±	18.2**	98.1	±	17.3**	
		40	60.5	±	4.0	92.2	±	7.1**	89.0	±	6.7**	152.6	±	10.9**	147.3	±	10.0**	
		V + EE	60.0	±	5.2	145.3	±	28.3**	121.4	±	18.2**	241.1	±	33.9**	201.6	±	16.7**	
		2 + EE	59.7	±	3.3	118.0	±	7.5	108.8	±	6.3	197.9	±	14.1#	182.5	±	12.4 #	
		10 + EE	60.9	±	3.0	127.2	±	13.2	121.7	±	12.6	208.9	±	20.0	199.9	±	19.0	
		40 + EE	61.0	±	2.4	136.4	±	16.4	129.6	±	16.8	223.0	±	19.6	211.9	±	20.4	
		TMX+EE	58.1	±	2.8	89.5	±	7.1##	86.7	±	6.3##	154.2	±	13.4##	149.3	±	11.8##	
3-005	4-n-Amylphenol (14938-35-3)	V.C.	57.8	±	4.8	30.8	±	3.9	29.7	±	3.8	53.2	±	4.8	51.2	±	4.7	FY2001
		100	56.5	±	2.7	26.9	±	2.1	25.8	±	2.3	47.7	±	3.2*	45.8	±	3.8	
		400	56.4	±	2.6	38.0	±	9.7	37.2	±	9.5	67.4	±	16.3	65.9	±	16.1	
		800	51.2	±	3.9 (5)a*	66.4	±	10.0 (5)a**	64.5	±	9.9 (5)a**	129.8	±	17.5(5)a**	126.1	±	18.0(5)a**	
		V + EE	57.1	±	2.0	114.8	±	10.5**	102.2	±	7.5**	201.2	±	17.5**	179.2	±	13.8**	
		100 + EE	57.4	±	3.0	93.0	±	37.0	83.2	±	25.1	160.2	±	56.9	143.7	±	37.1	
		400 + EE	56.8	±	2.7	103.5	±	27.2	93.1	±	19.4	181.4	±	42.8	163.4	±	30.0	
		800 + EE	54.7	±	3.5	121.0	±	33.2	100.3	±	17.7	219.8	±	53.7	182.6	±	24.1	
		TMX+EE	55.7	±	2.5	85.4	±	6.5##	83.2	±	6.1##	153.1	±	7.4##	149.3	±	7.0##	
3-009	4-Dodecyl-phenol (104-43-8)	V.C.	59.0	±	2.6	28.0	±	2.8	27.4	±	3.0	47.5	±	2.8	46.3	±	3.2	FY2001
		8	57.7	±	1.7	29.4	±	2.7	28.9	±	2.9	51.0	±	4.8	50.0	±	4.9	
		40	58.4	±	3.6	38.1	±	7.3*	37.6	±	7.2*	65.4	±	12.0*	64.5	±	11.8*	
		200	56.7	±	3.4	102.6	±	17.2**	97.3	±	15.1**	180.6	±	25.9**	171.5	±	23.8**	
		V + EE	56.3	±	2.6	114.1	±	36.0**	100.3	±	25.6**	201.3	±	54.8**	177.1	±	37.9**	
		8 + EE	56.0	±	2.8	119.2	±	19.1	99.6	±	10.8	212.7	±	30.4	177.7	±	15.4	
		40 + EE	57.2	±	1.6	118.2	±	14.2	100.6	±	11.5	206.4	±	22.9	175.6	±	17.6	
		200 + EE	58.2	±	3.3	146.1	±	40.1	116.1	±	11.7	249.8	±	58.4	199.5	±	15.9	
		TMX+EE	55.6	±	2.6	78.3	±	5.0	76.0	±	4.7	141.1	±	13.2#	137.1	±	12.7#	
3-013	p-(tert-Butyl)phenol (98-54-4)	V.C.	62.2	±	3.6	32.9	±	6.2	32.3	±	6.1	52.7	±	8.2	51.8	±	8.1	FY2002
		100	63.4	±	4.2	43.8	±	7.0*	43.3	±	6.8*	68.8	±	7.8**	68.1	±	7.6**	
		300	63.2	±	5.5	59.0	±	9.2**	58.1	±	8.9**	93.2	±	10.9**	91.8	±	10.6**	
		1,000	61.2	±	5.4	79.7	±	13.7**	78.6	±	13.3**	130.7	±	21.5**	128.9	±	21.3**	

		V + EE	63.7	±	3.0	157.5	±	39.4**	121.9	±	11.6**	246.9	±	57.1**	191.3	±	13.0**	
		100+EE	62.7	±	3.6	174.0	±	20.7	129.3	±	13.0	278.0	±	33.3	206.2	±	16.5	
		300+EE	62.2	±	5.0	124.9	±	15.7	109.4	±	8.9	200.6	±	18.4	175.9	±	7.5#	
		1,000+EE	60.3	±	4.4	101.8	±	29.4#	95.2	±	17.8#	166.9	±	34.7#	156.9	±	18.1##	
		TMX+EE	62.7	±	4.3	98.4	±	10.8#	97.1	±	10.4##	156.9	±	14.2#	154.9	±	14.0##	
3-014	p-(tert-Amyl)phenol (80-46-6)	V.C.	57.4	±	3.1	30.1	±	3.6	29.4	±	3.6	52.4	±	5.1	51.2	±	4.9	FY2001
		8	57.3	±	2.7	28.6	±	2.4	27.8	±	2.1	49.9	±	3.9	48.6	±	3.5	
		40	57.9	±	1.9	34.5	±	7.6	33.8	±	7.8	59.7	±	13.2	58.4	±	13.6	
		200	53.6	±	2.1*	73.9	±	7.2**	72.2	±	7.1**	137.6	±	10.1**	134.5	±	9.7**	
		V + EE	58.3	±	1.9	133.0	±	12.9**	110.8	±	6.2**	228.5	±	25.0**	190.4	±	13.8**	
		8 + EE	57.2	±	2.9	122.2	±	31.0	103.2	±	17.4	213.7	±	52.0	180.8	±	31.1	
		40 + EE	58.0	±	2.1	136.1	±	22.2	108.2	±	12.6	234.7	±	37.0	186.5	±	20.4	
		200 + EE	56.6	±	3.9	98.6	±	15.7##	91.2	±	9.0 ##	173.6	±	18.9##	161.0	±	10.9 ##	
		TMX+EE	56.4	±	0.9	87.4	±	3.0##	85.1	±	2.6##	155.1	±	5.7##	151.1	±	4.9##	

Mean ± S.D.
EE; ethynyl estradiol, TMX; tamoxifen
* Significantly different from vehicle control at P<0.05, ** Significantly different from vehicle control at P<0.01
# Significantly different from vehicle control + EE at P<0.05, ## Significantly different from vehicle control + EE at P<0.01
( )a Number of animals used for statistical analysis.

		V.C.	56.6	±	2.4	28.6	±	1.4	27.9	±	1.1	50.6	±	3.0	49.5	±	2.6	
		8	55.2	±	2.2	29.0	±	2.5	27.9	±	2.9	52.5	±	4.8	50.6	±	5.2	
		40	55.6	±	1.5	30.5	±	4.9	29.7	±	4.5	54.8	±	9.3	53.4	±	8.6	
		200	55.2	±	2.5	61.7	±	7.7**	60.2	±	7.9**	111.5	±	11.4**	108.9	±	11.8**	
3-019	4-Cyclohexyl phenol(1131-60-8)	V + EE	56.1	±	1.3	105.2	±	8.1**	96.8	±	5.9**	187.9	±	17.1**	172.7	±	11.5**	FY2001
		8 + EE	54.4	±	3.3	109.2	±	11.1	100.4	±	9.6	201.2	±	23.5	184.8	±	17.4	
		40 + EE	54.9	±	1.9	120.3	±	25.1	103.3	±	18.3	219.4	±	47.0	188.3	±	34.1	
		200 + EE	55.2	±	3.0	98.4	±	16.8	92.1	±	12.6	178.2	±	27.2	166.8	±	19.9	
		TMX+EE	54.6	±	3.1	82.6	±	4.2##	81.2	±	4.2##	152.0	±	14.1##	149.4	±	14.1#	
3-020	4-(1-Adamantyl) phenol (29799-07-3)	V.C.	59.0	±	3.7	32.0	±	3.6	31.4	±	3.3	54.2	±	4.3	53.1	±	4.0	FY2001
		8	57.4	±	3.1	53.4	±	14.3*	52.0	±	14.0*	93.0	±	24.2*	90.5	±	23.7*	
		40	57.5	±	2.9	108.5	±	21.9**	102.3	±	15.5**	189.2	±	40.9**	178.1	±	27.8**	
		200	54.9	±	4.2	335.3	±	38.3**	138.4	±	7.9**	616.6	±	105.2**	252.8	±	17.0**	

		V + EE	59.3	±	3.2	135.5	±	24.4**	112.9	±	18.6**	229.1	±	42.4**	191.0	±	33.0**	
		8 + EE	58.6	±	3.6	156.8	±	32.2	119.6	±	18..0	268.3	±	54.1	204.3	±	27.1	
		40 + EE	58.6	±	3.5	142.7	±	68.0	112.7	±	20.7	241.2	±	103.0	192.0	±	28.0	
		200 + EE	58.0	±	5.1	307.0	±	33.2##	132.7	±	7.7#	534.8	±	91.5##	230.6	±	26.7#	
		TMX+EE	57.8	±	3.2	87.2	±	10.2##	85.5	±	10.2#	151.2	±	19.3##	148.4	±	19.4#	
3-030	2,4-Di-tert-butylphenol (96-76-4)	V.C.	60.2	±	5.3	37.0	±	8.9	35.8	±	8.7	61.5	±	14.4	59.4	±	14.1	FY2003
		100	61.5	±	5.0	33.3	±	7.8	32.2	±	7.7	54.1	±	11.8	52.3	±	11.6	
		300	63.0	±	4.6	36.0	±	7.0	34.9	±	6.9	57.4	±	11.8	55.7	±	11.8	
		1,000	58.8	±	3.9	28.5	±	2.1	27.7	±	2.0	48.4	±	2.5	47.2	±	2.4	
		V + EE	63.8	±	5.2	116.9	±	7.9 **	104.5	±	7.7 **	184.1	±	16.5 **	164.2	±	11.0 **	
		100 + EE	62.4	±	5.9	135.9	±	26.4	116.0	±	16.4	216.9	±	29.0 #	185.8	±	18.6 #	
		300 + EE	62.0	±	4.7	106.6	±	21.1	97.5	±	16.8	172.8	±	35.6	157.8	±	27.4	
		1,000 + EE	58.7	±	3.2	123.1	±	25.6	106.9	±	14.9	210.1	±	43.9	182.2	±	24.1	
		TMX+EE	61.5	±	4.1	85.3	±	5.6 ##	83.7	±	6.0 ##	139.0	±	10.6 ##	136.4	±	10.6 ##	
3-041	p-Hydroxybenzoic acid (99-96-7)	V.C.	61.8	±	3.9	40.7	±	8.8	40.1	±	8.7	65.6	±	12.2	64.6	±	12.0	FY2003
		100	59.2	±	5.2	44.9	±	11.8	44.4	±	11.9	75.2	±	15.1	74.3	±	15.2	
		300	62.8	±	6.2	43.6	±	3.9	43.1	±	3.8	70.0	±	8.5	69.2	±	8.4	
		1,000	60.4	±	4.7	35.8	±	3.6	35.5	±	3.6	59.6	±	7.1	59.0	±	7.0	
		V + EE	60.6	±	4.5	167.2	±	29.2 **	136.7	±	14.9 **	275.0	±	37.2 **	225.6	±	16.9 **	
		100 + EE	58.3	±	2.8	132.1	±	23.3 #	117.6	±	12.4 #	226.1	±	32.2 #	201.6	±	14.7 #	
		300 + EE	59.7	±	3.1	166.9	±	34.8	137.9	±	12.3	279.6	±	55.3	231.5	±	24.0	
		1,000 + EE	59.1	±	4.4	143.5	±	38.4	123.8	±	21.1	241.8	±	54.2	209.0	±	26.8	
		TMX+EE	58.9	±	2.7	90.4	±	5.7 ##	89.8	±	5.5 ##	153.7	±	9.7 ##	152.7	±	9.5 ##	
3-042	Ethyl-p-hydroxybenzoate (120-47-8)	V.C.	59.7	±	3.8	43.5	±	18.0	42.5	±	17.6	72.2	±	27.4	70.4	±	26.7	FY2003
		100	58.3	±	3.0	35.7	±	4.9	34.8	±	4.9	61.2	±	6.8	59.6	±	7.0	
		300	59.8	±	5.4	37.0	±	6.5	36.1	±	6.4	62.1	±	10.3	60.5	±	10.0	
		1,000	58.3	±	4.2	49.2	±	9.7	48.2	±	9.3	84.4	±	16.3	82.6	±	15.5	
		V + EE	58.2	±	3.1	164.3	±	24.9 **	127.0	±	10.1 **	282.2	±	40.5 **	218.3	±	15.6 **	
		100 + EE	59.0	±	4.7	157.9	±	28.8	123.7	±	14.1	267.7	±	41.9	210.3	±	22.2	
		300 + EE	59.0	±	3.1	179.0	±	40.3	130.3	±	10.7	302.7	±	60.9	220.9	±	16.0	
		1,000 + EE	59.8	±	2.8	168.0	±	33.5	128.5	±	24.7	281.2	±	54.8	215.6	±	43.9	

		TMX+EE	58.0	±	4.7	89.5	±	8.1 ##	87.7	±	8.1 ##	154.7	±	12.5 ##	151.6	±	12.9 ##	
3-046	2-Ethylhexyl 4-hydroxybenzoate (5153-25-3)	V.C.	60.7	±	3.4	37.7	±	4.3	37.2	±	4.2	62.5	±	9.2	61.6	±	9.2	FY2002
		8	60.7	±	2.8	41.6	±	7.2	41.2	±	7.2	68.4	±	10.2	67.7	±	10.1	
		40	59.8	±	2.4	43.5	±	4.9	42.9	±	4.9	73.0	±	10.3	72.0	±	10.3	
		200	59.5	±	3.5	88.2	±	11.3**	86.1	±	10.8**	148.8	±	22.1**	145.2	±	20.5**	
		V + EE	61.3	±	2.0	156.3	±	20.1**	124.4	±	8.2**	255.5	±	37.2**	203.2	±	17.2**	
		8+EE	58.2	±	8.2	162.9	±	34.6	125.3	±	19.3	281.3	±	53.1	217.3	±	33.0	
		40+EE	61.5	±	1.9	157.2	±	9.8	131.9	±	6.9	255.8	±	13.1	214.7	±	12.5	
		200+EE	60.7	±	3.8	105.1	±	15.6##	101.6	±	15.1##	172.7	±	19.4##	167.0	±	18.4##	
		TMX+EE	58.5	±	2.0	89.8	±	3.0##	89.0	±	2.8##	153.8	±	9.1##	152.4	±	8.7##	
3-062	4-tert-Butyl catechol (98-29-3)	V.C.	63.9	±	4.0	38.6	±	6.9	38.0	±	6.8	60.7	±	12.4	59.7	±	12.2	FY2002
		100	61.8	±	2.5	40.2	±	7.4	39.5	±	7.2	64.9	±	10.4	63.9	±	10.0	
		300	59.8	±	4.1	43.4	±	5.5	42.9	±	5.6	72.6	±	7.8	71.7	±	7.9	
		1,000	50.7	±	5.9**	56.8	±	8.3**	56.0	±	8.2**	112.5	±	13.3**	111.1	±	13.5**	
		V + EE	62.4	±	3.2	153.5	±	18.6**	116.1	±	8.7**	246.5	±	32.6**	186.1	±	12.9**	
		100+EE	62.9	±	2.7	148.1	±	50.3	112.1	±	22.8	234.3	±	74.2	177.8	±	32.7	
		300+EE	59.1	±	3.3	96.4	±	21.1##	91.0	±	17.2##	164.3	±	40.8##	155.0	±	34.1	
		1,000+EE	53.7	±	4.6##	63.4	±	7.0##	62.6	±	7.0##	118.5	±	14.5##	116.9	±	14.3##	
		TMX+EE	62.7	±	3.8	91.7	±	4.9##	90.4	±	5.1##	146.5	±	7.2##	144.3	±	7.2##	
3-103	p-Dichlorobenzene (106-46-7)	V.C.	59.9	±	4.0	37.9	±	8.3	36.8	±	8.1	62.8	±	9.9	61.1	±	9.7	FY2002
		40	60.1	±	2.7	34.6	±	5.2	33.5	±	5.2	57.6	±	8.0	55.7	±	8.0	
		200	60.4	±	2.7	34.4	±	5.0	33.5	±	5.1	57.1	±	8.7	55.5	±	8.8	
		1,000	58.2	±	4.1	29.3	±	3.8*	28.4	±	3.7*	50.3	±	5.2*	48.8	±	4.9*	
		V + EE	60.5	±	1.2	141.4	±	42.8**	113.1	±	24.5**	233.6	±	70.5**	186.7	±	39.7**	
		40+EE	60.0	±	2.7	146.4	±	23.7	119.8	±	14.2	243.9	±	36.7	199.9	±	22.8	
		200+EE	60.5	±	2.4	140.6	±	12.3	116.8	±	6.1	232.8	±	23.8	193.2	±	12.8	
		1,000+EE	59.3	±	3.2	130.6	±	25.6	108.3	±	17.4	221.9	±	50.2	183.8	±	35.1	
		TMX+EE	60.3	±	1.5	91.5	±	9.7#	89.0	±	10.3	151.8	±	14.9#	147.6	±	15.6	

Mean ± S.D.
EE; ethynyl estradiol, TMX; tamoxifen
* Significantly different from vehicle control at P<0.05, ** Significantly different from vehicle control at P<0.01
# Significantly different from vehicle control + EE at P<0.05, ## Significantly different from vehicle control + EE at P<0.01
( )a Number of animals used for statistical analysis.

		V.C.	60.4	±	5.5	36.2	±	5.1	35.2	±	4.9	60.0	±	7.6	58.4	±	7.0	
		30	59.4	±	5.0	36.0	±	5.4	35.3	±	5.3	60.6	±	8.0	59.6	±	7.8	
		100	51.4 (1)a			27.9 (1)			27.3 (1)			54.3 (1)			53.1 (1)			
		300	—			—			—			—			—			
3-136	Pentachloro-phenol(87-86-5)	V + EE	60.7	±	5.3	154.6	±	46.6**	119.8	±	21.1**	254.1	±	70.5**	197.2	±	28.9**	FY2002
		30+EE	57.4	±	4.2	132.8	±	44.3	107.7	±	24.3	229.1	±	65.5	187.0	±	37.2	
		100+EE	52.5	±	3.1 (3)	102.7	±	29.6 (3)	91.3	±	18.5 (3)	194.0	±	45.1 (3)	173.1	±	25.4 (3)	
		300+EE	—			—			—			—			—			
		TMX+EE	56.5	±	3.2	93.4	±	6.9#	91.8	±	6.8#	165.5	±	11.0#	162.6	±	10.8#	
		V.C.	62.0	±	3.4	33.4	±	1.8	32.5	±	1.8	54.0	±	2.4	52.5	±	3.0	
		40	61.4	±	3.0	36.4	±	6.0	35.5	±	5.4	59.0	±	7.3	57.5	±	6.4	
		200	60.6	±	2.9	35.1	±	4.9	34.4	±	5.0	58.0	±	8.3	56.9	±	8.6	
		1000	61.4	±	2.4	32.8	±	4.6	32.1	±	4.6	53.5	±	7.9	52.4	±	7.9	
3-159	Diethyl phthalate (84-66-2)	V + EE	61.2	±	3.3	141.8	±	20.3**	117.1	±	11.1**	232.0	±	32.7**	191.7	±	18.3**	FY2001
		40 + EE	63.2	±	2.8	146.6	±	23.4	122.9	±	16.7	231.5	±	30.3	194.3	±	22.0	
		200 + EE	62.0	±	2.5(5)a	134.3	±	15.4 (5)a	112.8	±	7.8 (5)a	217.1	±	30.6 (5)a	182.0	±	13.7 (5)a	
		1000 + EE	61.0	±	3.4	133.9	±	18.1 (5)a	112.8	±	12.0 (5)a	223.3	±	34.6 (5)a	187.9	±	24.0 (5)a	
		TMX+EE	59.1	±	2.7	85.4	±	3.2##	82.7	±	3.2##	144.8	±	7.1##	140.2	±	6.9##	
		V.C.	63.8	±	4.2	33.5	±	5.0	33.2	±	4.9	52.4	±	5.3	51.9	±	5.3	
		100	64.2	±	1.2	39.0	±	5.6	38.6	±	5.6	60.6	±	7.8	59.9	±	7.8	
		300	64.7	±	3.2	42.8	±	9.2	42.3	±	9.3	65.7	±	11.5*	65.0	±	11.7*	
		1,000	63.9	±	3.2	41.1	±	8.7	40.6	±	8.5	64.2	±	13.1	63.5	±	12.8	
3-160	Di-n-propylphthalate (131-16-8)	V + EE	64.5	±	5.6	152.0	±	48.0**	119.8	±	23.5**	232.4	±	58.3**	184.8	±	27.2**	FY2002
		100+EE	63.0	±	3.6	169.3	±	20.8	130.8	±	5.8	270.2	±	41.1	208.5	±	17.7	
		300+EE	60.2	±	3.2	144.5	±	11.3(5)a	127.1	±	15.2	243.9	±	12.0(5)	210.5	±	14.8	
		1,000+EE	62.3	±	5.3	181.6	±	23.2	139.9	±	10.4	293.6	±	48.0	225.4	±	19.6#	
		TMX+EE	62.5	±	3.9	95.7	±	5.2#	93.9	±	5.1#	153.8	±	16.9#	150.9	±	16.4#	

		V.C.	60.9	±	3.8	44.4	±	6.1	43.0	±	5.9	73.0	±	8.2	70.6	±	7.8	
		100	61.4	±	3.4	37.9	±	8.1	36.9	±	7.8	61.4	±	10.6	59.8	±	10.1	
		300	60.5	±	4.2	40.5	±	8.0	39.4	±	7.7	66.9	±	11.4	65.0	±	10.9	
		1,000	59.5	±	4.3	34.0	±	4.1**	33.0	±	4.0**	57.4	±	7.7**	55.7	±	7.4**	
3-162	Di-n-pentylphthalate (131-18-0)	V + EE	61.1	±	5.4	167.5	±	22.5**	126.2	±	14.0**	274.9	±	35.2**	207.5	±	26.3**	FY2002
		100+EE	60.2	±	3.0	156.3	±	26.3	121.4	±	9.5	261.0	±	52.5	202.2	±	20.9	
		300+EE	60.3	±	3.6	150.6	±	43.9	114.5	±	24.7	248.5	±	66.8	189.5	±	37.1	
		1,000+EE	59.4	±	3.6	158.1	±	31.0	124.4	±	12.8	266.0	±	48.9	209.6	±	19.7	
		TMX+EE	60.2	±	2.6	87.3	±	1.8##	85.2	±	1.4##	145.3	±	5.5##	141.8	±	5.5##	
		V.C.	58.8	±	5.6	40.5	±	8.9	39.6	±	8.7	68.3	±	10.2	66.8	±	10.2	
		100	62.8	±	4.2	39.9	±	6.7	39.0	±	6.7	63.7	±	10.5	62.3	±	10.4	
		300	63.4	±	4.9	38.4	±	6.1	37.5	±	6.2	60.7	±	9.3	59.3	±	9.5	
		1,000	59.8	±	2.3	36.3	±	4.5	35.4	±	4.3	60.7	±	7.7	59.2	±	7.4	
3-163	Di-n-hexylphthalate (84-75-3)	V + EE	60.9	±	4.0	143.6	±	23.8**	122.7	±	15.7**	237.4	±	46.1**	202.2	±	28.5**	FY2002
		100+EE	59.8	±	4.9	154.7	±	24.5	121.2	±	13.9	258.2	±	28.7	203.3	±	22.6	
		300+EE	61.4	±	4.0	166.3	±	19.0	130.4	±	6.8	270.8	±	23.9	213.1	±	17.1	
		1,000+EE	62.7	±	5.7	149.1	±	25.3	121.9	±	13.3	238.8	±	43.1	195.3	±	23.3	
		TMX+EE	60.6	±	2.3	85.6	±	8.3##	84.0	±	8.2##	141.4	±	14.9##	138.8	±	14.8##	
		V.C.	62.3	±	3.9	31.4	±	4.2	30.9	±	4.2	50.3	±	5.2	49.5	±	5.1	
		100	61.2	±	2.5	32.2	±	5.6	31.5	±	5.7	52.6	±	8.2	51.4	±	8.5	
		300	63.7	±	3.2	29.6	±	2.3	28.7	±	2.6	46.6	±	5.4	45.3	±	5.7	
		1,000	64.0	±	3.9	36.1	±	9.8	35.4	±	9.7	56.0	±	11.7	54.9	±	11.5	
3-164	Diheptyl phthalate (3648-21-3)	V + EE	60.5	±	3.4	127.0	±	9.0 **	115.9	±	8.3 **	210.1	±	13.8 **	191.5	±	9.1 **	FY2003
		100 + EE	63.5	±	4.5	125.8	±	13.2	117.4	±	9.9	198.9	±	22.9	185.6	±	17.8	
		300 + EE	62.6	±	4.8	120.3	±	26.7	110.5	±	22.2	191.0	±	36.7	175.7	±	29.7	
		1,000 + EE	62.3	±	4.0	123.4	±	12.2	114.6	±	10.5	198.3	±	19.2	184.2	±	18.3	
		TMX+EE	60.7	±	2.6	82.6	±	5.7 ##	81.5	±	5.7 ##	136.0	±	9.2 ##	134.3	±	8.7 ##	
3-173	Diisononyl phthalate (28553-12-0)	V.C.	60.5	±	4.1	35.1	±	10.4	34.0	±	10.0	57.7	±	15.2	55.8	±	14.5	FY2003
		100	61.3	±	3.9	34.5	±	5.0	33.2	±	4.9	56.1	±	5.9	54.0	±	5.7	
		300	60.9	±	3.7	29.0	±	2.1	28.2	±	2.2	47.7	±	2.7	46.3	±	3.0	
		1,000	60.5	±	3.8	30.5	±	2.2	29.5	±	2.2	50.6	±	2.9	48.8	±	3.1	

		V + EE	60.8	±	2.8	107.5	±	7.0 **	101.9	±	6.0 **	177.0	±	15.2 **	168.0	±	14.2 **	
		100 + EE	62.2	±	3.7	112.1	±	13.5	105.2	±	14.1	180.0	±	17.0	168.8	±	17.7	
		300 + EE	62.0	±	3.2	119.6	±	21.5	105.8	±	13.9	192.9	±	32.5	171.1	±	23.3	
		1,000 + EE	59.6	±	3.8	110.6	±	7.9	.105.3	±	6.5	186.0	±	13.8	177.1	±	11.3	
		TMX+EE	59.5	±	3.9	85.4	±	8.1 ##	83.2	±	8.2 ##	143.3	±	6.0 ##	139.7	±	6.5 ##	
3-174	1,2-Benzenedicarboxylic acid diisodecyl ester (26761-40-0)	V.C.	61.6	±	3.4	34.5	±	3.2	33.8	±	3.2	56.0	±	4.8	54.9	±	4.5	
		100	60.4	±	2.7	36.0	±	5.5	35.1	±	5.5	59.6	±	8.8	58.2	±	8.7	
		300	62.3	±	3.2	34.4	±	5.8	33.5	±	5.9	55.1	±	7.8	53.6	±	7.8	
		1,000	61.6	±	3.6	31.6	±	2.7	30.9	±	2.6	51.3	±	2.6	50.2	±	2.5 *	
		V + EE	61.5	±	3.0	167.6	±	33.5 **	134.7	±	18.1 **	271.2	±	41.7 **	218.4	±	19.4 **	FY2003
		100 + EE	63.5	±	4.7	153.9	±	12.3	128.4	±	10.4	243.2	±	20.2	202.9	±	17.3	
		300 + EE	61.1	±	2.9	156.7	±	43.2	120.3	±	25.9	257.3	±	71.4	197.8	±	44.8	
		1,000 + EE	60.6	±	3.2	163.2	±	34.8	127.2	±	18.8	268.0	±	46.8	209.3	±	22.4	
		TMX+EE	61.6	±	4.3	93.5	±	4.9 ##	91.4	±	4.9 ##	152.5	±	14.2 ##	149.2	±	14 .0 ##	

Mean ± S.D.
EE; ethynyl estradiol, TMX; tamoxifen
* Significantly different from vehicle control at P<0.05, ** Significantly different from vehicle control at P<0.01
# Significantly different from vehicle control + EE at P<0.05, ## Significantly different from vehicle control + EE at P<0.01
( )a Number of animals used for statistical analysis.

3-175	Di-sec-octyl phthalate(= Di(2-ethylhexyl) phthalate)(117-81-7)	V.C.	56.7	±	2.1	31.6	±	4.7	30.6	±	4.5	55.6	±	8.1	53.9	±	7.7	
		40	57.1	±	2.7	29.5	±	4.2	28.5	±	4.3	51.5	±	5.7	49.7	±	6.0	
		200	56.8	±	2.5	29.1	±	2.7	28.3	±	2.6	51.4	±	6.0	50.0	±	5.9	
		1000	57.6	±	3.8	29.2	±	1.9	28.5	±	1.9	50.8	±	3.7	49.6	±	4.0	
		V + EE	56.8	±	4.4	131.3	±	28.3**	109.2	±	16.2**	232.4	±	51.7**	193.1	±	29.9**	FY2001
		40 + EE	56.7	±	3.2	145.6	±	31.0	116.4	±	18.2	255.9	±	46.4	205.0	±	26.2	
		200 + EE	55.8	±	3.1	108.2	±	15.8	98.8	±	12.1	195.0	±	36.6	177.8	±	26.7	
		1000 + EE	58.0	±	1.3	122.4	±	13.9	110.4	±	10.5	211.0	±	20.8	190.4	±	15.9	
		TMX+EE	54.5	±	2.3	86.7	±	9.0#	85.0	±	8.3##	158.8	±	11.5#	155.6	±	10.2#	
3-193	Diallyl terephthlate (1026-92-2)	V.C.	62.6	±	4.0	33.3	±	5.3	32.9	±	5.1	53.4	±	9.2	52.7	±	8.8	FY2002
		20	61.8	±	3.9	33.2	±	4.4	32.8	±	4.3	53.6	±	5.0	52.9	±	4.9	
		60	63.0	±	2.8	33.5	±	4.2	33.0	±	4.2	53.2	±	6.6	52.5	±	6.6	
		200	54.3	±	6.8*	30.5	±	6.2	30.2	±	6.2	56.0	±	7.8	55.3	±	7.8	

		V + EE	62.2	±	4.6	153.0	±	24.7**	124.1	±	13.9**	248.7	±	55.5**	201.2	±	35.7**	
		20+EE	63.2	±	4.3	157.9	±	26.1	122.6	±	13.6	250.6	±	43.8	194.7	±	24.1	
		60+EE	61.5	±	5.1	175.8	±	22.7	133.1	±	11.6	289.4	±	59.6	218.2	±	32.1	
		200+EE	59.4	±	5.8	136.5	±	20.3	114.5	±	11.3	230.9	±	35.8	193.4	±	17.0	
		TMX+EE	61.7	±	3.0	86.4	±	5.0##	85.1	±	5.3##	140.2	±	7.0##	138.1	±	7.6##	
3-212	Fluthamide (13311-84-7)	V.C.	57.1	±	5.2	30.4	±	4.3	29.6	±	4.5	53.5	±	8.1	52.2	±	8.5	
		20	56.9	±	4.6	29.3	±	1.9	28.5	±	2.0	51.6	±	3.4	50.2	±	3.5	
		60	53.9	±	4.6	26.7	±	4.7	26.0	±	4.8	49.3	±	6.4	48.1	±	6.7	
		200	42.7	±	8.8 **	25.2	±	2.5 *	24.5	±	2.4 *	61.1	±	13.7	59.6	±	13.2	
		V + EE	57.1	±	5.1	152.1	±	16.1 **	122.5	±	7.4 **	268.4	±	38.8 **	215.8	±	19.1 **	FY2004
		20 + EE	57.4	±	3.4	138.8	±	26.5	116.8	±	16.5	241.0	±	36.2	203.0	±	19.9	
		60 + EE	55.2	±	4.6	167.3	±	17.9	123.3	±	6.7	306.4	±	52.4	225.0	±	25.6	
		200 + EE	45.7	±	4.2 #	140.0	±	60.2	97.9	±	19.0	300.2	±	102.7	213.0	±	21.6	
		TMX+EE	56.6	±	4.9	91.6	±	6.1 ##	89.6	±	5.8 ##	162.2	±	9.1 ##	158.7	±	9.1 ##	
3-345	4-Diethylaminobenzaldehyde (120-21-8)	V.C.	66.5	±	3.1	35.5	±	3.8	34.7	±	3.6	53.5	±	5.5	52.2	±	5.3	
		60	64.5	±	1.9	37.2	±	6.6	36.3	±	6.5	57.7	±	10.4	56.2	±	10.3	
		200	65.3	±	2.2	47.4	±	10.3 *	46.5	±	10.1 *	72.4	±	14.0 *	70.9	±	13.7 *	
		600	64.6	±	2.8	42.8	±	5.1 *	42.0	±	5.2 *	66.2	±	5.2 **	64.9	±	5.4 *	
		V + EE	67.2	±	2.8	152.8	±	34.0 **	132.5	±	25.6 **	226.4	±	45.8 **	196.6	±	34.4 **	FY2004
		60 + EE	65.6	±	4.0	168.0	±	26.5	133.5	±	14.3	256.9	±	42.0	203.7	±	19.2	
		200 + EE	65.0	±	2.4	144.6	±	32.4	125.1	±	19.9	221.9	±	45.6	192.0	±	25.9	
		600 + EE	62.9	±	4.6	151.3	±	27.2	126.4	±	16.2	243.7	±	57.6	202.9	±	37.4	
		TMX+EE	64.8	±	3.5	98.5	±	8.5 #	96.4	±	8.2 #	152.5	±	17.8 ##	149.3	±	17.3 #	
4-011	4,4-Dihydroxydiphenyl (92-88-6)	V.C.	60.9	±	2.9	37.4	±	5.0	36.2	±	4.7	61.4	±	7.1	59.4	±	6.6	FY2002
		60	57.7	±	2.3	47.8	±	7.9*	46.8	±	7.7*	82.8	±	12.3**	81.0	±	12.0**	
		200	59.3	±	4.2	85.3	±	15.4**	83.0	±	15.3**	143.0	±	16.6**	139.1	±	16.6**	
		600	59.0	±	3.3	185.4	±	83.8**	130.9	±	21.7**	314.7	±	142.4**	222.3	±	38.0**	
		V + EE	59.8	±	3.3	148.4	±	12.7**	121.2	±	9.1**	249.1	±	29.3**	203.4	±	22.0**	
		60+EE	60.1	±	2.6	118.0	±	9.9##	103.0	±	6.4##	196.3	±	9.7##	171.5	±	7.1#	
		200+EE	58.8	±	5.1	107.4	±	48.8	92.4	±	25.1#	180.1	±	74.8	155.4	±	32.2#	
		600+EE	57.3	±	1.5	230.8	±	99.0	126.7	±	18.7	400.8	±	170.0	220.8	±	30.6	

		TMX+EE	58.4	±	2.6	84.2	±	7.3##	82.0	±	6.9##	144.7	±	17.4##	140.9	±	16.9##	
4-018	4'-Hydroxy-4-biphenylcarbonitrile (19812-93-2)	V.C.	62.2	±	2.7	56.4	±	21.7	55.5	±	21.5	91.9	±	38.2	90.5	±	37.9	
		100	61.0	±	3.9	31.9	±	4.7 *	31.2	±	4.7 *	52.6	±	8.6	51.4	±	8.6	
		300	62.0	±	5.3	39.5	±	3.3	38.8	±	3.2	64.2	±	9.5	63.1	±	9.0	
		1,000	60.7	±	5.4	75.7	±	23.8	74.4	±	23.5	127.1	±	47.5	125.0	±	46.9	
		V + EE	60.3	±	2.9	142.1	±	18.2 **	123.4	±	12.5 **	235.8	±	28.4 **	204.8	±	19.7 **	FY2004
		100 + EE	60.5	±	4.4	173.2	±	20.5 #	130.8	±	15.4	285.9	±	19.8 ##	216.1	±	17.5	
		300 + EE	62.5	±	2.8	145.9	±	29.1	124.4	±	11.8	233.3	±	41.8	199.4	±	18.7	
		1,000 + EE	58.5	±	3.6	177.8	±	39.0	136.6	±	19.2	304.0	±	67.3	233.2	±	27.0	
		TMX+EE	59.5	±	3.8	85.7	±	6.7 ##	84.6	±	6.5 ##	144.3	±	12.5 ##	142.5	±	12.3 ##	
4-019	3,3'5,5'-Tetramethyl-(1,1'-biphenyl)-4,4'-diol (2417-04-1)	V.C.	58.0	±	2.7	33.8	±	8.6	32.8	±	8.6	58.3	±	15.2	56.5	±	15.1	
		100	56.8	±	4.4	32.0	±	3.5	31.2	±	3.5	56.4	±	6.5	55.0	±	6.2	
		300	57.1	±	3.4	44.7	±	10.0	43.7	±	10.0	78.0	±	15.7	76.2	±	15.7	
		1,000	56.9	±	5.9	33.5	±	3.6	32.6	±	3.8	59.3	±	7.5	57.7	±	7.7	
		V + EE	58.3	±	5.9	154.7	±	34.8 **	124.9	±	21.3 **	263.2	±	35.6 **	213.2	±	19.1 **	FY2003
		100 + EE	57.0	±	4.1	129.3	±	46.3	106.4	±	29.5	228.5	±	85.9	187.6	±	54.6	
		300 + EE	55.9	±	3.3	126.8	±	29.1	110.3	±	12.4	225.2	±	39.3	196.8	±	12.5	
		1,000 + EE	56.7	±	2.9	139.3	±	22.7	115.4	±	12.6	245.7	±	36.1	203.8	±	22.0	
		TMX+EE	57.4	±	4.7	90.9	±	6.8 ##	88.6	±	6.4 ##	158.6	±	9.7 ##	154.8	±	10.8 ##	
4-023	4-(Phenylmethyl)-phenol (101-53-1)	V.C.	61.1	±	4.8	34.6	±	7.5	33.2	±	8.1	56.4	±	10.0	54.1	±	10.7	
		8	62.5	±	4.2	34.0	±	5.0	32.9	±	5.3	54.2	±	5.8	52.4	±	6.3	
		40	60.9	±	3.2	36.7	±	4.8	36.0	±	4.9	60.4	±	7.9	59.1	±	7.9	
		200	60.4	±	4.5	60.1	±	5.7**	58.3	±	5.6**	99.7	±	9.7**	96.8	±	9.2**	
		V + EE	62.9	±	2.9	141.3	±	23.6**	119.4	±	11.8**	224.4	±	35.2**	190.0	±	17.6**	FY2001
		8 + EE	62.1	±	3.3	158.4	±	15.8	130.7	±	8.7	255.1	±	21.0	210.6	±	12.6 #	
		40 + EE	62.8	±	3.5	142.8	±	16.3	120.6	±	9.1	227.2	±	21.0	192.3	±	13.6	
		200 + EE	62.3	±	3.3	110.0	±	20.0#	101.2	±	14.0 #	177.7	±	37.1#	163.6	±	28.4	
		TMX+EE	60.3	±	3.3	84.2	±	8.3##	81.6	±	8.0##	139.9	±	12.1##	135.5	±	11.9##	

Mean ± S.D.
EE; ethynyl estradiol, TMX; tamoxifen
* Significantly different from vehicle control at P<0.05, ** Significantly different from vehicle control at P<0.01
# Significantly different from vehicle control + EE at P<0.05, ## Significantly different from vehicle control + EE at P<0.01
( )a Number of animals used for statistical analysis.

4-024	4,4'-Dihydroxy diphenylmethane(620-92-8)	V.C.	61.4	±	3.8	30.9	±	3.9	30.3	±	3.8	50.5	±	7.6	49.6	±	7.4	FY2002
		100	59.8	±	2.0	41.5	±	2.9**	40.8	±	2.7**	69.4	±	4.1**	68.3	±	3.9**	
		300	59.1	±	2.7	52.5	±	3.9**	51.8	±	3.8**	88.9	±	5.3**	87.7	±	5.0**	
		1,000	58.9	±	2.7	134.8	±	31.2**	115.1	±	13.6**	228.8	±	52.5**	195.3	±	20.7**	
		V + EE	60.9	±	4.1	134.6	±	15.1**	109.6	±	9.0**	221.4	±	25.6**	180.4	±	15.8**	
		100 + EE	62.8	±	3.4	100.0	±	4.1##	95.4	±	3.9##	159.5	±	5.7##	152.2	±	3.8##	
		300+EE	60.3	±	3.5	62.7	±	4.8##	61.5	±	4.6##	104.3	±	10.8##	102.4	±	10.7##	
		1,000+EE	59.9	±	4.3	104.9	±	15.8##	102.6	±	15.4	175.7	±	30.1#	171.9	±	30.1	
		TMX+EE	61.1	±	3.5	89.4	±	4.7##	87.7	±	5.0##	147.0	±	13.6##	144.2	±	14.1##	
4-029	2,2-Bis (4-hydroxyphenyl)-4-methyl-n-pentane (6807-17-6)	V.C.	58.3	±	2.8	29.2	±	2.8	28.8	±	2.6	50.1	±	3.4	49.4	±	3.0	FY2001
		2	58.0	±	2.7	58.9	±	8.7**	58.4	±	8.8**	101.5	±	14.0**	100.5	±	14.2**	
		10	59.2	±	3.3	85.4	±	12.1**	84.0	±	11.9**	144.1	±	15.9**	141.6	±	15.3**	
		40	57.4	±	3.2	163.7	±	50.9**	115.1	±	16.6**	285.1	±	89.7**	200.3	±	25.3**	
		V + EE	59.7	±	2.7	125.5	±	10.8**	107.6	±	7.1**	210.2	±	17.3**	180.4	±	11.8**	
		2 + EE	58.0	±	3.8	108.9	±	21.6	96.2	±	10.3 #	187.1	±	30.0	165.7	±	13.3	
		10 + EE	59.0	±	3.0	95.0	±	7.7##	92.5	±	7.9 ##	161.5	±	16.4##	157.3	±	17.1 #	
		40 + EE	57.9	±	3.1	144.1	±	40.8	112.3	±	15.9	248.2	±	66.8	193.8	±	23.9	
		TMX+EE	58.1	±	2.4	88.8	±	5.5##	87.5	±	5.5##	153.2	±	14.1##	151.0	±	14.1##	
4-033	4,4'-(Hexafluoroisopropylidene)diphenol (1478-61-1)	V.C.	56.1	±	4.3	30.0	±	5.1	28.6	±	4.9	53.5	±	7.9	50.9	±	7.4	FY2001
		4	55.0	±	4.5	48.7	±	10.4**	47.2	±	9.9**	87.8	±	12.6**	85.1	±	11.9**	
		20	56.6	±	4.0	68.1	±	10.6**	65.9	±	9.8**	119.7	±	12.8**	116.0	±	11.7**	
		100	54.7	±	4.2	108.7	±	17.2**	96.4	±	9.0**	200.0	±	37.5**	177.2	±	22.2**	
		V + EE	56.5	±	3.8	120.8	±	19.0**	110.3	±	15.7**	214.0	±	30.2**	195.4	±	25.0**	
		4 + EE	56.7	±	3.8	105.8	±	8.7	95.1	±	6.1	186.8	±	13.5	168.0	±	9.4 #	
		20 + EE	55.5	±	2.1	76.6	±	9.5##	74.5	±	9.1 ##	138.1	±	15.9##	134.2	±	15.4 ##	
		100 + EE	55.9	±	3.2	107.9	±	15.1	99.3	±	11.9	193.2	±	24.5	177.8	±	18.9	
		TMX+EE	54.7	±	3.4	80.8	±	4.5##	79.5	±	5.2##	148.3	±	13.9##	145.9	±	14.4##	

4-034	4,4'-Cyclohexylidenebisphenol (843-55-0)	V.C.	55.7	±	4.5	40.8	±	12.0	40.4	±	12.1	72.8	±	18.7	72.0	±	18.9	FY2002
		6	54.7	±	1.8	42.4	±	12.4	41.5	±	12.2	77.4	±	22.1	75.9	±	21.7	
		30	55.3	±	3.1	58.4	±	4.9*	57.8	±	4.8*	105.8	±	8.8**	104.7	±	8.6**	
		150	55.3	±	3.3	107.7	±	19.0**	96.6	±	11.0**	195.3	±	35.2**	175.2	±	23.2**	
		V + EE	55.3	±	3.5	160.6	±	18.8 (5)a**	122.5	±	5.3 (5)**	293.9	±	47.9 (5)**	223.4	±	17.4 (5)**	
		6+EE	55.3	±	2.6	169.0	±	17.4	121.1	±	6.1	306.2	±	34.7	219.6	±	16.9	
		30+EE	56.8	±	4.3	84.3	±	6.2##	80.1	±	6.8##	149.0	±	14.8##	141.4	±	13.6##	
		150+EE	54.7	±	3.2	97.3	±	8.1##	91.9	±	8.1##	178.3	±	17.5##	168.2	±	16.7##	
		TMX+EE	52.1	±	2.3	81.1	±	8.0##	80.1	±	8.0##	155.8	±	12.0##	153.8	±	12.1##	
4-035	4,4'-(Octahydro-4,7-methano-5H-inden-5-ylidene) bisphenol (1943-97-1)	V.C.	54.9	±	3.2	29.2	±	4.7	28.3	±	4.4	53.1	±	7.7	51.6	±	7.1	FY2001
		2	56.2	±	3.1	64.9	±	5.8**	63.4	±	5.2**	115.4	±	8.8**	112.9	±	7.8**	
		10	53.2	±	3.8	88.6	±	6.3**	86.2	±	6.5**	166.9	±	12.8**	162.5	±	13.7**	
		40	54.6	±	1.7	95.6	±	7.9**	92.5	±	7.1**	174.9	±	11.6**	169.3	±	10.3**	
		V + EE	55.7	±	3.0	107.5	±	14.2**	97.4	±	8.3**	193.0	±	24.9**	174.9	±	12.6**	
		2 + EE	54.8	±	2.6	84.9	±	11.6#	78.7	±	8.4 ##	155.0	±	20.8#	143.9	±	16.7 ##	
		10 + EE	55.9	±	2.3	86.3	±	8.4#	82.3	±	9.4 #	154.6	±	14.1##	147.4	±	15.3 ##	
		40 + EE	53.0	±	4.2	96.9	±	8.6	93.5	±	8.3	183.3	±	17.2	176.8	±	16.6	
		TMX+EE	52.4	±	2.6	81.7	±	3.6##	79.7	±	3.6##	156.0	±	5.6#	152.2	±	4.7##	
4-041	4,4'-(1,3-Phenylene diisopropylidene)bisphenol (13595-25-0)	V.C.	59.6	±	3.3	38.6	±	6.8	37.4	±	6.4	64.6	±	10.4	62.8	±	9.6	FY2002
		2	59.8	±	2.3	37.2	±	4.5	36.0	±	4.4	62.4	±	8.9	60.3	±	8.7	
		10	60.6	±	5.2	32.3	±	4.3	31.5	±	4.3	54.0	±	10.4	52.6	±	10.3	
		50	59.2	±	3.2	50.8	±	9.2*	49.3	±	8.8*	85.7	±	14.8*	83.2	±	14.2*	
		V + EE	60.0	±	2.8	178.8	±	25.9**	142.6	±	11.9**	298.0	±	37.9**	237.8	±	13.3**	
		2+EE	61.3	±	2.8	116.1	±	46.0#	102.2	±	28.3#	190.5	±	80.7#	167.1	±	49.0#	
		10+EE	60.9	±	3.9	166.4	±	21.3	132.9	±	12.8	275.8	±	49.9	219.7	±	32.3	
		50+EE	60.2	±	2.8	163.1	±	41.0	128.0	±	17.5	269.8	±	58.4	212.4	±	22.0#	
		TMX+EE	59.3	±	3.4	95.6	±	6.3##	93.5	±	6.2##	161.8	±	17.3##	158.2	±	16.5##	
4-044	4,4'-Sulfonyldiphenol (80-09-1)	V.C.	58.3	±	3.4	37.4	±	6.9	36.8	±	7.0	64.0	±	9.3	63.0	±	9.4	FY2002
		20	59.7	±	3.8	48.8	±	9.0*	48.2	±	8.8*	81.6	±	13.3*	80.7	±	12.8*	
		100	57.6	±	1.9	42.6	±	6.2	41.9	±	6.0	73.8	±	8.5	72.6	±	8.3	
		500	58.0	±	2.8	62.1	±	13.3**	61.0	±	12.6**	107.5	±	25.0**	105.6	±	23.8**	

		V + EE	60.6	±	2.3	148.7	±	9.4**	117.5	±	8.8**	245.3	±	13.8**	193.8	±	13.2**	
		20+EE	58.9	±	4.0	181.1	±	23.9#	128.7	±	10.7	307.5	±	38.8##	218.4	±	10.6##	
		100+EE	59.0	±	2.8	151.6	±	43.3	118.6	±	12.2	257.0	±	73.7	201.4	±	21.2	
		500+EE	58.2	±	3.8	79.9	±	10.5##	78.5	±	10.4##	137.7	±	20.8##	135.4	±	20.3##	
		TMX+EE	57.5	±	3.6	91.1	±	9.8##	90.0	±	9.7##	158.5	±	15.2##	156.6	±	15.0##	
4-054	1,1,3-Tris(2-methyl-4-hydroxy-5-tert-butylphenyl)butane (1843-03-4)	V.C.	63.8	±	5.6	36.0	±	5.8	35.4	±	6.0	56.8	±	10.2	55.8	±	10.5	FY2004
		100	66.9	±	4.1	35.1	±	3.5	34.5	±	3.3	52.6	±	6.0	51.7	±	5.6	
		300	64.9	±	3.4	34.2	±	5.4	33.5	±	5.4	52.4	±	5.7	51.4	±	5.6	
		1,000	61.8	±	3.7	30.1	±	4.0	29.5	±	3.9	48.7	±	5.2	47.7	±	5.0	
		V + EE	65.1	±	3.1	153.9	±	16.8 **	131.1	±	8.9 **	236.8	±	25.6 **	201.5	±	11.1 **	
		100 + EE	64.1	±	4.0	146.2	±	37.6	125.2	±	22.8	227.4	±	53.6	195.0	±	30.4	
		300 + EE	66.8	±	4.2	143.4	±	41.7	123.5	±	24.5	216.9	±	69.0	186.4	±	41.9	
		1,000 + EE	61.2	±	4.4	173.4	±	23.7	142.6	±	7.2 #	284.2	±	36.2 #	234.2	±	20.7 ##	
		TMX+EE	66.1	±	2.9	98.4	±	5.4 ##	97.4	±	5.4 ##	149.0	±	9.5 ##	147.5	±	9.3 ##	

Mean ± S.D.
EE; ethynyl estradiol, TMX; tamoxifen
* Significantly different from vehicle control at P<0.05, ** Significantly different from vehicle control at P<0.01
# Significantly different from vehicle control + EE at P<0.05, ## Significantly different from vehicle control + EE at P<0.01
( )a Number of animals used for statistical analysis.

		V.C.	59.0	±	4.9	30.6	±	3.4	29.7	±	3.4	52.1	±	6.2	50.5	±	6.1	
		40	58.8	±	3.4	31.4	±	1.3	30.7	±	1.1	53.6	±	4.4	52.4	±	4.1	
		200	60.0	±	2.3	35.2	±	5.6	34.4	±	5.5	58.7	±	9.6	57.4	±	9.4	
		1000	58.8	±	3.5	40.5	±	11.5	39.4	±	10.6	69.8	±	23.4	67.7	±	21.5	
4-061	4,4'-Dimethoxy-triphenylmethane(7500-76-7)	V + EE	59.8	±	5.8	141.6	±	30.8**	112.5	±	16.5**	235.7	±	37.3**	188.0	±	18.6**	FY2001
		40 + EE	58.2	±	2.0	128.7	±	9.0	109.4	±	6.3	220.9	±	10.1	188.0	±	9.2	
		200 + EE	57.5	±	2.6	117.6	±	20.3	99.5	±	11.7	205.3	±	39.2	173.3	±	21.6	
		1000 + EE	60.3	±	2.9	120.0	±	12.4	100.9	±	10.4	199.6	±	24.2	167.5	±	16.8	
		TMX+EE	58.1	±	2.5	88.8	±	6.2##	86.4	±	6.5##	153.2	±	12.7##	149.1	±	13.0##	
4-103	4-Hydroxybenzophenone (1137-42-4)	V.C.	59.6	±	3.5	35.3	±	6.2	34.9	±	6.1	59.4	±	10.3	58.7	±	10.1	FY2001
		40	57.6	±	2.8	40.1	±	3.5	39.6	±	3.7	69.5	±	4.9	68.8	±	5.3	
		200	55.9	±	9.1	48.7	±	6.8**	48.3	±	6.3**	89.2	±	19.1**	88.7	±	19.2**	
		800	55.9	±	2.5	88.7	±	15.4**	86.1	±	12.7**	158.7	±	26.8**	154.1	±	22.3**	

		V + EE	57.5	±	2.5	130.3	±	21.5**	108.3	±	11.5**	226.9	±	37.9**	188.4	±	19.6**	
		40 + EE	59.5	±	2.4	155.7	±	27.9	115.1	±	12.2	263.1	±	55.3	194.2	±	26.9	
		200 + EE	57.2	±	2.9	89.1	±	18.2##	84.7	±	15.1 #	156.4	±	35.5##	148.7	±	29.2 #	
		800 + EE	57.4	±	2.2	98.8	±	13.2#	95.5	±	12.1	172.4	±	22.9#	166.4	±	20.0	
		TMX+EE	57.7	±	3.3	83.9	±	9.2##	82.5	±	8.9##	145.4	±	13.1##	142.9	±	12.2##	
4-106	4,4'-Dihydroxybenzophenone (611-99-4)	V.C.	56.3	±	4.2	32.0	±	6.7	31.0	±	6.6	56.8	±	9.8	55.0	±	9.7	FY2001
		8	54.3	±	6.1	30.0	±	4.3	28.9	±	3.9	55.8	±	9.6	53.8	±	8.9	
		40	53.7	±	4.2	30.2	±	4.7	29.3	±	4.5	56.1	±	7.1	54.5	±	6.8	
		200	56.1	±	3.9	46.5	±	5.4**	45.0	±	5.0**	82.9	±	7.7**	80.4	±	7.5**	
		V + EE	54.9	±	2.9	106.9	±	13.8**	95.0	±	5.4**	194.9	±	27.2**	173.1	±	9.2**	
		8 + EE	55.8	±	5.2	133.1	±	25.9	110.1	±	17.4	239.8	±	51.1	197.8	±	30.1	
		40 + EE	55.7	±	5.1	129.2	±	28.6	106.9	±	17.8	233.0	±	54.1	192.3	±	29.0	
		200 + EE	54.4	±	4.4	73.4	±	14.7##	69.8	±	14.8 ##	135.0	±	23.3##	128.3	±	23.5 ##	
		TMX+EE	54.2	±	4.5	87.6	±	7.7#	84.7	±	7.8#	162.3	±	16.4#	157.0	±	17.0	
4-107	2,4-Dihydroxy benzophenone (131-56-6)	V.C.	63.0	±	2.9	37.8	±	6.7	36.7	±	6.3	60.3	±	12.5	58.5	±	11.8	FY2002
		100	63.3	±	3.8	49.6	±	5.7**	48.2	±	5.5**	78.8	±	11.6*	76.5	±	11.2*	
		300	62.3	±	4.1	53.5	±	10.1*	52.0	±	9.9**	85.3	±	11.5**	83.0	±	11.4**	
		1,000	56.6	±	9.7	94.5	±	22.8**	86.7	±	16.8**	169.7	±	39.2**	155.6	±	29.2**	
		V + EE	63.1	±	3.0	158.1	±	25.9**	126.7	±	12.3**	251.4	±	46.1**	201.0	±	19.7**	
		100 + EE	62.1	±	3.0	197.5	±	38.5	144.6	±	15.6	317.3	±	56.8	232.6	±	19.0#	
		300+EE	60.9	±	3.7	99.4	±	12.6##	94.7	±	10.7##	164.1	±	24.6##	155.8	±	22.1##	
		1,000+EE	58.1	±	6.8	100.3	±	10.8##	95.6	±	10.0##	174.0	±	23.1##	165.7	±	20.8#	
		TMX+EE	61.4	±	2.7	96.9	±	7.7##	95.2	±	7.5##	158.4	±	16.7##	155.5	±	16.5##	
4-108	2,4,4'-Trihydroxybenzophenone (1470-79-7)	V.C.	59.2	±	4.5(5)a	35.1	±	2.4 (5)a	34.9	±	2.1 (5)a	59.5	±	4.5 (5)a	59.1	±	4.5 (5)a	FY2001
		8	59.9	±	3.7	38.3	±	3.6	37.8	±	3.5	63.9	±	3.8	63.1	±	3.4	
		40	58.2	±	4.3	45.6	±	4.2**	45.1	±	4.2**	78.6	±	9.2**	77.8	±	9.0**	
		200	58.2	±	5.9	73.5	±	10.3**	72.8	±	10.2**	126.4	±	12.3**	125.1	±	12.0**	
		V + EE	58.2	±	3.5	140.6	±	19.8**	116.5	±	12.6**	241.0	±	26.5**	199.7	±	12.1**	
		8 + EE	58.2	±	3.2(5)a	141.2	±	31.0 (5)a	109.5	±	15.0 (5)a	241.1	±	42.8(5)a	187.5	±	17.8 (5)a	
		40 + EE	58.1	±	2.8	107.1	±	14.6##	94.5	±	10.4 ##	184.8	±	26.6##	163.1	±	20.0 ##	
		200 + EE	55.2	±	3.3	69.3	±	12.2##	67.7	±	11.6 ##	126.3	±	26.6##	123.4	±	25.0 ##	

		TMX+EE	58.8	±	3.3	87.1	±	4.0##	84.2	±	3.8##	148.4	±	8.2##	143.4	±	8.7##	
4-111	4,4'-Dimethoxybenzophenone (90-96-0)	V.C.	58.7	±	2.7	32.4	±	4.6	31.6	±	4.3	55.2	±	7.6	53.9	±	6.9	
		40	58.0	±	4.3	32.2	±	9.5	31.5	±	8.8	55.1	±	13.0	54.0	±	11.8	
		200	58.6	±	2.0	28.4	±	3.5	28.1	±	3.5	48.5	±	6.1	48.1	±	6.1	
		1000	56.7	±	1.4	26.3	±	2.7*	25.9	±	2.6*	46.4	±	4.5*	45.7	±	4.3*	
		V + EE	55.9	±	2.7	108.9	±	17.2**	99.2	±	12.0**	194.1	±	22.3**	177.1	±	15.2**	FY2001
		40 + EE	58.3	±	1.6	128.9	±	11.7#	108.9	±	9.5	221.0	±	20.7	186.6	±	15.1	
		200 + EE	55.5	±	3.7	135.4	±	29.4	108.6	±	11.8	243.9	±	50.9	196.0	±	21.0	
		1000 + EE	57.1	±	3.1	124.0	±	44.7	105.8	±	28.2	215.0	±	67.8	183.9	±	41.7	
		TMX+EE	56.4	±	3.4	89.7	±	13.0	87.2	±	12.6	159.4	±	22.4#	154.9	±	21.3	
4-112	2,2',4,4'-Tetrahydroxybenzophenone (131-55-5)	V.C.	58.1	±	3.3	34.7	±	4.3	34.2	±	4.2	59.8	±	7.0	58.9	±	6.8	
		40	57.9	±	3.3	38.2	±	2.6	37.6	±	2.6	66.1	±	5.8	65.1	±	5.7	
		200	57.4	±	1.5	79.2	±	10.6**	77.2	±	10.4**	137.7	±	15.5**	134.2	±	15.3**	
		800	54.8	±	3.9	216.8	±	59.6**	119.7	±	12.1**	397.8	±	116.3**	218.9	±	20.2**	
		V + EE	57.6	±	3.5	137.3	±	14.4**	117.8	±	11.3**	239.0	±	29.2**	205.3	±	25.0**	FY2001
		40 + EE	57.4	±	2.9	106.4	±	17.7##	100.5	±	15.3 #	185.5	±	32.7#	175.1	±	27.6	
		200 + EE	56.8	±	3.4	68.3	±	10.1##	67.0	±	10.3 ##	120.2	±	16.0##	117.8	±	16.2##	
		800 + EE	53.0	±	2.4#	232.4	±	63.3#	124.3	±	9.2	438.6	±	118.1##	234.8	±	15.6#	
		TMX+EE	56.5	±	4.4	89.5	±	4.6##	87.8	±	4.6##	159.0		12.5##	156.1	±	12.6##	
4-114	4-Fluoro-4'-hydroxybenzophenone (25913-05-7)	V.C.	52.7	±	3.2	29.5	±	4.1	29.0	±	4.4	56.0	±	6.8	54.9	±	7.1	
		100	50.7	±	4.3	37.7	±	3.2 **	37.1	±	3.1 **	74.7	±	5.8 **	73.3	±	5.7 **	
		300	53.1	±	2.6	60.8	±	12.7 **	59.4	±	11.9 **	115.3	±	26.9 **	112.6	±	25.4 **	
		1,000	52.3	±	3.8	136.5	±	35.5 **	113.9	±	14.8 **	263.5	±	78.8 **	218.8	±	34.6 **	
		V + EE	52.4	±	3.4	121.2	±	17.7 **	107.2	±	11.0 **	230.4	±	22.3 **	204.5	±	16.2 **	FY2004
		100 + EE	52.0	±	3.1	143.5	±	18.6	119.8	±	10.8	276.1	±	31.6 #	230.9	±	20.9 #	
		300 + EE	51.1	±	3.0	102.1	±	10.2 #	96.8	±	8.0	200.4	±	23.3 #	190.1	±	20.3	
		1,000 + EE	51.2	±	4.1	138.4	±	35.7	112.4	±	20.7	270.6	±	63.9	221.4	±	48.8	
		TMX+EE	52.8	±	2.5	92.0	±	5.0 ##	90.0	±	5.1 ##	174.5	±	9.5 ##	170.6	±	9.8 ##	

Mean ± S.D.
EE; ethynyl estradiol, TMX; tamoxifen
* Significantly different from vehicle control at P<0.05, ** Significantly different from vehicle control at P<0.01
# Significantly different from vehicle control + EE at P<0.05, ## Significantly different from vehicle control + EE at P<0.01
( )a Number of animals used for statistical analysis.

		V.C.	63.9	±	1.8	36.9	±	5.8	36.5	±	5.7	57.7	±	8.7	57.1	±	8.6	
		100	64.0	±	2.7	35.9	±	4.0	35.4	±	3.9	56.1	±	4.7	55.3	±	4.6	
		300	63.4	±	3.1	52.9	±	8.0 **	52.2	±	7.9 **	83.4	±	9.5 **	82.3	±	9.4 **	
		1,000	62.5	±	4.9	67.7	±	11.2 **	66.9	±	10.9 **	108.1	±	14.0 **	106.8	±	13.5 **	
4-115	2,3,4-Trihydroxybenzophenone(1143-72-2)	V + EE	63.9	±	3.6	143.4	±	30.4 **	121.6	±	18.8 **	226.0	±	51.4 **	191.5	±	33.7 **	FY2004
		100 + EE	64.1	±	3.8	183.2	±	47.0	135.6	±	18.8	285.5	±	67.4	211.5	±	24.6	
		300 + EE	62.6	±	4.2	178.7	±	33.9	137.1	±	16.0	284.3	±	41.0	218.7	±	14.3	
		1,000 + EE	61.1	±	3.8	106.1	±	16.2 #	100.5	±	13.3 #	173.0	±	17.2	164.0	±	13.2	
		TMX+EE	62.3	±	2.8	92.7	±	4.9 ##	91.0	±	4.7 ##	149.0	±	8.9 #	146.3	±	8.5 #	
		V.C.	57.8	±	3.7	28.4	±	2.7	27.5	±	2.6	49.4	±	7.0	47.9	±	6.9	
		8	57.0	±	4.0	30.5	±	4.4	29.5	±	4.2	53.5	±	7.4	51.8	±	7.0	
		40	56.8	±	2.7	43.7	±	7.0**	43.2	±	6.9**	77.1	±	12.1**	76.1	±	11.9**	
		200	56.4	±	2.4	57.0	±	6.3**	56.2	±	6.0**	101.1	±	9.8**	99.6	±	9.6**	
4-137	4-Hydroxyazobenzene (1689-82-3)	V + EE	57.8	±	2.6	121.3	±	22.9**	104.2	±	13.5**	210.3	±	42.0**	180.8	±	26.7**	FY2001
		8 + EE	58.0	±	3.1	136.8	±	30.5	112.5	±	19.1	236.2	±	51.5	194.4	±	32.8	
		40 + EE	57.6	±	3.5	128.4	±	9.6	108.8	±	4.3	223.5	±	18.4	189.3	±	7.2	
		200 + EE	57.0	±	2.1	108.9	±	9.2	93.4	±	3.7	191.8	±	22.9	164.4	±	11.8	
		TMX+EE	56.1	±	4.3	83.0	±	7.1##	81.1	±	7.1##	149.0	±	20.5##	145.5	±	19.9#	
		V.C.	58.3	±	3.8	29.5	±	6.8	28.8	±	6.9	50.9	±	13.5	49.8	±	13.6	
		32	55.5	±	3.0	28.7	±	3.3	27.9	±	3.3	51.8	±	6.1	50.3	±	5.8	
		160	57.4	±	3.0	29.2	±	1.9	28.6	±	2.0	51.0	±	4.6	50.0	±	4.7	
		800	57.0	±	3.2	29.3	±	3.9	28.7	±	3.8	51.4	±	5.0	50.2	±	4.8	
4-144	3,3,3',3'-Tetramethyl-1,1'-spirobisindane-5,5',6,6'-tetrol (77-08-7)	V + EE	55.6	±	3.7	135.2	±	30.5**	113.7	±	12.2**	243.7	±	55.3**	204.9	±	22.1**	FY2001
		32 + EE	55.3	±	5.1	118.0	±	10.2	108.8	±	6.1	213.9	±	16.3	197.5	±	11.6	
		160 + EE	55.5	±	3.5	120.9	±	24.8	104.2	±	11.8	217.4	±	37.4	187.8	±	15.6	
		800 + EE	55.1	±	3.3	107.7	±	12.1	99.6	±	8.7#	196.3	±	29.3	181.5	±	23.4	
		TMX+EE	54.1	±	4.9	84.8	±	6.8##	83.0	±	7.0##	157.1	±	12.1#	153.8	±	11.8##	

		V.C.	55.2	±	2.0	29.1	±	1.6	28.6	±	1.5	52.7	±	3.4	51.8	±	2.9	
		2	55.8	±	3.7	34.2	±	5.1	33.7	±	4.9	61.1	±	6.7*	60.2	±	6.7*	
		10	55.5	±	2.2	37.0	±	3.2**	36.5	±	3.2**	66.8	±	6.4**	66.0	±	6.6**	
		40	55.0	±	2.9	42.8	±	2.5**	42.1	±	2.6**	77.9	±	4.4**	76.5	±	4.7**	
4-147	4,4'-Thiobis-phenol (2664-63-3)	V + EE	56.4	±	3.0	133.6	±	13.3**	111.0	±	7.4**	236.9	±	20.7**	197.0	±	12.1**	FY2001
		2 + EE	55.2	±	1.9	136.8	±	21.7	112.1	±	11.3	248.7	±	44.0	203.5	±	23.7	
		10 + EE	56.8	±	3.6	113.1	±	15.1#	100.0	±	8.8#	199.0	±	24.2#	176.3	±	17.9#	
		40 + EE	56.1	±	2.8	79.0	±	17.2##	77.1	±	15.8##	140.2	±	25.9##	136.8	±	23.8##	
		TMX+EE	54.9	±	2.1	80.9	±	6.4##	79.8	±	6.5##	147.2	±	10.9##	145.4	±	11.5##	
		V.C.	52.8	±	3.4	25.0	±	2.9	24.6	±	2.8	47.4	±	5.7	46.6	±	5.5	
		100	55.2	±	4.6	30.1	±	4.9	29.8	±	4.9*	54.5	±	7.8	53.9	±	7.6	
		400	52.8	±	1.3	34.6	±	4.8**	33.4	±	4.7**	65.7	±	9.4**	63.4	±	9.3**	
		800	52.0	±	2.9	50.0	±	6.7**	48.9	±	6.9**	96.2	±	12.7**	94.2	±	12.9**	
4-150	Diphenyl-p-phenylenediamine (74-31-7)	V + EE	54.2	±	3.0	124.5	±	50.5**	98.2	±	7.5**	231.5	±	100.5**	181.8	±	18.4**	FY2001
		100 + EE	54.3	±	1.3	147.4	±	30.4	113.6	±	13.7#	272.0	±	58.1	209.5	±	27.4	
		400 + EE	53.9	±	2.4	135.0	±	29.1	104.2	±	14.6	251.6	±	58.5	194.1	±	31.7	
		800 + EE	50.8	±	7.2	143.8	±	28.2	106.1	±	17.5	286.3	±	55.9	209.8	±	25.6	
		TMX+EE	53.2	±	2.4	84.2	±	6.4	82.2	±	6.7##	158.5	±	12.5	154.5	±	12.7#	
		V.C.	58.5	±	3.5	35.3	±	4.5	34.8	±	4.6	60.3	±	7.2	59.5	±	7.4	
		2	56.5	±	2.7	88.6	±	8.5**	87.2	±	8.7**	157.3	±	17.9**	154.8	±	17.9**	
		10	57.5	±	3.9	87.2	±	4.7**	86.0	±	4.6**	151.9	±	6.8**	149.9	±	6.8**	
		50	54.5	±	5.1	91.3	±	5.3**	89.8	±	5.2**	168.5	±	14.7**	165.8	±	14.6**	
4-172	Clomiphene citrate (cis and trans mixture) (50-41-9)	V + EE	58.6	±	2.6	185.3	±	34.0**	132.1	±	16.1**	315.5	±	49.9**	225.1	±	21.2**	FY2002
		2+EE	56.5	±	4.2	91.7	±	4.3##	90.4	±	4.3##	163.3	±	18.4##	161.0	±	18.0##	
		10+EE	56.0	±	4.0	87.1	±	6.9##	85.5	±	6.4##	155.6	±	10.2##	152.8	±	9.6##	
		50+EE	55.7	±	3.6	95.0	±	6.3##	93.7	±	6.0##	170.9	±	8.8##	168.4	±	8.0##	
		TMX+EE	58.4	±	4.6	84.9	±	5.9##	83.5	±	5.7##	145.8	±	10.0##	143.4	±	10.2##	
4-408	3,3'-Dichlorobenzidine dihydrochloride (612-83-9)	V.C.	63.2	±	4.2	34.9	±	7.8	34.3	±	7.9	55.1	±	11.0	54.1	±	11.1	FY2004
		100	59.7	±	2.7	31.4	±	2.4	30.9	±	2.5	52.8	±	5.6	51.9	±	5.8	
		300	57.6	±	4.3 *	31.5	±	4.2	30.9	±	4.1	54.8	±	8.0	53.8	±	7.9	
		1,000	53.7	±	3.9 **	28.3	±	5.8	27.6	±	5.8	52.4	±	8.2	51.1	±	8.2	

		V + EE	65.0	±	3.2	159.4	±	30.1 **	129.2	±	19.6 **	246.1	±	48.4 **	198.9	±	29.0 **	
		100 + EE	58.8	±	4.7 #	151.4	±	33.3	127.3	±	20.3	258.8	±	60.2	217.6	±	37.8	
		300 + EE	54.1	±	3.6 ##	137.4	±	13.4	126.0	±	13.4	253.7	±	11.5	232.6	±	14.3 #	
		1,000 + EE	53.2	±	4.4 ##	96.2	±	24.5 ##	92.2	±	21.5 #	182.7	±	53.1	174.9	±	46.6	
		TMX+EE	60.5	±	3.9	92.4	±	10.2 ##	91.1	±	10 ##	152.5	±	10.2 ##	150.4	±	9.9 ##	
5-021	2-Naphthol (135-19-3)	V.C.	61.5	±	4.4	37.6	±	5.7	36.7	±	5.6	61.0	±	6.4	59.6	±	6.2	FY2003
		30	61.8	±	4.8	34.9	±	8.2	34.0	±	8.1	56.2	±	10.6	54.7	±	10.3	
		100	59.1	±	4.1	35.5	±	6.8	34.6	±	6.7	60.0	±	10.1	58.4	±	9.9	
		300	50.4	±	5.2 **	27.8	±	4.9 **	27.1	±	4.9 **	54.8	±	4.8	53.4	±	4.8	
		V + EE	61.7	±	5.7	149.4	±	21.8 **	123.8	±	15.2 **	243.3	±	37.4 **	201.0	±	19.8 **	
		100 + EE	60.2	±	3.9	158.3	±	30.2	126.5	±	19.1	263.0	±	47.2	210.3	±	29.8	
		300 + EE	58.8	±	4.0	130.8	±	31.0	113.7	±	22.8	222.5	±	52.4	193.7	±	40.1	
		1,000 + EE	52.6	±	6.9 #	136.1	±	19.5	114.6	±	6.7	266.6	±	76.2	221.8	±	38.2	
		TMX+EE	60.1	±	3.5	89.3	±	7.1 ##	87.3	±	7.0 ##	148.6	±	5.5 ##	145.1	±	5.3 ##	

Mean ± S.D.
EE; ethynyl estradiol, TMX; tamoxifen
* Significantly different from vehicle control at P<0.05, ** Significantly different from vehicle control at P<0.01
# Significantly different from vehicle control + EE at P<0.05, ## Significantly different from vehicle control + EE at P<0.01
( )a Number of animals used for statistical analysis.

5-136	Benzoanthrone(82-05-3)	V.C.	64.7	±	3.5	30.9	±	5.9	30.2	±	5.9	47.6	±	7.7	46.5	±	7.7	FY2004
		100	65.1	±	4.6	30.9	±	4.6	30.1	±	4.5	47.6	±	7.7	46.3	±	7.6	
		300	65.3	±	3.3	29.5	±	2.3	28.7	±	2.3	45.2	±	3.7	44.1	±	3.7	
		1,000	62.3	±	3.9	31.2	±	2.5	30.4	±	2.3	50.3	±	4.9	48.9	±	4.6	
		V + EE	67.2	±	4.8	145.3	±	27.0 **	121.1	±	15.6 **	216.4	±	35.9 **	180.6	±	21.7 **	
		100 + EE	65.4	±	4.1	112.3	±	32.2	101.7	±	27.0	173.5	±	54.3	156.6	±	44.2	
		300 + EE	63.9	±	4.4	122.6	±	16.3	113.2	±	15.3	192.8	±	30.4	177.8	±	26.7	
		1,000 + EE	58.9	±	7.4 #	91.6	±	23.3 ##	86.5	±	19.2 ##	159.8	±	52.3	150.6	±	44.5	
		TMX+EE	65.3	±	3.6	92.5	±	8.7 ##	90.2	±	8.4 ##	141.6	±	11.7 ##	138.1	±	11.3 ##	
6-008	Atrazine (1912-24-9)	V.C.	60.9	±	4.9	32.4	±	3.0	31.8	±	2.9	53.4	±	6.1	52.4	±	6.0	FY2004
		20	59.4	±	5.1	30.4	±	4.3	29.5	±	4.0	51.3	±	5.7	49.6	±	5.4	
		60	57.8	±	3.7	30.5	±	3.5	29.8	±	3.4	52.7	±	4.3	51.5	±	4.4	
		200	42.6	±	4.3 **	21.9	±	1.8 **	21.5	±	1.7 **	51.7	±	2.4	50.6	±	2.5	

		V + EE	59.8	±	4.7	162.6	±	29.2 **	134.2	±	14.2 **	274.7	±	62.9 **	225.5	±	27.4 **	
		20 + EE	59.4	±	4.2	157.0	±	21.8	126.6	±	10.6	263.9	±	27.4	213.8	±	19.5	
		60 + EE	56.6	±	5.5	130.3	±	40.6	110.9	±	21.4 #	227.7	±	55.0	195.0	±	24.6	
		200 + EE	46.4	±	4.6 ##	90.7	±	22.3 ##	85.7	±	16.6 ##	194.7	±	37.0 #	184.5	±	27.5 #	
		TMX+EE	58.7	±	4.5	93.6	±	6.1 ##	92.1	±	6.0 ##	160.5	±	17.3 ##	157.9	±	17.2 ##	
6-026	Amitrol (61-82-5)	V.C.	60.0	±	4.5	34.6	±	6.2	33.8	±	5.9	58.0	±	11.8	56.6	±	11.2	FY2002
		100	61.8	±	4.3	44.4	±	9.4	43.3	±	9.2	71.7	±	13.6	69.9	±	13.3	
		300	59.2	±	4.4	34.2	±	5.9	33.2	±	5.4	57.5	±	6.7	55.9	±	6.1	
		1,000	59.0	±	2.0	29.8	±	3.6	29.0	±	3.5	50.6	±	6.3	49.2	±	6.2	
		V + EE	59.6	±	3.7	140.9	±	19.2**	111.8	±	10.4**	237.3	±	35.4**	188.1	±	20.0**	
		100+EE	59.8	±	4.0	125.8	±	15.4	106.8	±	10.4	210.7	±	23.6	179.1	±	18.4	
		300+EE	60.5	±	5.7	182.3	±	31.5#	129.8	±	20.1	301.9	±	46.4#	214.1	±	20.3#	
		1,000+EE	58.8	±	5.7	162.5	±	17.9	119.5	±	7.5	277.5	±	30.5	204.2	±	17.1	
		TMX+EE	58.8	±	3.3	81.1	±	4.9##	79.2	±	5.0##	138.5	±	12.3##	135.3	±	12.5##	
6-032	Benomyl (17804-35-2)	V.C.	62.4	±	2.6	34.4	±	2.8	33.6	±	2.9	55.2	±	3.5	53.8	±	3.7	FY2004
		100	58.4	±	4.3	31.0	±	3.2	30.1	±	3.3	53.2	±	4.8	51.7	±	5.0	
		300	58.8	±	4.1	30.1	±	5.1	29.3	±	5.1	51.2	±	8.1	49.9	±	8.1	
		1,000	57.8	±	3.2 *	30.2	±	3.2 *	29.4	±	3.2 *	52.5	±	7.3	51.1	±	7.3	
		V + EE	59.5	±	3.4	202.2	±	48.3 **	142.1	±	22.0 **	342.0	±	88.7 **	240.4	±	43.9 **	
		100 + EE	59.5	±	4.1	170.9	±	27.7	130.4	±	13.3	286.6	±	38.6	219.4	±	20.1	
		300 + EE	58.1	±	4.8	179.4	±	24.0	130.5	±	17.6	309.6	±	40.1	225.0	±	27.1	
		1,000 + EE	57.1	±	3.9	175.0	±	47.4	132.1	±	27.1	304.3	±	75.8	230.6	±	41.7	
		TMX+EE	57.7	±	3.5	95.3	±	12.2 ##	93.1	±	11.9 ##	165.0	±	15.6 ##	161.1	±	15.1 ##	
6-067	Captafol (ISO) (= 1,2,3,6-Tetrahydro-N-(1,1,2,2-tetrachloroethylthio)phthalimide) (2425-06-1)	V.C.	64.5	±	3.7	33.8	±	4.4	33.0	±	4.2	52.6	±	8.6	51.4	±	8.1	FY2004
		100	65.3	±	2.3	33.2	±	3.3	32.6	±	3.3	50.8	±	3.8	49.8	±	3.8	
		300	63.6	±	5.7	31.1	±	3.0	30.4	±	3.0	49.1	±	4.0	48.0	±	4.2	
		1,000	63.6	±	3.6	31.1	±	3.6	30.5	±	3.6	48.9	±	5.1	48.0	±	5.0	
		V + EE	63.8	±	4.1	133.2	±	40.8 **	114.7	±	24.7 **	210.7	±	69.1 **	180.8	±	41.8 **	
		100 + EE	64.1	±	3.5	159.6	±	37.0	129.8	±	23.1	251.0	±	65.4	203.8	±	42.4	
		300 + EE	62.5	±	5.6	130.0	±	35.7	109.6	±	19.4	210.9	±	68.4	176.9	±	38.3	
		1,000 + EE	61.0	±	2.6	120.8	±	33.8	106.0	±	23.2	199.6	±	61.1	174.8	±	42.4	

		TMX+EE	61.1	±	3.9	96.0	±	4.1	93.8	±	3.8	157.2	±	5.2	153.7	±	5.3	
6-071	N-Cycrohexyl-2-benzothiazolesulfenamide (95-33-0)	V.C.	62.7	±	2.0	43.6	±	16.7	42.5	±	16.5	69.2	±	25.4	67.6	±	25.1	FY2003
		100	63.4	±	4.0	33.3	±	3.7	32.5	±	3.5	52.7	±	6.9	51.5	±	6.5	
		300	63.0	±	2.6	31.8	±	1.9	31.1	±	1.7	50.6	±	4.3	49.4	±	4.0	
		1,000	60.0	±	2.7	31.6	±	2.8	30.7	±	2.6	52.9	±	6.5	51.3	±	6.2	
		V + EE	62.5	±	3.5	141.8	±	23.0 **	120.6	±	14.1 **	226.4	±	28.9 **	192.6	±	14.0 **	
		100 + EE	63.2	±	5.3	162.0	±	20.4	131.4	±	14.3	256.3	±	24.2	207.9	±	14.4	
		300 + EE	61.6	±	4.4	152.8	±	14.4	129.5	±	7.9	249.6	±	33.2	211.1	±	19.6	
		1,000 + EE	61.8	±	3.4	152.5	±	22.0	132.6	±	9.0	247.7	±	40.9	215.2	±	20.0 #	
		TMX+EE	62.5	±	3.0	89.6	±	5.8 ##	87.7	±	5.6 ##	143.7	±	13.1 ##	140.8	±	12.3 ##	
6-072	2,2'-Dithiobis[benzothiazole] (120-78-5)	V.C.	60.9	±	5.0	31.6	±	4.9	30.8	±	4.7	51.8	±	6.6	50.5	±	6.3	FY2003
		100	60.5	±	3.9	34.4	±	4.5	33.3	±	4.3	56.8	±	5.7	55.0	±	5.6	
		300	59.9	±	5.0	34.9	±	5.9	34.1	±	5.7	59.2	±	14.1	57.8	±	13.6	
		1,000	59.8	±	3.6	35.9	±	4.7	35.2	±	4.4	60.3	±	8.5	59.0	±	8.0	
		V + EE	60.3	±	3.9	139.4	±	25.9 **	120.8	±	13.3 **	230.3	±	32.5 **	200.1	±	13.4 **	
		100 + EE	60.3	±	4.8	149.2	±	22.1	124.3	±	9.6	246.8	±	27.3	206.3	±	10.7	
		300 + EE	59.8	±	3.8	135.5	±	30.4	115.8	±	15.9	226.7	±	46.2	194.0	±	25.5	
		1,000 + EE	61.5	±	3.0	146.7	±	17.4	124.0	±	11.1	239.2	±	31.3	201.8	±	16.5	
		TMX+EE	59.4	±	3.1	91.6	±	4.8 ##	89.6	±	4.4 ##	154.7	±	11.7 ##	151.2	±	10.5 ##	
6-074	2-Benzothiazolethiol (149-30-4)	V.C.	64.8	±	4.5	37.5	±	7.2	36.7	±	7.1	57.9	±	11.2	56.8	±	11.0	FY2004
		100	65.5	±	4.0	36.7	±	6.7	36.0	±	6.5	56.5	±	13.0	55.4	±	12.7	
		300	62.5	±	4.3	33.7	±	6.4	33.1	±	6.3	54.6	±	12.7	53.5	±	12.6	
		1,000	61.0	±	8.3	29.2	±	4.5 *	28.7	±	4.5 *	48.2	±	6.1	47.3	±	6.1	
		V + EE	65.7	±	3.9	178.3	±	20.8 **	143.7	±	13.7 **	271.8	±	30.3 **	218.8	±	16.9 **	
		100 + EE	63.6	±	4.7	151.8	±	33.0	126.1	±	19.1	238.4	±	47.8	198.3	±	26.4	
		300 + EE	63.7	±	4.5	168.8	±	27.3	132.2	±	14.7	265.3	±	42.2	207.6	±	19.6	
		1,000 + EE	61.3	±	4.6	147.4	±	28.0	126.7	±	19.8	240.4	±	42.8	206.9	±	31.1	
		TMX+EE	63.7	±	3.8	92.9	±	4.5 ##	91.4	±	4.4 ##	146.2	±	11.4 ##	144.0	±	11.1 ##	

Mean ± S.D.
EE; ethynyl estradiol, TMX; tamoxifen
* Significantly different from vehicle control at P<0.05, ** Significantly different from vehicle control at P<0.01
# Significantly different from vehicle control + EE at P<0.05, ## Significantly different from vehicle control + EE at P<0.01
( )a Number of animals used for statistical analysis.

6-087	Hexachlorocyclopentadiene(77-47-4)	V.C.	63.9	±	3.0	27.8	±	1.8	27.0	±	1.6	43.6	±	3.8	42.4	±	3.5	FY2004
		100	63.4	±	3.1	29.2	±	2.4	28.5	±	2.4	46.0	±	3.1	44.9	±	3.1	
		300	60.6	±	5.1	24.8	±	1.6 *	24.2	±	1.5 *	41.1	±	3.2	40.1	±	3.3	
		1,000	55.1	±	2.6 **	24.2	±	1.2 **	23.6	±	1.3 **	44.0	±	3.0	43.0	±	3.0	
		V + EE	62.2	±	3.3	138.0	±	15.2 **	123.4	±	11.3 **	222.5	±	28.0 **	199.1	±	23.8 **	
		100 + EE	61.3	±	5.6	145.2	±	35.7	110.6	±	15.7	239.7	±	70.3	181.0	±	25.5	
		300 + EE	60.1	±	5.1	111.5	±	47.3	98.8	±	39.0	184.1	±	73.1	163.2	±	59.9	
		1,000 + EE	56.5	±	5.7	127.3	±	16.1	106.3	±	13.2 #	224.8	±	9.2	187.8	±	8.7	
		TMX+EE	60.9	±	3.3	86.8	±	7.1 ##	85.4	±	7.0 ##	142.9	±	14.4 ##	140.7	±	14.4 ##	
no-cord	Triphenyltin chloride (639-58-7)	V.C.	58.4	±	3.9	32.3	±	3.7	31.0	±	3.3	55.7	±	8.1	53.4	±	7.6	FY2001
		0.4	59.1	±	5.6	29.5	±	3.9	28.8	±	3.5	50.1	±	6.9	48.9	±	6.3	
		2	57.4	±	4.4	26.2	±	1.3**	24.8	±	0.9**	45.9	±	4.3*	43.4	±	3.8*	
		10	54.9	±	3.2	22.0	±	2.0**	20.9	±	1.5**	40.1	±	3.4**	38.1	±	2.1**	
		V + EE	58.2	±	3.0	145.6	±	25.0**	122.1	±	14.0**	249.6	±	33.3**	209.7	±	16.0**	
		0.4 + EE	58.8	±	3.4	136.6	±	16.4	118.5	±	12.5	231.9	±	18.2	201.2	±	11.2	
		2 + EE	59.4	±	5.0	118.2	±	31.5	104.0	±	23.5	196.9	±	41.7#	173.7	±	29.5 #	
		10 + EE	54.2	±	4.1	105.0	±	11.8##	95.1	±	9.1 ##	194.4	±	23.3##	176.3	±	20.1 ##	
		TMX+EE	58.0	±	4.0	86.8	±	5.5##	84.7	±	4.1##	150.5	±	16.7##	146.8	±	14.7##	

Mean ± S.D.
EE; ethynyl estradiol, TMX; tamoxifen
* Significantly different from vehicle control at P<0.05, ** Significantly different from vehicle control at P<0.01
# Significantly different from vehicle control + EE at P<0.05, ## Significantly different from vehicle control + EE at P<0.01
( )a Number of animals used for statistical analysis.

ANNEX 2: RESULTS OF ONE-GENERATION TESTS IN EVALUATION OF THE ENDOCRINE DISRUPTING ACTIVITIES IN RODENT (MOE, JAPAN)

Aldrin

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.1	0.5	2.5	12.5	1	
C F1 pups: High values of male sex ratio F2 pups: High values of male sex ratio	C F0 dams: High values of food consumption F1 pups: Low values of viability F1 males: Low values of thymus (absolute/relative) weight F1 females: Low values of thymus (absolute/relative) weight F2 pups: High values of body weight, body weight gain	C F0 dams: High values of food consumption F1 pups: High values of male sex ratio	D	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F1 pups*: High values of liver relative weight. Low values of viability and air righting reflux rate in behavior test

F1 females*: High values of average section movement frequency, average glooming frequency and average rearing behavior frequency in open field test. Low values of body weight gain, body weight and thymus (absolute/relative) weight

Amitrole

One-Generation test

Concentration in drinking water [ppb]			Concentration in drinking water [ppm]		Comments
0.5	5	50	100	1,000	
Dose [μg/kg/day]			Dose [mg/kg/day]		
0.084-0.273	0.702-2.438	7.253-25.61	14.02-37.90	145.5-372.4	
D	D	C F1 females: Low values of body weight gain	A*[1]	A*[2]	Drinking water for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*[1]> (The underlined findings have already been reported.)

F0 dams*: High values of frequency of reddish or enlarged thyroid, thyroid (absolute/relative) weight, serum TSH level, frequency of follicular cell enlargement with colloid decrease or follicular capillary hyperemia or vascular degeneration of follicular cells in thyroid, serum T3 level, serum T4 level and frequency of chromophobic cell with enlargement or hyaline/vascular degeneration in pituitary gland. Low values of kidney (absolute/relative) weight, adrenal gland (absolute/relative) weight, food consumption and water intake.

F1 males*: High values of thyroid (absolute/relative) weight, follicular cell count in thyroid, frequency of enlarged/squamous follicular cell or follicular cell filled with lumen colloid or follicular capillary hyperemia in thyroid, frequency of chromophobic cell increase or acidophilic cell decrease in pituitary gland, frequency of depopulation or necrosis of reproductive cells in testis, frequency of outer cerebellar granular cell layer, serum T3 level and serum T4 level

F1 females*: High values of follicular cell number, frequency of enlarged follicular cell or capillary hyperemia in thyroid and frequency of chromophobic cell increase or acidophilic cell decrease or vascular/cystoid degeneration in pituitary gland. Low values of pituitary gland (absolute/relative) weight, serum T3 level, serum T4 level and rate of air righting reflux (delay).

<Findings observed at A*[2]> (The underlined findings have already been reported.)

F0 dams*: High values of frequency of reddish/enlarged thyroid, thyroid (absolute/relative) weight, serum TSH level, frequency of follicular cell enlargement/increase, capi1llary hyperlemina in thyroid and frequency of chromophobic cell increase or hyaline/vascular degeneration of chromophobic cells in pituitary gland. Low values of body weight gain, body weight, kidney (absolute/relative) weight, adrenal gland (absolute/relative) weight, food consumption, water intake, serum T3 level and serum T4 level

F1 males*: High values of frequency of enlarged or reddish thyroid, frequency of brain deformation, thyroid (absolute/relative) weight, follicular cell count in thyroid, frequency of enlarged follicular cell or squamous follicular cells or follicular cells filled with lumen colloid, and capi1llary hyperlemina in thyroid, frequency of chromophobic cell increase or acidophilic cell decrease in pituitary gland, frequency of depopulation or necrosis of reproductive cells in testis, frequency of outer cerebellar granular cell layer and frequency of partial

cerebellum deficiency in brain. Low values of body weight, body weight gain, liver (absolute/relative) weight, serum T3 level, serum T4 level, brain size (absolute length), the rate of incisor eruption (delay), the rate of eyelid opening (delay), the rate of righting reflux (delay) and the rate of preputial separation (delay). High or Low values of testis (absolute/relative) weight

F1 females*: High values of thyroid (absolute/relative) weight, serum TSH level, frequency of enlarged/reddish thyroid, frequency of follicular cell increase or of enlarged follicular cell or squamous follicular cells or follicular cells filled with lumen colloid, and capi1llary hyperlemina in thyroid, frequency of brain deformation, body weight, frequency of chromophobic cell increase or acidophilic cell decrease in pituitary gland, frequency of outer cerebellar granular cell layer and the day of vaginal opening (delay). Low values of body weight, body weight gain, pituitary gland (absolute/relative) weight, the rate of incisor eruption (delay), the rate of eyelid opening (delay), the rate of air righting reflux (delay), serum T3 level, serum T4 level, ovary (absolute/relative) and weight, brain size (absolute length, length/width)

Benzophenone

One-generation test

Dose [μg/kg/day]			Dose [mg/kg/day]		Comments
2	10	50	20	100	
C F0 dams: High values of food consumption	C F0 dams: High values of food consumption P F1 males: High values of serum LH level	C F1 males: Low values of dorsal prostate (absolute/ relative) weight P F1 males: Low values of spleen (absolute/relative) weight and serum FSH level F1 females: Low values of serum E2 level	A*[1]	A*[2]	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*[1]> (The underlined findings have already been reported.)

F0 dams*: High values of water intake

F1 males*: High values of kidney (absolute/relative) weight and serum LH level. Low values of serum E2 level and serum FSH level.

F1 females*: High values of adrenal (absolute/relative) weight and serum LH level. Low values of serum E2 level.

<Findings observed at A*[2]> (The underlined findings have already been reported.)

F0 dams*: High values of food consumption. Low values of number of delivered pups and gestation period.

F1 pups*: Low values of number of viable pups and viability of pups.

F1 males*: High values of AGD (absolute/relative) and serum LH level. Low values of residual nipples and serum FSH level. Degeneration of seminiferous tubule.

F1 females*: High values of kidney (absolute/relative) weight and serum LH level.

Bisphenol A

One-generation test

Concentration in drinking water [ppb]				Dose [mg/kg/day]	Comments
2	10	50	250		
Dose [μg/kg/day]					
0.473	2.24	11.8	53.8	500	
D	D	D	D	A*	2-250 ppb: Drinking water for 42 days 500 mg/kg/day: Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of number of individuals of muck on the meatal fur skin and kidney relative weight. Low values of food consumption and body weight. High or low values of body weight gain

F1 males*: High values of epididymis relative weight and testis relative weight

F1 females*: Low values of liver relative weight

Butylbenzyl phthalate

One-generation test

Dose [μg/kg/day]				Dose [mg/kg/day]	Comments
2	12	60	300	500	
D	D	P F1 males: Low values of AR mRNA expression level in prostate and epididymis F1 females: Low values of AR mRNA expression level in ovary	P F1 females: High values of ERβ mRNA expression level in uterus	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

D: No statistically significant difference from the control was observed.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: Low values of food consumption

F1 pups*: Low values of number of viable pups

F1 males*: High values of serum levels of LH and FSH. Severity of necrotic seminiferous tubule atrophy. Low values of body weight, body weight gain, AGD (absolute/relative), epididymis size, vas deference size, testicular sperm cell count, epididymis sperm cell count, spermatic duct (absolute/relative) weight, testis (absolute/relative) weight, epididymis (absolute/relative) weight, seminal vesicle (absolute/relative) weight, prostate (absolute/relative) weight, conception rate after second mating with untreated female, ERβ mRNA expression level in testis and AR mRNA expression level in prostate

F1 females*: High values of AGD (absolute/relative). Low values of body weight, body weight gain, conception rate, number of implantation, implantation rate, number of delivered pups, number of viable pups after second mating with untreated male and AR mRNA expression level in ovary

F2 pups* (from F1 female and untreated male): Low values of body weight gain.

cis-Chlordane

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.1	0.5	2.5	12.5	10	
C F0 dams: Low values of kidney (absolute/relative) weight F1 females: High values of adrenal gland (absolute/relative) weight	C F1 males: Low values of pituitary gland (absolute/ relative) weight	C F0 dams: Low values of food consumption, frequency of mineralization of the corticomedullary junction in the kidney F1 males: Low values of pituitary gland (absolute/ relative) weight	C F1 males: Low values of pituitary gland (absolute/ relative) weight	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of food consumption. Low values of body weight gain and kidney relative weight

F1 pups*: Low values of viability

F1 males*: High values of frequency of vascular degeneration in liver cell. Low values of pituitary gland (absolute/ relative) weight and kidney relative weight

F1 females*: High values of liver (absolute/relative) weight and frequency of vascular degeneration in liver cell

p,p'-DDD

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	
0.2	1	5	25	300	Comments
D	D	D	D	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of liver relative weight. Low values of body weight and food consumption. High or low values of body weight gain

F1 males*: High values of AGD relative, liver relative weight and frequency of vascular degeneration in cell around hepatic portal vein. Low values of body weight and pinna unfolding rate

F1 females*: High values of liver relative weight and spleen relative weight. Low values of body weight, pinna unfolding rate and brain absolute weight

p,p'-DDE

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.03	0.30	3	30	50	
C F1 pups: High values of male sex ratio P F1 males: Low values of C3mRNA expression level in prostates	C F1 pups: High values of male sex ratio P F1 males: Low values of C3mRNA expression level in prostates	C F1 pups: High values of male sex ratio P F1 males: Low values of C3mRNA expression level in prostates C F1 females: High values of the day of vaginal opening (delay), body weight and food consumption	P F1 males: Low values of C3mRNA expression level in prostates	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of liver (absolute/relative) weight and frequency of darkish hepatic color. Low values of food consumption

F1 males*: High values of liver (absolute/relative) weight, frequency of centrilobular hypertrophy of the hepatocyte and food consumption. Low values of epididymis absolute weight, seminal vesicle absolute weight, C3mRNA expression level in prostates and thymus relative weight

F1 females*: High values of liver (absolute/relative) weight, frequency of centrilobular hypertrophy of the hepatocyte, rate of incisor eruption and food consumption

o,p'-DDT

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.2	1	5	25	50	
D	D	P F0 dams: High values of frequency of thymic lymphatic tissue atrophy	P F0 dams: High values of frequency of thymic lymphatic tissue atrophy	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

D: No statistically significant difference from the control was observed.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: Low values of conception rate (0%)

p,p'-DDT

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.2	1	5	25	10	
D	C F0 dams: High values of food consumption	D	D	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of liver absolute weight

F1 males*: High values of liver (absolute/relative) weight, frequency of vascular degeneration in hepatocyte and rate of sperm head abnormality

F1 females*: High values of liver (absolute/relative) weight and frequency of vascular degeneration in hepatocyte

Di-*n*-butyl phthalate

One-generation test

Dose [µg/kg/day]					Dose [mg/kg/day]	
31	63	125	250	500	250	Comments
C F1 males: High values of seminal vesicle (absolute/relative) weight	D	D	D	D	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F1 males*: High values of spleen (absolute/relative) weight

2,4-Dichlorophenol

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.8	4	20	100	400	
D	D	C F1 males: Low values of platelet count	D	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings of 1) and 2) have already been reported for F1 and F0, respectively.)

F0 dams*: High values of liver (absolute/relative) weight[1)] and body weight gain and percentage of monocyte. Low values of food consumption

F1 males*: Low values of body weight gain[2)] and body weight.

F1 females*: Low values of body weight gain[2)] and body weight.

Dicyclohexyl phthalate

One-generation test

Dose [μg/kg/day]				Dose [mg/kg/day]	Comments
1.6	8	40	200	500	
C F1 males: Low values of pituitary gland (absolute/ relative) weight F1 females: High values of fetal death rate	P F1 females: High values of ERα and AR mRNA expression level in uterus	P F1 females: High values of ERα and AR mRNA expression level in uterus	C F0 dams: Low values of implantation sites P F1 females: High values of ERα and AR mRNA expression level in uterus	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of liver (absolute/relative) weight and adrenal (absolute/relative) weight. Hypertrophy of the centrilobular hepatocyte. Prolongation of gestation period. Low values of body weight, food consumption and number of delivered pups

F_1 males*: High values of mRNA expression level in AR in the prostate. Low values of body weight, testis (absolute/relative) weight, seminal vesicle (absolute/relative) weight, epididymides (absolute/relative) weight, kidney (absolute/relative) weight, prostate (absolute/relative) weight and levator ani muscle (absolute/relative) weight. Defects of the kidney, epididymis, ureter or seminal vesicle, small testis or epididymis, hypoplasia/agenesis of the epididymis, disappearance of the germ cell in the seminiferous tubule, hyperplasia/giant cell formation of Leydig cell, disappearance of the sperm in the lumen of the epididymis, cell debris in the lumen of the epididymis.

F_1 females*: Low values of body weight. Small uterus, hypoplasia/agenesis of the uterine horn, defects of the kidney, ureter, ovary, oviduct and uterine horn, and mineralization of the corticomedullary junction in the kidney.

Dieldrin

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.1	0.5	2.5	12.5	1	
C F0 dams: Low values of food consumption F1 males: Low values of sperm head amplitude F1 females: Low values of body weight	C F1 females: Low values of body weight	D	C F0 dams: High values of body weight gain	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F1 pups*: Low values of viability

F1 females*: High values of liver relative weight

Di-(2-ethylhexyl) adipate

One-generation test

Dose [μg/kg/day]		Dose [mg/kg/day]			Comments
15	150	1.5	15	600	
D	C F0 dams: Low values of body weight gain	C F1 males: Low values of serum testosterone level	C F1 males: Low values of serum testosterone level	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of liver (absolute and relative) weight.

F1 pups*: High values of number of stillborns. Low values of weaning rate.

F1 males*: Low values of serum testosterone level.

F1 females*: Low values of ERα mRNA expression level in ovaries.

Di-(2-ethylhexyl) phthalate

One-generation test

Dose [µg/kg/day]			Dose [mg/kg/day]		Comments
10	50	250	1.25	100	
D	C F1 females: High values of serum FSH level	D	D	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of liver (absolute/relative) weight and centrilobular hypertrophy of the hepatocyte. Increase of eosinophilic granule of the hepatocyte. Enlargement of liver.

Diethyl phthalate

One-generation test

Dose [μg/kg/day]				Dose [mg/kg/day]	Comments
0.4	2	10	50	2,000	
C F0 dams: Low values of pituitary gland (absolute/ relative) weight	C F0 dams: Low values of pituitary gland (absolute/ relative) weight	C F0 dams: Low values of pituitary gland (absolute/ relative) weight and thyroid (absolute/ relative) weight	C F0 dams: Low values of pituitary gland (absolute/ relative) weight and thyroid (absolute/ relative) weight F1 females: delay of vaginal opening	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: Low values of body weight, body weight gain, pituitary gland (absolute/relative) weight, food consumption, thyroid weight (absolute/relative) and Eosinophilic granular change of liver.

F1 pups*: Low values of viability rate and number of live offspring.

F1 males*: Low values of body weight, sperm motility (straight line velocity), body weight gain, thymus (absolute/relative) weight, testis (absolute/relative) weight; delays of behavioral development (negative geotaxis), physical development (pinna unfolding and eyelid opening) and preputial separation. Changed FSH level in serum (decreased; day 21 of lactation, increased; after mating), and histopathological changes in the testis (day 21 of lactation: decreased number of the germ cells and appearance of the elongated nuclear cells, after weaning: focal tubular atrophy).

F1 females*: High values of AGD (absolute and relative). Low values of body weight, body weight gain, thymus (absolute/relative) weight, kidney (absolute/relative) weight and motor activity (horizontal movement and rearing behavior). Delays of behavioral development (cliff aversion and negative geotaxis) and physical development (pinna unfolding, eyelid opening).

Dihexyl phthalate

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
2	10	50	250	500	
P F1 males: Low values of average glooming frequency in open field test	D	D	C F1 males: High values of frequency of minor or moderate lymphocyte infiltration of prostate interstitial tissue	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: Low values of number of delivered pups and food consumption

F1 males*: Low values of body weight and AGD (absolute/relative)

F1 females*: Low values of implantation sites

Dipentyl phthalate

One-generation test

Dose [μg/kg/day]				Dose [mg/kg/day]	
2	10	50	250	1,000	Comments
D	C F1 females: Low values of backing error frequency at the second trial on the first day of T-shaped water maize test	C F1 males: High values of select error frequency at the first and third trials on the first day of T-shaped water maize test	C F1 females: High values of spleen (absolute/relative) weight	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: Low values of body weight, fertility rate, number of delivered pups (all dead), body weight gain and food consumption.

Dipropyl phthalate

One-generation test

Dose [μg/kg/day]				Dose [mg/kg/day]	Comments
2	10	50	250	2,000	
C F1 females: Low values of body weight, body weight gain and food consumption	C F0 dams: High values of body weight gain F1 males: Low values of the day of preputial separation (advance) F1 females: High values of brain absolute weight	D	C F1 males: Low values of body weight and body weight gain F1 females: Low values of food consumption and body weight P F1 males: Low values of body weight and body weight gain	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of liver (absolute/relative) weight and frequency of sialorrhea. Low values of food consumption

F1 pups*: Low values of viability

F1 males*: High values of mortality. Low values of body weight, body weight gain, brain absolute weight, spleen (absolute/relative) weight, food consumption and error frequency on the third day of learning test

F1 females*: High values of mortality, frequency of incisive malocclusion, the day of pinna unfolding and the day of vaginal opening. Low values of body weight, air righting reflux rate, spleen (absolute/relative) weight, body weight gain, food consumption and frequency of rearing behavior

Endrin

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.1	1	5	25	0.4	
D	C F0 dams: Low values of food consumption	D	D	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of frequency of all newborns died. Low values of body weight gain, food consumption

F1 males*: High values of AGD relative

F1 pups*: Low values of liver relative weight

F1 females*: Low values of pituitary gland (absolute/ relative) weight

Heptachlor

One-generation test

Dose [µg/kg/day]			Dose [mg/kg/day]		Comments
0.05	0.5	5	1	3	
D	D	C F1 males： Low values of frequency of thyroid follicles epithelial edema	A*[1]	A*[2]	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*[1]> (The underlined findings have already been reported.)

F1 pups*: High values of liver (absolute/relative) weight and frequency of hypertrophy of the centrilobular hepatocyte

F1 females*: High values of body weight at vaginal opening day. Delay of vaginal opening day

<Findings observed at A*[2]> (The underlined findings have already been reported.)

F0 dams*: High values of frequency of all newborns died

F1 pups*: High values of frequency of died, liver (absolute/relative) weight and frequency of hypertrophy of the centrilobular hepatocyte. Low values of viability

Hexachlorobenzene

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	
0.04	0.4	4	40	40	Comments
C F1 males: Low values of sperm motility (path velocity, straight line velocity)	C F1 males: Low values of sperm motility (path velocity, straight line velocity) F1 females: Low values of serum levels of triglyceride	C F1 males: Low values of blood phospholipid level, serum levels of triglyceride and sperm motility (path velocity, straight line velocity, curvilinear velocity)	C F1 males: Low values of blood phospholipid level, serum levels of triglyceride and sperm motility (path velocity, straight line velocity, curvilinear velocity) F1 females: Low values of serum levels of triglyceride	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

serum levels of triglyceride

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of frequency of dysfunctional gestation (neglected pup, improper placental care, poor suckling behavior), frequency of tremor, frequency of autopsy finding (mammary gland developmental deficiency and defects of thymus), frequency of hypertrophy of the centrilobular hepatocyte, frequency of hepatic steatosis, frequency of hepatocellular necrosis, frequency of hepatocellular cell division, frequency of proximal tubular steatosis, frequency of defects of thymus, frequency of agalactosis and frequency of all pups died

F1 pups*: Low values of fertility rate, number of live offspring and viability

F1 males*: Low values of body weight and frequency of developed righting reflex

F1 females*: Low values of frequency of developed righting reflex

beta-Hexachlorocyclohexane

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.1	0.5	2.5	12.5	5	
C F1 males: High values of endurance in swimming behavior test(first time) and liver (absolute/relative) weight	D	D	D	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

endurance in swimming behavior test(first time)

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: Low values of body weight gain and food consumption

F1 pups*: Low values of number of live weaning pups and viability

F1 males*: High values of liver relative weight. Low values of testis absolute weight, brain absolute weight and prostate (absolute/relative) weight

F1 females*: High values of liver relative weight, pituitary gland relative weight, kidney relative weight, adrenal gland relative weight, frequency of reduction of corpora lutea in ovary, frequency of uterine squamous metaplasia, frequency of endometrial squamous metaplasia and frequency of vaginal cornification. Low values of thymus (absolute/relative) weight, body weight gain, brain absolute weight, ovary (absolute/relative) weight. Delay of vaginal opening day and first estrus. Persistent estrus. High or low values of uterus (absolute/relative) weight. High or low values of body weight

Malathion

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	
1	5	25	125		Comments
D	C F0 dams: Low values of body weight gain and food consumption	C F0 dams: Low values of body weight gain and food consumption	C F0 dams: Low values of food consumption	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: Low values of acetylcholine esterase activity in brain

Mirex

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.02	0.2	2	20	2	
D	D	C F1 females: High values of body weight and body weight at autopsy	C F1 females: High values of body weight at autopsy	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of frequency of hypertrophy of the centrilobular hepatocyte and frequency of all newborns died. Low values of body weight gain and food consumption

F1 pups*: High values of frequency of cataract. Low values of viability, fertility rate number of live offspring and rate of developed righting reflex

F1 females*: Low values of rate of developed auditory canal and body weight gain. Delay of vaginal opening day

4-Nitrotoluene

One-generation test

Dose [μg/kg/day]				Dose [mg/kg/day]	
1	5	25	125	100	Comments
C F0 dams: Low values of erythrocyte count and hematocrit	C F0 dams: Low values of hematocrit	C F0 dams: Low values of erythrocyte count, hemoglobin content and hematocrit P F1 males: Low values of reticulocyte count F1 females: High values of neutrophil percentage	C F0 dams: Low values of hematocrit F1 male: Low values of body weight gain P F1 females: Low values of reticulocyte count	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of body weight gain and average erythrocyte pigment level. Low values of erythrocyte count, hemoglobin content and hematocrit.

F1 males*: Low values of body weight gain, liver (absolute) weight and body weight and reticulocyte count.

F1 females*: High values of the day of incisor eruption (delay). Low values of body weight, hemoglobin content, reticulocyte count and leukocyte count

trans-Nonachlor

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.05	0.5	5	50	10	
D	D	C F0 dams: Low values of body weight gain F1 males: High values of brain absolute weight F1 females: High values of brain absolute weight	C F1 males: High values of brain absolute weight	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (Underlined findings were only observed in preliminary tests.)

F0 dams*: High values of liver relative weight, frequency of hypertrophy of the centrilobular hepatocyte and frequency of hepatocellular ground glass appearance in central lobule. Low values of body weight gain

F1 males*: High values of brain absolute weight, liver (absolute/relative) weight, frequency of hypertrophy of the centrilobular hepatocyte and frequency of hepatocellular vacuolization in lobular intermediate zone

F1 females*: High values of brain absolute weight, liver (absolute/relative) weight, ovary relative weight, frequency of hypertrophy of the centrilobular hepatocyte and frequency of hepatocellular vacuolization in lobular intermediate zone

4-**Nonylphenol(branched)**

One-generation test

Concentration [ppb]				Dose of EE [μg/kg/day]	Comments
30	100	300	1,000		
Dose [μg/kg/day]					
6.9	23.2	70.9	234	0.1	
C F1 pups: Low values of male sex ratio F1 males: High values of body weight gain	C F0 dams: High values of food consumption	P F1 males: Low values of GAPDH mRNA expression level in prostate. High values of ERβ and AR mRNA expression levels in prostate	C F0 dams: High values of food consumption P F0 dams: High values of spleen (absolute/ relative) weight F1 males: High values of ERβ and AR mRNA expression level in prostate F1 females: High values of GAPDH mRNA expression level in uterus	A*	Drinking water for 42 days EE: Subcutaneous injection for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (Underlined findings were only observed in preliminary tests.)

F0 dams*: High values of food consumption

F1 males*: High values of body weight, body weight gain and water intake..

F1 females*: High values of food consumption and water intake. Low value of the first estrus cycle period

Octachlorostyrene

One-generation test

Dose [μg/kg/day]				Dose [mg/kg/day]	Comments
2.4	12	60	300	50	
C F1 females: High values of corpora lutea	D	C F1 females: Low values of uterus (absolute/relative) weight P F1 females: High values of ERβ mRNA expression level in uterus	C F1 males: High values of epididymis (absolute/relative) weight P F1 females: High values of ERβ mRNA expression level in uterus	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: Hypertrophy of the centrilobular hepatocyte. Slight proliferation of mammary gland lobule. Low values of food consumption and number of implantations.

F_1 pups*: Low values of viability rate (all newborns died before Day 9 of lactation) and body weight.

4-*t*-Octylphenol

One-generation test

Dose [μg/kg/day]				Dose of EE [μg/kg/day]	Comments
3	10	30	100	0.1	
D	C F1 females: Low values of spleen (absolute/relative) weight P F1 males: High values of GAPDH mRNA expression level in prostate	D	P F1 females: Low values of IGF-1 mRNA expression level in uterus	A*	Gavage for 42 days EE: subcutaneous injection for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (Underlined findings were only observed in preliminary tests.)

F1 males*: High values of ERβ and GAPDH mRNA expression level in prostate and testicular sperm count. Low values of AR mRNA expression level in prostate, body weight and spleen (absolute/relative) weight.

F1 females*: Low values of body weight spleen (absolute/relative) weight, IGF-1 mRNA expression level in uterus and the day of vaginal opening (advance).

Pentachlorophenol

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	Comments
0.5	5	50	500	30	
C F1 females: Low values of body weight gain. High values of error frequency at the first time of the third trial of T-shaped water maize tests	P F1 females: High values of spleen (absolute/ relative) weight	C F1 males: Low values of seminal vesicle (absolute/ relative) weight	C F1 males: Low values of seminal vesicle (absolute/relative) weight. High values of error frequency at the third time of the first trial of T-shaped water maize tests and endurance in swimming behavior test F1 females: Low values of body weight gain	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of liver (absolute/relative) weight. Low values of body weight, body weight gain and fertility rate

F1 pups*: High value of male sex ratio. Low values of number of delivered pups and number of viable pups

F1 males*: High values of testicular sperm cell count, the day of incisor eruption (delay) and the day of preputial separation (delay). Low values of body weight, body weight gain, pinna unfolding rate, testis (absolute/relative) weight, prostate (absolute/relative) weight and brain (absolute/relative) weight.

F1 females*: High values of ovary (absolute/relative) weight, thymus (absolute/relative) weight, spleen (absolute/relative) weight and the day of incisor eruption (delay). Low values of body weight, body weight gain, pinna unfolding rate, endurance in swimming behavior test (at the first time of the first trial), error frequency in the T-shaped water maize test (at the first time of the third trial) and brain absolute weight.

F2 pups*: Low values of number of delivered pups and number of viable pups.

Permethrin (mixture)
(*cis*-Permethrin:*trans*-Permethrin=40:60)

One-generation test

Dose [µg/kg/day]				Dose [mg/kg/day]	
0.5	5	50	500	100	Comments
D	C F1 females: Low values of pituitary gland (absolute/ relative) weight	D	D	A*	Gavage for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: High values of frequency of tremor or hypersensitivity

F1 females*: Low values of pituitary gland (absolute/ relative) weight

Tributyltin chloride

One-generation test

Concentration in diet [ppm]					Comments
0.15	0.45	1.5	4.5	30	
Dose [µg/kg/day]				Dose [mg/kg/day]	
10	30	100	300	2	
D	D	C F1 males: Low values of spleen (absolute/ relative) weight	C F0 dams: High values of food consumption F1 males: Low values of spleen (absolute/relative) weight P F1 males: High values of neutrophil percentage	A*	Dietary for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: Low values of body weight, thymus (absolute/relative) weight, spleen (absolute/relative) weight, ovary (absolute/relative) weight and uterus (absolute/relative) weight.

F1 males*: Low values of spleen (absolute/relative) weight.

F1 females*: Low values of body weight and thymus (absolute/relative) weight.

Triphenyltin chloride

One-generation test

Concentration in diet [ppm]					Comments
0.015	0.15	1.5	5	15	
Dose [µg/kg/day]				Dose [mg/kg/day]	
1.1	11	107	370	1.117	
D	D	C F1 males: Low values of body weight	C F0 dams: High values of food efficiency P F1 females: Low values of serum levels of T3	A*	Dietary for 42 days

A: Substantial changes (statistically significant differences from the control) that have already been reported were observed around the LOEL or LOAEL.

C: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. In the present study, however, these changes are suggested to be within the range of biological/physiological deviation.

D: No statistically significant difference from the control was observed.

P: Statistically significant differences from the control were observed in certain endpoints below the LOEL or LOAEL at which some effects have already been reported. However, their biological/toxicological significance remains to be elucidated at present (pending).

<Findings observed at A*> (The underlined findings have already been reported.)

F0 dams*: Low values of food consumption.

F1 pups*: Low values of viability.

ANNEX 3

ER-mediated mechanism

Updated results

		ER-binding		Total
		+	−	
Utero-trophic assay	+	33*1	5	38
	−	21*2	15	36
Total		54	20	74

Concordance	65
Positive Predictivity	61
Negative Predictivity	75
False Positive Rate	58
False Negative Rate	13

*1 :The lowest RBA in ER-binders, which showed uterotrophic activity was 0.0023.

*2: RBAs of 12 of 21 chemicals were lower than 0.0023.

Put chemicals with RBA<0.002% "negative".

		ER-binding		Total
		+	−	
Utero-trophic assay	+	33	5	38
	−	9*3	27	36
Total		42	32	74

Concordance	81
Positive Predictivity	79
Negative Predictivity	84
False Positive Rate	25
False Negative Rate	13

*3 : Negative in ER-TA assay

Relationships between *in vitro* and *in vivo* screening assays - Chemicals Evaluation and Research Institute, Japan (CERI) – presented December 2006 at the VMG-Non Animal.

PART II

VALIDATION OF THE UTEROTROPHIC BIOASSAY IN MICE BY BRIDGING DATA TO RATS

Part II of this document includes additional data on the mouse Uterotrophic Bioassay. The report relies on data generated by Cellular and Molecular Toxicology Division of the National Institute of Health Sciences (NIHS), Japan. It is gratefully acknowledged that NIHS made this information available to the OECD.

Background

1. The OECD has developed a Test Guideline for the Uterotrophic Bioassay in rodents (TG 440) for *in vivo* screening for oestrogenic properties of potential endocrine disrupters. This short-term screening test originated in the 1930's (1, 2) and is based on the increase in uterine weight or uterotrophic response (3). The uterus responses in rats and mice qualitatively are comparable.

2. The Test Guideline (TG 440) is based on the results of an international validation programme under the umbrella of the OECD including extensive intra- and interlaboratory studies to show the reliability and reproducibility of the bioassay. The validation studies were restricted to the rat as test species, using two different basic test protocols, i.e. immature intact or young adult ovariectomized rats, by oral or subcutaneous substance administration.

3. Notwithstanding that the validation studies were only carried out with rats, but taking into consideration that a vast historical data base exists for mice, too, the Test Guideline for the Uterotrophic Bioassay (TG 440) offers the option to also use mice. But there is a restriction stating (cf. §19 of TG 440) that "in some cases mice may be used instead of rats" and "a rational should be given for this species (mice)". It is pointed out that "modifications of the protocol may be necessary for mice".

4. Such modifications may relate to the higher food consumption of mice on a body weight basis as compared to that of rats and therefore the phytooestrogen content in food should be lower for mice than for rats (4, 5, 6). In addition, because of the earlier onset of puberty, dosing of immature mice should start earlier than for rats. Finally, the smaller size of mice and a more demanding dissection or trimming procedure may lead to a higher coefficient of variation of the uterine weight for mice than for rats.

5. To broaden the scope of the Uterotrophic Bioassay Test Guideline in rodents (TG 440) to the use of mice as test species, a limited validation study was carried out in mice by the "Cellular and Molecular Toxicology Division of NIHS" (Tokyo) (Annex). The results thereby obtained are used as bridging data in conjunction with the previous rat validation studies.

Basic design of the bridging validation studies

6. Two laboratories, that had already participated in the OECD rat Uterotrophic Bioassay validation activities (phases I and II) (7, 8) were involved in the mouse validation bridging studies.

7. Test animals were C57BL/6 female adult mice. Test substances were given in corn oil or 0.5%

CMC-Na (for genistein per os study). The diet was CRF-1 (Oriental Yeast, Co., Tokyo) with a phytooestrogen content of approximately 130µg of genistein (aglycon) equivalents/g of diet .

8. The basic study design used ovariectomized adult female animals both by the subcutaneous and oral application route. Ovariectomy was carried out at the age of 7 weeks according to the procedure described for rats in TG 440. Substance administration started at the age of 8 weeks.

9. The time span between ovariectomy and substance application generally was only one week in mice, in contrast to the two weeks time interval specified for rats (TG 440, § 30) . The justification is given in Fig.0, since after ovariectomy mice show a slightly faster decline in uterine weight than rats.

10. The duration of test material administration varied between 1 – 14 days and the weight of the blotted uterus was determined. The increase of uterine weight was the parameter for oestrogenic activity.

11. In addition, some studies focused on antioestrogenic activity of the test chemicals (bisphenol A and genistein). Antioestrogenic activity was measured against ethinyl estradiol as reference oestrogen with a dose of 0.6 µg/kg body weight subcutaneously.

Results (all the figures are presented in Annex)

12. In Fig. 1 and 2 the uterotrophic response is given after an administration of 17ß-estradiol over 1 – 14 days. A clear increase in uterine weight is recorded already after three daily administrations of 1 and 5 µg/kg body weight/day. The prolongation of treatment gives some further increase in response, but the three days administration period is considered to be sufficient for this *in vivo* screening test. This corresponds well to the specification of TG 440 recommending for rats daily dosing over three consecutive days which may be extended up to seven consecutive days for ovariectomized female rats (cf. §39). Obviously, in mice similar to rats there is no significant advantage of a 7-day vs. a 3-day treatment, at least for potent oestrogenic compounds.

13. Fig. 3 shows the uterus weights of rats after daily subcutaneous applications of 17ß-estradiol over 3, 7 and 14 days at dose levels of 0.1, 0.3, 0.7, and 1.0 µg/kg body weight/day. Fig. 4 compares the uterine weight increase after 7 and 14 days of administration in rats (17ß-estradiol and ethinyl estradiol (7)) and in mice (17ß-estradiol). At a dose level of 0.3 µg/kg body weight/day there is a clear and almost identical increase in uterine weight for rats and mice dosed with 17ß-estradiol. The response to 17α-ethinyl estradiol in rats is even more pronounced. At higher dose levels (1 µg/kg body weight/day) the uterine weight increase in mice is higher as compared to that of rats. Therefore, the uterotrophic response to 17ß-estradiol is qualitatively well comparable to that in rats and the threshold dose is very similar in both species.

14. In Fig. 5 the dose response relationship for the uterus weight after administration of bisphenol A (50, 100, 300, and 600 mg/kg body weight/day) over seven days is given. (Note: in this experiment the time span between ovariectomy and substance application was eleven days.) Already with a dose of 50 mg/kg body weight/day there was a significant increase in uterine weight. The uterine weight increase at 600 mg/kg body weight/day was nearly twice as high as that obtained with a reference dose of 1 µg 17ß-estradiol/kg body weight/day. This data shows that this mouse bioassay clearly detected the weak oestrogen agonist activity of bisphenol A.

15. Qualitative similar results are presented in Fig. 6. After application of 10, 30, 100, and 300 mg/kg body weight/day (s.c.) over seven days there was a non-significant uterine weight increase at a dose level of 10 and a significant increase at 30, 100 and 300 mg/kg body weight/day. The uterine weight increase at 100 mg/kg body weight/day was about 1/3 of that obtained with 17α-ethinyl estradiol at a dose level of 0.2 µg/kg (s.c.) body weight/day. (Fig. 6' shows the antagonistic response of bisphenol A monitored concurrently).

16. Fig. 7 compares the uterine weight increase of ovariectomized rats and mice after seven days of subcutaneous administration of bisphenol A obtained by the two participating laboratories. In the dose range below 500 mg/kg body weight/day rats are roughly twice as sensitive as mice, but around 500 mg/kg body weight/day and above the response of both species is similar. Notwithstanding the lower sensitivity of mice in the low dose region, for in vivo screening purposes by the Uterotrophic Bioassay the response of mice is comparable to that of rats.

17. In Fig. 8, the uterotrophic response is given after oral application of genistein (20, 60, 200, and 600 mg/kg body weight/day) over seven days. There is a significant increase in blotted uterine weight at 60, 200 and 600 mg/kg body weight/day demonstrating the sensitivity of mice for the detection of this weak oestrogen agonist. (Fig. 8' shows the antagonistic response of genistein monitored concurrently).

18. In order to compare the mouse genistein data (oral, 7 days) to rat, OECD phase II validation study data were referred (8). There were four protocols in the OECD phase II validation studies, i.e. immature p.o. 3 days (Protocol A), immature s.c. 3 days (Protocol B), adult ovx s.c. 3 days (Protocol C) and adult ovx s.c. 7 days (Protocol CX). As there was no ovx 7day p.o. protocol in OECD validation, virtual ovx 7day p.o. genistein data were created from available ovx 7day s.c. data by estimating the sensitivity difference between the two different routes of exposure. The sensitivity difference due to the route of exposure was estimated by comparing the response of immature p.o. 3 day and immature s.c. 3 day, i.e. Protocol A and Protocol B. Data from three labs are shown in Figure 9. The log transformed graphs allowed to estimate the sensitivity difference by route as horizontal distance (average) between Protocol A and B, as shown in Figures 10 and 11. The sensitivity difference was about 10 fold. The data of ovx 7 day s.c. before transforming to p.o. data is shown in Figure 12. In Figure 13, the transformed rat data from s.c. to p.o. by a 10 fold difference in sensitivity is co-plotted with the mouse data shown in Figure 8. Thereby the excellent concordance between ovariectomised rats and mice after oral exposure is substantiated.

19. In addition to the agonistic activities of bisphenol A and genistein, the antagonistic activities were also investigated:

- Bisphenol A (fig.6') was given subcutaneously daily for seven days at dose levels of 10, 30, 100, and 300 mg/kg body weight/day after administration of a single subcutaneous dose of 17α-ethinyl estradiol (0.6 µg/kg) as reference oestrogen agonist. This compares well to the reference agonist dose proposed for rats (1 µg/kg body weight of 17α-ethinyl estradiol) (cf. § 31 of the Guidance Document on the Uterotrophic Bioassay – Procedure to test for antioestrogenicity). A significant antagonistic activity was found at dose levels of 100 and 300 mg bisphenol A/kg body weight/day: at these dose levels there was a significant reduction of the increased uterus weight elicited by the reference oestrogen agonist.

- Qualitatively similar results were obtained with genistein (fig. 8'): again 17α-ethinyl estradiol was used as reference oestrogen agonist at a dose level of 0.6 µg/kg body weight subcutaneously. Oral administration of genistein at dose levels of 0, 20, 60, 200, and 600 mg/kg body weight/day over seven days led to a significant reduction of the blotted uterus weight at dose levels of 60 mg/kg body weight/day and higher as compared to the uterus weight obtained by the reference oestrogen agonist.

- In conclusion, the Uterotrophic Bioassay in young adult ovariectomized mice clearly showed an antioestrogenic activity of bisphenol A and genistein using a similar protocol as proposed for rats with similar dose levels for the reference oestrogen agonist.

20. Tab. 1 lists the coefficients of variation obtained in the experiments mentioned here for mice (fig.

1, 2, 5, 6, and 8) and rats (fig. 3, 7, and 12) with 17ß-estradiol, bisphenol A, and genistein. The mean coefficient of variation for mice was 12.6 % (SD +/- 7.7) and that for rats 10 % (SD +/- 4.5). There is a slight tendency for a larger coefficient of variation in mice, but as a whole the CVs of both species are considered to be equivalent. Therefore the overall sensitivity is comparable despite the smaller size of mouse uteri.

21. Apart from the studies mentioned above, to obtain an indication for the inter-laboratory variation, a limited comparison has also been carried out for bridging data obtained after oral and subcutaneous administration in two laboratories (A and B). Although these two laboratories did not conduct the assays on a same chemical to avoid unnecessary overlap, a vehicle control and two ethinyl estradiol control groups, either for agonist (either given s.c. or p.o.) or antagonist detection (always given via s.c. route) were commonly conducted. The data of four separate experiments each, from both laboratories, are compared for the evaluation of inter-laboratory variation. In Figure 14, "(s.c.)" is for subcutaneous route protocol and "(p.o.)" for oral route protocol. In s.c. protocol, EE0.2 and EE0.6 indicate that 0.2 and 0.6 µg/kg of ethinyl estradiol were given s.c. In p.o. protocol, EE6 indicates that 6 µg/kg of ethinyl estradiol were given orally and EE0.6 indicates that 0.6 µg/kg of ethinyl estradiol were given subcutaneously. The four columns represent four experiments carried out separately. Vertical axes are the blotted uterine weight in mgs. In conclusion, the blotted uterine weights obtained by both laboratories (Lab A and Lab B, (s.c.) and (p.o.)) are within good concordance taking different breeder of mouse, diet, water and animal facility into consideration.

A good inter-laboratory reproducibility can also be deferred qualitatively from the data depicted in Fig.7 for bisphenol A.

22. Most of these investigations were carried out with a one week interval between ovariectomy and application of the test chemical and clear uterotrophic responses could be demonstrated. Therefore, such a one week interval may be sufficient for mice although a two week interval may be preferable to allow for complete uterus regression. (Cf. Fig. 0)

23. Most of these investigations were carried out with a 7-day application duration. Comparative data for a 3- and 7-day duration are available only for 17ß-oestradiol, but not for the reference weak oestrogen agonists, bisphenol A and genistein. In two experiments with the strong agonist 17ß-oestradiol different application durations were used. In Fig. 2 there is an indication for an increase in uterine response when the duration is prolonged from 3 to 7 days, but this prolongation would not change the overall interpretation. In contrast, in rats, according to Fig. 3 there was virtually no difference in response when 17ß-oestradiol was given over 3 or 7 days.

Conclusion

24. The results presented here are meant to bridge the extensive validation for the rat Uterotrophic Bioassay so that mice can be used in a corresponding screening procedure. This bridging approach with a limited number of test chemicals, participating laboratories and without coded sample testing has been selected for animal welfare reasons not to use an unnecessary large number of experimental mice. This bridging validation study shows for the Uterotrophic Bioassay in young adult ovariectomized mice that:

- a one week interval between ovariectomy and substance application may be sufficient, but a longer interval of two weeks as proposed for rats may also be used;

- for weak oestrogens and chemicals without information on their potency the test material should be administered over seven days;

- for strong oestrogen agonists an application duration of three days should be sufficient without a significant advantage by an extension of up to seven days;

- weak oestrogen agonists will be detected with a sensitivity comparable to that of experiments with rats;

- a protocol modification (comparable to that given in the GD on the protocol for antioestrogenicity of the Uterotrophic bioassay (10)) with prior administration of a reference oestrogen agonist will allow for the detection of antagonistic activities;

- the oral and subcutaneous application route are both acceptable;

- there is a good interlaboratory concordance;

- and in summary that qualitatively and quantitatively, the data obtained in rats and mice correspond well with each other.

25. These bridging validation studies are limited to young adult ovariectomized mice.

26. In conclusion, these data give strong support to the use of young adult ovariectomized mice in the context of the OECD Test Guideline on the "Uterotrophic Bioassay in rodents" (TG 440).

Recommendations for the future

27. There are two points that may be addressed in the future to expand the applicability of this procedure:

a) application duration of three days in ovariectomised mice for chemicals with weak or unknown oestrogenic activity;

b) use of intact immature mice.

28. ad a): whether a three day application duration may be sufficient for weak oestrogen agonists might be solved by a literature search or by data collected within future studies.

29. ad b): major problems to establish an OECD test guideline for immature intact mice may be:

- what is the most appropriate postnatal day to start dosing?

- is there an influence of different phytooestrogen concentrations in the diet?

- what are the CVs to be expected for the uterine weights taking account of the small uteri in immature mice?

  Again these issues might be solved by data from literature or by results obtained in the future with immature intact mouse test animals.

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ANNEX: SOME CONSIDERATIONS ON MOUSE UTEROTROPHIC ASSAY

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Division of Cellular and Molecular Toxicology, National Institute of Health Sciences

Abstract

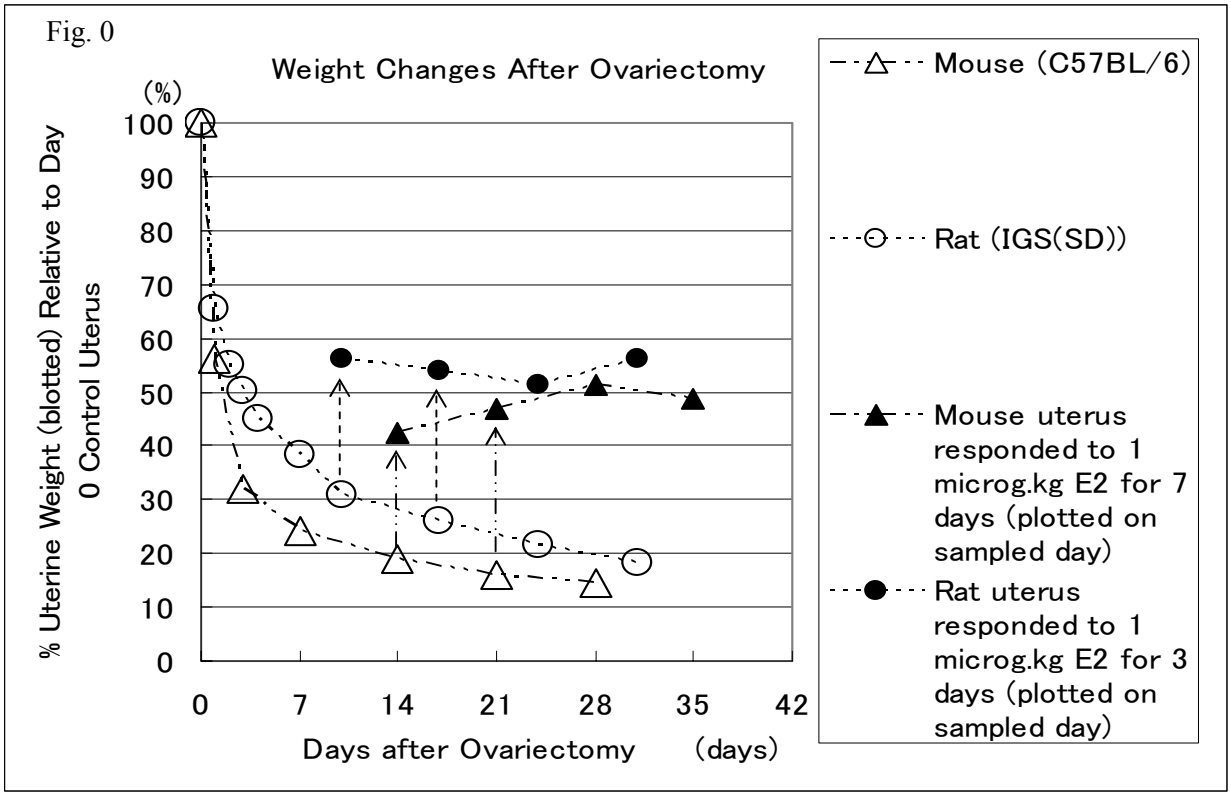
Data generated by Cellular and Molecular Toxicology Division of NIHS, Tokyo to reinforce the biological plausibility that mouse uterotrophic assay should be equivalent to rat uterotrophic assay are shown along with brief explanation on the protocols. Two laboratories, who had participated in OECD Rat Uterotropic Assay Validation activities (Phase I and II), have been conducting Mouse Uterotrophic protocol assays (Ovariectomized adult female protocol, oral and subcutaneous (s.c.) route) under MHLW contract, have shown comparable data on vehicle control and two ethinyl estradiol (EE) control groups. As a conclusion, overall performance between rat and mouse were considered equivalent in terms of sensitivity and robustness.

i. Comparison of Uterotrophic responses in Rat and Mouse

(0) Uterine weight change after ovariectomy (time course)

The uterine weight change was monitored after ovariectomy up to 4 weeks (@NIHS). As shown in Figure 0, mouse shows slightly faster decline in uterine weight than rat. Although a 7-day interval between ovariectomy and dosing was considered to be acceptable, 14 day interval was slightly better in response to Estradiol and recommended by the OECD validation protocols (Figure 0).

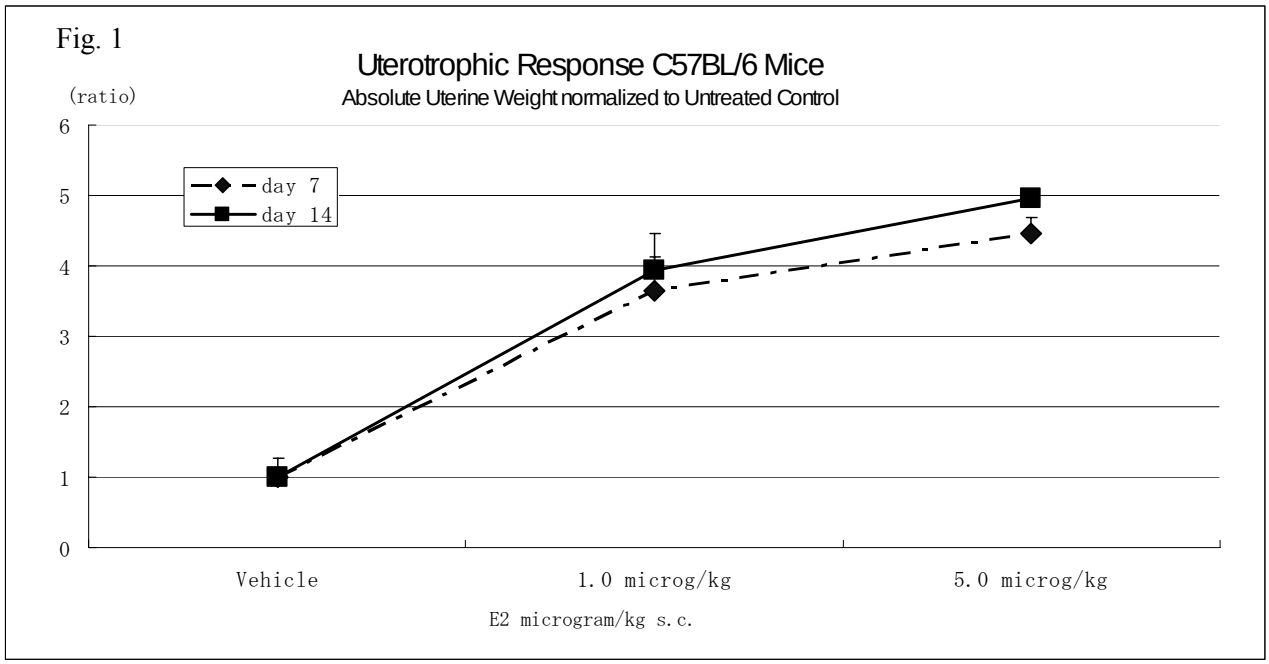
Fig. 0



(1) 17-beta-estradiol

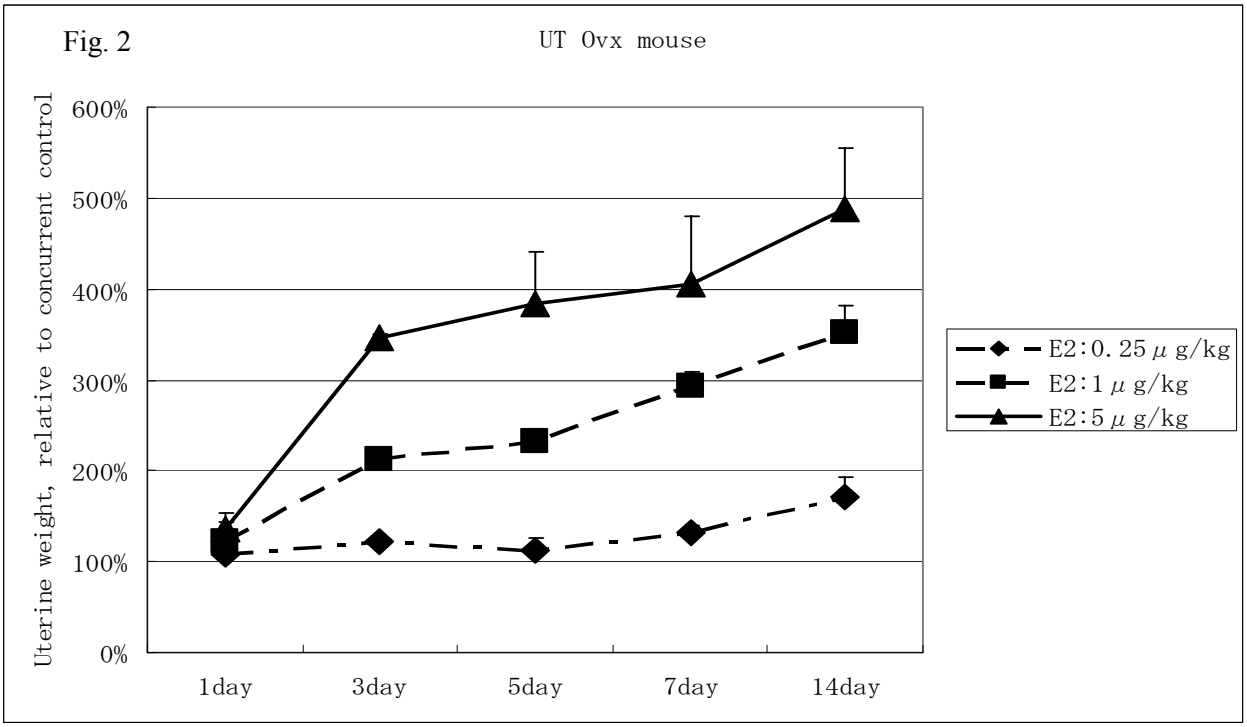
a) 17-beta estradiol at a dosage of 0, 1.0, or 5.0 microgram/kg, were given subcutaneously for 7 or 14 days from the age of 8 weeks to C57BL/6 female adult mice ovariectomized at the age of 7 weeks, and uterus weights (blotted) were measured (vehicle = corn oil, @NIHS). Horizontal axis, estradiol dosage (s.c.); vertical axis, fold increase of uterus weights against concurrent vehicle control (Figure 1).

Fig. 1



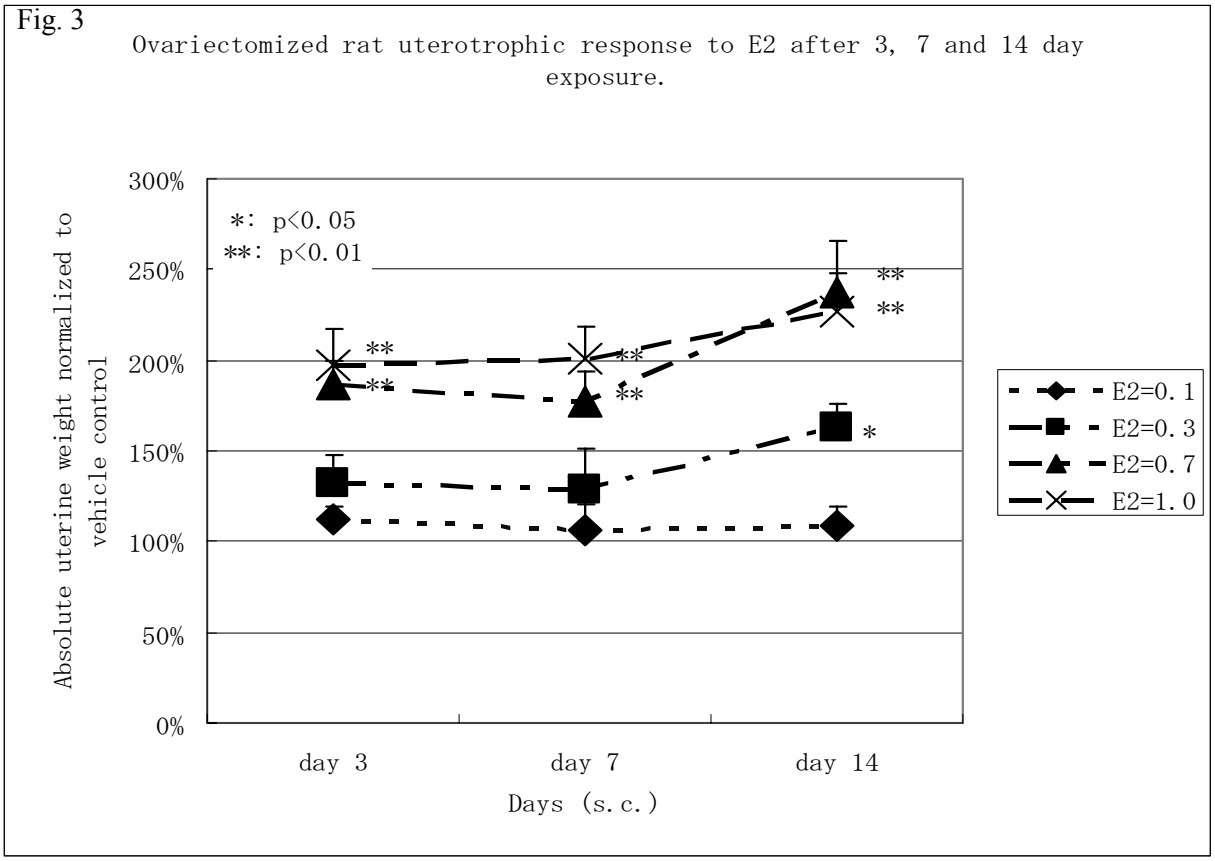
b) 17-beta estradiol at a dosage of 0, 0.25 or 1.0 or 5.0 microgram/kg, was given subcutaneously for 1, 3, 5, 7 or 14 days from the age of 8 weeks to C57BL/6 female adult mice ovariectomized at the age of 7 weeks, and uterus weights (blotted) were measured (vehicle = corn oil, @NIHS). Horizontal axis, days of estradiol administration (s.c.); vertical axis, percent increase of uterus weights against the concurrent vehicle control (Figure 2).

Fig. 2



c) SD(IGS) rats were given of 17-beta-estradiol subcutaneously at dosage levels of 0, 0.1, 0.3, 0.7, or 1.0 microgram/kg for 3, 7 or 14 days, and uterine weights (blotted) were measured (vehicle = corn oil, @NIHS). Horizontal axis, days of estradiol administration (s.c.); vertical axis, percent increase of uterus weights against the concurrent vehicle control (Figure 3).

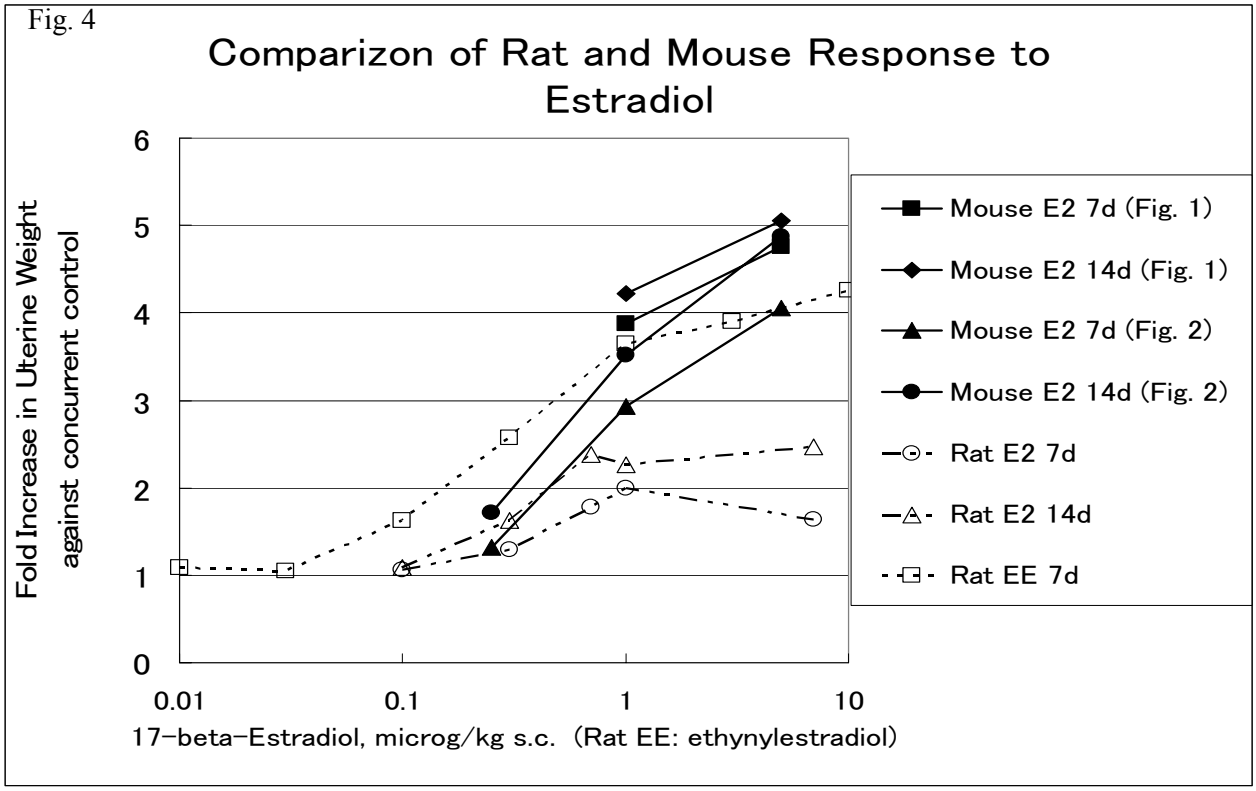
Fig. 3



d) The above three data obtained by NIHS are combined to a single graph for comparison of rat and mouse performance on 17-beta estradiol (7 day and 14 day exposure by subcutaneous route are shown). A rat ethinyl estradiol data, subcutaneously administered for 7 days is included as a reference (OECD Phase I data, CX protocol). Ethinyl estradiol is known to be similar to, or slightly more potent than 17-beta-estradiol in estrogenicity. Horizontal axis, dosage of 17-beta estradiol and ethinyl estradiol; vertical axis, fold increase of uterine weights against the concurrent vehicle control (Figure 4).

As a conclusion, despite a slight difference in slope steepness, mouse responded similar to, or slight better than rat in response to 17-beta estradiol.

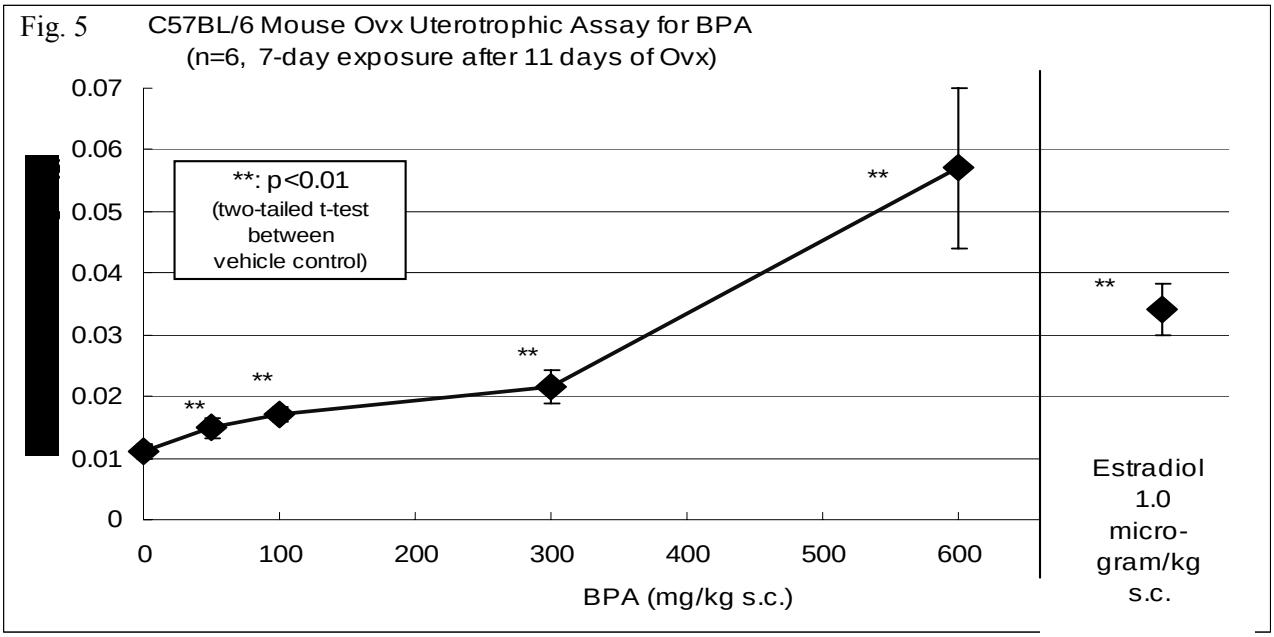
Fig. 4



(2) Bisphenol A

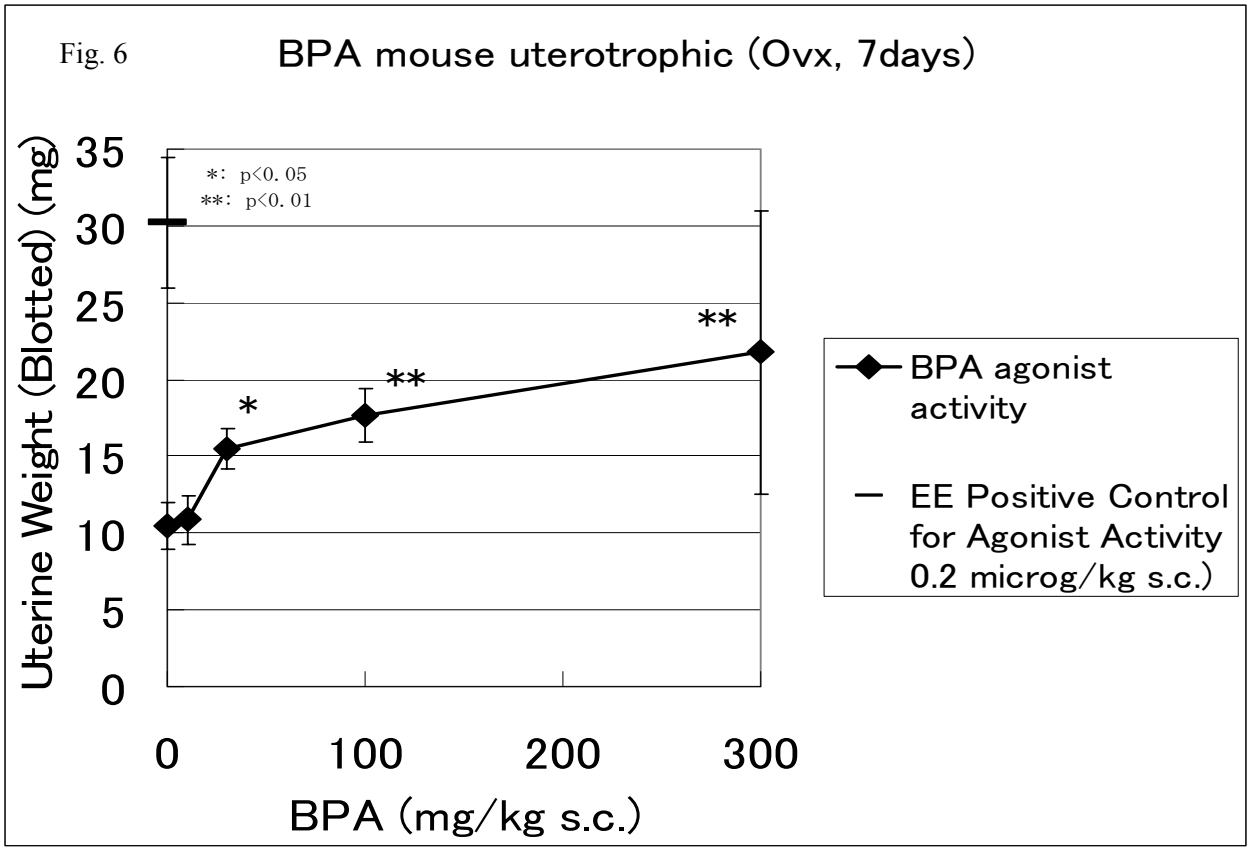
e) Bisphenol A (BPA) at a dosage of 50, 100, 300 or 600 mg/kg was given s.c. for 7 days from the age of 8 weeks to C57BL/6 female adult mice ovariectomized 11 days prior to dosage, and uterine weights (blotted) were measured (vehicle = corn oil, @ NIHS). 17-beta estradiol of 1 microgram/kg s.c. was administered as concurrent positive control. Horizontal axis, dosage of BPA; vertical axis, absolute uterine weights (Figure 5).

Fig. 5 C57BL/6 Mouse Ovx Uterotrophic Assay for BPA
(n=6, 7-day exposure after 11 days of Ovx)



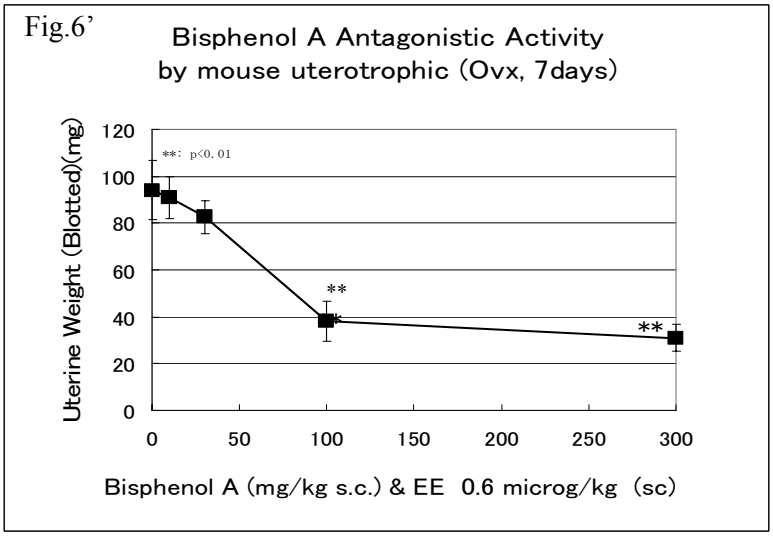
f) Bisphenol A at a dosage of 10, 30, 100 or 300 mg/kg was given s.c. for 7 days from the age of 8 weeks to C57BL/6 female adult mice ovariectomized at the age of 7 weeks, and uterus weights (blotted) were measured (vehicle = corn oil, @FDSC). Ethinyl estradiol of 0.2 microgram/kg s.c. was administered as a positive control. (Figure 6)

Fig. 6



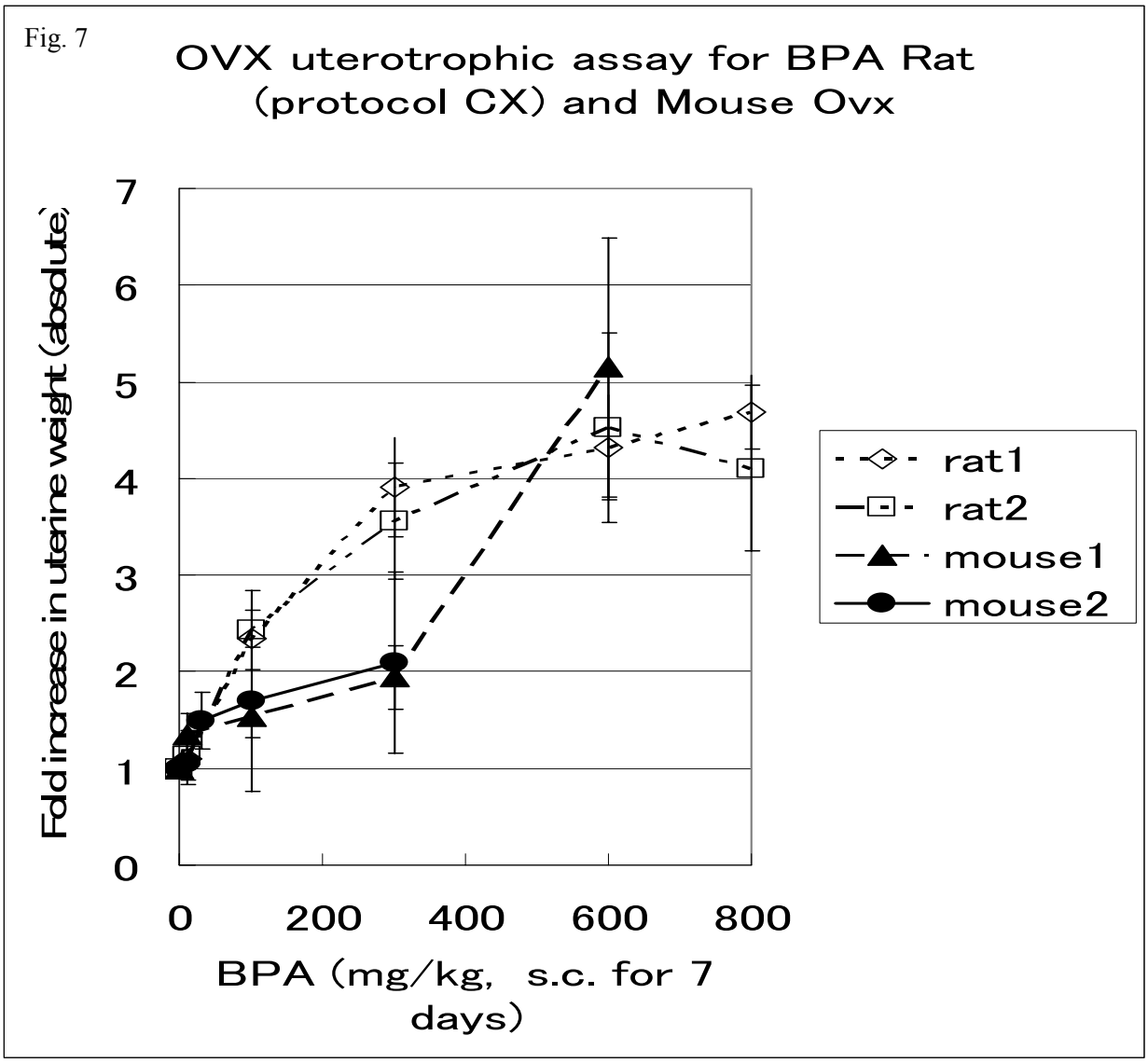
Just for reference, antagonistic activity of Bisphenol A (s.c.) monitored concurrently is shown in Figure 6'. It indicates that Bisphenol A acts as partial agonist to ovx mouse uterus (reference estrogen: EE 0.6 microgram /kg s.c.).

Fig.6'



g) The mouse data above obtained by the two participating laboratories are plotted together with comparable rat data in OECD rat phase 2 validation paper (ref 1), from studies using similar protocols, i.e. the protocol CX (ovariectomized rat, 7 day s.c. administration) (Figure 7).

Fig. 7

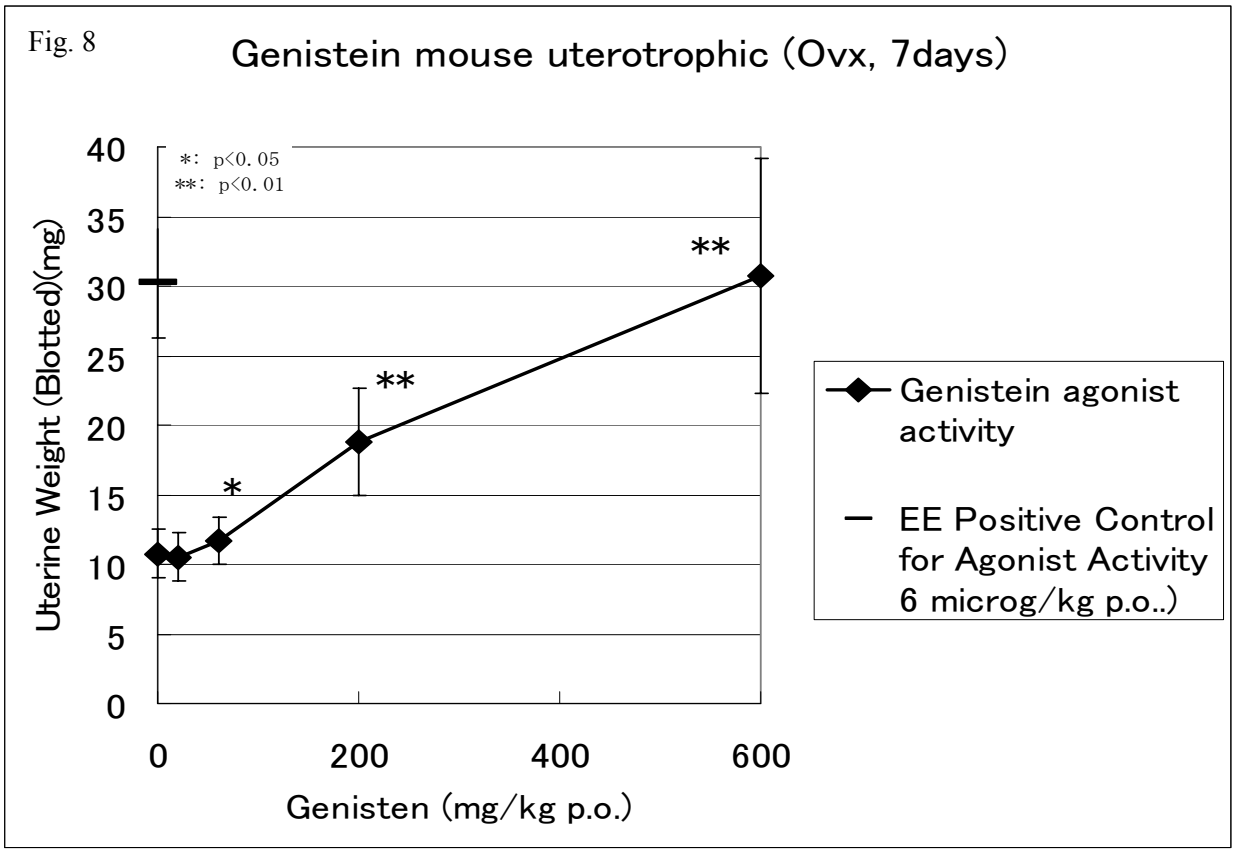


Rats are roughly twice as sensitive as mice to BPA in lower dose range. Assumed lowest detectable doses of rat and mouse would be around 20 mg/kg and 40mg/kg, respectively. In this particular comparison, mouse are slightly less sensitive at lower dose ranges, but catches up at higher dose range, assuring equal overall sensitivity.

(3) Genistein

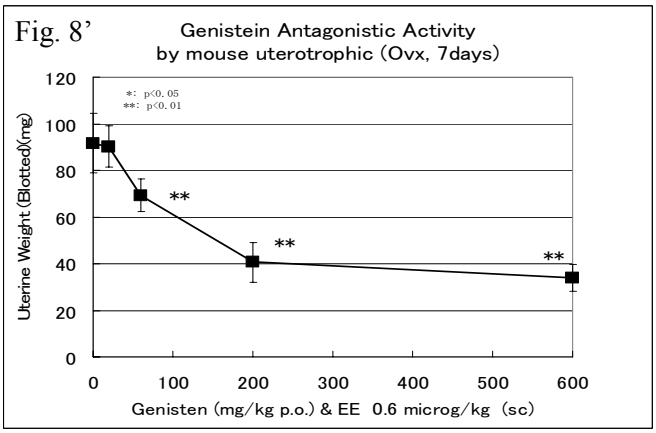
h) Genistein at a dosage of 20, 60, 200 or 600 mg/kg, was given orally for 7 days from the age of 8 weeks to C57BL/6 female adult mice ovariectomized at the age of 7 weeks, and uterus weights (blotted) were measured (vehicle = 0.5% CMC-Na). Ethinyl estradiol of 6 microgram/kg p.o. was administered as positive control (Figure 8).

Fig. 8



Just for reference, antagonistic activity of genistein (p.o.) monitored concurrently is shown in Figure 8'. It indicates that genistein acts as partial agonist to ovx mouse uterus (reference estrogen: EE 0.6 microgram /kg s.c.).

Fig. 8'



In order to compare the mouse genistein data (oral, 7 days) to rat, OECD phase II validation study data were referred. There were four protocols in the OECD phase II validation studies, i.e. immature p.o. 3 days (Protocol A), immature s.c. 3 days (Protocol B), adult ovx s.c. 3 days (Protocol C) and adult ovx s.c. 7 days (Protocol CX). To compare mouse ovx p.o. 7 day data with rat ovx s.c. 7 day data, here the sensitivity difference due to the route of exposure is estimated by comparing the response of immature p.o. 3 day and immature s.c. 3 day, i.e. Protocol A and Protocol B. Data from three labs are shown in Figure 9.

Fig. 9

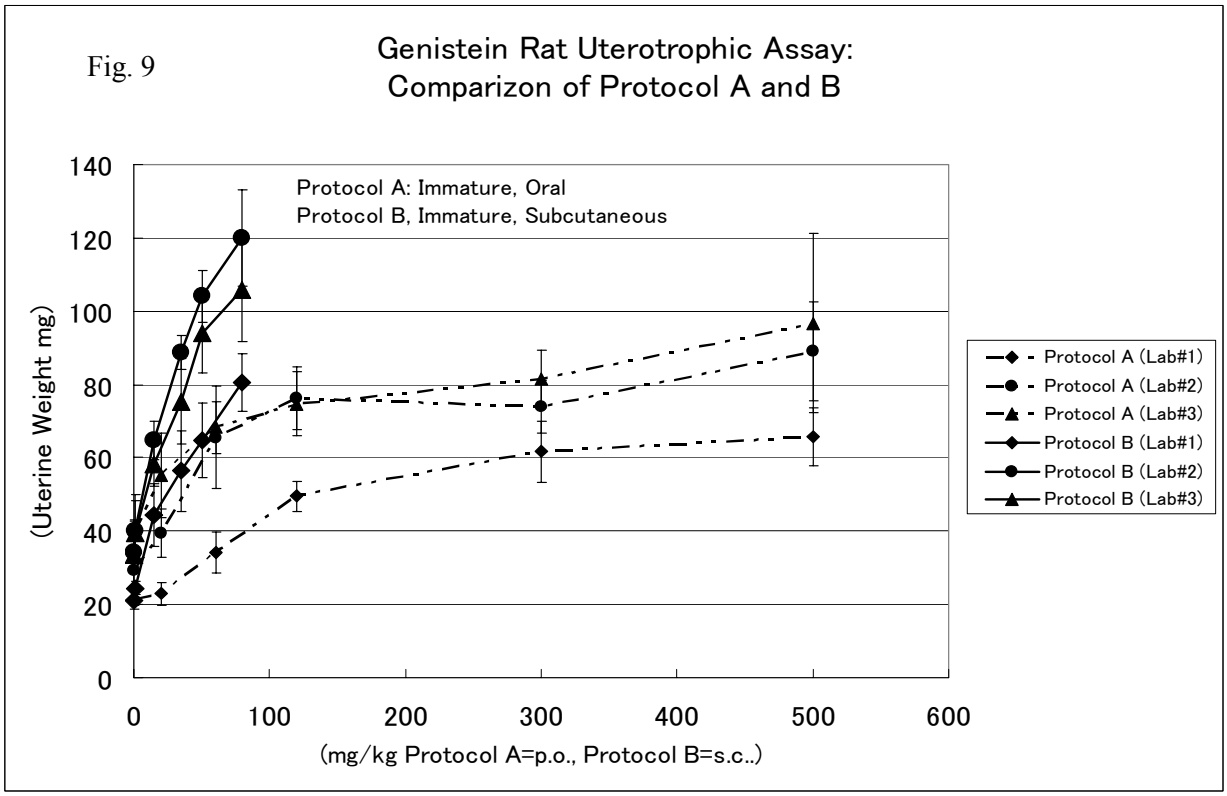


Figure 10 is the same graph in log scale, and Figure 11 is the log regression curve of those six dose-response curves to indicate the overall sensitivity difference between oral and subcutaneous route of exposure to genistein in three labs. The approximate difference was 10 fold in average indicating that the s.c. route is 10 fold more sensitive compared to the oral route in rat immature protocol.

Fig. 10

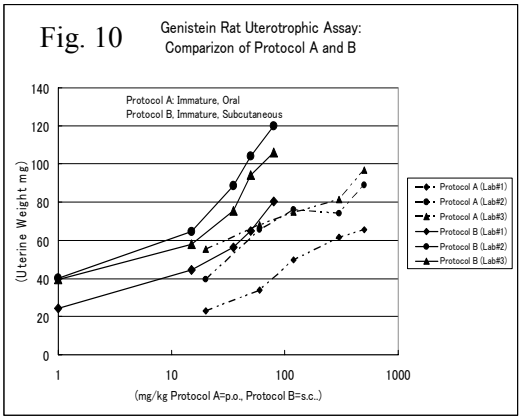


Fig. 11

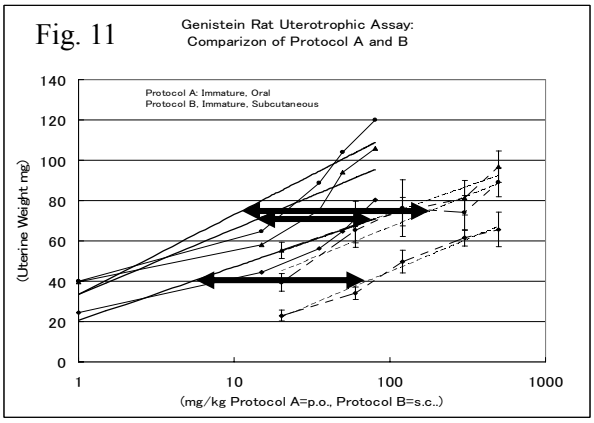
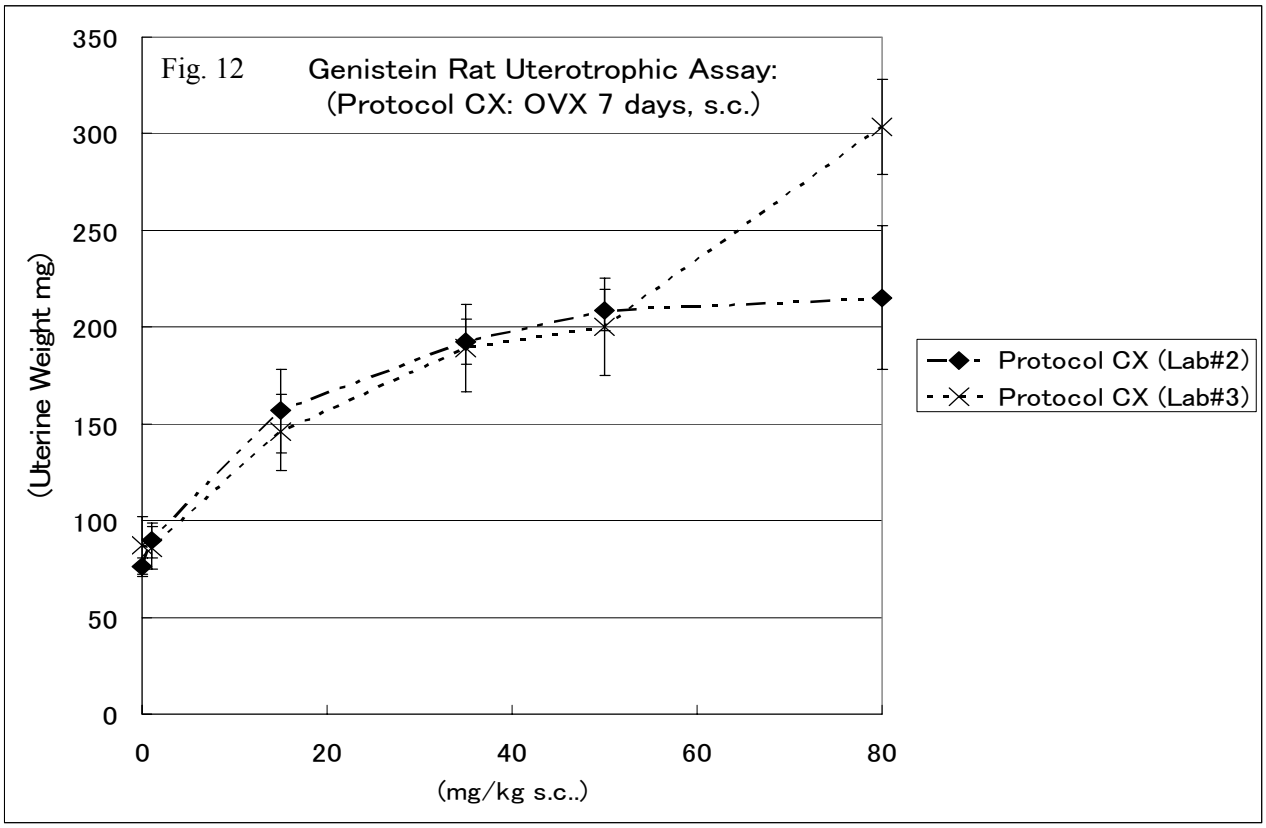
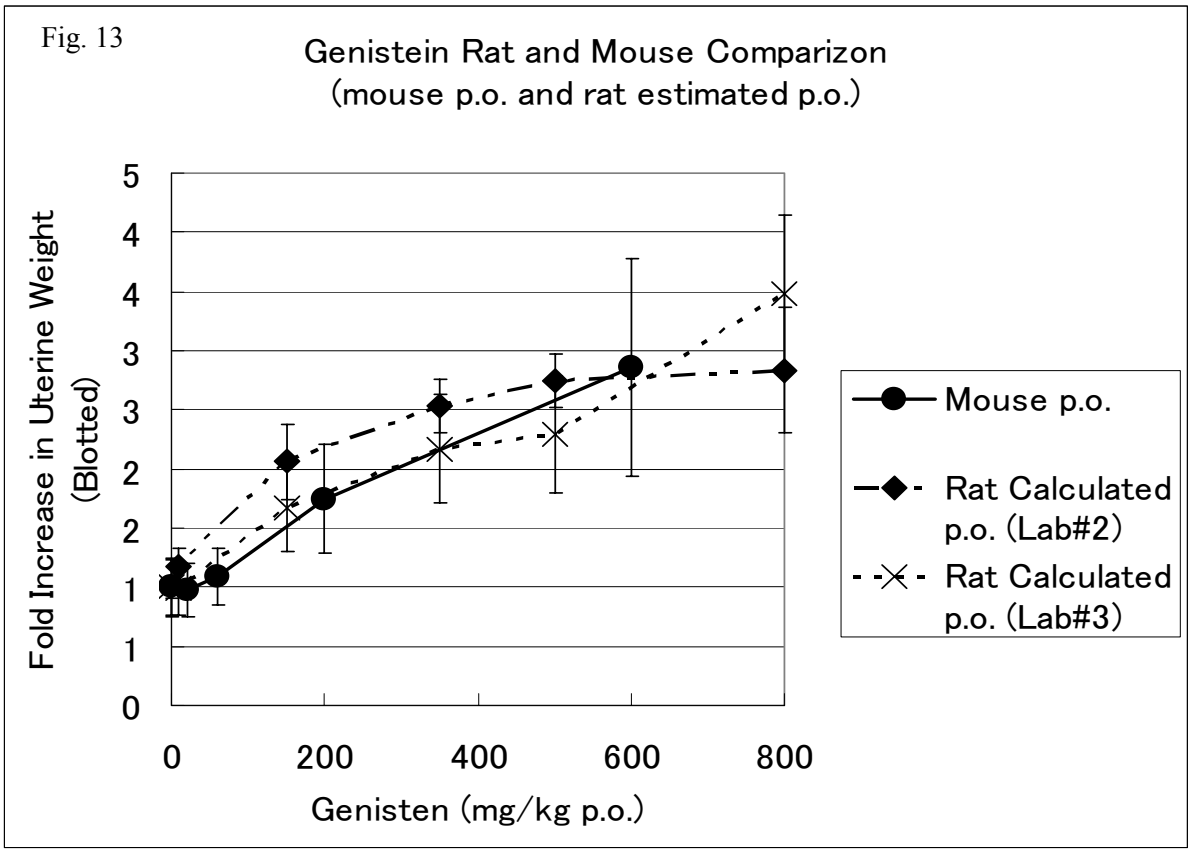


Figure 12 shows the genistein data by rat ovx 7 day subcutaneous protocol (Protocol CX). It is expected from the immature uterotrophic assays mentioned above, and from the literatures and our experiences in both rat and mouse uterotrphic assay (data not shown) that the sensitivity of rat ovx 7 day exposure protocol will also show 10 fold difference depending on the route of exposure, that is oral and subcutaneous.



Under this assumption, mouse ovx p.o. 7 day data on Genisten is compared to presumptive rat data calculated from s.c. data by applying 10 fold sensitivity difference. The result is shown in Figure 13.

Fig. 13



The result indicates that the ovariectomized mouse and rat would respond virtually identical to Genistein given orally for 7 days.

ii. C.V. values compared

The coefficient of variance (C.V.) of the data shown in this document is listed in table 1.

Table 1.

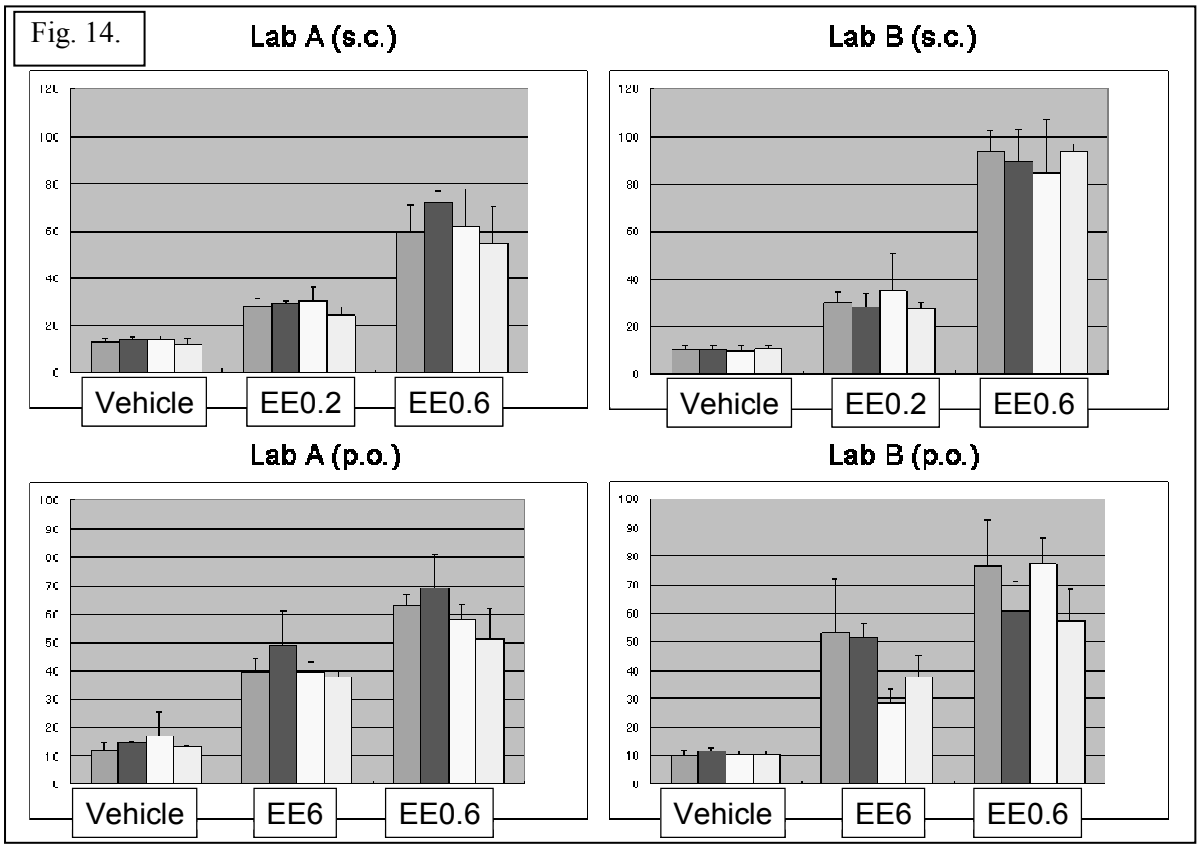
	Treatment	Chemical	dose	unit	route	exposure (days)	C.V. (%)	n
Figure 1	Mouse (B6) Ovx	17-beta-estradiol	0 (vehicle)	microg/kg	s.c.	7	7.8	3
	Mouse (B6) Ovx	17-beta-estradiol	1	microg/kg	s.c.	7	16.5	3
	Mouse (B6) Ovx	17-beta-estradiol	5	microg/kg	s.c.	7	4.9	3
	Mouse (B6) Ovx	17-beta-estradiol	0 (vehicle)	microg/kg	s.c.	14	20.5	3
	Mouse (B6) Ovx	17-beta-estradiol	1	microg/kg	s.c.	14	15.8	3
	Mouse (B6) Ovx	17-beta-estradiol	5	microg/kg	s.c.	14	7.3	3
Figure 2	Mouse (B6) Ovx	17-beta-estradiol	0 (vehicle)	microg/kg	s.c.	1	13.6	3
	Mouse (B6) Ovx	17-beta-estradiol	0.25	microg/kg	s.c.	1	14.4	2
	Mouse (B6) Ovx	17-beta-estradiol	1	microg/kg	s.c.	1	3.7	3
	Mouse (B6) Ovx	17-beta-estradiol	5	microg/kg	s.c.	1	7.8	3
	Mouse (B6) Ovx	17-beta-estradiol	0 (vehicle)	microg/kg	s.c.	3	1.5	2
	Mouse (B6) Ovx	17-beta-estradiol	0.25	microg/kg	s.c.	3	7.4	2
	Mouse (B6) Ovx	17-beta-estradiol	1	microg/kg	s.c.	3	0.3	2
	Mouse (B6) Ovx	17-beta-estradiol	5	microg/kg	s.c.	3	8.8	3
	Mouse (B6) Ovx	17-beta-estradiol	0 (vehicle)	microg/kg	s.c.	5	10.9	3
	Mouse (B6) Ovx	17-beta-estradiol	0.25	microg/kg	s.c.	5	5.8	3
	Mouse (B6) Ovx	17-beta-estradiol	1	microg/kg	s.c.	5	21.8	2
	Mouse (B6) Ovx	17-beta-estradiol	5	microg/kg	s.c.	5	8.1	3
	Mouse (B6) Ovx	17-beta-estradiol	0 (vehicle)	microg/kg	s.c.	7	6.3	3
	Mouse (B6) Ovx	17-beta-estradiol	0.25	microg/kg	s.c.	7	10.2	3
	Mouse (B6) Ovx	17-beta-estradiol	1	microg/kg	s.c.	7	24.2	3
	Mouse (B6) Ovx	17-beta-estradiol	5	microg/kg	s.c.	7	4.6	3
	Mouse (B6) Ovx	17-beta-estradiol	0 (vehicle)	microg/kg	s.c.	14	14.5	3
	Mouse (B6) Ovx	17-beta-estradiol	0.25	microg/kg	s.c.	14	8.4	3
	Mouse (B6) Ovx	17-beta-estradiol	1	microg/kg	s.c.	14	12.6	3
	Mouse (B6) Ovx	17-beta-estradiol	5	microg/kg	s.c.	14	10.2	3
Figure 5	Mouse (B6) Ovx	BisphenolA	0 (vehicle)	mg/kg	s.c.	7	12.0	7
	Mouse (B6) Ovx	BisphenolA	50	mg/kg	s.c.	7	12.4	6
	Mouse (B6) Ovx	BisphenolA	100	mg/kg	s.c.	7	11.0	6
	Mouse (B6) Ovx	BisphenolA	300	mg/kg	s.c.	7	7.8	6
	Mouse (B6) Ovx	BisphenolA	600	mg/kg	s.c.	7	12.0	6
	Mouse (B6) Ovx	17-beta-estradiol	1	microg/kg	s.c.	7	23.0	6
Figure 6	Mouse (B6) Ovx	BisphenolA	0 (vehicle)	mg/kg	s.c.	7	14.8	6
	Mouse (B6) Ovx	BisphenolA	10	mg/kg	s.c.	7	14.1	6
	Mouse (B6) Ovx	BisphenolA	30	mg/kg	s.c.	7	8.5	6
	Mouse (B6) Ovx	BisphenolA	100	mg/kg	s.c.	7	9.8	6
	Mouse (B6) Ovx	BisphenolA	300	mg/kg	s.c.	7	42.4	6
Figure 8	Mouse (B6) Ovx	Genistein	0 (vehicle)	mg/kg	s.c.	7	16.4	6
	Mouse (B6) Ovx	Genistein	20	mg/kg	s.c.	7	16.4	6
	Mouse (B6) Ovx	Genistein	60	mg/kg	s.c.	7	14.1	6
	Mouse (B6) Ovx	Genistein	200	mg/kg	s.c.	7	20.5	6
	Mouse (B6) Ovx	Genistein	600	mg/kg	s.c.	7	27.5	6
						Average of C.V.	12.6	
						Standard deviation of C.V.	7.7	
	Treatment	Chemical	dose	unit	route	exposure (days)	C.V. (%)	n
Figure 3	Rat (SD (IGS) Ovx	17-beta-estradiol	0 (vehicle)	microg/kg	s.c.	3	5.1	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	0.1	microg/kg	s.c.	3	12.0	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	0.3	microg/kg	s.c.	3	8.2	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	0.7	microg/kg	s.c.	3	10.0	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	1	microg/kg	s.c.	3	8.6	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	0 (vehicle)	microg/kg	s.c.	7	9.9	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	0.1	microg/kg	s.c.	7	18.2	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	0.3	microg/kg	s.c.	7	7.8	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	0.7	microg/kg	s.c.	7	2.1	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	1	microg/kg	s.c.	7	2.2	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	0 (vehicle)	microg/kg	s.c.	14	7.4	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	0.1	microg/kg	s.c.	14	10.0	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	0.3	microg/kg	s.c.	14	16.1	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	0.7	microg/kg	s.c.	14	5.6	3
	Rat (SD (IGS) Ovx	17-beta-estradiol	1	microg/kg	s.c.	14	10.7	3
Figure 7	Rat 1 (SD (IGS) Ovx	BisphenolA	0 (vehicle)	mg/kg	s.c.	7	3.4	6
	Rat 1 (SD (IGS) Ovx	BisphenolA	10	mg/kg	s.c.	7	8.7	6
	Rat 1 (SD (IGS) Ovx	BisphenolA	100	mg/kg	s.c.	7	2.6	6
	Rat 1 (SD (IGS) Ovx	BisphenolA	300	mg/kg	s.c.	7	12.8	6
	Rat 1 (SD (IGS) Ovx	BisphenolA	600	mg/kg	s.c.	7	12.1	6
	Rat 1 (SD (IGS) Ovx	BisphenolA	800	mg/kg	s.c.	7	7.3	6
	Rat 2 (SD (IGS) Ovx	BisphenolA	0 (vehicle)	mg/kg	s.c.	7	15.7	6
	Rat 2 (SD (IGS) Ovx	BisphenolA	10	mg/kg	s.c.	7	16.5	6
	Rat 2 (SD (IGS) Ovx	BisphenolA	100	mg/kg	s.c.	7	6.3	6
	Rat 2 (SD (IGS) Ovx	BisphenolA	300	mg/kg	s.c.	7	6.0	6
	Rat 2 (SD (IGS) Ovx	BisphenolA	600	mg/kg	s.c.	7	14.8	6
	Rat 2 (SD (IGS) Ovx	BisphenolA	800	mg/kg	s.c.	7	13.6	6
Figure 12	Rat Lab#2 SD (IGS)	Genistein	0 (vehicle)	mg/kg	s.c.	7	6.6	6
	Rat Lab#2 SD (IGS)	Genistein	1	mg/kg	s.c.	7	10.2	6
	Rat Lab#2 SD (IGS)	Genistein	15	mg/kg	s.c.	7	13.9	6
	Rat Lab#2 SD (IGS)	Genistein	35	mg/kg	s.c.	7	6.1	6
	Rat Lab#2 SD (IGS)	Genistein	50	mg/kg	s.c.	7	5.1	6
	Rat Lab#2 SD (IGS)	Genistein	80	mg/kg	s.c.	7	17.3	6
	Rat Lab#3 SD (IGS)	Genistein	0 (vehicle)	mg/kg	s.c.	7	17.4	6
	Rat Lab#3 SD (IGS)	Genistein	1	mg/kg	s.c.	7	12.9	6
	Rat Lab#3 SD (IGS)	Genistein	15	mg/kg	s.c.	7	13.5	6
	Rat Lab#3 SD (IGS)	Genistein	35	mg/kg	s.c.	7	11.9	6
	Rat Lab#3 SD (IGS)	Genistein	50	mg/kg	s.c.	7	12.6	6
	Rat Lab#3 SD (IGS)	Genistein	80	mg/kg	s.c.	7	8.0	6
						Average of C.V.	10.0	
						Standard deviation of C.V.	4.5	

The C.V. value of mouse was about 12.6% and that of the rat was 10.0%. There is a slight tendency that mouse data has larger C.V. value, especially at higher dosage. As a whole, the C.V. values of mouse and rat uterotrophic assay (ovx 7 day s.c. protocol) are considered to be equivalent, and therefore the overall sensitivity was considered to be comparable, especially when the smaller size of mouse uterus is considered.

iii. Inter-laboratory variation in Mouse Uterotrophic assay

Two laboratories, who had participated in OECD Rat Uterotropic Assay Validation activities (Phase I and II), have been conducting, under MHLW contract, Mouse uterotrophic assay using ovx adult female C57BL/6 mice with 7 day exposure via oral and s.c. route. Although these two laboratories do not conduct assay on a same chemical to avoid unnecessary overlap, a vehicle control and two ethinyl estradiol control groups, that is for agonist (given via same route to the test chemical, i.e. either s.c. or p.o.) and antagonist detection (always given via s.c. route) are commonly conducted. Here, these data, that are available for four experiments each from both laboratories, are compared for the evaluation of inter-laboratory variation. In Figure 14, (s.c.) indicates the data are from subcutaneous route protocol and (p.o.) indicates oral route protocol. In s.c. protocol, EE0.2 and EE0.6 indicate that 0.2 and 0.6 microg/kg of ethinyl estradiol were given s.c. In p.o. protocol, EE6 indicates that 6 microg/kg of ethinyl estradiol were given orally and EE0.6 indicates that 0.6 microg/kg of ethinyl estradiol were given subcutaneously. Four columns represent four experiments carried out separately. Vertical axes are the blotted uterine weight in mgs. In conclusion, the blotted uterine weights obtained by both laboratories (Lab A and Lab B, (s.c.) and (p.o.)) are within good concordance taking different breeder of mouse, diet, water and animal facility into consideration.

Fig. 14.



Overall Conclusion

Very small numbers of chemicals are tested by both species to avoid unnecessary overlaps and use of experimental animals. Those few chemicals tested by both species include a strong estrogen such as 17-beta estradiol / ethinyl estradiol and weak estrogens such as Bisphenol A and Genistein. Interlaboratory variation was checked between two laboratories on vehicle control and two ethinyl estradiol control groups. As a whole, the sensitivity and specificity of the response as well as robustness of the Mouse uterotrophic assay were comparable to Rat uterotrophic assay.

Reference

Kanno J, Onyon L, Peddada S, Ashby J, Jacob E, Owens W. The OECD program to validate the rat uterotrophic bioassay. Phase 2: dose-response studies. Environ Health Perspect. 2003 Sep;111(12):1530-49.